135G>C and 172G>T polymorphism in the 5' untranslated region of *RAD51* and sporadic endometrial cancer risk in Polish women

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Background: Despite advanced diagnostic and therapeutic procedures, endometrial cancer (EC) is still responsible for high morbidity and mortality of women. The genetic variability in *RAD51* may contribute to the appearance and progression of various cancers including EC.
Aim: We investigated the association of polymorphisms in the DNA repair genes *RAD51* 135G>C and 172G>T with endometrial cancer risk.
Material and methods: The genotypes of *RAD51* 135G>C and 172G>T polymorphism were determined by PCR-RFLP methods in endometrial tissue of 240 cancer subjects and 240 healthy subjects who served as controls.
Results: In the present work we demonstrated a significant positive association between the *RAD51* C/C genotype and endometrial carcinoma, with an adjusted odds ratio (OR) of 13.0 ($p < 0.0001$). The distribution of genotypes for 135G>C SNP in endometrial cancer patients vs. controls was: 10% vs. 27% for GG, 13% vs. 58% for GC and 77% vs. 15% for CC genotype, respectively. Variant 135C allele of *RAD51* increased the cancer risk (OR = 1.81; 95% CI 0.11-2.93, $p = 0.022$). The higher risk of EC occurrence was associated with the combined C135C-G172T genotype (OR = 7.69; 95% CI 3.45-17.12).
Conclusion: The results indicated that the polymorphism 135G>C of the *RAD51* gene may be positively associated with endometrial carcinoma in the Polish population. Further studies, conducted on a larger group, are required to clarify this point.

Key words: RAD51, endometrial cancer, gene polymorphism.

Introduction

Endometrial carcinoma (EC) is the most common gynaecologic malignancy, with estimated 40 100 incident cases and 7470 deaths in 2009 [1]. In Poland, corpus uteri cancer in 2006 was diagnosed in 4376 women [2]. Uterine cancer is the fourth cancer site for incidence cases among women in Poland. The number of deaths caused by corpus uteri cancer amounts to 814 (12th cause of death among women). The share of corpus uteri cancer in morbidity is 7.1%, and in mortality – 2% [2].

The main cause of cancer development is the age and hormonal condition. Diabetes, hypertension, obesity,

Table I. Characteristics of endometrial cancer patients (n = 240)

CHARACTERISTICS	NUMBER OF CASES	(%)
Menopause status:		
Postmenopausal	240	100
BMI (kg/m^2):		
<24.9	50	21
25-29.9	74	31
>30	116	48
Number of pregnancies:		
1	74	31
2-3	156	65
> 4	10	4
Use of HRT:		
Yes	144	60
No	96	40
Grading:		
I	71	30
II	159	66
III	10	4
Uterine bleeding:		
Yes	150	63
No	90	90
Endometrial TVU:		
> 5 mm	130	54
Diabetes mellitus:		
Yes	35	14
No	205	86
Hypertension:		
Yes	91	38
No	149	62

BMI – body mass index, HRT – hormone replacement therapy, TVU – transvaginal ultrasonography

sterility, low birth number, late menopause and genetic factors increase the risk of cancer development.

The relationships between risk factors and EC development are not exactly known. Therefore, the identification of new risk factors for endometrial cancer is urgently needed, and an analysis of some gene polymorphisms could be an interesting option.

Mutations in DNA double-strand breaks (DSB) repair genes are involved in the pathogenesis of tumours. Defects in this pathway may play a role in development and progression of endometrial cancer. DSB in DNA may be rectified by either homologous recombination (HR) or nonhomologous end joining (NHEJ) [3, 4].

RAD51 is involved in homologous recombination and repair of double-strand breaks in DNA and DNA cross-links and for the maintenance of chromosome stability [5]. *RAD51* gene is highly polymorphic. 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 [6]. This SNP is located in the regulatory element of the *RAD51* promoter and is suggested to be associated with messenger RNA expression. It is known that the *RAD51* gene 135G>C and 172G>T polymorphisms have been studied as a risk factor for various cancers. In the literature, the variant 135C allele has been linked to an increased risk of head and neck cancer [7]. Many researches suggest that the *RAD51* gene 135G>C polymorphism may contribute to mammary carcinogenesis [8-13]. Lu *et al.* found that the *RAD51* 172TT homozygous variant genotype of the *RAD51* 172G>T SNP was associated with a significantly reduced risk of squamous cell carcinoma of the head and neck (SCCHN) [14].

In addition, the variant T allele of the *RAD51* 172G>T SNP was shown to be associated with a non-significantly decreased risk of sporadic breast cancer in women [15, 16]. However, little is known about the interconnections between *RAD51* polymorphisms and endometrial carcinoma occurrence [17]. Therefore, in the present work the association between the *RAD51* 135G>C and 172G>T polymorphism and endometrial carcinoma in the Polish population were investigated.

Material and methods

Endometrial cancer patients

Tumour tissues were obtained from 240 women with endometrial carcinoma (mean age 63.80 ±7.1) treated at the Department of Surgical Gynaecology, Institute of Polish Mother's Memorial Hospital between 2004 and 2010 (Table I). The endometrial cancer tissue samples were fixed routinely in formalin and embedded in paraffin. All tumours were graded according to the criteria of the International Federation of Gynaecology and Obstetrics (FIGO). DNA from normal endometrial tissue (n = 150) served as control (mean age 54.42 ±19.22). The Local Ethic Committee approved the study and each patient gave her written consent.

The 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 an archival pathological paraffin-embedded tumour and healthy endometrial samples which were deparaffinized in xylene and rehydrated in ethanol and distilled water. In order to ensure that the chosen histological material is representative for cancerous and non-cancerous tissue, every tissue sample qualified for DNA extraction was initially checked by a pathologist. DNA was extracted from material using commercially available QIAmp Kit (Qiagen GmbH, Hilden, Germany) DNA purification kit according to the manufacturer's instructions.

Genotype determination

Single nucleotide polymorphisms 135G>C and 172G>T of the *RAD51* gene was 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 analyzed 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 product. The 135C allele was not digested by the enzyme, resulting in a single 157-bp product.

The PCR was carried out in a GeneAmp PCR system 9700 (Applied Biosystems) thermal cycler. PCR amplification was performed in a final volume of 25 µl. The reaction mixture contained 5 ng genomic DNA, 0.2 µmol of each appropriate primer (ARK Scientific GmbH Biosystems, Darmstadt, 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 s, 54°C for 30 s, then 72°C for 40 s, 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.

The 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, Darmstadt, 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 s, 65°C for 45 s and 72°C for 50 s and a final extension step of 72°C for 10 min. The product after PCR was digested with *Ngo*MIV (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).

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.

Results

Table II shows genotype distribution *RAD51* 135G>C polymorphism between endometrial cancer patients and controls. It can be seen from the Table that there were significant differences ($p < 0.05$) between the two investigated groups. We observed an association between endometrial carcinoma occurrence and the presence of the C/C genotypes. A stronger association was observed for the C/C homozygotes than for the G/C heterozygotes. Variant 135C allele of *RAD51* increased the cancer risk. 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.

Table III displays the distribution of genotypes and frequency of alleles of the 172G>T polymorphism in patients with EC and controls. All distributions of genotypes and alleles were in Hardy-Weinberg equilibrium. We did not find any significant difference in genotype and allele frequencies in patients with cancer and controls ($p > 0.05$).

Table II. Distribution of *RAD51* 135G>C genotype frequencies in patients with endometrial cancer and control group

	Endometrial cancer n = 240		Controls n = 240		OR (95% CI)[a]	P[b]
	Number	(%)	Number	(%)		
G/G	25	10	65	27	1.00 Ref	
G/C	30	13	138	58	0.56 (0.31-1.04)	0.090
C/C	185	77	37	15	**13 (7.27-23.24)**	**<0.0001**
G	80	17	268	56	1.00 Ref	
C	400	83	212	44	**1.81 (1.11-2.93)**	**0.022**

Data in boldface are statistically significant

[a]*Crude odds ratio (OR), 95% CI = confidence interval at 95%,* [b]χ^2

Table III. Distribution of 172G>T *RAD51* genotype frequencies in patients with endometrial cancer and control group

	ENDOMETRIAL CANCER N = 240		CONTROLS N = 240		OR (95% CI)[a]	P[b]
	NUMBER	(%)	NUMBER	(%)		
G/G	55	23	58	24	1.00 Ref	
G/T	124	52	116	48	1.12 (0.72-1.76)	0.680
T/T	61	25	66	28	0.97 (0.58-1.61)	0.920
G	234	49	232	48	1.00 Ref	
T	264	51	248	52	1.09 (0.85-1.41)	0.502

[a]Crude odds ratio (OR), 95% CI = confidence interval at 95%, [b]χ^2

We also investigated the association between the haplotypes analysis of *RAD51* and endometrial cancer. The haplotype analysis according to wild-type of G135G-G172G showed high association with EC (Table IV). The findings indicated that a statistically significantly increased risk of endometrial cancer was associated with the combined C/C-G/G genotype and C/C-T/T genotype. The higher risk of EC occurrence was associated with the combined C135C-G172T genotype but no altered risk was associated with other haplotypes.

FIGO grading was related to the *RAD51* 135G>C and 172G>T polymorphism. The histological grade was evaluated in all cases (n = 240). 71 cases were stage I, 159 cases were stage II, 10 cases were stage III. Grade II and III were grouped together for the purposes of statistical analysis (Table V). We did not observe any difference between *RAD51* 135G>C and 172G>T genotype distributions in these groups. There was no correlation between genotypes of the polymorphisms and endometrial cancer invasiveness.

No statistically significant differences were observed in the alleles or in the genotype frequencies of the *RAD51* 135G>C and 172G>T gene polymorphisms between risk factors for endometrial cancer such as BMI (body mass index), HRT (hormone replacement therapy), uterine bleeding, endometrial ultrasound transvaginal, diabetes and hypertension and the women with endometrial cancer.

Discussion

As mentioned above in the Introduction, little is known about association of the homologous recombination repair *RAD51* single nucleotide polymorphism and sporadic endometrial cancer. Therefore, we analysed the role of 135G>C and 172G>T genetic variation in homologous recombination repair gene and risk of this cancer.

In the present work, we investigated the frequencies of the alleles of the *RAD51* 135G>C and 172G>T polymorphism in samples from patients with endometrial cancer and from healthy individuals. We investigated the relationship between genotype and the risk for endometrial cancer. In our study, we found an association between endometrial cancer occurrence and 135G>C polymorphism in this study population. *RAD51* C/C

Table IV. Haplotypes distribution and frequencies of *RAD51* gene polymorphisms in the endometrial cancer patients and the controls

HAPLOTYPES *RAD51* 135-172	PATIENTS (N = 240) N (%)	CONTROLS (N = 240) N (%)	OR (95% CI)[a]	P[b]
G/G-G/G	12 (5%)	29 (12.1%)	1.00 Ref.	
G/G-G/T	13 (5.4%)	12 (5%)	2.61 (0.93-7.35)	0.113
G/G-T/T	12 (5%)	20 (8.3%)	1.45 (0.54-3.87)	0.624
G/C-G/G	15 (6.3%)	29 (12.1%)	1.25 (0.49-3.12)	0.806
G/C-G/T	13 (5.4%)	57 (23.8%)	0.55 (0.22-1.35)	0.285
G/C-T/T	12 (5%)	30 (12.5%)	0.96 (0.37-2.49)	0.862
C/C-G/G	36 (15%)	18 (7.5%)	**4.83 (2.00-11.6)**	**0.0006**
C/C-G/T	86 (35.8%)	27 (11.3%)	**7.69 (3.45-17.12)**	**<0.0001**
C/C-T/T	41 (17.1%)	18 (7.5%)	**5.50 (2.30-13.16)**	**0.0001**

Data in boldface are statistically significant

[a]Crude odds ratio (OR), 95% CI = confidence interval at 95%, [b]χ^2

Table V. Dependence of genotypes and frequencies of the alleles of the *RAD51* gene polymorphism on the tumour grade in patients with endometrial cancer (n = 240)

GRADE[a]	I (N = 71)	II + III (N = 169)	OR (95% CI)[b]	P[c]
***RAD51* 135G>C**	**NUMBER (%)**	**NUMBER (%)**		
G/G	19 (27%)	32 (19%)	1.00 Ref	
G/C	9 (13%)	19 (11%)	0.79 (0.30-2.12)	0.841
C/C	43 (60%)	118 (70%)	0.61 (0.31-1.19)	0.205
G	47 (33%)	83 (25%)	1.00 Ref	
C	95 (67%)	255 (75%)	0.65 (0.43-1.01)	0.070
***RAD51* 172G>T**				
G/G	21 (30%)	40 (24%)	1.00 Ref	
G/T	10 (14%)	19 (11%)	1.00 (0.39-2.54)	0.823
T/T	40 (56%)	110 (65%)	0.69 (0.36-1.31)	0.337
G	52 (37%)	99 (29%)	1.00 Ref	
T	90 (63%)	239 (71%)	0.71 (0.47-1.08)	0.141

[a]according to FIGO criteria, [b]Crude odds ratio (OR), 95% CI = confidence interval at 95%, [c]χ^2

genotype and C allele were associated with an elevated risk of endometrial cancer in the Polish population. There was a 13-fold increased risk of endometrial carcinoma for individuals carrying the *RAD51* C/C genotype, compared with subjects carrying the *RAD51* G/G, G/C genotype, respectively. 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 the RAD51 concentration in plasma.

We also analysed the distribution of genotypes and frequency of alleles in groups of patients suffering from endometrial cancer according to different cancer grading by the FIGO classification. In the present study, the *RAD51* 135G>C and 172G>T polymorphism was not related to the cancer grade. The reason for this can be a relatively small group of I, II and III grade enrolled in our study.

The effect of the *RAD51* 135G>C and 172G>T polymorphism on endometrial cancer occurrence in Poland has not been investigated before. We identified the combined genotype of C135C-G172G, C135C-G172T and C135C-T172T, which was associated with EC risk and may have an impact on identification of a high-risk population.

The present work was performed on an ethnically homogenous population, which may improve our knowledge regarding to what extent the genotype-phenotype relationship variations are population-related.

Unfortunately, it is difficult to find in the literature reports directly binding SNPs in the DNA repair gene by HR with clinicopathological features of the tumour. Only in single studies, the researches suggest that the homologous recombination repair gene polymorphism may play a role in carcinoma of the endometrial occurrence [17].

Krupa *et al*. found that the C135C genotype increased the risk of endometrial cancer in the Polish population [17].

Weiss *et al*. examined the role of SNPs in nucleotide excision repair genes and risk of endometrial cancer. The presence of the *XPA* G23A variant allele was associated with a modest decrease in the risk of endometrial cancer [18].

The researches found no association between the *h0GG1* gene Ser326Cys polymorphism (base excision repair – BER) and EC [17, 19].

Few studies have investigated the association between the *RAD51* 172G>T SNP and risk of cancer. In a large European 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 [20]. Similar results were reported in a Korean case-control study of breast cancer [16, 20].

Conversely, in a recent case-control study of epithelial ovarian cancer, none of the 135G>C and 172G>T variants of *RAD51* were associated with a reduction in risk [21].

To our knowledge, this is the first study that linked the 172G>T polymorphism of the *RAD51* gene with endometrial cancer.

In conclusion, the present study provides another proof for the significance of *RAD51* polymorphisms in endometrial cancer.

The obtained data suggest that both C allele and homozygous CC genotype of *RAD51* are associated with the endometrial cancer risk. Finally, it is postulated that the 135G>C polymorphism of *RAD51* may be used as a predictive factor for endometrial cancer in Poland. Further studies, conducted on a larger group, are suggested to clarify this point.

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