hMSH2 Gly322Asp (rs4987188) Single nucleotide polymorphism and the risk of breast cancer in the Polish women

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Abstract—

Aim: Breast cancer *is the most common cancer in women both in the developed and less developed* world*. The reported study was designed to explore associations between hMSH2 - Gly322Asp (1032G>A, rs4987188) single nucleotide polymorphism (SNP) and the risk of breast carcinoma in the Polish women.*

Material and methods: *Blood samples were obtained from women with breast cancer (n=225), treated at the Department of Oncological Surgery and Breast Diseases, Polish Mother's Memorial Hospital – Research Institute between the years 2005 and 2012. A control group included 220 cancer-free women. Genomic DNA was isolated and the SNP Gly322Asp of hMSH2 was determined by High-Resolution Melter method. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for each genotype and allele.*

Results: *This study revealed that single nucleotide polymorphism Gly322Asp of hMSH2 is associated with both breast cancer risk and grading. Moreover, it can be linked with breast carcinoma tumor size and lymph node status. The Asp allele in patients may be a risk factor for breast carcinoma (OR 5.12; 95% CI 3.77 –6.97, p<.0001).*

Conclusions: *Gly322Asp single nucleotide polymorphism of hMSH2 may be a risk factor of breast cancer in the Polish women.*

Keywords— breast cancer, single nucleotide polymorphism, hMSH2, mismatch repair genes.

I. INTRODUCTION

Carcinoma of the breast is the most common cause of death among women worldwide. 1.7 million of new cases was diagnosed in 2012. Same year half a million women died due to breast cancer (GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012). Every year over 17 thousand women in Poland are diagnosed with breast cancer with more than 5 thousand of them eventually succumbing to the illness [i].

According to authors of "White Book. Overcoming colorectal and breast cancer in Poland in comparison to other European countries" by 2020 the incidence should increase to almost 20 thousand patients yearly [2]. 5year survival rate among breast cancer patients in Poland is one of the lowest in whole Europe. Analyzing European average survival rate of breast cancer patients versus Poland rate, in first year after diagnose the values are 4% lower, and after 5 years they rise to 10% (EUROCARE5, online database – years 2000–2007).

The system of DNA repair takes part in maintaining the genomic integrity which undergoes changes under exo- and endogenous factors. In man, the protein products of genes are directly involved in the repair process, taking part in several

repair systems. The repair process usually encompasses two stages: the excision of lesion and the repair synthesis. This is how repair system act via base-excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR). Totally converse is the repair system activity by direct lesion reversal, in which there is merely a single-stage process with maintained integrity of the DNA phosphodiester chain and the system of recombination repair (HR). Defects of the proteins, which directly participate in DNA repair and its control, are associated with an increased susceptibility to malignant changes.

The alternations in the oncogenes and tumor suppressor genes as well as DNA mismatch repair genes have been associated with cancers development [3, 4, 5]. There are seven major DNA mismatch repair genes in humans: *MLH1*, *MLH3*, *PMS1*, *PMS2*, *MSH2*, *MSH3* and *MSH6*. *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].

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].

A positive correlation between polymorphisms of the *hMSH2* gene and occurrence of cancer was reported in 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 role of *hMSH2* polymorphism and breast cancer development is still unknown. The primary goal of the study was an identification of genetic variants which increase the risk of breast cancer in the population of Polish women.

II. MATERIALS AND METHODS

2.1 Patients

Blood samples from patients with breast cancer (n=225) treated in the Department of Oncological Surgery and Breast Diseases, Polish Mother's Memorial Hospital – Research Institute between 2005-2012 were selected to the test group the study. All the tumors were graded by a method, based on the criteria of Scarf-Bloom-Richardson. The age of the patients ranged in from 38 to 85 (mean age: 54.2 ± 9.11). Controls consisted of the DNA extracted from blood samples from age-matched 440 cancer-free women (age range: 35–83, mean age: 52.27±10.16). The full clinicopathological characteristics of the study group are presented in Table 1.

TABLE 1
THE CLINICOPATHOLOGICAL CHARACTERISTICS OF 225 PATIENTS WITH BREAST CANCER

Characteristics	Number of patients (%)
Lymph node status	
N0	80 (35%)
N1	90 (40%)
N2	40 (18%)
N3	15 (7%)
Tumor size grade	
T1	64 (28%)
T2	149 (66%)
T3	12 (48%)
Bloom-Richardson grading	
I	49 (22%)
II	166 (74%)
III	10 (4%)

All of the studied individuals were Caucasians and constituted a homogenous population from the same ethnic and geographical origins. Genetic studies were performed at the Laboratory of Cancer Genetics, Department of Clinical Pathomorphology, Institute of the Polish Mother's Memorial Hospital in Lodz, Poland. The study was approved by the Research Ethics Committee at the Polish Mother's Memorial Hospital Research Institute (Approval number, No 10/2012).

2.2 DNA isolation

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

2.3 Determination of hMSH2 genotype

Polymorphism Gly322Asp of the *hMSH2* gene was determined by High-Resolution Melter analysis. The analysis of the SNPs was performed with support of a Light Cycler® 480 High Resolution Melting Master Kit (Roche, Mannheim, Germany), according to the manufacturer's recommendations. A non-template control contained water, instead of genomic DNA, as a negative control. Additionally, positive controls (DNA samples with known genotype) were employed in each run of HRM analysis. All PCR was carried out in a LightCycler® 96 (Roche, Mannheim, Germany) Thermocycler. The collected data were analysed, using the LightCycler® 96 software version SW 1.1 (Roche, Mannheim, Germany).

2.4 Statistical Analysis

A standard $\chi 2$-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 $\chi 2$-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.

III. RESULTS

All the recruited breast cancer subjects and control were successfully genotyped for the *hMSH2* polymorphisms. From the PCR analysis, all patients were classified into three genotypes of the *hMSH2* polymorphism: Gly/Gly, Gly/Asp and Asp/Asp.

In case of the Gly322Asp polymorphism of *hMSH2* gene the distribution of the genotypes in the patients differed significantly from one expected from the Hardy-Weinberg equilibrium ($p < 0.05$). The observed genotype frequency of Gly322Asp polymorphism in the controls group were in agreement with Hardy-Weinberg equilibrium ($p>0.05$). It is caused by the very low abundance of the Gln/Gln genotype in the examined population of Polish women.

The study of a series of 225 DNA samples from the test group followed by a comparison with controls revealed a relationship of the studied SNP with the risk of breast cancer (Table 2).

TABLE 2
DISTRIB0075TIONS OF GLY/GLY, GLY/ASP AND ASP/ASP GENOTYPES AND GLY AND ASP ALLELES OF THE Gly322Asp POLYMORPHISM OF *hMSH2* GENE IN THE GROUP OF BREAST CANCER PATIENTS AND CONTROLS.

	Patients (n = 225)		**Controls (n = 220)**			
hMSH2- Gly322Asp	Number	(%)	Number	(%)	OR (95% CI)[a]	P^b
Gly/Gly	25	11	44	20	1.00 Ref	
Gly/Asp	28	12	140	64	0.35 (0.181-0.66)	0.001
Asp/Asp	172	77	36	16	8.41 (4.57-15.44)	<.0001
Gly	78	17	228	52	1.00 Ref	
Asp	372	83	212	48	5.12 (3.77-6.97)	<.0001

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

We have proven that Asp variant may increase the risk of cancer (OR 5.12; 95% CI 3.77 –6.97, $p<.0001$). Moreover, we observed that Asp/Asp genotype was strongly associated with the risk of breast carcinoma (OR 8.41; 95% CI 4.57 – 15.44, $p<.0001$). Furthermore, an association was observed between the studied polymorphism and cancer grading (Table 3): Grade 1 (G1) patients generally demonstrated higher frequencies of Gly/Asp heterozygotes (p=0.039). Additionally, the studied SNP was statistically related to tumor size and lymph node status (Table 4).

TABLE 3
DEPENDENCE OF *hMSH2* GENE POLYMORPHISM GENOTYPES AND ALLELE FREQUENCY ON TUMOUR GRADE IN BREAST CANCER PATIENTS[a]

	breast cancer patients			
grade[b]	**GI (n = 49)**	**GII + GIII (n = 176)**	**OR (95% CI)[c]**	***p***
hMSH2- Gly322Asp	Number (%)	Number (%)		
Gly/Gly	9 (18)	16 (9)	1.00 Ref	
Gly/Asp	18 (37)	10 (6)	3.20 (1.04-9.85)	0.039
Asp/Asp	22 (45)	150 (85)	0.26 (0.10-0.66)	0.006
Gly	36 (37)	42 (12)	1.00 Ref	
Asp	62 (63)	310 (88)	0.23 (0.13 – 0.39)	<.0001

[a]n =450; [b]according to Scarf-Bloom-Richardson criteria

TABLE 4
***hMSH2* GENE POLYMORPHISM AND BREAST CANCER PROGRESSION[a]**

T	**T1 (n=64)**		**T2+T3 (n=161)**		**OR (95% CI)[b]**	***p*[c]**
	number	**(%)**	**number**	**(%)**		
Gly/Gly	15	23	10	6	1.00 Ref.	-
Gly/Asp	10	16	18	11	0.37 (0.12-1.12)	0.135
Asp/Asp	39	61	133	83	0.19 (0.08-0.46)	0.0002
Gly	40	31	38	12	1.00 Ref.	-
Asp	88	69	284	88	0.29 (0.17-0.48)	<.0001
N	N- (n=80)		N+ (n=145)		OR (95% CI)	*p*
	number	(%)	number	(%)		
Gly/Gly	11	14	14	10	1.00 Ref.	-
Gly/Asp	18	23	10	7	2.29 (0.75-6.91)	0.228
Asp/Asp	51	63	121	83	0.53 (0.22-1.26)	0.225
Gly	40	25	38	13	1.00 Ref.	-
Asp	120	75	252	87	0.45 (0.27-0.74)	0.002

[a]T1 vs. T2 + T3, [b]N - (node negative) vs. N + (node positive), [b]Crude odds ratio (OR), 95 % CI = confidence interval at 95%, [c]Chi square

IV. DISCUSSION

The genes of the DNA lesion repair systems play the key role in maintaining the genome integrity and control the repair of mutation-affected DNA. Without the genes, DNA would continue the accumulation of errors which would within a short time prevent further cell survival. Proper DNA repair ensures genomic integrity and plays a significant role in its protection against effects of carcinogenic factors. The polymorphism of repair genes may influence the performance of the process, by which defects of genetic material are removed, thus influencing the individual susceptibility to formation of neoplastic disease.

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 [16]. 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 86% of the entire breast cancer population shows a substantial loss in expression of DNA mismatch repair genes [16].

There were more than 130 DNA repair genes identified, in which a series of single nucleotide polymorphisms (SNPs) were discovered. In order to define the role, which may be played by these variants in modulating the risk of cancer formation, it is necessary to define their functional significance. The variability, perceived in DNA repair genes, may be of clinical importance for evaluation of the risk of occurrence of a given type of cancer, its prophylactics and therapy [17]. Literature data suggest that genetic variation of *hMSH2* gene may increase the risk of ovarian, breast and endometrial cancers [18, 19].

In our study we have demonstrated that both the Asp allele and the polymorphic homozygote Asp/Asp of the analysed SNP strongly increase the risk of breast carcinoma. 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 tumor size and lymph node status of breast cancer.

Our study, however, has certain limitations: patients group and controls may be quantitatively unsatisfactory. 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 single nucleotide polymorphism Gly322Asp of *hMSH2* and other SNPs in DNA mismatch repair genes could shed a new light on the role of SNPs in breast cancer.

V. CONCLUSIONS

The obtained results demonstrate a possibility of a relationship between the Gly322Asp polymorphism of *hMSH2* and breast cancer in the population of the Polish women. However, it requires further studies on much larger groups of patients.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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