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Association between single nucleotide polymorphisms (SNPs) of *XRCC2* and *XRCC3* homologous recombination repair genes and ovarian cancer in Polish women

Magdalena M. Michalska [a], Dariusz Samulak [a,b], Hanna Romanowicz [c], Filip Jabłoński [c], Beata Smolarz [c,*]

[a] Department of Obstetrics and Gynaecology, Regional Hospital in Kalisz, Poland
[b] Cathedral of Mother's and Child's Health, Poznan University of Medical Sciences, Poznań, Poland,
[c] Laboratory of Cancer Genetics, Department of Pathology, Institute of Polish Mother's Memorial Hospital, Lodz, Poland

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ABSTRACT

The variability, perceived in DNA repair genes, may be of clinical importance for evaluation of the risk of occurrence of a given type of cancer, its prophylactics and therapy. The aim of the present work was to evaluate associations between the risk of ovarian cancer and polymorphisms in the genes, encoding for two key proteins of homologous recombination: *XRCC2* Arg188His (c. 563 G > A; rs3218536) and *XRCC3* Thr241Met (c. 722 C > T; rs861539). The study consisted of 700 patients with ovarian cancer and 700 healthy subjects. Analysis of the gene polymorphisms was performed using PCR-RFLP (restriction length fragment polymorphism). We found a statistically significant increase of the 188His allele frequency (OR = 4.01; 95% CI = 3.40–4.72; $p < .0001$) of *XRCC2* in ovarian cancer compared to healthy controls. There were no differences in the genotype and allele distributions and odds ratios of the *XRCC3* Thr241Met polymorphism between patient and control groups. Association of these genetic polymorphisms with histological grading showed increased *XRCC2* 188Arg/His (OR = 33.0; 95% CI = 14.51–75.05; $p < .0001$) and 188His/His genotypes (OR = 9.37; 95% CI = 4.79–18.32; $p < .0001$) and *XRCC3* 241Thr/Met (OR = 24.28; 95% CI = 12.38–47.61; $p < .0001$) and 241Met/Met genotype frequencies (OR = 17.00; 95% CI = 8.42–34.28; $p < .0001$) in grading 1 (G1) as well as 188His (OR = 2.78; 95% CI = 2.11–3.69; $p < .0001$) and 241Met allele overrepresentation (OR = 2.59; 95% CI = 2.08–3.22; $p < .0001$) in G1 ovarian patients. Finally, with clinical FIGO staging under evaluation, an increase in *XRCC2* 188His/His homozygote and 188Arg/His heterozygote frequencies in staging I (SI) and *XRCC3* Thr/Met heterozygote frequencies in SI was observed. The obtained results indicate that *XRCC2* Arg188His and *XRCC3* Thr241Met polymorphisms may be positively associated with the incidence of ovarian carcinoma in the population of Polish women.

1. Introduction

The system of DNA repair takes part in maintaining the genomic integrity which undergoes changes under exo and endogenous factors. In man, the protein products of genes are directly involved in the repair process, taking part in several repair systems. The repair process usually encompasses two stages: the excision of lesion and the repair synthesis. This is how a repair system acts via base-excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR). Totally converse is the repair system activity by direct lesion reversal, in which there is merely a single-stage process with maintained integrity of the DNA phosphodiester chain and the system of recombination repair.

The repair by recombination enables removal of a number of serious DNA lesions, including double-stranded breaks (DSBs). These breaks may bring about a loss of some chromosomes, causing translocation of genetic material between them. DSBs are repaired by two mechanisms: homologous recombination (HR) and nonhomologous end joining (NHEJ) (Jackson, 2002 and Helleday, 2003).

Double strand DNA breaks are the most dangerous DNA damage. If not repaired leads to down-regulation of transcription and cancers development (Helleday, 2003 and Khanna and Jackson, 2001).

A recent study on the Caucasian population has provided the first epidemiological evidence, supporting the association between DSBs repair gene variants and ovarian cancer development (Liang et al., 2014; Shi et al., 2014 and Yuan et al., 2014).

Abbreviations: CI, Confidence interval; DSBs, Double-stranded breaks; FIGO, International Federation of Gynaecology and Obstetrics; FFPE, Formalin-fixed paraffin-embedded; HWE, Hardy–Weinberg equilibrium; OR, Odds ratio; PCR-RFLP, Polymerase chain reaction-Restriction Fragment Length Polymorphism; SNP, Single nucleotide polymorphism; XRCC, X-ray repair cross-complementing group.

* Corresponding author at: Laboratory of Cancer Genetics, Department of Pathology, Institute of Polish Mother's Memorial Hospital, Rzgowska 281/289, 93-338 Lodz, Poland.
E-mail address: smolbea@wp.pl (B. Smolarz).

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RAD51 (RAD51 homolog protein) is the key protein, taking part in repair via homologous recombination and being the main component of the recombination process. The recombination complex formation is facilitated by auxiliary proteins, which are paralogues of RAD51, including X-ray repair cross-complementing group (XRCC) 2 and XRCC3. These proteins are encoded by genes with the same name, which demonstrate high polymorphism.

The Arg188His substitution is the most thoroughly analyzed polymorphism in the *XRCC2* gene (c. 563 G > A; rs3218536, GenBank accession number NT 007914). Some studies have reported that the Arg188His genotype is associated with risk of ovarian cancer (He et al., 2014 and Zhang et al., 2014).

Single nucleotide polymorphism (SNP) Thr241Met (c. 722 C > T; rs861539, GenBank accession number NT 026437) is one of the most widely studied genetic markers in *XRCC3* and its role in various cancers development is evident (Du et al., 2014; Kayani et al., 2014; Nissar et al., 2014 and Ramadan et al., 2014).

However, the correlation of this SNP of *XRCC3* and ovarian cancer is controversial (Yan et al., 2014; Gonzalez-Hormazabal et al., 2012; Beesley et al., 2007; Auranen et al., 2005 and Webb et al., 2005). Yet, to our knowledge, there are no reports that assess the effect of this genetic alteration on the risk of ovarian cancer in Poland.

In the present study the association between the Arg188His polymorphism of *XRCC2* gene and Thr241Met polymorphism of *XRCC3* gene and ovarian cancer in the population of Polish women was investigated.

2. Materials and methods

2.1. Patients

Formalin-fixed paraffin-embedded (FFPE) tumor tissue specimens were obtained from women (n = 700) with ovarian carcinoma, treated at the Department of Surgical Gynaecology and Gynaecologic Oncology, Institute of Polish Mother's Memorial Hospital, between 2000 and 2013. The age of the patients ranged from 38 to 80 years (the mean age 53.2 ± 9.11). All the diagnosed tumors were graded by criteria of the International Federation of Gynaecology and Obstetrics (FIGO). The full characteristics of the study group are presented in Table 1. We enrolled only women born and living in central Poland (Lodz region). All of the studied individuals were Caucasians and constituted a homogenous population from the same ethnic and geographical origins. The control population (n = 700), matched for age (age range 39–84, mean age 50.42 ± 17.22), with no previous or concurrent malignant disease, was recruited at the same hospital. Control samples were used for a validation set. In addition normal ovarian tissue was obtained from women undergoing laparoscopy for non-malignant conditions. In order to ensure that the chosen histological material was representative for cancerous and non-cancerous tissues, each tissue sample, qualified for DNA extraction, was initially checked by a pathologist. The Local Ethic Committee approved the study and each patient gave written informed consent.

2.2. DNA isolation

Genomic DNA was prepared using QIAamp DNA FFPE Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer instruction.

2.3. Genotype determination

The PCR-Restriction Fragment Length Polymorphism method (PCR-RFLP) was used to detect the genotypes of the Arg188His and Thr241Met polymorphisms as described previously (Romanowicz-Makowska et al., 2012). Polymorphism of *XRCC2* and *XRCC3* genes was determined by PCR-RFLP, using the appropriate primers (Table 2).

Table 1
Characteristics of ovarian cancer patients and controls.

Characteristics	Cases (n, %) (n = 700)	Controls (n, %) (n = 700)
Histology of tumor		
Serous	190 (27)	
Mucinous	62 (9)	
Endometrioid	161 (23)	
Clear cell	71 (10)	
Undifferentiated	151 (22)	
Other	65 (9)	
Grading		
G1	300 (43)	
G2	380 (54)	
G3	20 (3)	
Staging		
I	340 (49)	
II	330 (47)	
III	30 (4)	
Size of tumor		
>5 cm	270 (39)	
<5 cm	430 (61)	
Number of pregnancy		
1	272 (39)	262 (37)
2–3	248 (35)	232 (33)
>4	180 (26)	206 (30)
Ascites		
Present	298 (43)	288 (41)
Absent	402 (57)	412 (59)
Use of hormone replacement therapy — HRT		
Yes	418 (60)	402 (57)
No	282 (40)	298 (43)
Tumor wall infiltration/injury		
Present	254 (36)	204 (29)
Absent	446 (64)	496 (71)
Menarche		
<12 years old	370 (53)	335 (48)
>12 years old	330 (47)	365 (52)

2.4. Determination of XRCC2 genotype

The PCR reaction (total volume 25 μl) contained 100 ng of DNA, 12.5 pmol of each primer, 0.2 mmol/l of dNTPs, 2 mmol/l of $MgCl_2$ and 1 U of Taq DNA polymerase. PCR conditions were as follows: initial denaturation step at 94 °C, 30 cycles at 94 °C for 30 s and 30 s at 60 °C annealing temperature, and at 72 °C for 1 min. The final extension was performed at 72 °C for 7 min. The PCR was carried out in a MJ Research, INC thermal cycler, model PTC-100 (Waltham, MA, USA). The 290 bp amplified product was digested overnight with 1 U of *Hpn*I (New England Biolabs, Ipswich, MA, USA) at 37 °C. The wild-type allele Arg was identified by the presence of single band of 290 bp, while the mutant allele His was represented by 148 and 142 bp bands.

2.5. Determination of XRCC3 genotype

The 25 μl PCR mixture contained 100 ng of DNA, 12.5 pmol of each primer, 0.2 mmol/l of dNTPs, 2 mmol/l of $MgCl_2$ and 1 U of Taq DNA

Table 2
Primers used to analyze Arg188His polymorphism of the *XRCC2* gene and Thr241Met polymorphism of the *XRCC3* gene.

Gene	Polymorphism	Primers
XRCC2	Arg188His	Forward 5′-TGTAGTCACCCATCTCTCTGC-3′ Reverse 5′-AGTTGCTGCCATGCCTTACA-3′
XRCC3	Thr241Met	Forward 5′-GCCTGGTGGTCATCGACTC-3′ Reverse 5′-ACAGGGCTCTGGAAGGCACTGCTCAGCTCACG CACC-3′