A single nucleotide polymorphism in the 5' untranslated region of *RAD51* and ovarian cancer risk in Polish women

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Summary

Background. DNA repair gene polymorphisms are known to influence cancer risk. The *RAD51* gene encodes proteins essential for maintaining genomic stability by playing a central role in holmology-dependent recombinational repair of the DNA double-strand breaks. *Aims*. We investigated the association of polymorphisms in the DNA repair genes *RAD51*-135G>C and 172G>T with ovarian cancer risk. *Methods*. 120 Polish ovarian cancer patients and 120 healthy controls were genotyped for *RAD51* (135G>C and 172G>T) by PCR-RFLP. *Results*. In the present work no association was detected between ovarian cancer risk and 172G>T polymorphism of the *RAD51* gene. The 135G>C polymorphism was associated with ovarian cancer risk. We found evidence of an increased ovarian cancer risk in CC homozygotes (OR 12.97 [95% confidence interval {CI} (5.73 - 29.36)]) but not in heterozygotes (OR 0.55 [95% CI 0.23 - 1.29]). We demonstrated a significant positive association between the *RAD51* variant 135C allele and ovarian carcinoma, with an adjusted odds ratio (OR) of 6.24 ($p < .0001$). *Conclusion*. The results indicated that the polymorphism 135G>C of *RAD51* may be positively associated with ovarian carcinoma in the Polish population. Further studies on the role of the *RAD51* gene on ovarian cancer are warranted.

Key words: RAD51; Ovarian cancer; Gene polymorphism.

Introduction

Ovarian cancer is detected in more than 3,000 women a year and two-thirds of these die within the five subsequent years [1].

There are no valuable diagnostic methods used worldwide for early recognition of ovarian cancer, and the first unspecific signs of the disease are frequently ignored by patients. Hence, tumours are detected mostly in advanced clinical stages. The risk of ovarian cancer is increased by several factors such as age, childbearing status, infertility, dietary factors, gynaecological diseases (endometriosis, ovarian cysts, pelvic inflammatory disease), and gene mutations [2, 3].

So far the most significant and recognized risk factor for ovarian cancer has been the presence of breast cancer-1 (BRCA1) or breast cancer-2 (BRCA2) gene mutations. They are responsible for about 5-10% of ovarian cancers [4].

Despite the growing knowledge about ovarian cancer, no effective screening program has been discovered so far. Therefore, the identification of new risk factors for ovarian cancer in the population of women is urgently needed, and an analysis of some gene polymorphisms could be an interesting option.

It is known that defects in the DNA double-strand breaks (DSB) repair pathway may play a role in development and progression of various cancers. DSB in DNA may be rectified by either homologous recombination (HR) and nonhomologous end joining (NHEJ) [5, 6].

RAD51 is involved in homologous recombination and repair of double-strand breaks in DNA and DNA crosslinks and for the maintenance of chromosome stability [7].

Two common *RAD51* SNP (single nucleotide polymorphism), 135G>C and 172G>T in the 5 UTR have been reported to be associated with altered gene transcription [7].

This SNP is located in the regulatory element of the *RAD51* promoter and is suggested to be associated with messenger RNA expression.

RAD51 gene 135G>C and 172G>T polymorphism have been studied as risk factors for various cancers such as breast, colorectal, head and neck and ovarian cancer [8-12].

It is known that variants in the *RAD51* gene that interact biologically with *BRCA1* and/or *BRCA2* may be associated with modified ovarian cancer risk in women who carry *BRCA1/2* mutations [13-15].

However, little is known about the interconnections between *RAD51* polymorphisms and ovarian carcinoma occurrence in patients without BRCA1/2 mutations [12].

Therefore, the purpose of this study was to determine the frequency of *RAD51* 135G>C and 172G>T polymorphism in ovarian tissue of Polish women treated for ovarian cancer and the possible influence on the risk of development of this neoplasm.

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Materials and Methods

Ovarian cancer patients

The study group consisted of Polish women (n = 120) belonging to the Caucasian population, with recognized ovarian cancer, who qualified for tumor debulking surgery at the Department of Gynaecological Surgery of the Institute of Polish Mother's Memorial Hospital between 2000 and 2007. All tumours were staged according to the criteria of the International Federation of Gynaecology and Obstetrics (FIGO). The full characteristics of the examined group are presented in Table 1.

One hundred and twenty age- and ethnically-matched women with normal ovaries served as controls. The healthy ovaries were removed during the hysterectomy procedure and bilateral salpingo-oophorectomy was performed due to the presence of uterine fibroids. The Local Ethics Committee approved the study and each patient gave written informed consent.

The ovarian tissue samples (cancerous and non-cancerous) were fixed routinely in formaldehyde, embedded in paraffin, cut into thin slices and stained with hematoxylin/eosin for pathological examination. DNA for analysis was obtained from an archival pathological paraffin-embedded tumour and healthy ovarian samples which were deparaffinised in xylene and rehydratated in ethanol and distilled water. To ensure that the chosen histological material was representative of cancerous and non-cancerous tissue, every tissue sample which qualified for DNA extraction was initially checked by a pathologist. The DNA samples were extracted using QIAmp Kit (Qiagen GmbH, Hilden, Germany). DNA purification was achieved according to the manufacturer's instructions.

Genotype determination

Single nucleotide polymorphisms 135G>C and 172G>T of *RAD51* gene were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), using primers 5'-TGG GAA CTG CAA CTC ATC TGG-3' (forward) and 5'-GCT CCG ACT TCA CCC CGC CGG-3' (reverse).

RAD51 135G>C genotyping was analysed by PCR amplification of a 175-bp region around nucleotide 135. This region contained a single *Mva*I site that was abolished in the 135C allele. Wild type alleles were digested by *Mva*I resulting in 86- and 71-bp products. The 135C allele was not digested by the enzyme, resulting in a single 157-bp product.

PCR was carried out in a GeneAmp PCR system 9700 (Applied Biosystems) thermal cycler. PCR amplification was performed in a final volume of 25 l. The reaction mixture contained 5 ng genomic DNA, 0.2 μmol of each appropriate primer (ARK Scientific GmbH Biosystems, Darmstad, Germany), 2.5 mM $MgCl_2$, 1 mM dNTPs and 1 unit of Taq polymerase (Qiagen GmbH, Hilden, Germany). The PCR cycle conditions were 94°C for 60 sec, 54°C for 30 sec, then 72°C for 40 sec, repeated for 35 cycles. After digestion with *Mva*I for 4 h at 37°C samples were run on 7% polyacrylamide gel and visualised by ethidium bromide staining. Each subject was classified into one of the three possible genotypes: G/G, G/C or C/C.

PCR for 172G>T SNPs was performed in 25 μl reaction systems containing 5 ng genomic DNA, 0.2 μmol of each appropriate primer (ARK Scientific GmbH Biosystems, Darmstad, Germany), 2.5 mM $MgCl_2$, 1 mM dNTPs and 1 unit of Taq polymerase (Qiagen GmbH, Hilden, Germany). The PCR profile consisted of an initial melting step at 95°C for 5 min; 30 cycles of 95°C for 30 sec, 65°C for 45 sec, and 72°C for 50 sec, and a final extension step of 72°C for 10 min. The product after PCR was digested with *NgoM*IV (New England BioLabs) overnight. The products were separated in 7% polyacrylamide gel. The 172G/G genotype produced two bands (110 and 21 bp), whereas the 172T/T genotype produced only one band (131 bp) and the 172G/T heterozygote displayed all three bands (131, 110 and 21 bp).

Table 1. — *Characteristics of the 120 ovarian cancer patients*.

Characteristics	Number of cases (%)
Age [years]	
Median	54
Range	37-79
Histology of tumor	
Serous	46 (38.3)
Mucinous	6 (5.0)
Endometrioid	33 (27.5)
Clear cell	7 (5.8)
Undifferentiated	23 (19.2)
Other	5 (4.2)
FIGO stage	
I	35 (29.2)
II	0 (0)
III	77 (64.2)
IV	6 (5.0)
no data	2 (1.7)
Grading	
G1	2 (1.7)
G2	34 (28.3)
G3	70 (58.3)
No data	14 (11.7)
Ascites	
present	50 (41.7)
absent	70 (58.3)
Tumor wall infiltration/injury	
present	70 (58.3)
absent	50 (41.7)
Size of tumor	
< 5 cm	32 (26.7)
> 5 cm	88 (73.3)
Menarche	
< 12 years old	26 (21.7)
> 12 years old	94 (78.3)
Number of pregnancies	
0	10 (8.3)
1	22 (18.4)
2 and more	88 (73.3)
Number of deliveries	
0	16 (13.3)
1	34 (28.3)
2 and more	70 (58.4)

Statistical analysis

For each polymorphism, deviation of the genotype frequencies in the controls from those expected under Hardy–Weinberg equilibrium was assessed using the standard χ^2-test. Genotype frequencies in cases and controls were compared by χ^2-tests. The genotypic-specific risks were estimated as odds ratios (ORs) with associated 95% intervals (CIs) by unconditional logistic regression; p values < 0.05 were considered to be significant. STATISTICA 6.0 software (Statsoft, Tulsa, OK, USA) was used to perform analyses.

Table 2. — *Distribution of* RAD51 *135G>C genotype frequencies in patients with ovarian cancer and control groups*.

	Ovarian cancer n = 120		Controls n = 120		OR (95% CI)[a]	*p*[b]
	Number	(%)	Number	(%)		
G/G	13	10.8	33	27.5	1.00 Ref	
G/C	15	12.5	69	57.5	0.55 (0.23 - 1.29)	0.247
C/C	92	76.7	18	15.0	12.97 (5.73 - 29.36)	< .0001
G	41	17.1	135	56.3	1.00 Ref	
C	199	82.9	105	43.7	6.24 (4.09 - 9.51)	< .0001

Data in boldface are statistically significant.
[a]Crude odds ratio (OR), 95% CI = confidence interval at 95%, [b]Chi square.

Results

Table 2 shows genotype distribution *RAD51* 135G>C polymorphism between ovarian cancer patients and controls. It can be seen from the table that there were significant differences ($p < 0.05$) between the two investigated groups. The distribution of genotypes for 135G>C SNP in ovarian cancer patients vs controls was: 10.8 % vs 27.5% for GG, 12.5% vs 57.5 % for GC and 76.7% vs 15.0% for CC genotype, respectively. A strong association of the C/C genotype [OR = 12.97 (5.73-29.36)] and the C allele [OR = 6.24 (4.09-9.51)] and ovarian cancer occurrence was observed. In patients the observed frequencies of the G/G, G/C and C/C genotypes differed significantly ($p < 0.05$) from the distribution expected from the Hardy-Weinberg equilibrium.

The distribution of genotypes of 172G>T polymorphism in ovarian cancer patients did not differ significantly compared to that predicted by the Hardy-Weinberg distribution. No significant deviation from Hardy-Weinberg distribution of genotypes characterising controls was revealed either. The distribution of genotypes for ovarian cancer patients was estimated as: 19.2 % for GG, 51.7% for GT and 29.1% for TT genotype. The controls distribution was: for CC- 24.2 %, CT- 48.3 %, TT- 27.5%, respectively. We did not find any significant difference in genotype and allele frequencies in patients with cancer and controls. The frequency of genotype and allele distribution in ovarian cancer patients in comparison to controls are summarised in Table 3.

The association between the haplotype analysis of *RAD51* and ovarian cancer was also investigated. Haplotype analysis according to wild-type of G135G-G172G showed a high association with ovarian cancer (Table 4). The findings indicated that a statistically significantly increased risk of ovarian cancer was associated with the combined C/C-G/G genotype (OR, 5.00; 95% CI, 1.44–17.27) and C/C-T/T genotype 5.55 [1.62-19.02]. The higher risk of ovarian cancer occurrence was associated with the combined C135C-G172T genotype (OR, 8.26; 95% CI, 1.62–19.02) but no altered risk was associated with other haplotypes.

FIGO staging were related to the *RAD51* 135G>C and 172G>T polymorphism. The histological stage was evaluated in all cases (n = 120). Stage II, III and IV were grouped together for the purposes of statistical analysis (Table 5). No differences between *RAD51*-135G>C and 172G>T genotype distributions in these groups were observed. There was a lack of correlation between genotypes of the polymorphisms and ovarian cancer invasiveness.

Table 3. — *Distribution of 172G>T* RAD51 *genotype frequencies in patients with ovarian cancer and control groups*.

	Ovarian cancer n = 120		Controls n = 120		OR (95% CI)[a]	*p*[b]
	Number	(%)	Number	(%)		
G/G	23	19.2	29	24.2	1.00 Reference	
G/T	62	51.7	58	48.3	1.34 [0.70-2.59]	0.466
T/T	35	29.1	33	27.5	1.33 [0.64-2.76]	0.548
G	108	45.0	116	48.3	1.00 Ref	
T	132	55.0	124	51.7	1.14 [0.79-1.63]	0.521

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

Table 4. — *Haplotype distribution and frequencies of* RAD51 *gene polymorphisms in the ovarian cancer patients and controls*.

Haplotypes *RAD51-135–172*	Patients (N = 120) N (%)	Controls (N = 120) N (%)	OR (95% CI)[a]	p[b]
G/G-G/G	6 (5.0%)	15 (12.5%)	1.00 Reference	
G/G-G/T	7 (5.8%)	7 (5.8%)	2.5 [0.60- 10.26]	0.353
G/G-T/T	6 (5.0%)	10 (8.3%)	1.5 [0.37- 5.99]	0.823
G/C-G/G	8 (6.6%)	14 (11.7%)	1.42 [0.39- 5.16]	0.823
G/C-G/T	6 (5.0%)	28 (23.3%)	0.53 [0.14- 1.95]	0.266
G/C-T/T	6 (5.0%)	15 (12.5%)	1.00 [0.26- 3.81]	0.729
C/C-G/G	18 (15.0%)	9 (7.5%)	5.00 [1.44- 17.27]	0.019
C/C-G/T	43 (35.8%)	13 (10.8%)	8.26 [2.66- 25.64]	0.0002
C/C-T/T	20 (16.7%)	9 (7.5%)	5.55 [1.62- 19.02]	0.0112

Data in boldface are statistically significant
[a]Crude odds ratio (OR), 95% CI = confidence interval at 95%, [b]Chi square.

Table 5. — *Dependency of genotypes and frequencies of the alleles of* RAD51 *gene polymorphism on tumour grade in patients with ovarian cancer*[a].

Stage[b]	I (n = 35)	II + III +IV (n = 85)	OR (95% CI)[c]	*p*[d]
RAD51 135G>C	Number (%)	Number (%)		
G/G	10 (28.6%)	16 (18.8%)	1.00 Ref	
G/C	4 (11.4%)	10 (12.9%)	0.64 (0.15-2.60)	0.394
C/C	21 (60.0%)	59 (69.3%)	0.56 (0.22-1.44)	0.345
G	24 (34.3%)	42 (24.7%)	1.00 Ref	
C	46 (65.7%)	128 (75.3%)	0.62 (0.34-1.15)	0.176
RAD51 172G>T				
G/G	10 (28.6%)	20 (23.5%)	1.00 Ref	
G/T	5 (14.3%)	10 (11.8%)	1.00 [0.26-3.72]	0.740
T/T	20 (57.1%)	55 (64.7%)	0.72 [0.29-1.81]	0.654
G	25 (35.7%)	50 (29.4%)	1.00 Ref	
T	45 (64.3%)	120 (70.6%)	0.75 [0.41-1.35]	0.420

[a]n = 120; [b]according to FIGO criteria; [c]Crude odds ratio (OR), 95% CI = confidence interval at 95%, [d]Chi square

Discussion

As mentioned before RAD51 is an important component of double-stranded DNA-repair mechanisms that interacts with both BRCA1 and BRCA2.

The contribution of polymorphisms of DNA repair

genes in developing ovarian cancer is still being investigated. Therefore we analysed the role of 135G>C and 172G>T genetic variation in homologous recombination repair gene *RAD51* and risk of this cancer. A single nucleotide polymorphism was identified in the 5' untranslated region of the *RAD51* gene and was shown to influence gene transcription activity. *RAD51* expression is often increased in various malignancies.

A single-nucleotide polymorphism (SNP) in the 5' untranslated region (UTR) of *RAD51*, 135G>C has been suggested as a possible modifier of ovarian cancer risk in *BRCA1* and *BRCA2* mutation carriers [13-16].

In the Polish population *RAD51* 135C allele showed a protective tendency against ovarian cancer in women harboring *BRCA1* mutations. The results reveal that women who harbor the C allele have almost twice the reduction in breast and ovarian cancer risk compared with women who harbor only the G allele [14].

Unfortunately, it is difficult to find reports directly binding *RAD51* 172G>T with ovarian cancer in the literature.

Few studies have investigated the association between *RAD51* 172G>T SNP and risk of breast cancer. In a large European and Korean case-control study of patients with breast cancer, the 172T variant genotypes of *RAD51* were found to be associated with a non-significantly reduced risk of breast cancer [17, 18].

Conversely, in a recent case-control study of epithelial ovarian cancer (EOC), none of the 135G>C and 172G>T variants of *RAD51* were associated with a reduction in risk [12]. Only some polymorphisms in *XRCC2* and *XRCC3* genes were associated with EOC risk.

A study performed in Australian women did not shown any association between variants in *RAD51* and *XRCC2* and ovarian cancer etiology [19].

Finally, in a study examining ovarian cancer cases, an association was observed with the *RAD51* 135G>C allele, suggesting that this polymorphism is associated with disease risk in the context of *BRCA1* and *BRCA2* [14, 13, 20]. *RAD51* is the first gene to be reliably identified as a modifier of risk among BRCA1/2 mutation carriers.

There is a lack of significant data for *RAD51* 135G>C and 172G>T polymorphisms in ovarian cancer without BRCA1/2 mutations. Therefore in our study we investigated these polymorphisms in women with this cancer without any BRCA1/2 mutation context.

In our earlier studies we analysed *RAD51* polymorphism in sporadic breast cancer [8]. Our results suggest that the 135G>C polymorphism of the *RAD51* gene may not be linked to breast cancer nor be considered as an additional marker of this disease.

In the present work our results have shown the important role of *RAD51* 135G>C polymorphism for ovarian carcinoma occurrence in Poland. In this study *RAD51* C/C genotype increased the risk of ovarian cancer in a Polish population. There was a 13-fold increased risk of ovarian carcinoma for individuals carrying *RAD51*-C/C genotype compared with subjects carrying *RAD51*-G/G, G/C genotype, respectively. The combined genotype of C135C-G172G, C135C-G172T and C135C-T172T was associated with ovarian cancer risk and may have an impact on identification of a high-risk population. In the present study the frequency of 135C allele among ovarian cancer patients was higher than the 135G allele (82.9% vs 17.1%). We realise that this may be due to the small population enrolled in the study or *de novo* mutations in ovarian cancer. It is possible that the presence of the C allele is in linkage disequilibrium with another, so far unknown, mutation located outside the coding region in the *RAD51* gene, which may be of importance for RAD51 concentration in plasma. *RAD51* G135C polymorphism was not related to cancer grade. The reason for this can be a relatively small group of A, B and C grade subjects enrolled in our study.

To our knowledge this is the first study linking the 172G>T polymorphism of the *RAD51* gene with ovarian cancer.

Finally we suggest that *RAD51* 135G>C might be used as a predictive factor of precancerous lesion for ovarian cancer in a Polish population. Further studies on the role of these genes on ovarian cancer are warranted.

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