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POLYMORPHISM OF DNA MISMATCH REPAIR GENES IN ENDOMETRIAL CANCER

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Endometrial cancer (EC) is the second most common malignancy associated with hereditary non-polyposis colorectal cancer (HNPCC) family. The development of HNPCC is associated with defects in DNA mismatch repair (MMR) pathway resulting in microsatellite instability (MSI). MSI is present in a greater number of EC than can be accounted for by inherited MMR mutations, therefore alternative mechanisms may underline defective MMR in EC, including polymorphic variation. *Aim*: We checked the association between EC occurrence and two polymorphisms of MMR genes: a 1032G>A (rs4987188) transition in the hMSH2 gene resulting in a Gly22Asp substitution and a –93G>A (rs1800734) transition in the promoter of the hMLH1 gene. *Material and methods*: These polymorphisms were genotyped in DNA from peripheral blood lymphocytes of 100 EC patients and 100 age-matched women by restriction fragment length polymorphism PCR. *Results*: A positive association (OR 4.18; 95% CI 2.23–7.84) was found for the G/A genotype of the –93G>A polymorphism of the hMLH1 gene and EC occurrence. On the other hand, the A allele of this polymorphism was associated with decreased EC occurrence. The Gly/Gly genotype slightly increased the effect of the –93G>A-G/A genotype (OR 4.52; CI 2.41–8.49). Our results suggest that the –93G>A polymorphism of the hMLH1 gene singly and in combination with the Gly322Asp polymorphism of the hMSH2 gene may increase the risk of EC.
***Key Words*: hMSH2, hMLH1, endometrial cancer, genetic polymorphism, MMR.**

Endometrial cancer (EC) is the most common gynecologic malignancy among women in the Europe, Asia and North America [1, 2]. As with all solid tumors, it is a heterogeneous disease underlined by combination of genetic and environmental influences. Factors involved in the etiology of endometrial malignancy are obesity, hypertension, diabetes and hormonal elements. Family history may also be a risk factor as familial clustering of EC has been reported [3]. These cases are associated with colon cancer as a part of hereditary non-polyposis colorectal cancer (HNPCC) family [4]. EC is the second most common cancer associated with HNPCC [5, 6]. A part of ECs shares many of the molecular characteristics of HNPCC, including DNA mismatch repair (MMR) deregulation associated with mutations in MMR genes [7–9]. These high-penetrance, hereditary mutations are linked with the development of approximate 10% of all EC [10]. More commonly occurring, low-penetrance variant alleles may influence susceptibility to sporadic carcinogenesis through their effects on protein function and expression [11]. Single nucleotide polymorphisms (SNPs) that affect cancer transformation may be particularly relevant [12]. A number of pathways are involved in the EC development, including DNA damage repair, carcinogen metabolism, steroid metabolism, and steroid receptor activation pathways. Polymorphisms in MMR genes are considered to be candidate risk factors, because of the crucial role played by these genes in the maintenance of genomic integrity. The loss or serious alterations in MMR pathway may result in microsatellite instability (MSI), a feature occurring in a subset of ECs. However, this subset is larger than that expected from the number of high-penetrance mutations in MMR genes, which implies the involvement of other mechanism(s) of EC transformation [6]. Mutations in low-penetrance genes, sometimes having a form of polymorphisms, may underline EC, since cancer transformation is a multi-gene process andcombined effect of even small changes in these genes may affect it.

In the present work, we searched for an association between EC occurrence and 2 polymorphisms of MMR genes: a 1032G>A transition in the MSH2 gene, resulting in a Gly322Asp substitution (the Gly322Asp polymorphism, rs4987188) and a –93G>A transition in the MLH1 gene (the –93G>A polymorphism, rs1800734). We have recently correlated the Gly322Asp polymorphism with breast cancer [13]. The –93G>A polymorphism, due to its location in the core promoter may influence the level of transcription of the MLH1 gene. An association of this polymorphism with malignant phenotype for colon and lung cancers has been shown [14, 15].

MATERIALS AND METHODS

Patients. Blood was obtained from 100 women (median age 48 years) with EC treated in 2010–2012 in the Polish Mother's Memorial Hospital (Lodz, Poland). All patients had histologically confirmed endometrial carcinoma and agreed to complete a risk factor questionnaire. The characteristics of the patients and controls is presented in Table 1. Control

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Abbreviations used: EC – endometrial cancer; HNPCC – hereditary non-polyposis colorectal cancer; MMR – mismatch repair; MSI – microsatellite instability; SNPs – single nucleotide polymorphisms.

samples consisted of DNA extracted from blood cells from age-matched 100 cancer-free women. The study was approved by the Local Bioethical Committee and each patient gave a written consent.

Table 1. Characteristics of EC patients and controls enrolled in the study

Characteristics	Cases (n = 100)	Controls (n = 100)
Age, years		
Mean	61	55
Min	43	45
Max	83	84
Education		
Elementary school	23	22
Secondary technical school	17	15
High school	38	43
More than high school	22	20
No. of birds		
0	16	17
1	30	32
> 1	54	51
Body mass index		
< 19	0	0
19–25	27	33
26–29	41	38
> 30	32	29
First menarche		
Before 11 years	5	10
12–13 years	35	42
14–15 years	43	30
After 16 years	9	11
Missing	8	7
Hypertension	51	43
Hormone replacement therapy		
Yes	19	31
No	81	60
Missing	0	9
Smoking		
No	64	62
Past or Current	26	33
Missing	10	5
Alcohol consumption		
Yes	49	46
No	49	48
Missing	2	6
Family cancer		
Yes	29	12
No	63	77
Missing	8	11
FIGO stage		
I	71	–
II	14	–
III	13	–
IV	2	–
FIGO grade		
G1	45	–
G2	30	–
G3	25	–

Genotype determination. Genomic DNA was prepared using GeneMatrix Blood DNA purification Kit (EURx, Gdansk, Poland) according to the manufacturer instruction. Genotypes were determined by restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR). The following primers were used for the Gly322Asp polymorphism — forward: 5'GTTTTCACTAATGAGCTTGC-3' and reverse: 5'-AGTGGTATAATCATGTGGGT-3'; for the –93G>A polymorphism — forward: 5'-CTCGTCGAGCCGAATAA-3' and reverse: 5'AGTAGCCGCTTCAGGGA-3'. The PCR reaction was run with a mixture containing 100 ng genomic DNA, 5 mM dNTPs, 5 pmol each primer and 1U (in 25 µl) Taq DNA polymerase (Biotools, Madrid, Spain) which was added into PCR buffer containing 10 mMTris-HCl, 1.5 mM $MgCl_2$ and 50 mM KCl. PCR conditions were as follows: initial denaturation step at 95 °C for 5 min, 30 cycles at 95 °C for 30 s and 30 s at the 62 °C annealing temperature, and at 72 °C for 30 s. The final extension step was performed at 72 °C for 5 min. The PCR was carried out in a MJ Research, INC thermal cycler, model PTC-100 (Waltham, MA, USA). Aliquots of 20 µl were taken and subjected to restriction digestion with HinfI (the Gly322Arg polymorphism) or PvuII (the –93G>A polymorphism, both restriction enzymes were from Fermentas, Vilnius, Lithuania). The digested products were resolved on an 8% polyacrylamide (the Gly322Arg polymorphism) or 2% agarose gel (the –93G>A polymorphism) and stained with 0.5 mg/ml ethidium bromide. 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. The PvuII restriction enzyme acting on the promoter region of hMLH1 produced bands of 259, 259/134/125 and 134/125 bp corresponding to the G/G, G/A and A/A genotypes, respectively at –93.

Data analysis. Genotype frequencies were tested for Hardy — Weinberg equilibrium using the χ^2 test. The association between genotype and the risk of EC was estimated by odds ratios (OR) and 95% confidence intervals (95% CI), calculated by unconditional logistic regression models. All statistical analyses were performed with Statistica (Statsoft, Tulusa, OK, USA).

RESULTS

Genotyping was successfully performed for all samples in the study. Allele and genotype frequencies among the patients and controls were in the Hardy — Weinberg equilibrium. No substantial differences in environmental risk factors (see Table 1) between EC patients and controls were observed. A strong association (OR 4.18; 95% CI 2.23–7.84) was found between the G/A genotype of the –93G>A polymorphism and EC occurrence (Table 2). On the other hand, the A allele of this polymorphism was associated with decrease in EC occurrence. There were no differences in the genotype distributions between cancer patients and controls for the Gly322Asp polymorphism (Table 3), but the Gly/Gly genotype enhanced a positive effect of the G/A genotype of the –93G>A polymorphism (Table 4).

Table 2. The allele and genotype frequency and OR of the –93G>A polymorphism of the hMLH1 gene in EC

Genotype or Allele	Patients (n = 100)		Controls (n = 100)		OR (95% CI)
	Number	Frequency	Number	Frequency	
G/G	18	0.18	9	0.09	2.16 (0.93–4.99)
G/A	81	0.81	50	0.50	4.18* (2.23–7.84)
A/A	1	0.01	41	0.41	0.02 (0.004–0.11)
G	117	0.59	68	0.31	2.71* (1.81–4.08)
A	83	0.41	132	0.69	0.36 (0.24–0.55)

*ORs values with $p < 0.001$.

Table 3. The allele and genotype frequencies and OR of the Gly322Asp polymorphism of the hMSH2 gene

Genotype or Allele	Patients (n = 100)		Controls (n = 100)		OR (95% CI)
	Number	Frequency	Number	Frequency	
Gly/Gly	98	0.98	96	0.96	1.84 (0.38–8.84)
Gly/Asp	2	0.02	4	0.04	0.54 (0.11–2.62)
Asp/Asp	0	–	0	–	–
Met	198	0.98	196	0.96	1.82 (0.38–8.64)
Thr	2	0.02	4	0.04	0.55 (0.11–2.61)

"–" not estimated.

Table 4. The distribution of combined genotypes of the Gly322Asp polymorphism of the hMSH2 gene and the –93G>A polymorphism of the hMLH1 gene in EC

Genotype or Allele	Patients (n = 100)		Controls (n = 100)		OR (95% CI)
	Number	Frequency	Number	Frequency	
Gly/Gly – G/G	17	0.17	8	0.08	2.28 (0.95–5.45)
Gly/Gly – G/A	81	0.81	48	0.48	4.52* (2.41–8.49)
Gly/Gly – A/A	0	–	40	0.40	–
Gly/Asp – G/G	1	0.01	1	0.01	1.00 (0.10–9.78)
Gly/Asp – G/A	1	0.01	2	0.02	0.59 (0.08–4.58)
Gly/Asp – A/A	0	–	1	0.01	–
Asp/Asp – G/G	0	–	0	–	–
Asp/Asp – G/A	0	–	0	–	–
Asp/Asp – A/A	0	–	0	–	–

*OR value with $p < 0.001$.

DISCUSSION

The main function of the MMR system is to correct mismatches left by DNA polymerase during DNA replication. MMR is also responsible for removal of DNA lesions (particularly small DNA loops) formed in homologous recombination (reviewed in [16]). To date, six DNA MMR genes have been identified in human hMLH1, hMSH2, hPMS1, hPMS2, hMLH6, and hMSH3. Except hMSH3, all remaining MMR genes have been linked to HNPCC susceptibility [17–19]. MMR is initiated by the heterodimeric complexes hMSH2-hMSH6 (hMutSα) and hMSH2-hMSH3 (hMutSβ). Base-base mismatches and small loops with up to eight unpaired nucleotides are recognized by MutSα. hMutSβ mediates the repair of small loops with 2–8 unpaired nucleotides. The hMLH1, hPMS1 and hPMS2 form a protein complexes that interact with MutSα or hMutSβ. The results obtained in the present work suggest that the –93G>A polymorphism of the hMLH1 gene singly and in combination with the Gly322Asp polymorphism of the hMSH2 gene may increase the risk of EC. The role of the –93G>A polymorphism in cancer development, if any, has not been clarified. The hMLH1 –93A variant has previously been associated with an increased risk of developing hyperplastic colonic polyps in smokers [20], and colorectal cancers [21], particularly in persons with a family history of this disease [14]. It was also associated with risk for squamous cell lung cancers [15]. Using immunochemistry methods, an association between the hMLH1 –93A variant and somatic loss of hMLH1 protein in MMR deficient colorectal cancer was shown [22]. The molecular mechanism which lead to disturbance of hMLH1 protein level in the –93A variant carriers of the –93G>A polymorphism remains unknown. The A allele of this polymorphism did not change the level of expression of the gene in luciferase assay [23]. It was hypothesized that the –93 variant promoted hypermethylation of the promoter sequence of the hMLH1 gene, and in this manner disturbed its expression. A similar relationship between gene variant and silencing carrier gene by promoter methylation in cancer cells was observed for MGMT gene encoding O-6methylguanine-DNA methyltransferase in colorectal cancer [24]. Another large cohort study showed that the –93G>A polymorphism of hMLH1 gene was associated with hMLH1 promoter methylation and silencing the gene, but additional unknown genetic factors contributed also in this process [21]. Indeed, –93G>A is located in the promoter region of hMLH1 binding an unknown factor that is required for the optimal expression [25]. It is not known whether variation at –93 has direct or indirect effect on methylation of the promoter region, but it looks that this effect is very strong, what was confirmed by the association of the –93A allele for both AA homozygotes and AG heterozygotes. This might be explained by a dominant trans effects similar to transactivation seen in yeast [26]. In a multicenter, large cohorts study it was shown that this polymorphism was not associated with EC occurrence in Polish and British populations [27]. However, it was positively correlated with the risk of EC in a large Canadian (Ontario) population [28].

We consider that the Gly/Gly genotype of the Gly322Asp polymorphism of the hMSH2 gene could be one of these factors that enhance observed effect of the –93G>A polymorphism of hMLH1 on EC occurrence. A large study population showed that the Gly322Asp mutation was detected in 2 out of 19 patients with hereditary EC [29]. In our work we showed that the EC occurrence slightly increased in subjects with –93 A and Gly322Gly genotypes carriers (ORs 4.18–4.52).

In summary, our results suggest that the –93G>A polymorphism of the hMLH1 gene can be associated with the occurrence of EC and the Gly322Asp polymorphism of the hMSH2 gene increased the risk of EC in individuals with the –93A allele. Therefore, genetic variations in the DNA MMR system may play a role in the pathogenesis of EC.

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