Introduction

There are several biochemical pathways that can lead to cancerogenesis, one of which involves DNA damage induced by exogenous carcinogens or by endogenous metabolic processes. The double-strand break DNA repair pathway, including XRCC2 gene, is implicated in maintaining genomic stability and therefore could affect cancer risk. Common genetic polymorphisms in DNA repair genes might affect protein function and thus the capacity of repair DNA damage, which in turn could lead to genetic instability [1,2].

Single nucleotide polymorphisms (SNPs) were found in nearly all human DNA repair genes that have been investigated so far, and some of them were shown to modulate levels of DNA damage, individual DNA repair capacity and cancer risk. Among them, polymorphisms of X-ray repair cross complementing group 2 (XRCC2) have been studied extensively [3-5].

The XRCC2 gene, located at 7q36.1, is an essential part of the homologous recombination repair pathway and a functional candidate for involvement in cancer progression. Common variants within XRCC2, including Arg188His polymorphism, have been identified as potential cancer susceptibility loci in recent studies, although association results are controversial. The Arg188His polymorphism has been proposed to be a genetic modifier for pancreatic cancer and was associated with an increased risk of breast, laryngeal and oral cancers [6-9].

Recently, a large number of studies have attempted to identify the association between this polymorphism and other human cancers such as ovarian cancer, thyroid cancer, and colorectal cancer. However, results of these studies still remain inconsistent rather than conclusive [4,10].

XRCC2 -41657C/T polymorphism was associated with the risks of many cancers, such as esophageal squamous cell carcinoma (ESCC), gastric cardia adenocarcinoma (GCA), smoking- drinking-related laryngeal cancer or ovarian cancer [8,11,12].

Some reports provide the proof that the XRCC2 41657C/4234C and 41657T/4234G haplotypes were related to increased risk of GCA [11]. To find reports that directly link SNP 4234G/C in DNA repair genes with endometrial cancer risk, the current study failed to show the association between the 4234G/C XRCC2 polymorphism and clinical data of patients with endometrial cancer.
gene XRCC2 with endometrial cancer (EC) incidence poses a serious difficulty. Yet, to our knowledge, there are no reports that assess the effect of XRCC2 4234G/C polymorphism on the risk of EC.

The purpose of this study was to evaluate the clinical significance of the 4234G/C (rs3218384) XRCC2 gene polymorphism in patients with EC.

Materials and Methods

Patients

The study included 808 patients with endometrial cancer. Analysed patients were hospitalized in Department of Surgical Gynaecology and Gynaecologic Oncology, Institute of Polish Mothers Memorial Hospital, between 2000-2013. All patients involved into the study were Caucasians. The age of patients ranged in from 50 to 85 years (the mean age 59.1 ± 10.12). 824 individuals treated in the parallel period for uterine fibroids constituted the control group (age range 49–83, mean age 54.2±11.16).

Only patients with confirmed pathology diagnosis of endometrial carcinoma were included into the study. The associations of the analysed genotypes and clinical data at diagnosis have been evaluated. The following demographic and clinical data have been analysed: age, histological grade, body mass index, number of pregnancies, uterine bleeding, endometrial ultrasound transvaginal, diabetes and hypertension.

All the diagnosed tumours were graded by criteria of the International Federation of Gynaecology and Obstetrics (FIGO). Histological grade was based on the degree of glandular differentiation, and tumors were graded as: G1 (percentage of solid growth in the tumor mass up to 5%); G2 (percentage of solid growth between 6 and 50%); G3 (percentage of solid growth above 50%). Histological typing and grading were done according to the WHO classification. The Local Ethic Committee approved the study and each patient gave a written consent (No 4/2011).

DNA isolation

Formalin-fixed paraffin-embedded (FFPE) endometrial tissue samples were obtained from all analysed patients. Genomic DNA was prepared using QIAamp DNA FFPE Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer instruction.

Determination of XRCC genotype

Polymorphism 4234G/C of the XRCC2 gene was determined by PCR-RFLP (restriction fragment-length polymorphism) [11]. The primers: forward 5’- GTGCGCAGGGCAGGCGGCTGGGAC-3’ and reverse 5’- GCGCCGCCCAAGCTGCTCCCAATC-3’ were used to amplify the region containing the 4234G/C XRCC2 variant. PCR amplification was performed in a final volume of 25 μl containing 100 ng of DNA, 1.5 mM of MgCl₂, 10mM Tris-HCl (pH 8.3), 50 mM KCl, 0.2 mM of dNTP, each primer at 1.0 μM and 1.0 unit of Taq polymerase (Takara, Japan) in PTC-100 TM (MJ Research, INC, Waltham, MA, USA) Thermocycler. PCR cycle conditions were the following: 95°C for 45s, 70°C for 45s and 72°C for 60s, repeated in 35 cycles. PCR products were electrophoresed in a 2% agarose gel and visualized by ethidium bromide staining. The cleavage with MluI (New England BioLabs, Frankfurt am Main, Germany) produced fragments of 251, 251/230/21 and 230/21 bp corresponding to the G/G, G/C and C/C genotypes of the XRCC2 gene, respectively (21 bp have been out of the gel) (Figure 1).

Statistics

To determine differences between groups, standard Chi square test (χ²) or Fisher’s exact tests were used. Clinical significance of analyzed polymorphism was determined using logistic regression analysis and presented in tables as odds ratios (OR) with their 95% confidence intervals. The deviations from Hardy-Weinberg equilibrium were analyzed using the χ² test. Differences with a p value less than 0.05 were considered significantly.

Results

The genotype distributions of analyzed the 4234G/C XRCC2 gene polymorphism are summarized in Table 1. All allele distributions were consistent with Hardy–Weinberg equilibrium. The distribution of gene variants was similar between all cases and controls, the maximal difference of 3.5% was found for the XRCC2 polymorphism with the homozygous C/C genotype being less frequent in cases (19.8%) than in controls (23.3%). This difference was, however, not significant (p > 0.05).

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The potential relationship between XRCC2 genotype distribution and clinical data of EC patients was investigated. However, the current study failed to show the correlation between analysed gene polymorphism and histological grading. XRCC2 gene polymorphism was also unrelated to the patient’s age, body mass index, number of pregnancies, uterine bleeding, endometrial ultrasound transvaginal, diabetes and hypertension (Table 2).

Discussion

Several studies have investigated the possible role of XRCC2 gene polymorphisms in neoplastic diseases. The 188His allele and 188His/His homozygous variant of XRCC2 Arg188His polymorphism were associated with increased risk of breast cancer in Polish population [7].

Other authors observed that XRCC2 polymorphisms might play important role in colorectal cancer tumorigenesis, conferring susceptibility to rectal tumors [13].

This polymorphism may be also associated with increased risk of gastric and pharyngeal cancers [14,15].

However, in the recently published metaanalysis, statistical significant association between XRCC2 Arg188His polymorphisms and neoplastic diseases was found in ovarian cancer but not in other studied cancers [16].

According to the recently published analysis, the XRCC2 -41657C/T polymorphism might be also a risk factor for ESCC, GCA, laryngeal and ovarian cancer [8,11,12].

The 41657C/4234C and 41657T/4234G haplotypes of this gene may be responsible for significantly increased risk of gastric adenocarcinoma.

Until now, there have been no studies that analyse the association between 4234G/C polymorphism of the XRCC2 gene and EC.

Table 2: Relationship between the 4234G/C XRCC2 polymorphism and clinical data of patients with endometrial cancer.

| Group                      | C(*) allele (G/C and C/C) n=504 |  | C(*) allele (G/G) n=304 |  |
|----------------------------|---------------------------------|---|------------------------|---|
| Age                       | ns                              |  | 144 (47.4%) 160 (52.6%) |  |
| Number of pregnancy       |                                 |  | 136 (44.7%). 168 (55.3%) |  |
| Body mass index (BMI)     |                                 |  | 152 (50%). 152 (50%)    |  |
| FIGO grade                |                                 |  | 216 (71.1%). 88 (28.9%) |  |
| FIGO stage                |                                 |  | 96 (31.6%). 208 (68.4%) |  |
| Uterine bleeding           |                                 |  | 216 (71.1%). 88 (28.9%) |  |
| Diabetes mellitus         |                                 |  | 160 (52.6%). 144 (47.4%) |  |
| Hypertension              |                                 |  | 136 (44.7%). 160 (52.6%) |  |
| Endometrial ultrasound transvaginal - TVU |  | 208 (41.3%). 160 (52.6%) |  |
| NS                        |                                 |  | 144 (47.4%). 160 (52.6%) |  |
| NS                        |                                 |  | 216 (71.1%). 88 (28.9%) |  |

Because a proper functioning of the XRCC2 gene is important for the genomic stability, its alternations may be associated with higher cancer susceptibility.

In the current study, the XRCC2 4234G/C genotype distribution was similar in patients with endometrial carcinoma and control group. According to our data, the analysed polymorphisms were also unrelated to the histologic grade, age, number of pregnancies, body mass index, uterine bleeding, endometrial ultrasound transvaginal, diabetes and hypertension.

To our best knowledge, only a few authors investigated the genetic variants of XRCC2 gene and the risk of developing endometrial cancer [17,18].

Earlier, Han et al., demonstrated that the heterozygous and homozygous variant alleles of XRCC2 Arg188His are not associated with risk of endometrial cancer [17].

No significant associations were observed between the Arg188His genotype and endometrial cancer in Polish population [18].

This is the first article of XRCC2 4234G/C polymorphism and endometrial cancer risk. A limitation of the study was the relatively small population of participants. The sample for the present study comprised of 808 EC patients. This sample is only a very small proportion of the entire population of endometrial cancer women in the country. Therefore the obtained results cannot be considered as definitive and require further, more extensive evaluations, performed on bigger groups of patients.

In conclusion, current evidence did not suggest that analysed XRCC2 polymorphism was directly associated with endometrial cancer risk. Our study failed to show any correlation between 4234G/C polymorphism and clinical data of endometrial cancer patients. Our results should be explained with some caution and be re-evaluated in the future when more studies with larger sample size are conducted.

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