Association between Arg399Gln polymorphism of X-ray repair cross-complementing 1 (XRCC1) gene and sporadic endometrial cancer in the Polish population

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Summary

Background: Endometrial cancer is one of the most common malignant neoplasms which appear in the uterine body. X-ray repair cross-complementing 1 *(XRCC1)* protein can be involved in the repair of DNA lesions, which are known to contribute to endometrial cancer. *Material and Methods:* The genotype analysis of *XRCC1* Arg399Gln gene polymorphisms for 456 endometrial cancer patients and 300 controls of cancer-free subjects in the Polish population were performed using the PCR-based restriction fragment length polymorphism (PCR-RFLP). *Results:* The association between endometrial cancer occurrence and the Gln/Gln genotype of the Arg399Gln polymorphism (odds ratio, OR 2.28; 95% confidence interval, CI 2.02-2.54) was found. The Gln/Gln genotype of *XRCC1* increased the risk of type I endometrial cancer occurrence (OR = 2.42, 95% CI = 2.12-2.72). No statistically significant association was found between gene polymorphisms and endometrial cancer risk factors such as BMI, HRT, uterine bleeding, endometrial ultrasound transvaginal, diabetes and hypertension. *Conclusion:* The results support the hypothesis that the Arg399Gln polymorphism of the *XRCC1* gene may be associated with the incidence of sporadic endometrial cancer in Polish women.

Key words: XRCC1; Endometrial cancer; Gene polymorphism.

Introduction

Endometrial cancer is the most common malignancy of the female genital tract. Annually 150,000 new cases of this cancer are noted worldwide. Every year in the age group 65-75 years, 65 new cases of endometrial cancer are diagnosed among every 100,000 women [1].

Uterine cancer is the fourth cancer site for incidence cases among women in Poland. The number of deaths caused by uterine corpus cancer amounts to 814 (12th cause of death among women). Morbidity is 7.1% and mortality 2% for uterine corpus cancer [2].

The number of morbidity cases rises dramatically beginning with the age of 45; thereafter it stabilizes to the level of 600-700 new cases in subsequent 5-year age groups and after the age of 70 it quickly diminishes Increase in the number of deaths with age is similar to the one observed for morbidity.

Endometrial cells are constantly under oxidative stress during menstrual cycles [3]. The stress is generated in the metabolic reactions of estrogens, producing reactive oxygen species (ROS), which can cause damage to biomolecules, including DNA. ROS may induce mutations in proto-oncogenes and tumor suppressor genes, as well as in other genes important for induction, promotion and progression of cancer, thus accelerating malignant transformation [4].

Oxidative damage to the DNA bases are mainly removed by the base excision repair (BER) pathway. BER is the repair mechanism for small lesions such as single-strand breaks, non-bulky adducts, oxidative damage, alkylation, or methylation [5]. X-ray repair cross-complementing 1 (*XRCC1*) and the human oxoguanine glycosylase 1 (*hOGG1*) and genes are key genes in the BER pathway.

XRCC1 is a multidomain protein that repairs single-strand breaks in DNA. Two major single nucleotide polymorphisms (SNPs) of the *XRCC1* gene have been identified at codon 194 (C → T substitution at position 26304, exon 6, Arg to Trp) and 399 (G → A substitution at position 28152, exon 10, Arg to Gln). Genetic polymorphisms of DNA repair genes have been reported to lead to amino acid substitution in various cancers. There were some reports about the relation between *XRCC1* polymorphisms and risk for several cancers: breast, prostate, laryngeal and bladder cancer [6-14].

Little is known about *XRCC1* polymorphism in endometrial cancer risk. In the available literature not many researchers have investigated an association of *XRCC1* polymorphism and endometrial carcinoma [15-17].

In the present work we performed a hospital based case control study using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay to genotype polymorphism of gene *XRCC1* Arg399Gln in relation to endometrial cancer susceptibility.

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Materials and Methods

Endometrial cancer patients

Four hundred and fifty-six patients with histologically proven diagnoses of endometrial cancer were included in the study (Table 1). Paraffin-embedded tumor tissues were obtained from postmenopausal women with endometrial carcinoma treated at the Department of Menopausal Diseases, Institute of Polish Mother's Memorial Hospital between 2002 and 2009. All tumors were staged according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO). DNA from normal endometrial tissue (n = 300) served as a control. Detailed information on demographic factors, menstrual and reproductive history, hormone use, prior disease history, physical activity, tobacco and alcohol use, diet, weight history, and family history of cancer was collected for all participants. Body weight, height, and circumferences of the waist and hips were measured according to a standarized protocol at the time of interview. Menopause was defined as cessation of the menstrual period for at least 12 months before the reference date (diagnosis date for the cases and interview date for the controls), excluding lapses caused by pregnancy, breastfeeding, or estrogen hormone use. Body mass index (BMI, weight in kilograms/height in meters^2) and waist-to-hip circumference ratio (WHR) was calculated using measured anthropometrics.

DNA isolation

DNA was extracted from material using the commercially available QIAmp Kit (Qiagen GmbH, Hilden, Germany) for DNA purification according to the manufacturer's instructions.

Determination of XRCC1 genotype

Genotypic analysis of the *XRCC1* polymorphism was determined by the PCR-based restriction fragment length polymorphism (PCR-RFLP) method. Polymorphism Arg399Gln of the *XRCC1* gene was determined by PCR-RFLP, using primers 5'-TTGTGCTTTCTCTGTGTCCA-3' and 5'- TCCTCCAGCCTTTTCTGATA-3'. The PCR was carried out in a GeneAmp PCR system 9700 (Applied Biosystems) thermal cycler. The 25 µl PCR mixture contained about 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. The PCR cycle conditions were 94°C for 30 sec, 62°C for 30 sec then 72°C for 30 sec, repeated for 35 cycles. The PCR products were digested overnight with 10 U of *Msp*I at 37ºC.

The wild-type Arg allele for codon 194 is identified by the presence of a 293 bp band, and the mutant Trp allele by the presence of a 313 bp band (indicative of the absence of the *Msp*I cutting site). For codon 399, the presence of two bands of 375 and 240 bp, respectively, identifies the wild-type Arg allele, while the uncut 615 bp band identifies the mutant Gln allele (indicative of the absence of the *Msp*I cutting site).

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 square 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% confidence intervals (CIs) by unconditional logistic regression; p values < 0.05 were considered to be significant.

Table 1. — *Characteristic of endometrial cancer (n=456) patients.*

Characteristics	Number of cases (%)
Age (years)	
Median	64
Range	52-83
BMI (body mass index) (kg/m^2)	
< 24.9	96 (21%)
25-29.9	147 (32%)
> 30	213 (47%)
Number of pregnancies	
1	144 (32%)
2-3	312 (68%)
> 4	0
Use of hormone replacement therapy - HRT	
Yes	288 (63%)
No	168 (37%)
Staging	
I	249 (54%)
II	102 (22%)
III	105 (23%)
Grading	
G1	249 (55%)
G2	180 (39%)
G3	27 (6%)
Menopause status	
Postmenopausal	456
Uterine bleeding	
Yes	300 (65%)
No	156 (35%)
Endometrial transvaginal sonography - TVS	
> 5 mm	345 (75%)
Diabetes mellitus	84 (18%)
Hypertension	240 (53%)

Table 2. — *Allele and genotype frequencies and odds ratio (OR) of Arg399Gln polymorphisms of the XRCC1 gene in patients with endometrial cancer (n = 456) and controls (n = 300).*

	Endometrial cancer patients		Controls		OR (95% CI)[a]	p^b
	Number	(%)	number	(%)		
Arg/Arg	72	16	72	24	0.66 (0.39-0.92)	0.53
Arg/Gln	90	20	144	48	0.41 (0.15-0.67)	0.29
Gln/Gln	294	64	84	28	**2.28 (2.02-2.54)**	**0.03**
Arg	234	26	288	48	0.54 (0.28-0.80)	0.10
Gln	678	74	312	52	1.42 (1.16-1.68)	0.10

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

Table 3. — *Dependency of genotypes and frequencies of the alleles of the XRCC1 gene Arg399Gln polymorphism on tumor grade in patients with endometrial cancer.*

Polymorphism *XRCC1-Arg399Gln*	Grade I (%) (n = 249)	Grade II+III (%) (n = 207)	OR (95% PU)[a]	p^b
Arg/Arg	39 (16%)	33 (29%)	0.48 [0.24-0.94]	0.051
Arg/Gln	39 (16%)	51 (26%)	1.34 [0.77-2.3]	0.292
Gln/Gln	171 (68%)	123 (44%)	**2.42 [2.12-2.72]**	**0.013**
Arg	117 (23%)	117 (28%)	0.87 [0.67-1.12]	0.296
Gln	381 (77%)	297 (72%)	1.19 [0.90-1.56]	0.204

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

Table 4. — *Distribution of genotypes and frequencies of the alleles of XRCC1 gene Arg399Gln polymorphisms and endometrial cancer risk factors.*

BMI	< 24.99 kg/m² (n = 96)		25-29.99 kg/m² (n = 147)		> 30 kg/m² (n = 213)	
	number	frequency	number	frequency	number	frequency
Arg/Arg	24	0.25	45	0.31	36	0.17
Arg/Gln	15	0.16	21	0.14	39	0.18
Gln/Gln	57	0.60	81	0.55	138	0.65
Arg	63	0.32	111	0.38	111	0.26
Gln	129	0.68	183	0.62	315	0.74
χ^2	3.683[a]		2.15[a]		3.43[a]	
Hormone replacement therapy - HRT	yes		no			
	number	frequency	number	frequency		
Arg/Arg	84	0.29	36	0.21		
Arg/Gln	108	0.37	30	0.18		
Gln/Gln	96	0.33	102	0.61		
Arg	276	0.48	102	0.30		
Gln	300	0.52	234	0.70		
χ^2	0.001[a]		2.74[a]			
Uterine bleeding	Metrorrhagia (+) (n = 156)		Metrorrhagia (–) (n = 300)			
Arg/Arg	42	0.18	63	0.21		
Arg/Gln	63	0.05	63	0.21		
Gln/Gln	51	0.77	174	0.58		
Arg	147	0.47	189	0.32		
Gln	165	0.53	411	0.68		
χ^2	0.051[a]		9.68[a]			
TVS	< 5 mm (n = 111)		> 5 mm (n = 345)			
Arg/Arg	30	0.27	66	0.19		
Arg/Gln	33	0.29	60	0.17		
Gln/Gln	48	0.43	219	0.63		
Arg	93	0.42	192	0.28		
Gln	129	0.58	498	0.72		
χ^2	0.001[a]		0.169[a]			
Hypertension	yes (n = 240)		no (n = 216)			
Arg/Arg	42	0.18	33	0.15		
Arg/Gln	30	0.13	45	0.21		
Gln/Gln	168	0.70	138	0.64		
Arg	114	0.24	111	0.26		
Gln	366	0.76	321	0.74		
χ^2	1.434[a]		1.350[a]			
Diabetes mellitus	yes (n = 84)		no (n = 372)			
Arg/Arg	15	0.18	57	0.15		
Arg/Gln	18	0.18	72	0.19		
Gln/Gln	51	0.60	243	0.65		
Arg	48	0.29	186	0.25		
Gln	120	0.71	558	0.75		
χ^2	2.082[a]		3.280[a]			

[a] $p > 0.05$ as compared with Hardy-Weinberg distribution.

Results

Table 2 shows genotype distribution of XRCC1 (Arg399Gln) 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. The women with endometrial cancer showed an incidence of 16, 20 and 64%, respectively, for the Arg/Arg, Arg/Gln, and Gln/Gln genotypes of the *XRCC1* gene, whereas the control group showed 24, 48, and 28% for the same genotypes. In patients the observed frequencies of the Arg/Arg, Arg/Gln and Gln/Gln genotypes differed significantly ($p < 0.05$) from the distribution expected from the Hardy-Weinberg equilibrium. The Gln/Gln genotype frequency was statistically significant with an OR of 2.28 and 95% CI of (2.02-2.54) (Table 2).

Because we were interested in the association between the distribution of genotypes and frequencies of alleles of investigated polymorphisms on the tumor stage evaluated according to FIGO criteria, these data were also analyzed (Table 3). The histological grade was evaluated in all cases (n = 456); 249 cases were grade 1, 180 cases were grade 2 and 27 cases were grade 3. Grade 2 and 3 were

grouped together for the purposes of statistical analysis.

The homozygous Gln/Gln genotype was also associated with type I endometrial cancer (OR = 2.42, 95% CI = 2.12-2.72)

No statistically significant differences were observed in the alleles or in the genotype frequencies of the *XRCC1* gene polymorphisms between risk factors of endometrial cancer such as BMI, HRT, uterine bleeding, endometrial transvaginal sonography (TVS), diabetes and hypertension and the women with endometrial cancer (Table 4).

Discussion

In this study, we aimed to verify a possible association between DNA repair gene *XRCC1* Arg399Gln polymorphisms with histological characteristics and risk factors such as BMI, HRT, uterine bleeding, endometrial TVS, diabetes and hypertension in women with endometrial cancer.

The *XRCC1*-Arg399Gln gene polymorphism has been studied as a risk factor for various cancers. It was suggested that SNPs in the *XRCC1* gene may alter the ability of XRCC1 to repair damaged DNA, especially SNPs at codon 399.

XRCC1-Arg399Gln has been associated with increased risks for lung cancer [18, 19], head and neck cancer [20] and possibly stomach cancer [21].

In contrast, no increased risk was observed for bladder cancer [22], esophageal cancer [23] and non-melanoma skin cancer [24].

We showed previously that the *XRCC1*-Arg399Gln polymorphism was not an independent marker in breast cancer [25] Similar results came from other laboratories [26-28].

In the literature little is known about *XRCC1* Arg399Gln polymorphisms in endometrial cancer risk.

Only De Ruyck *et al*. showed that SNPs in *XRCC1* with a combination of different polymorphisms in DNA repair genes (*XRCC3* and *hOGG1*) are associated with an enhanced clinical radiosensitivity in endometrial cancer patients treated with late radiotherapy (RT) [15, 16].

We found an association between endometrial cancer and Arg399Gln polymorphisms in this study population. Our results obtained for the Arg399Gln polymorphism of the *XRCC1* gene indicated that the Gln/Gln genotype was associated with an increased risk for the development of endometrial cancer compared with the Arg/Gln and Gln/Gln genotype. The 399Gln allele also increased the risk of endometrial cancer (OR = 1.42, 95% CI = 1.16 to 1.68) compared with the Arg allele, but no statistical difference was found (p = 0.100).

We also analyzed the distribution of genotypes and frequency of alleles in groups of patients with endometrial cancer according to different cancer staging by FIGO classification (Table 3). The homozygous Gln/Gln genotype was associated with type I endometrial cancer (OR = 2.42, 95% CI = 2.12-2.72, $p < 0.05$).

The present data confirm our previous suggestion that Arg399Gln polymorphisms of the *XRCC1* gene have a phenotypic effect, manifested in changes in the extent of DNA damage [25].

These results suggest that homozygous Gln/Gln genotype of *XRCC1* may be a risk factor for postmenopausal and type I endometrial cancer in a Polish population. Further studies, conducted on a larger group, are required to clarify this point.

Taken together our findings contribute to a better current understanding of the pathogenesis of endometrial carcinoma and the function of the SNP DNA repair gene polymorphism in this type of neoplasm.

These findings could be helpful for clinicians in the assessment and counselling of patients affected by these cancers or for scientists to consider new potential therapeutic agents for the treatment of these tumors.

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