

ORIGINAL PAPER

ASSOCIATION BETWEEN -41657C/T SINGLE NUCLEOTIDE POLYMORPHISM OF DNA REPAIR GENE XRCC2 AND ENDOMETRIAL CANCER RISK IN POLISH WOMEN

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Aim of the study: The *XRCC2* gene plays a crucial role in double-strand DNA break repair by homologous recombination. Current literature provides clear evidence that *XRCC2* polymorphisms may be associated with the development of certain types of cancer; however, still little is known about their association with endometrial cancer (EC).

Material and methods: The single nucleotide polymorphism (SNP) -41657C/T (rs718282) of the *XRCC2* gene was investigated by PCR-RFLP in 304 patients with EC and in 200 age- and sex-matched non-cancer controls.

Results: The analysis revealed a relationship between *XRCC2* -41657C/T polymorphism and the incidence of EC. Endometrial cancer patients showed overrepresentation of the T allele of the SNP. The T/T homozygous variant increased the cancer risk. There were no significant differences between the distribution of *XRCC2* -41657C/T genotypes in the subgroups according to histological grade.

Conclusions: This is the first study that links the SNP -41657C/T (rs718282) of the *XRCC2* gene with EC in Polish women. The results support the hypothesis that this polymorphism may be positively correlated with the incidence of EC.

Key words: endometrial cancer, *XRCC2* gene, genetic polymorphism.

Introduction

Endometrial cancer (EC) is one of the most common female malignancies in developed countries [1, 2]. Age, hormonal status, diabetes, hypertension, obesity, sterility, low parity, late menopause and genetic factors (including mutations in *TP53* and *P16* and single nucleotide polymorphisms) [3, 4] are the classical risk factors of this disease. Despite advanced diag-

nostic and therapeutic protocols, EC still carries high morbidity and mortality. As effective clinical screening has not been found yet, the genetic approach seems to be appropriate to identify high-risk subjects. Therefore, there is a clear need to identify new tools that could provide risk and predictive factors of EC.

XRCC2 (X-ray repair cross-complementing group 2) with *RAD51* (RecA homolog, *E. coli*) (*S. cerevisiae*), *XRCC3* (X-ray repair cross-complementing group 3),

Table I. Characteristics of endometrial cancer patients (n = 304).

| CHARACTERISTICS | NUMBER OF CASES (%) |
|---|---------------------|
| Age (years) | |
| median | 69 |
| range | 51-84 |
| BMI (body mass index) (kg/m²) | |
| 24-29.99 | 162 (53) |
| > 30 | 142 (47) |
| Number of pregnancies | |
| 1 | 96 (32) |
| 2-3 | 208 (68) |
| > 4 | 0 |
| Use of hormone replacement therapy | |
| yes | 192 (63) |
| no | 112 (37) |
| Grading | |
| I | 166 (54) |
| II | 68 (22) |
| III | 70 (23) |
| Menopause status | |
| postmenopausal | 304 (100) |
| Uterine bleeding | |
| yes | 200 (65) |
| no | 104 (35) |
| Endometrial transvaginal ultrasound | |
| > 5 mm | 230 (75) |
| < 5 mm | 74 (25) |
| Diabetes mellitus | |
| yes | 56 (18) |
| no | 248 (82) |
| Hypertension | |
| yes | 160 (53) |
| no | 144 (47) |

BRCA1 (breast cancer-1), BRCA2 (breast cancer-2) and other DNA repair proteins are involved in the homologous recombination and repair of double-strand DNA breaks (DSBs) and DNA cross-links, as well as in the maintenance of chromosome stability.

The *XRCC2* gene (7q36.1) is an essential part of the homologous recombination repair pathway and a functional candidate for involvement in cancer progression [5]. Moreover, it has been proven that poly-

morphisms in *XRCC2* may modify individual susceptibility to various types of cancer [6-13].

However, according to up-to-date literature, no significant association has been observed yet between the Arg188His genotype of *XRCC2* and endometrial cancer [14-16]. Nevertheless, this study was performed on a relatively small group; thus the results cannot be considered representative for the target population. Further large group studies are warranted for more representative outcomes.

Some reports provide proof that the *XRCC2* -41657C/T genotype was related to increased risk of oesophageal squamous cell carcinoma (ESCC), gastric cardia adenocarcinoma (GCA) and of smoking- and drinking-related laryngeal cancer [11, 17].

Recent literature provides limited data on a direct association of the SNP -41657C/T (rs718282) in the DNA repair gene *XRCC2* and EC. This encouraged us to seek a link between EC development and the -41657C/T (rs718282) polymorphism in the *XRCC2* gene.

Material and methods

Patients

Three hundred and four patients with histologically proven diagnosis of EC were included in the study (Table I). Paraffin-embedded tumour tissue was obtained from postmenopausal women with EC treated in the Department of Gynaecological Surgery, Institute of Polish Mother's Memorial Hospital (Lodz, Poland) between 2000 and 2014. All tumours were graded according to the International Federation of Gynaecology and Obstetrics (FIGO) criteria [18]. DNA extracted from normal endometrial tissue obtained from patients who had undergone hysterectomy for intramural leiomyomas (n = 200) served as controls. The Local Ethic Committee approved the study and each patient provided written consent (No. 4/2011).

Endometrial tissue samples (cancerous and non-cancerous) were fixed routinely in formaldehyde, embedded in paraffin, cut into thin slices and stained with haematoxylin/eosin for pathological examination. DNA for analysis was obtained from archival pathological paraffin-embedded both tumour and normal endometrial samples which were deparaffinized in xylene and rehydrated in ethanol and in distilled water. In order to ensure that the chosen histological material was representative for cancerous and non-cancerous tissue, each sample qualified for DNA extraction was initially checked by a pathologist (Department of Pathology, Institute of Polish Mother's Memorial Hospital, Lodz, Poland). DNA was extracted from the material using the commercially available QIAamp DNA Kit (Qiagen GmbH,

Hilden, Germany) according to the manufacturer's instructions.

Genotype determination

The PCR-restriction fragment length polymorphism method (PCR-RFLP) was used to detect the genotypes of the -41657C/T polymorphism as described above [17].

Polymorphism -41657C/T of the *XRCC2* gene was determined by PCR-RFLP using primers (forward 5'-GGAGGCCGCAATGAGCTGAGATG-3' and reverse 5'-TCGGGAAGCTGAGGTGGGAGGA-3'). The PCR was carried out in a PTC-100 (MJ Research, INC, Waltham, MA, USA) thermal cycler. PCR amplification was performed in the final volume of 25 μ l of reaction mixture, which contained 100 ng of genomic DNA, 0.2 μ mol of each primer (ARK Scientific GmbH Biosystems, Darmstadt, Germany), 2.5 mM of MgCl₂, 1 mM of dNTPs and 1 unit of Taq Polymerase (Qiagen GmbH, Hilden, Germany). PCR cycle conditions were as follows: 95°C for 45 s, 72°C for 45 s and 72°C for 60 s, repeated in 35 cycles. PCR products were electrophoresed in a 2% agarose gel and visualised by ethidium bromide staining. Cleavage with *Mva*I (New England BioLabs, Frankfurt am Main, Germany) produced fragments of 315/59/42, 357/315/59/42 and 357/59 bp corresponding to the C/C, C/T and T/T genotypes of the *XRCC2* gene, respectively (42 and 59 bp have been out of the gel) (Fig. 1).

Statistical analysis

For each polymorphism departure from Hardy-Weinberg equilibrium was assessed by the standard χ^2 test. Genotype frequencies in cases and controls were compared by the χ^2 test. Genotype specific risks were estimated as odds ratios (ORs) with associated 95% intervals (CIs) by unconditional logistic regression. P-values < 0.05 were considered significant. All the statistical analyses were performed using the STATISTICA 6.0 software (StatSoft, Tulsa, Oklahoma, USA).

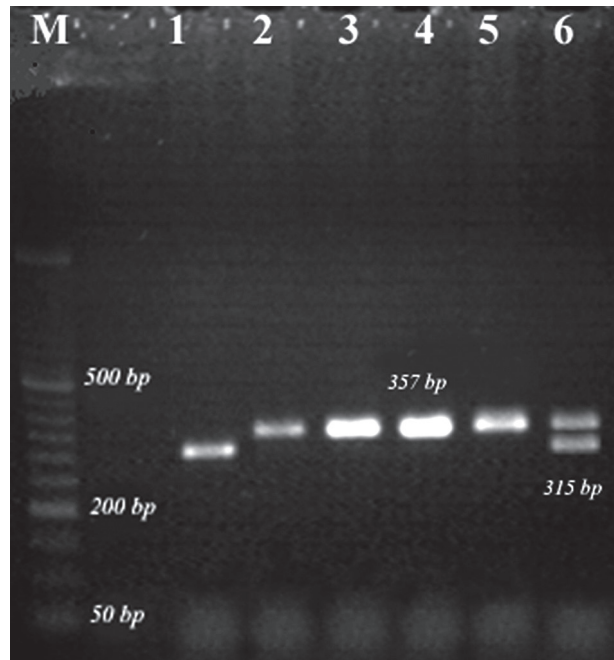


Fig. 1 . Representative PCR-RFLP analyses on 2% agarose gel containing ethidium bromide. The -41657C/T polymorphism of the *XRCC2* gene. Lane M – 50 bp DNA ladder (Fermentas); lanes 1 – the C/C homozygote; lane 2-5 – the T/T homozygote; lane 6 – the C/T heterozygote

Results

All the recruited samples were successfully genotyped for *XRCC2* polymorphisms. Cases and controls were classified into three genotypes of the -41657C/T polymorphism: C/C, C/T and T/T. Table II shows the genotype distribution of the -41657C/T *XRCC2* polymorphism in EC patients and in controls. The table presents significant differences ($p < 0.05$) between the two investigated groups. A stronger correlation was observed for homozygous T/T than the heterozygous C/T variant. The T allele variant of *XRCC2* statistically significantly increased the EC risk ($p < 0.05$). The genotype distribution in cases differed significantly from the one expected from the Hardy-Weinberg equilibrium ($p < 0.05$).

Table II. Allele and genotype frequency and odds ratio (OR) of -41657C/T polymorphism of the *XRCC2* gene in endometrial cancer (n = 304) and controls (n = 200)

| XRCC2 -41657C/T | ENDOMETRIAL CANCER PATIENTS | | CONTROLS | | OR (95% CI) ^a | p ^b |
|--------------------|-----------------------------|-----|----------|-----|--------------------------|--------------------|
| | NUMBER | (%) | NUMBER | (%) | | |
| C/C | 48 | 16 | 48 | 24 | 1.00 Ref. | |
| C/T | 60 | 20 | 96 | 48 | 0.62 (0.37-1.05) | 0.095 |
| T/T | 196 | 64 | 56 | 28 | 3.50 (2.12-5.76) | < 0.0001 |
| C | 156 | 26 | 192 | 48 | 1.00 Ref. | |
| T | 452 | 74 | 208 | 52 | 2.67 (2.04-3.49) | < 0.0001 |

Data in boldface are statistically significant.

^aCrude odds ratio (OR), 95% CI – confidence interval at 95%

^b χ^2

Moreover, we assessed the correlation of histological grading and *XRCC2* polymorphism. Grading in cases ($n = 304$) was as follows: grade I – 166 cases, grade II – 68 cases and grade III – 70 cases (Table I). Grades II and III were analysed jointly for statistical calculations. However, we did not find any association of the *XRCC2* polymorphisms in the cases with cancer progression assessed by EC grading ($p > 0.05$).

Furthermore, our results did not reveal any statistically significant correlation between *XRCC2* -41657C/T polymorphism and the risk factors of endometrial cancer, such as BMI (body mass index), HRT (hormone replacement therapy), uterine bleeding, endometrial transvaginal ultrasound findings, diabetes and hypertension.

Discussion

Besides mutations in proto-oncogenes and suppressor genes, genetic polymorphisms – including SNP – may also play an important role in neoplastic transformation. Our study assessed the role of SNP -41657C/T of the DNA repair gene *XRCC2* in EC risk.

The *XRCC2* gene is highly polymorphic. The involvement of *XRCC2* in DNA repair determines its potential role in maintaining genetic stability, which is obviously disturbed in cancer. Therefore, genetic variability of the *XRCC2* gene in cancer is a promising target for further research. Recent studies have identified common variants within *XRCC2*, including SNP in exon 3 (Arg188His or R188H or rs3218536 or 31479G>A) as potential cancer susceptibility *loci*, although the final conclusions are controversial. The Arg188His polymorphism has been proposed to be a genetic modifier for smoking-related pancreatic cancer and was associated with an increased risk of pharyngeal cancer and oral cancer [19-21]. The 188His allele of this polymorphism may be responsible for significantly increased risk of breast cancer, but not with the risk of bladder cancer, colorectal adenoma, or skin cancer [5, 22-24].

Han *et al.* did not find any statistically significant association between *XRCC2* Arg188His polymorphism and EC [15]. Furthermore, the study of the Polish population suggests that Arg188His genotype is not correlated with EC risk [14].

It has already been proven that *XRCC2* -41657C/T polymorphism is associated with increased risk of oesophageal squamous cell carcinoma (ESCC), gastric cardia adenocarcinoma (GCA) and smoking- and drinking-related laryngeal cancer [11, 16]. However, the functional consequences of -41657C/T *XRCC2* polymorphism in the response to different DNA damaging agents still remains unclear.

Until this moment, there have been no studies that analyse the association between alterations in

this region of the *XRCC2* gene and EC. Due to the crucial role that the *XRCC2* gene plays in maintaining genomic stability, its alterations may be associated with increased cancer risk.

Therefore, we analysed the role of -41657C/T genetic variations in the homologous recombination repair gene and the impact they have on EC risk.

In this study, the PCR-RFLP technique was used to screen 304 endometrial cancer cases for *XRCC2* polymorphisms. A significant difference was found in the incidence of allele distribution among investigated groups. In the present study the incidence of the T allele in cases was higher than in controls (74% vs. 52%, respectively). Moreover, homozygous T/T genotype increased the risk of EC. It is possible that the presence of the T allele remains in some linkage disequilibrium with a distinct, not yet discovered mutation, located outside of the coding region in the *XRCC2* gene, which may be responsible for the *XRCC2* concentration in plasma and cancer development.

In conclusion, this study provides clear evidence of the significance of -41657C/T genotypes in EC risk. Therefore, our results may be important in encouraging further studies on the role of *XRCC2* in EC development.

The authors declare no conflicts of interests.

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