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Original Article

Association between Gly322Asp polymorphism of *hMSH2* (1032G>A, rs4987188) and endometrial cancer

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Abstract: *Aim*: The aim of this study was to analyse the frequency of genotypes and alleles of single nucleotide polymorphism (SNP) Gly322Asp (1032G>A, rs4987188) of *hMSH2* gene and to investigate the significance this genetic variation exerts on endometrial cancer (EC). *Material and methods*: 450 paraffin embedded EC tissue samples were included into the test group and 440 blood samples obtained from cancer-free women formed the controls. Genomic DNA was isolated and the SNP Gly322Asp of *hMSH2* was determined by PCR-RFLP method. *Results*: This study revealed that SNP Gly322Asp of *hMSH2* is associated with both EC risk and grading. Moreover, it can be linked with certain typical EC risk factors like obesity, hormone replacement therapy, abnormal uterine bleeding and diabetes but neither with transvaginal ultrasound finding nor with arterial hypertension. *Conclusions*: SNP Gly322Asp of *hMSH2* may be a risk factor of EC.

Keywords: Endometrial cancer, single nucleotide polymorphism, *hMSH2,* mismatch repair genes

Introduction

Endometrial cancer (EC) is one of the most common malignancies in females and its morbidity is still growing. This phenomenon is particularly visible in developed societies what can be scientifically explained by the rising prevalence of common risk factors of EC such as obesity, diabetes and hypertension in these populations. Moreover, the positive correlation between the abovementioned conditions and the risk of EC is one of the mostly proven clinical interactions in entire oncology. Clinical practice and histology separate this malignancy into two autonomous groups: endometrioid endometrial carcinoma (EEC) and non-endometrioid endometrial carcinoma (NEEC) which both highly differ in clinical manifestation, symptomatology, treatment and prognosis [1].

Besides clinical features that predispose towards the development of EC, the study of DNA has also given clear evidence of interconnections that link this malignancy with genetic variations. As EC is heterogenic itself-like it was already said before-the qualities of genetic findings in both types of the disease are obviously distinct: mutations of *PTEN*, *K-ras* and β-catenin together with defects in mismatch repair (MMR) genes are typical of EEC, but-on the contrary-aneuplodies and *p53* mutations are common in NEEC [2].

There are seven major DNA mismatch repair genes in humans: *MLH1, MLH3, PMS1, PMS2, MSH2, MSH3* and *MSH6*. Loss of expression of the abovementioned results in increased genetic instability leading to impairment of crucial mechanisms regulating cell proliferation and death [3-5]. *hMSH2*, also known as MutS protein homolog 2, is a tumor suppressor gene that encodes a protein which plays a crucial role in DNA mismatch repair, but also holds activity in other versatile types of DNA repair such as: homologous recombination, transcription-coupled repair or even base excision repair. Microsatellite instability, which is a notorious effect of *hMSH2* mutations, is also an axis feature of Hereditary Nonpolyposis Colorectal Cancer (HNPCC) alias Lynch syndrome [6].

Table 1. Characteristics of endometrial cancer patients (n=450) and controls (n=440)

Characteristics	Number of patients (%)	Number of controls (%)
First menarche		
Before 11 years	87 (19%)	81 (18.4%)
12-13 years	140 (31%)	134 (30.5%)
14-15 years	137 (30%)	133 (30.2%)
After 16 years	86 (20%)	92 (20.9%)
BMI (body mass index) (kg/m^2)		
<24.9	66 (15%)	62 (14%)
25-29.9	167 (37%)	159 (36%)
>30	217 (48%)	219 (50%)
Number of pregnancies		
1	145 (32%)	138 (31%)
2-3	305 (68%)	302 (69%)
>4	0	0
Use of hormone replacement therapy (HRT)		
Yes	332 (74%)	313 (71%)
No	118 (26%)	127 (29%)
Uterine bleeding		
Yes	353 (78%)	320 (73%)
No	97 (22%)	120 (27%)
Endometrial transvaginal ultrasound (TVU)		
>5 mm	408 (91%)	332 (75%)
Diabetes mellitus		
Yes	113 (25%)	102 (23%)
No	337 (75%)	338 (77%)
Hypertension		
Yes	271 (60%)	254 (58%)
No	179 (40%)	186 (42%)
FIGO grade		
G1	98 (22%)	
G2	315 (70%)	
G3	37 (8%)	

State-of-the-art research focuses on the analysis of versatile genetic aspects and on the attempt to associate these with clinical manifestation of carcinogenesis. Large effort has been lately put into investigation of single nucleotide polymorphisms (SNPs), which may underline the differences in ones susceptibility and natural history of diseases. According to NCBI dbSNP there are more than 150 million SNPs in human already discovered [7] and as many as 380 SNPs in *hMSH2* only [8]. Furthermore, the latter have been already linked with the increased risk of colorectal cancer [9, 10], gastroesophageal cancer [11, 12], lung cancer [13] or even gallbladder cancer [14]. In addition, polymorphisms of *hMSH2* may also have en effect on cellular response to radiation therapy in breast cancer patients [15].

Gly322Asp (1032G>A, rs4987188) is a missense SNP resulting in a Glycine to Aspartic Acid switch at codon 322. However, the exact clinical significance of this alteration still remains unclear. The aim of this study was to analyse the frequency of genotypes and alleles of SNP Gly322Asp (1032G>A, rs4987188) of *hMSH2* and to investigate the significance this genetic variation exerts on EC.

Materials and methods

Patients

Paraffin embedded tumor tissue samples from patients with EC (n=450) treated in the Department of Surgical Gynaecology and Gynaecologic Oncology, Polish Mother's Memorial Hospital-Research Institute between 2000-2014 were selected to the test group the study. All the specimens were graded according to the International Federation of Gynaecology and Obstetrics (FIGO). The age of the patients ranged in from 48 to 85 (mean age: 59.2±10.11). Controls consisted of the DNA extracted from blood samples from age-matched 440 cancer-free women (age range: 49-83, mean age: 54.27±11.18). The full profile of both groups is presented in **Table 1**. The Local Ethic Committee approved the study and each patient gave a written consent (No 4/2011).

DNA isolation

Genomic DNA was obtained using DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) and QIAamp DNA FFPE Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer instruction.

Table 2. Primers used to analyse Gly322Asp polymorphism of the *hMSH2* gene

Gene	Polymorphism	Primer sequence
HMSH2	Gly322Asp, (1032G>A, rs4987188)	forward 5'-GTTTTCACTAATGAGCTTGC-3' reverse 5'-AGTGGTATAATCATGTGGGT-3'

Table 3. The allele and genotype frequency of the Gly322Asp polymorphism of the *hMSH2* gene in endometrial cancer patients and controls

hMSH2- Gly322Asp	Patients (n=450)		Controls (n=440)		OR (95% CI)[a]	*P*[b]
	Number	(%)	Number	(%)		
Gly/Gly	45	10	88	20	1.00 Ref	
Gly/Asp	48	11	280	64	0.34 (0.21-0.53)	<.0001
Asp/Asp	357	79	72	16	9.67 (6.24-15.04)	<.0001
Gly	138	15	456	52	1.00 Ref	
Asp	762	85	424	48	5.93 (4.74-7.43)	<.0001

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

Table 4. Dependence of *hMSH2* gene polymorphism genotypes and allele frequency on tumour grade in patients with endometrial cancer[a]

Grade[b]	Endometrial cancer patients		OR (95% CI)[c]	*p*
	G1 (n=98)	G2 + G3 (n=352)		
HMSH2-Gly322Asp	Number (%)	Number (%)		
Gly/Gly	13 (13)	32 (9)	1.00 Ref	
Gly/Asp	28 (29)	20 (6)	3.44 (1.45-8.17)	0.008
Asp/Asp	57 (58)	300 (85)	0.47 (0.23-0.95)	0.051
Gly	54 (28)	84 (12)	1.00 Ref	
Asp	142 (72)	620 (88)	0.36 (0.24-0.52)	<.0001

[a]n=450; [b]according to FIGO criteria. [c]Crude odds ratio (OR), 95% CI = confidence interval at 95%.

Determination of hMSH2 genotype

Polymorphism Gly322Asp of the *hMSH2* gene was determined by PCR-RFLP, using primers specified in **Table 2**. 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). PCR products were electrophoresed in a 2% agarose gel and visualised by ethidium bromide staining. All PCR was carried out in a DNA Thermal Cycler PTC-100 TM (MJ Research, INC, Waltham, MA, USA). After an initial denaturation at 95°C for 5 min, 30 cycles of amplification with denaturation at 95°C for 30 s, annealing at 63°C for 30 s, and extension at 72°C for 30 s were performed, followed by a final extension step of 5 min at 72°C. The PCR product was digested with 1 unit of *Hinf*I (Fermentas, Vilnius, Lithuania). The cleavage of the *hMSH2* polymorphic site with HinfI produced bands of 252, 252/182/70 and 182/70 bp corresponding to the Gly/Gly, Gly/Asp and Asp/Asp genotypes, respectively.

Statistical analysis

A standard χ^2-test was used to assess the departure from Hardy-Weinberg equilibrium in the analysed SNP. Genotype and allele frequencies in patients and controls were correlated by χ^2-test. The general risks were illustrated as odds ratios (ORs) with associated 95% intervals (CIs) by unconditional logistic regression. *P*-values<0.05 were considered significant.

Results

The study of a series of 450 DNA samples from the test group followed by a comparison with controls revealed a relationship of the studied SNP with the risk of EC (**Table 3**). We have proven that Asp variant may increase the risk of cancer (OR 5.93; 95% CI 4.74-7.43, *P*<.0001). Moreover, we observed that Asp/Asp genotype was strongly associated with the risk of EC (OR 9.67; 95% CI 6.24-15.04, *P*<.0001). Furthermore, an association was observed between the studied polymorphism and cancer grading (**Table 4**): Grade 1 (G1) patients generally dem-

Table 5. Distribution of genotypes and frequencies of the alleles of *hMSH2* gene Gly322Asp polymorphism and the endometrial cancer risk factors

BMI	24-29, 99 kg/m^2 (n=233)		>30 kg/m^2 (n=217)		OR (95% CI)[a]	*p*[b]
	Number	(%)	Number	(%)		
Gly/Gly	35	15	10	5	1.00 Ref.	-
Gly/Asp	20	9	28	13	0.20 (0.08-0.51)	0.0008
Asp/Asp	178	76	179	82	0.28 (0.13-0.59)	0.0007
Gly	90	19	48	11	1.00 Ref.	-
Asp	376	81	386	89	0.52 (0.35-0.75)	0.0008
HRT	Yes (n=332)		No (n=118)		OR (95% CI)	*p*
	Number	(%)	Number	(%)		
Gly/Gly	14	4	31	26	1.00 Ref.	-
Gly/Asp	38	12	10	9	8.41 (3.28-21.54)	<.0001
Asp/Asp	280	84	77	65	8.05 (4.08-15.88)	<.0001
Gly	66	10	72	31	1.00 Ref.	-
Asp	598	90	164	69	3.97 (2.73-5.79)	<.0001
Uterine bleeding	Yes (n=353)		No (n=97)		OR (95% CI)	*p*
	Number	(%)	Number	(%)		
Gly/Gly	30	8	15	15	1.00 Ref.	-
Gly/Asp	29	8	19	20	0.76 (0.32-1.78)	0.680
Asp/Asp	294	84	63	65	2.33 (1.18-4.59)	0.021
Gly	89	13	49	25	1.00 Ref.	-
Asp	617	87	145	75	2.34 (1.58-3.47)	<.0001
TVU	<5 mm (n=408)		>5 mm (n=42)		OR (95% CI)	*p*
	Number	(%)	Number	(%)		
Gly/Gly	41	10	4	10	1.00 Ref.	-
Gly/Asp	40	10	8	19	0.48 (0.13-1.74)	0.420
Asp/Asp	327	80	30	71	1.06 (0.35-3.17)	0.544
Gly	122	15	16	19	1.00 Ref.	-
Asp	694	85	68	81	1.33 (0.75-2.38)	0.406
Hypertension	Yes (n=271)		No (n=179)		OR (95% CI)	*p*
	Number	(%)	Number	(%)		
Gly/Gly	25	9	20	11	1.00 Ref.	-
Gly/Asp	34	13	14	8	1.94 (0.82-4.57)	0.188
Asp/Asp	212	78	145	81	1.16 (0.62-2.18)	0.740
Gly	84	15	54	15	1.00 Ref.	-
Asp	458	85	304	85	0.96 (0.66-1.40)	0.920
Diabetes mellitius	Yes (n=113)		No (n=337)		OR (95% CI)	*p*
	Number	(%)	Number	(%)		
Gly/Gly	17	15	28	8	1.00 Ref.	-
Gly/Asp	18	16	30	9	0.98 (0.42-2.28)	0.862
Asp/Asp	78	69	279	83	0.46 (0.23-0.88)	0.029
Gly	52	23	86	13	1.00 Ref.	-
Asp	174	77	588	87	0.48 (0.33-0.71)	0.0003

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

onstrated higher frequencies of Gly/Asp heterozygotes (*P=0.008*). Additionally, the studied SNP was statistically related to such EC risk factors as obesity, hormone replacement therapy, abnormal uterine bleeding and diabetes (**Table 5**). However, SNP Gly322Asp of *hMSH2*

(1032G>A, rs4987188) proved not to be related to other typical clinical phenomena of EC like abnormal transvaginal ultrasound finding or arterial hypertension.

Discussion

Defective DNA mismatch repair that results in microsatellite instability (MSI) is the axis feature of Lynch Syndrome, which is characterized by increased hereditary incidence of colorectal, ovarian and endometrial cancers [6]. MSI itself is defined by changes in the lengths of dinucleotide repeats-mostly cytosine and adenine. Almost 60% HNPCC families carry the burden of genetic alterations in *hMSH2*. Although only a minimal share of all abovementioned cancers are directly linked with classical Lynch Syndrome [18], almost 30% of the entire EC population shows a substantial loss in expression of DNA mismatch repair genes where more than 12% represents for *hMSH2* alone [16].

It has already been demonstrated that SNPs in DNA repair genes may independently contribute to increased risk of carcinogenesis due to the disorders they provoke in maintaining genome integrity [17]. Such a genetic variation of *hMSH2* has already been proposed to coincide-among others-with ovarian, breast and endometrial cancers [18, 19]. Yet to our knowledge, until now, SNP Gly322Asp of *hMSH2* was not analysed in EC.

In our study we have demonstrated that both the Asp allele and the polymorphic homozygote Asp/Asp of the analysed SNP strongly increases the risk of EC. Interestingly, these results are not consistent with the analysis of the SNP Gly322Asp of *hMSH2* in breast cancer, where the Asp allele demonstrated rather a weak protective role or no clinical significance at all [19, 20]. Additionally, a correlation has been found between the studied polymorphism and cancer grading: G1 was statistically associated with Gly/Asp heterozygotes. The study revealed that SNP Gly322Asp of *hMSH2* could be correlated with classical risk factors of EC like obesity, hormone replacement therapy, abnormal uterine bleeding and diabetes, but rather neither with transvaginal ultrasound finding nor with arterial hypertension.

Our study, however, has certain limitations. Among the abundance of genetic polymorphisms in versatile MMR genes only one polymorphism of *hMSH2* was here analysed without respect to linkage disequilibrium (LD), which is a crucial feature of population genetics. A joint analysis of SNP Gly322Asp of *hMSH2* and other SNPs in MMR could shed a new light on the role of SNPs in endometrial cancer.

Conclusions

SNP Gly322Asp of *hMSH2* may be related to the risk of endometrial cancer. Further studies are warranted to determine the clinical impact of SNPs in DNA mismatch repair genes on endometrial cancer.

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Disclosure of conflict of interest

None.

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