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Research Article

Lack of Association between the 4234G/C X-Ray Repair Cross-Complementing 2 (*XRCC2*) Gene Polymorphism and the Risk of Endometrial Cancer among Polish Population

Abstract

Objective: One of the major causes of carcinogenesis is loss of genome stability. The double-strand break DNA repair pathway, including X-ray repair cross complementing group 2 (*XRCC2)* gene, is implicated in maintenance integrity of genome and therefore could affect endometrial cancer (EC) risk. The purpose of this study was to evaluate the clinical significance of the *XRCC2* 4234G/C (rs3218384) gene single nucleotide polymorphism (SNP) in endometrial cancer patients.

Material and Methods: The study included 1632 patients: 808 with endometrial cancer and 824 healthy controls. *XRCC2* 4234G/C (rs3218384) polymorphism was genotyped by the PCR-RFLP (restriction fragment-length polymorphism) method. The associations of the analysed genotypes and clinical data at diagnosis have been evaluated.

Results: The frequencies of genotype of the 4234G/C *XRCC2* polymorphism did not differ significantly between patients and controls. The current study failed to show the correlation between *XRCC2* genotypes and histological grading. Analyzed polymorphism was also unrelated to the patient age, body mass index, number of pregnancies, uterine bleeding, endometrial ultrasound transvaginal, diabetes and hypertension.

Conclusion: This is the first article of 4234G/C polymorphism in *XRCC2* gene and EC risk. The current study failed to show the association between the 4234G/C *XRCC2* polymorphism and clinical data of patients with endometrial cancer.

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

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 54.24±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'- GTGCGCACGCGCGCGGGTGGAC-3' and reverse 5'- GCGCCGCCCCAAGCCTCCCAATC-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 $MgCl_2$, 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 (χ^2) 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 χ^2 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).

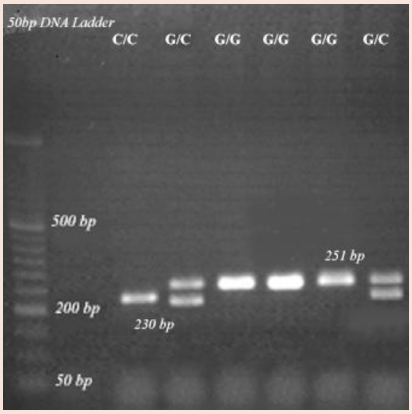

Figure 1: MluI RFLP for 4234G/C XRCC2 polymorphism of endometrial carcinoma cases. Figure shows the typical bands for G/G homozygote variant, G/C heterozygote variant, G/G homozygote variant and 50 bp DNA Ladder (Bioron Gmbh, Ludwigshafen, Germany).

Table 1: Distribution of -4234G/C *XRCC2* genotype in patients (n=808) and control group (n=824).

	Patients		Controls		OR (95% CI)[a]	p[b]
XRCC2 4234G/C	number	(%)	number	(%)		
G/G	304	37,6	304	36.9	1.00 Ref.	
G/C	344	42,6	328	39.8	1.05 (0.84-1.31)	0.708
C/C	160	19,8	192	23.3	0.83 (0.64-1.08)	0.196
G	952v	58.9	936	56.8	1.00 Ref.	
C	664	41.1	712	43.2	0.91 (0.79-1.05)	0.235

[a]Crude odds ratio (OR), 95 % CI = confidence interval at 95%, [b]Chi square

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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 cardia adenocarcinoma.

Until this moment, 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	*p*	C(-) allele (G/G) n=304	*p*
Age	< 59 ≥59	232 (46%) 272 (54%)	NS	144 (47.4%) 160 (52.6%)	NS
Number of pregnancy	1 2-3	240 (47.6%) 264 (52.4%)	NS	136 (44.7%) 168 (55.3%)	NS
Body mass index (BMI)	24-29,99 >30	216 (42.8%) 288 (57.2%)	NS	152 (50%) 152 (50%)	NS
FIGO grade	G1 G2+G3	312 (61.9%) 192 (38.1%)	NS	216 (71.1%) 88 (28.9%)	NS
FIGO stage	SI SII+SIII	224 (44.4%) 296 (55.6%)	NS	96 (31.6%) 208 (68.4%)	NS
Uterine bleeding	present absent	360 (71.4%) 144 (28.6%)	NS	216 (71.1%) 88 (28.9%)	NS
Diabetes mellitius	present absent	248 (49.2%) 256 (50.8%)	NS	160 (52.6%) 144 (47.4%)	NS
Hypertension	present absent	136 (26.9%) 368 (73.1%)	NS	80 (26.3%) 224 (73.7%)	NS
Endometrial ultrasound transvaginal - TVU	> 5 mm < 5 mm	208 (41.3%) 296 (58.7%)	NS	144 (47.4%) 160 (52.6%)	NS

NS – not statistically significant.

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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