Association between *MDM2* SNP309 polymorphism and endometrial cancer risk in Polish women

Agnieszka Zając[1], Grzegorz Stachowiak[1], Tomasz Pertyński[2], Hanna Romanowicz[3], Jacek Wilczyński[1], Beata Smolarz[3]

[1]Department of Gynecological Surgery, Institute of Polish Mother's Memorial Hospital, Lodz, Poland
[2]Department of Menopausal Diseases, Institute of Polish Mother's Memorial Hospital, Lodz, Poland
[3]Laboratory of Molecular Genetics, Department of Pathology, Institute of Polish Mother's Memorial Hospital, Lodz, Poland

The prognostic value of the *MDM2* gene amplification/expression in many types of cancer remains unclear. Polymorphisms in the promoter region of the *MDM2* gene have been shown to alter the protein expression and thus, may play a role in carcinogenesis. The aim of the present study was to evaluate the association between the risk of endometrial cancer and SNP309 polymorphisms in the *MDM2* gene.

The genotype analysis of SNP309 *MDM2* gene polymorphisms in 152 endometrial cancer patients and 100 controls of cancer-free subjects, in the Polish population, was performed using the PCR-based restriction fragment length polymorphism (PCR-RFLP).

In the presented study, an association between *MDM2* SNP309 polymorphisms and the incidence of endometrial cancer was identified. Our results obtained for the SNP309 polymorphisms of the *MDM2* gene indicated that both the G/G genotype and the G allele are strongly associated with endometrial cancer. We did not observe any relationship between gene polymorphism and endometrial cancer progression assessed by FIGO grade. This is the first study linking single nucleotide polymorphisms of the *MDM2* gene with endometrial cancer incidence in the population of Polish women.

The results support the hypothesis that the SNP309 polymorphism of the *MDM2* gene may be associated with the incidence of endometrial cancer in the female population.

Key words: *MDM2*, endometrial cancer, gene polymorphism.

Introduction

Endometrial cancer is the fourth most common carcinoma in women, and due to its growing incidence it is nowadays the leading gynecologic malignancy, especially in well-developed countries [1]. Approximately 150 000 new cases are noted worldwide annually, 80% of which being diagnosed in post menopausal patients. In the past the highest incidence was noted at the age of 57-58 years, however, recent observations confirm that the peak incidence occurs at the 6-7th decade of life. In 2006, endometrial cancer was diagnosed in 4376 Polish women [2] and was indicated as a cause of 814 deaths, presenting as the 12th cause of deaths among women in Poland. The share of endometrial cancer in general morbidity and mortality in our country is estimated at 7.1% and 2%, respectively. Apart from age, there are plenty of recognized risk factors, especially for endometrioid endometrial cancer, including: hyperestrogenism, obesity, diabetes, hypertension, the history

of sterility, low parity and late menopause. A growing amount of data seem to support the notion of genetic predisposition to endometrial cancer.

The human *MDM2* (murine double minute) oncogene has been mapped to chromosome 12q13-14 and possesses a role of down regulator of *p53* suppressor gene. The product of the *MDM2* gene promotes a rapid degradation of p53 protein, and through this mechanism protects the cell from p53-mediated growth arrest or apoptosis. It is also capable of inhibiting the p53 transactivation domain which interacts with the transcriptional machinery, resulting in p53 inactivation [3]. Furthermore, *MDM2* plays a regulatory role for many tumour-related genes that are important for cell-cycle control. It also contributes to carcinogenesis independently of p53 through interaction with transcriptional factors of the E2F family, inhibition of the Rb growth regulatory function and inhibition of G0/G1-S-phase transition in normal cells [4-6].

In endometrial cancer tissue, p53 and MDM2 levels are correlated, suggesting that p53 is inactivated by MDM2 in endometrial cancer [7, 8]. Furthermore, Stewart *et al.* sequenced the TP53 gene in a series of endometrial cancer cases overexpressing p53 and found no mutations, suggesting that overexpression was due to another source such as *MDM2* abnormalities [9]. A T $\rightarrow$ G polymorphism found in the promoter region of *MDM2* (SNP309) increases MDM2 expression and thereby attenuates p53 activity [10]. The variant allele of SNP309 has been associated with an earlier age at cancer diagnosis [11-14], and an increased risk of both sporadic and hereditary cancers. Terry *et al.* suggested that SNP309 polymorphism may be associated with the risk of endometrial cancer [15].

In the present study the association between the *MDM2* SNP309 polymorphisms and endometrial cancer risk in the Polish population was investigated.

Material and methods

Endometrial cancer patients

152 patients with histologically-proven diagnosis of endometrial cancer were included in the study (Table I). Paraffin-embedded tumour tissues were obtained from postmenopausal women (mean age 64.9 $\pm$8.2) with endometrial carcinoma treated at the Department of Menopausal Diseases, Institute of Polish Mother's Memorial Hospital in 2004-2009. All tumours were staged according to the criteria of the International Federation of Gynaecology and Obstetrics (FIGO). DNA from normal endometrial tissue (n = 100) served as control (mean age 54.42 $\pm$19.22). The Local Ethic Committee approved the study and each patient gave 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 hematoxylin/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 a commercially available QIAmp Kit (Qiagen GmbH, Hilden, Germany) DNA purification kit according to the manufacturer's instructions.

Table I. Characteristic of endometrial cancer (n = 152) patients

CHARACTERISTICS	NUMBER OF CASES (%)
Age [years]	
Median	64
Range	52-83
BMI (body mass index) (kg/m^2)	
< 24.9	32 (21%)
25-29.9	49 (32%)
> 30	71 (47%)
Number of pregnancy	
1	48 (32%)
2-3	104 (68%)
> 4	0
Use of hormone replacement therapy (HRT)	
Yes	96 (63%)
No	56 (37%)
Staging	
I	83 (54%)
II	34 (22%)
III	35 (23%)
Grading	
G1	62 (57%)
G2	38 (35%)
G3	9 (8%)
Menopause status	
Postmenopausal	152
Uterine bleeding	
Yes	100 (65%)
No	52 (35%)
Endometrial ultrasound transvaginal (TVU)	
> 5 mm	115 (75%)
Diabetes mellitus	28 (18%)
Hypertension	80 (53%)

Determination of *MDM2* genotype

Genotypic analysis of the *MDM2* SNP309 polymorphism was determined by the PCR-based restriction fragment length polymorphism (PCR-RFLP) method. Polymorphism SNP309 of the *MDM2* gene was determined by PCR-RFLP, using primers 5'-CGCGGGAGTTCAGGGTAAAG-3' and 5'-AGCTGGAGACAAGTCAGGACTTAAC-3'. The PCR was carried out in a GeneAmp PCR system 9700 (Applied Biosystems) thermal cycler. The reaction mixture contained 5 ng genomic. 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 (TaKaRa, Japan). The PCR cycle conditions were 94°C for 30 s, 62°C for 30 s, then 72°C for 30 s, repeated for 35 cycles. The 237 bp amplified product was digested overnight with 1 U of MspA1I (BioLaps, New England) at 37°C. The wild-type allele T was identified by the presence of 237 bp band, while the mutant allele G was represented by 189 and 48 bp bands.

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 genotype-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.

Results

Table II shows the genotype distribution of *MDM2* SNP309 polymorphisms between endometrial cancer patients and controls. The Table shows that there were significant differences ($p < 0.05$) between the two investigated groups. Women with endometrial cancer showed an incidence of 16%, 20% and 64%, respectively, for the T/T, T/G, and G/G genotypes of the *MDM2* gene, whereas the control group showed 24%, 48%, and 28% for the same genotypes. We observed an association between the endometrial carcinoma occurrence and the presence of the G/G genotypes. Variant G allele of *MDM2* increased the cancer risk. In patients, the observed frequencies of the T/T, T/G and G/G genotypes differed significantly ($p < 0.05$) from the distribution expected from the Hardy-Weinberg equilibrium (Table II).

Because we were interested in the association between the distribution of genotypes and frequencies of alleles of the investigated polymorphism on the tumour grade evaluated according to FIGO criteria, these

Table II. The allele and genotype frequencies and odds ratio (OR) of the SNP309 polymorphism of the *MDM2* gene in patients with endometrial cancer (n = 152) and controls (n = 100)

ENDOMETRIAL	CANCER PATIENTS		CONTROLS		OR (95% CI)[c]	P
	NUMBER	(%)	NUMBER	(%)		
T/T	24	16	24	24	0.66 (0.39-0.92)	0.53
T/G	30	20	48	48	0.41 (0.15-0.67)	0.29
G/G	98	64	28	28	2.28 (2.02-2.54)	0.03
χ^2	8.019[a]		0.245[b]			
T	78	26	96	48	0.54 (0.28-0.80)	0.10
G	226	74	104	52	1.42 (1.16-1.68)	0.10

[a]$p < 0.05$ as compared with Hardy-Weinberg distribution; [b]$p > 0.05$ as compared with Hardy-Weinberg distribution; [c]Crude odds ratio (OR) 95% CI – confidence interval at 95%

Table III. Dependency of genotypes and frequencies of the alleles of *MDM2* gene SNP309 polymorphism on the tumour stage in patients with endometrial cancer[a]

STAGE	I (N = 83)		II (N = 34)		III (N = 35)	
	NUMBER (%)	OR (95% CI)[D]	NUMBER (%)	OR (95% CI)	NUMBER (%)	OR (95% CI)
T/T	13 (16%)	0.66 (0.36-0.96)	10 (29%)	1.20 (1.11-1.29)	1 (3%)	0.12 (0.01-0.48)
T/G	13 (16%)	0.33 (0.03-0.63)	9 (26%)	0.54 (0.45-0.63)	8 (23%)	0.47 (0.11-0.83)
G/G	57 (68%)	2.42 (2.12-2.72)	15 (44%)	1.57 (1.48-1.66)	26 (74%)	1.64 (1.28-2.0)
T	39 (23%)	0.47 (0.17-0.77)	29 (43%)	0.89 (0.8-0.98)	10 (14%)	0.29 (0.01-0.65)
G	127 (77%)	1.48 (1.18-1.78)	39 (57%)	1.09 (1.0-1.18)	60 (86%)	1.65 (1.29-1.91)
χ^2	6.463[b]		0.001[c]		1.29[c]	

[a]n = 152; [b]$p < 0.05$ as compared with Hardy-Weinberg distribution, [c]$p > 0.05$ as compared with Hardy-Weinberg distribution; [d]Crude odds ratio (OR) 95% CI – confidence interval at 95%

Table IV. Distribution of genotypes and frequencies of the alleles of *MDM2* gene SNP309 polymorphism and the endometrial cancer risk factors

BMI	< 24.99 kg/m² (n = 32)		25-29.99 kg/m² (n = 49)		> 30 kg/m² (n = 71)	
	NUMBER	FREQUENCY	NUMBER	FREQUENCY	NUMBER	FREQUENCY
T/T	8	0.25	15	0.31	12	0.17
T/G	5	0.16	7	0.14	13	0.18
G/G	19	0.60	27	0.55	46	0.65
T	21	0.32	37	0.38	37	0.26
G	43	0.68	61	0.62	105	0.74
χ^2	3.683[a]		2.15[a]		3.43[a]	
HORMONE REPLACEMENT THERAPY (HRT)			YES (n = 96)		NO (n = 56)	
			NUMBER	FREQUENCY	NUMBER	FREQUENCY
T/T			28	0.29	12	0.21
T/G			36	0.37	10	0.18
G/G			32	0.33	34	0.61
T			92	0.48	34	0.30
G			100	0.52	78	0.70
χ^2			0.001[a]		2.74[a]	
UTERINE BLEEDING			METRORRHAGIA (+) (n = 52)		METRORRHAGIA (–) (n = 100)	
T/T			14	0.18	21	0.21
T/G			21	0.05	21	0.21
G/G			17	0.77	58	0.58
T			49	0.47	63	0.32
G			55	0.53	137	0.68
χ^2			0.051[a]		9.68[a]	
TRANSVAGINAL ULTRASOUND (TVU)			< 5 mm (n = 37)		> 5 mm (n = 115)	
T/T			10	0.27	22	0.19
T/G			11	0.29	20	0.17
G/G			16	0.43	73	0.63
T			31	0.42	64	0.28
G			43	0.58	166	0.72
χ^2			0.001[a]		0.169[a]	
HYPERTENSION			YES (n = 80)		NO (n = 72)	
T/T			14	0.18	11	0.15
T/G			10	0.13	15	0.21
G/G			56	0.70	46	0.64
T			38	0.24	37	0.26
G			122	0.76	107	0.74
χ^2			1.434[a]		1.350[a]	
DIABETES MELLITUS			YES (n = 28)		NO (n = 124)	
T/T			5	0.18	19	0.15
T/G			6	0.18	24	0.19
G/G			17	0.60	81	0.65
T			16	0.29	62	0.25
G			40	0.71	186	0.75
χ^2			2.082[a]		3.280[a]	

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

data were also analyzed. Histological grades were evaluated in all the cases (n = 152); grade I – 83 cases, grade II – 34 cases and grade III – 35 cases. Grades II and III were accounted together for statistical analysis (see Table III).

We did not observe any difference between SNP309 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 *MDM2* SNP309 gene polymorphisms between risk factors of 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

The literature data suggest that the identification of new risk factors for endometrial cancer in a population of women is urgently needed, and an analysis of some gene polymorphisms could be an interesting option.

In the present work we investigated the frequencies of the alleles of the *MDM2* SNP309 polymorphism in samples from patients with endometrial cancer and from healthy individuals. We investigated the relationship between genotype and the risk of endometrial cancer. In our study we found an association between the endometrial cancer occurrence and SNP309 polymorphism. The *MDM2* G/G genotype and G allele were associated with an elevated risk of endometrial cancer in this study population. There was a 3.5-fold higher risk of endometrial carcinoma for the individuals carrying the *MDM2*-G/G genotype, compared with subjects carrying the *MDM2*-T/T, G/T genotype, respectively. It is possible that the presence of the G allele is in linkage disequilibrium with another, so far unknown, mutation located outside the coding region in the *MDM2* gene, which may be of importance for the MDM2 concentration in plasma.

We also analyzed the distribution of genotypes and frequency of alleles in groups of patients suffering from endometrial cancer according to different cancer grading by FIGO classification. In the present study, the *MDM2* SNP309 polymorphism was not related to the cancer grade. The reason for this can be a relatively small group of I, II and III grade cases enrolled in our study.

The effect of *MDM2* polymorphism on endometrial cancer occurrence in Poland has not been investigated before.

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

Our results are in line with the data from other reports, introducing an important role of the *MDM2* SNP309 polymorphism for endometrial carcinoma occurrence. SNP309 polymorphisms, chosen for the study, have been identified in the 5' untranslated region of the *MDM2* gene and have been shown to influence gene transcription activity [10]. It is known that single nucleotide polymorphism SNP309 in the promoter region of *MDM2* gene plays an important role in human tumorigenesis [16-26].

MDM2 gene SNP309 polymorphisms have been studied as a risk factor for endometrial cancer [15, 19, 27-29]. Terry *et al.* showed that women carrying GG genotype of *MDM2* SNP309 polymorphism may be at greater risk of endometrial cancer [15]. Walsh *et al.* found an association between a functional single nucleotide polymorphism in the *MDM2* gene and sporadic endometrial cancer risk [19].

In a large American case-control study of patients with endometrial cancer, the SNP309 G/G variant genotypes of *MDM2* were found to be associated with an increased risk of endometrial cancer [15, 19].

Similar results were reported in a Australian case-control study of endometrial cancer [27]. Ashton *et al.* suggested that the combination of the *MDM2* SNP309 and the three TP53 polymorphisms appear to be related to a higher grade of endometrial cancer.

The homozygous variants of wild p53 codon 72 and mutant *MDM2* promoter 309 may cooperatively increase the risk of endometrial cancer in a Japanese population [28]. The homozygous GG genotype of SNP309 polymorphism was also associated with the postmenopausal status and type I endometrial cancer in Japanese women [29].

In conclusion, the present study provides evidence for the significance of *MDM2* polymorphism in endometrial cancer.

The obtained data suggest that both G allele and homozygous GG genotype of *MDM2*-SNP309 are associated with the endometrial cancer risk. Finally, it is postulated that the SNP309 polymorphism of the *MDM2* gene may be used as predictive factors for endometrial cancer in Poland. Further studies conducted on a larger group are suggested to clarify this point.

The authors declare no conflict of interest.

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Address for correspondence

Beata Smolarz
Laboratory of Molecular Genetics
Department of Pathology
Institute of Polish Mother's Memorial Hospital
Rzgowska 281/289
93-338 Lodz, Poland
tel. +48 42 271 20 71
e-mail: smolbea@wp.pl