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The polymorphisms of genes involved in DNA methylation in patients with malignancies from West Ukraine

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Aim. To determine a potential role of single-nucleotide polymorphisms in genes involved in DNA methylation (*MTHFR*, *MTR*, *TYMS*) in the breast cancer risk and risk of leukemia in a case-control study from West Ukraine. **Methods.** Genotyping of *MTHFR* 677 C>T, *MTR* 2756 A>G and *TS* 3R2R, *TS* 3RG>C was performed in 60 patients with leukemia, 90 patients with breast cancer and in 100 persons from a control group. The molecular-genetic analysis was performed by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism analysis. A statistical analysis was conducted by Chi-square tests and odds ratio (OR) calculation. **Results.** We did not observe any significant difference in genotype frequencies of the *MTHFR* and *TYMS* polymorphisms between the patients and the controls. The *MTR* 2756AA genotype frequency was significantly higher in the breast cancer patients vs. control (0.67 vs. 0.50, $p = 0.02$) while the difference between the leukemia patients and the control group was not statistically significant. The increased risk of breast cancer development was associated with the *MTR* 2756AA genotype (OR = 2.00, CI – 95 %: 1.11 – 3.60) and the *MTR* 2756A allele (OR = 1.75, CI – 95 %: 1.08 – 2.84). **Conclusions.** Our findings show that West Ukrainian inhabitants carrying at least one *MTR* 2756A allele have a significantly increased risk of breast cancer.

Keywords: methylation, polymorphism, gene, leukemia, breast cancer

Introduction

Aberrant DNA methylation is a key epigenetic factor in tumorigenesis [1]. The genomic methylation patterns depend of the one-carbon metabolizing enzymes, that involved in the provision of methyl groups, and DNA methyltransferases (DNMT) that transfer methyl groups from S-adenosylmethionine to DNA CpG dinucleotides [2, 3].

A crucial role for the control on DNA methylation as well as DNA synthesis and repair mechanisms belongs to the provision of adequate pools of methyl groups through the functional activity of methylenetetrahydrofolate reductase (*MTHFR*), methionine synthase (*MTR*) and thymidylate synthase (*TYMS*) in one-carbon metabolism. *MTHFR* catalyzes the ir-

reversible conversion of 5,10 – methylenetetrahydrofolate (THF) to 5-methyl-THF. The 5,10-methylenetetrahydrofolate is used by *TYMS* in the methylation of dUMP to dTMP, which is the only *de novo* source of thymidine required for DNA synthesis and repair. The 5-methyl-THF is a methyl group donor for the remethylation of homocysteine to methionine catalysed by *MTR* [4].

The genetic variants of *MTHFR*, *MTR*, and *TYMS* have been shown to affect the function of corresponding proteins [5–8]. A single nucleotide polymorphism 677C>T of the *MTHFR* gene is correlated with the enzyme thermolability and reduced activity [6]. A common polymorphism 2756A>G in the *MTR* gene is associated with a lower enzyme activity, causing DNA hypomethylation [7]. The individuals with polymorphisms in the *TYMS* 5' untranslated enhanc-

er region (TSER), that contains a 28-bp tandem repeat sequence, and those carrying alleles with three repeats (3R) have higher *TYMS* mRNA levels than the individuals with two repeats (2R) [9]. An additional polymorphism mapping to the second repeat sequence of the 3R allele, 3R *G>C*, may also affect the *TYMS* expression, 3RC alleles being associated with a comparable expression to 2R alleles [10]. There are a large number of molecular epidemiological studies about a role of the folate metabolic enzyme gene polymorphism in different kinds of malignancy: lung cancer [11], breast cancer [12–15], lymphoma [16, 17]. However, the association between polymorphism and cancer risk is still controversial.

It is well known that methylation of the promoter and exon regions is one of the major regulatory mechanisms of gene expression; thus, we hypothesized that a gene involved in the folate metabolism may be responsible for regulating the methylation status of other genes associated with cancer risk. Therefore, the aim of this study was to determine the occurrence of single-nucleotide polymorphisms in the genes encoding the folate metabolizing enzymes (*MTHFR*, *MTR*, *TYMS*) in a case-control study from West Ukraine for their potential role in the breast cancer risk and risk of leukemia.

Materials and Methods

Patients. For molecular genetic testing the DNA samples, that were obtained from the venous blood nuclear cells of the patients with cancer, were used. The patients were hospitalized in the Regional Specialized Children's Hospital, Lviv Regional Specialized Oncological Clinic and Lviv regional hospital. To form the patient groups a detailed medical anamnesis was obtained and clinical pictures of the oncological disease were estimated. The data were analyzed among 150 patients, who were separated into 2 groups in accordance with their diagnosis. The first research group contains 60 patients with leukemia and lymphoma (65 % - boys, 35 % - girls) aged 2 to 16 years. The diagnosis of oncohematological pathology was established for the first time. The group includes the 17 patients with acute

leukemia, 22 persons with Hodgkin's lymphoma and 21 patients with non-Hodgkin's lymphoma. 90 patients with breast cancer compose the second research group: women with breast cancer (II – IV stages) aged 35 to 75 years with burdened family history of breast or ovarian cancer. The control group consist of 100 healthy patients without cancer pathology in anamnesis. All persons from the research and control groups were from West Ukraine.

All patients signed the informed consent for using their DNA samples for molecular-genetic testing. The experimental protocol was approved by the Bioethics Committee of SI "Institute of hereditary pathology NAMS of Ukraine" and complied with international standards.

Genotyping. The extraction and refinement of DNA from the leukocytes of the peripheral blood were conducted according to the salting-out protocol extraction [18]. The amplification of DNA chain *in vitro* was performed using the method of polymerase chain reaction (PCR) [11, 12]. To detect the variable number tandem repeats (VNTR) for the *TYMS* gene, PCR product was separated on a 2 % agarose gel (Fig.1). The samples showing the 2R/3R or 3R/3R genotypes were further analyzed for the 3RG>C polymorphism by the method of restriction fragment length polymorphism (RFLP) using *HaeIII* restriction enzyme (Fig.2).

Genotyping of the *MTHFR* 677C>T and *MTR* 2756 A>G polymorphisms was performed by RFLP using the restriction enzymes *HinfI* and *HaeIII* respectively. The obtained restriction fragment was detected by 2 % agarose gels electrophoresis.

Statistics. The results were processed with the methods of variation statistics suitable for the biological studies and recommended for processing the molecular genetic research results: Chi-square analysis (χ^2 tests), Hardy – Weinberg equilibrium, odds ratio (OR) with 95 % confidence interval (CI).

Results and Discussion

There are many data on the interrelation between polymorphisms of the gene involved in the methylation process and the cancer susceptibility but the results vary depending of the patient ethnicity.

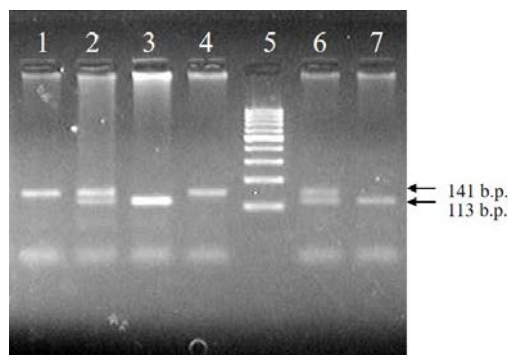


Fig. 1. Molecular genetic analysis of *TYMS* 3R/2R VNTR polymorphism, 2 % agarose gel. Lane 1,4 - genotype 3R3R; lane 2, 6 – genotype 3R2R; lane 3, 7 – genotype 2R2R; lane 5 – Marker pUC19 DNA/MspI.

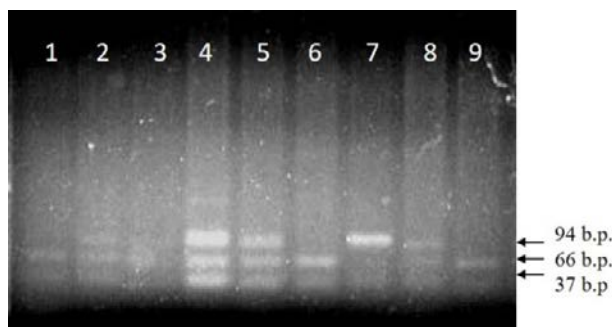


Fig. 2. Restriction analysis of *TYMS* 3R G>C polymorphism, 4 % agarose gel. Lane 1, 6, 9 – genotype GG; lane 2, 3, 4, 5, 8 – genotype GC; lane 7 – genotype CC.

Table 1. Genotypes and alleles frequency of *MTHFR* 677C>T, *MTR* 2756 A>G and *TYMS* 3R2R, 3R G>C polymorphisms among patients with different types of cancer and control group.

Genotype/ allele	Frequency of genotypes and alleles, %														
	Patients with breast cancer					Patients with leukemia					Group of control				
	N	Actual frequency	HWE	χ^2	P	N	Actual frequency	HWE	χ^2	P	N	Actual frequency	HWE	χ^2	P
<i>MTHFR</i> 677 C>T															
677CC	41	46	47.5			32	53	53.8			40	40	43.6		
677CT	42	47	42.9			24	40	39.1			52	52	44.9		
677TT	7	7	9.7	0.71	0.40	4	7	7.1	0.03	0.86	8	8	11.6	2.52	0.11
677C	124	69	–			88	73	–			132	66	–		
677T	56	31	–			32	27	–			68	34	–		
<i>MTR</i> 2756 A>G															
2756AA	60	67	65.8			37	62	61.4			50	50	50.4		
2756AG	26	29	30.6			20	33	33.9			42	42	41.2		
2756GG	4	4	3.6	0.29	0.59	3	5	4.7	0.02	0.89	8	8	8.4	0.04	0.84
2756A	146	81	–			94	78	–			142	71	–		
2756G	34	19	–			26	22	–			58	29	–		
<i>TYMS</i> 3R2R, 3R G>C															
HH	1	1	2.1			1	2	2.3			0	0	2.0		
HL	24	27	24.7			16	27	25.5			28	28	24.1		
LL	65	72	73.2	0.56	0.45	43	71	72.3	0.13	0.72	72	72	74.0	2.65	0.1
H	26	14	–			18	15	–			28	14	–		
L	154	86	–			102	85	–			172	86	–		

Note: N – quantity of observation; HWE (Hardy-Weinberg equilibrium) – theoretically expected frequency; * $p > 0.05$ – frequency distribution of genotypes conformed to the HWE; High (H) and low (L) TS expression genotypes based on TSER and TSER 3R G>C polymorphisms (high: 3RG/3RG, 3RG/3RC, 3RG/2R; low: 3RC/3RC, 3RC/2R, 2R/2R); HH – 3RG3RG genotype; HL – 3RG2R, 3RG3RC genotypes; LL – 2R2R, 3RC2R, 3RC3RC genotypes.

In this study we analyse functional polymorphisms in the genes encoding the one-carbon metabolism enzymes and compare it with other population study. We carried out the molecular genetic testing of the genotype and allele frequency of the *MTHFR 677 C>T*, *MTR 2756 A>G* and *TYMS 2R/3R*, *TYMS 3R G>C* polymorphisms among the patients with breast cancer, leukemia and of a control group. The established genotypes and alleles distribution of testing polymorphic loci in the research and control groups did not significantly differ from the theoretically expected frequency conformed to Hardy-Weinberg equilibrium (Table 1). We have determined and compared the alleles and genotypes frequency of the studied polymorphic loci in the control and case groups (Table 2).

The genotypes and allele distribution of the *MTHFR 677 C>T* polymorphic loci did not show significant difference between the case and control groups. (Table 2.). The frequency of low-expression 677 TT genotype was 8 % in the group of breast cancer and 7 % in the control group as much as in the group of patients with leukemia. The frequency of 677 T allele did not differ in the case groups compared to the control (34 %, 27 % vs. 31 %). OR calculation also did not show statistically significant result (Table 2). Our findings coincide with the results of German and Korean researches [19, 20]. The decreased susceptibility to acute lymphoblastic leukaemia was found in the West European populations [21]. It was found that the 677C>T polymorphism is strongly associated with breast cancer in the East

Table 2. Genotypes and alleles distribution of *MTHFR 677C>T*, *MTR 2756 A>G*, *DNMT3B -149C>T*, *DNMT3B -579 G>T* and *TYMS 3R2R*, *3R G>C* polymorphisms among the patients with different types of cancer compared to the control group

Genotype/ allele	Group of control (n=100)	Patients with breast cancer (n=90)				Patients with leukemia (n=60)			
	n (%)	n (%)	χ^2	P	OR (95 % CI)	n (%)	χ^2	p	OR (95 % CI)
<i>MTHFR 677 C>T</i>									
677CC	40 (40)	41 (46)	0.41	0.52	1.26 (0.71 – 2.23)	32 (53)	2.10	0.15	1.71 (0.90 – 3.27)
677CT	52 (52)	42 (47)			0.81 (0.46 – 1.43)	24 (40)			0.62 (0.32 – 1.18)
677TT	8 (8)	7 (7)			0.97 (0.34 – 2.79)	4 (7)			0.82 (0.24 – 2.85)
677C	132 (66)	124 (69)	0.36	0.55	1.14 (0.74 – 1.75)	88 (73)	1,88	0,17	1.42 (0.86 – 2.33)
677T	68 (34)	56 (31)			0.88 (0.57 – 1.35)	32 (27)			0.71 (0.43 – 1.16)
<i>MTR 2756 A>G</i>									
2756AA	50 (50)	60 (67)	5.15	0.02*	2.00* (1.11 – 3.60)	37(62)	2.09	0.15	1.61 (0.84 – 3.09)
2756AG	42 (42)	26 (29)			0.56 (0.31 – 1.03)	20(33)			0.69 (0.35 – 1.35)
2756GG	8 (8)	4 (4)			0.53 (0.16 – 1.84)	3(5)			0.61 (0.15 – 2.38)
2756A	142 (71)	146 (81)	5.28	0.02*	1.75* (1.08 – 2.84)	94(78)	2.08	0.15	1.48 (0.87 – 2.51)
2756G	58 (29)	34 (19)			0.57 (0.35 – 0.92)	26 (22)			0.68 (0.40 – 1.15)
<i>TYMS 3R2R, 3R G>C</i>									
H/H	1 (1)	1 (1)	0.26	0.88	1.11 (0.07 – 18.05)	1 (2)	0.32	0.85	1.68 (0.10 – 27.33)
H/L	30 (30)	24 (27)			0.85 (0.45 – 1.60)	16 (27)			0.85 (0.42 – 1.73)
L/L	69 (69)	65 (72)			1.17 (0.62 – 2.19)	43 (71)			1.14 (0.56 – 2.30)
H	32 (16)	26 (14)	0.18	0.67	0.89 (0.51 – 1.55)	18 (15)	0.06	0.81	0.92 (0.49 – 1.75)
L	168 (84)	154 (86)			1.13 (0.64 – 1.98)	102 (85)			1.08 (0.57 – 2.06)

Note: n – quantity of observation; * $p < 0.05$ – statistically significant difference; **OR*** – statistically significant association between genotype and alleles polymorphism and cancer susceptibility; High (H) and low (L) TS expression genotypes based on TSER and TSER 3R G>C polymorphisms (high: 3RG/3RG, 3RG/3RC, 3RG/2R; low: 3RC/3RC, 3RC/2R, 2R/2R); HH – 3RG3RG genotype; HL – 3RG2R, 3RG3RC genotypes; LL – 2R2R, 3RC2R, 3RC3RC genotypes

Table 3. Genotype and allele frequency of the *TYMS* 3R2R, 3R G>C polymorphisms among the patients with different types of cancer and the control group

<i>TYMS</i> 3R2R, 3R G>C		Group of control (N=100) n (%)		Patients with breast cancer (N = 90) n (%)		Patients with leukemia (N=60) n (%)	
Genotypes							
3R3R	3RG3RG	27 (27)	1 (1)	26 (29)	1 (1)	14 (23)	1 (2)
	3RG3RC		17 (17)		14 (16)		8 (13)
	3RC3RC		9 (9)		11(12)		5 (8)
3R2R	2R3RG	40 (40)	13 (13)	43 (48)	10 (11)	29 (48)	8 (13)
	2R3RC		27 (27)		33 (37)		21 (35)
2R2R		33 (33)		21 (23)		17 (29)	
Allele							
3R	3RG	94 (47)	32 (16)	95 (53)	26 (15)	57 (48)	18 (15)
	3RC		62 (31)		69 (38)		39 (33)
2R		106 (53)		85 (47)		63 (52)	

Note: N – quantity of observation

Asian populations [22]. Therefore, our results seem to be more relevant to other population studies: the lack of association of the *MTHFR* polymorphisms with the breast cancer risk was observed in Indian women [23] and in the residents of Scotland [13], Finland [24] and Greece [25].

For better understanding of the clinical significance of the SNP and VNTR of *TYMS*, we analysed the frequency of comprehensive genotypes of 3R2R and 3R G>C polymorphisms in case and control groups. The allele and genotype frequency is summarized in Table 3.

The *TYMS* genotypes were each stratify into three groups because the statistical power was insufficient by analysis with more than three genotype groups. The *TYMS* functional groups were classified into the H-group (H/H and H/L) and L-group (L/L). The *TYMS* 3G allele was considered a high-expression allele and 2R or 3RC as low-expression alleles according to the *in vitro* functional analysis [26]. Therefore, the TS genotypes of 2R/3G, 3C/3G, or 3G/3G were considered as high expression (H) type and 2R/2R, 2R/3C or 3C/3C – low expression (L) type. According to our results, there was no significant association between the genotype groups and cancer pathology (Table 2.). Thus, the frequency of H-genotype group amounts 15 % in the patients with leukemia, 14 % in

the control group and the same in the patients with breast cancer. The following frequencies were detected in the L-genotype functional group: 85 %, 86 % and 86 % for the patients with leukemia, breast cancer and control group respectively.

Our results are very similar to those reported for the Spanish [27] and Mexican [28] women. The studies on other populations have found the significant associations between the *TYMS* gene *TSER* variations and breast cancer development [14]. For the *TYMS* 2R3R polymorphisms was found a decreased susceptibility to acute lymphoblastic leukemia in the Western European [15] and Brazilian studies [30]. Furthermore, the frequency of TS genotypes in the patients with leukemias United Kingdom [16, 29] did not show any significant difference compared to the controls.

The *MTR* 2756 A>G genotypes analysis show a significantly lower frequency of *MTR* 2756 GG genotype in the group of patients with breast cancer (4 %) compared to the control group (8 %, P<0.05).

The results of OR calculation indicate 2-fold increase in the breast cancer risk for people with the *MTR* 2756 AA genotype (OR=2.00, CI – 95 %: 1.11 – 3.60). The 2756 GA genotype difference between the group of patients with breast cancer and the control group was statistically significant (0.29

Table 4. Distribution of two genotypes combination of *MTHFR* 677C>T, *MTR* 2756 A>G, *DNMT3B* -149C>T, *DNMT3B* -579 G>T and *TYMS* 3R2R, 3R G>C polymorphisms among the patients with different types of cancer compared to the control group.

Genotypes	Group of control	Patients with breast cancer (n=90)				Patients with leukemia (n=60)			
		n (%)	χ^2	P	OR (95 % CI)	n (%)	χ^2	p	OR (95 % CI)
<i>MTR</i> 2756 A>G / <i>MTHFR</i> 677 C>T									
2756AA/677CC	27 (54)	27 (45)	2.50	0.29	0.70 (0.33 – 1.48)	20 (54)	0.05	0.98	1.00(0.43 – 2.35)
2756AA/677CT	22 (44)	28 (47)			1.11 (0.52 – 2.37)	16 (43)			0.97(0.41 – 2.29)
2756AA/677TT	1 (2)	5 (8)			4.45 (0.50 – 39.46)	1 (3)			1.36(0.08 – 22.50)
2756AG/677CC	12 (29)	12 (46)	2.21	0.33	2.14 (0.77 – 5.95)	11 (55)	4.17	0,12	3.06(1.01 – 9.24)
2756AG/677CT	25 (59)	12 (46)			0.58 (0.22 – 1.56)	7 (35)			0.37(0.12 – 1.11)
2756AG/677TT	5 (12)	2 (8)			0.62 (0.11 – 3.44)	2 (10)			0.82(0.15 – 4.66)
2756GG/677CC	1 (13)	2 (50)	2.57	0.28	7.00(0.40 – 123.35)	1 (34)	0.92	0.63	3.50(0.14 – 84.70)
2756GG/677CT	5 (62)	2 (50)			0.60 (0.05 – 0.80)	1 (33)			0.30(0.02 – 4.91)
2756GG/677TT	2 (25)	0 (0)			0.29 (0.01 – 7.57)	1 (33)			1.50(0.08 – 26.86)
<i>MTR</i> 2756 A>G / <i>TYMS</i> 3R2R, 3R G>C									
2756AA/HH	1 (2)	0 (0)	3.17	0.21	0.27 (0.01 – 6.84)	0(0)	1.67	0.43	0.44(0.02 – 11.11)
2756AA/HL	9 (18)	18 (30)			1.95 (0.79 – 4.84)	10(27)			1.69(0.61 – 4.69)
2756AA/LL	40 (80)	42 (70)			0.58 (0.24 – 1.41)	27(73)			0.68(0.25 – 1.84)
2756AG/HH	0 (0)	1 (4)	5.24	0.07	5.00(0.20 – 127.43)	1(5)	3.63	0.16	6.54(0.25 – 167.83)
2756AG/HL	18 (43)	5 (19)			0.32 (0.10 – 1.00)	5 (25)			0.44(0.14 – 1.45)
2756AG/LL	24 (57)	20 (77)			2.50(0.83 – 7,50)	14 (70)			1.75(0.56 – 5.44)
2756GG/HH	0 (0)	0 (0)	0.19	0.91	1.89(0.03 – 112.07)	0(0)	0.02	0.99	2.43(0.04 – 148.44)
2756GG/HL	3 (38)	1 (25)			0.56 (0.04 – 8.09)	1(33)			0.83(0.05 – 13.63)
2756GG/LL	5 (62)	3 (75)			1.80 (0.12 – 26.20)	2(67)			1.20(0.07 – 19.63)
<i>MTHFR</i> 677 C>T / <i>TYMS</i> 3R2R, 3R G>C									
677CC/HH	0 (0)	1 (3)	1.04	0.59	3.00 (0.12 – 75.85)	0 (2)	1.00	0.61	1.25(0.02 – 64.53)
677CC/HL	13 (33)	14 (34)			1.08 (0.43 – 2.71)	7 (22)			0.58(0.20 – 1.69)
677CC/LL	27 (67)	26 (63)			0.83 (0.33 – 2.09)	25 (78)			1.72(0.59 – 5.00)
677CT/HH	0 (0)	0 (0)	22.99	1×10⁻⁵	1.24(0.02 – 63.57)	1 (4)	2.21	0.33	6.70(0.26–170.69)
677CT/HL	15 (29)	33 (79)			9.04 (3.50 – 23.39)	7 (29)			1.02(0.35 – 2.95)
677CT/LL	37 (71)	9 (21)			0.11 (0.04 – 0.29)	16 (67)			0.81(0.29 – 2.29)
677TT/HH	1 (12)	0 (0)	1.36	0.51	0.33 (0.01 – 9.57)	0 (0)	1.07	0.59	0.56(0.02 – 16.77)
677TT/HL	2 (25)	1 (14)			0.50 (0.04 – 7.10)	2 (50)			3.00(0.24 – 37.67)
677TT/LL	5 (63)	6 (86)			3.60(0.28 – 46.36)	2 (50)			0.60(0.05 – 6.80)

Note: *n* – quantity of observation; *p* < 0.05 – statistically significant difference; **OR**– statistically significant association between genotype and alleles polymorphism and cancer susceptibility; High (H) and low (L) TS expression genotypes based on TSER and TSER 3R G>C polymorphisms (high: 3RG/3RG, 3RG/3RC, 3RG/2R; low: 3RC/3RC, 3RC/2R, 2R/2R); HH – 3RG3RG genotype; HL – 3RG2R, 3RG3RC genotypes; LL – 2R2R, 3RC2R, 3RC3RC genotypes

Table 5. Distribution of three genotypes combination of *MTHFR* 677C>T, *MTR* 2756 A>G, *DNMT3B* -149C>T, *DNMT3B* -579 G>T and *TYMS* 3R2R, 3R G>C polymorphisms among the patients with different types of cancer compared to the control group.

Genotypes	Group of control	Patients with breast cancer (n=90)				Patients with leukemia (n=60)			
	n (%)	n (%)	χ^2	P	OR (95 % CI)	n (%)	χ^2	p	OR (95 % CI)
<i>MTHFR</i> 677 C>T/ <i>MTR</i> 2756 A>G / <i>TYMS</i> 3R2R, 3R G>C									
CC/AA/HH	0 (0)	0 (0)			0.75(0.01 – 39.16)	0 (0)			1.00 (0.02 – 52.85)
CC/AA/HL	2 (10)	10 (37)	4.42	0.11	5.29(1.01 – 27.75)	6 (30)	2.50	0.29	3.86 (0.67 – 22.11)
CC/AA/LL	18 (90)	17 (63)			0.19(0.04 – 0.99)	14(70)			0.26 (0.05 – 1.49)
CT/AA/HH	0 (0)	0 (0)			0.89(0.02 – 46.76)	0 (0)			1.55 (0.03 – 81.77)
CT/AA/HL	7 (28)	8 (29)	0.00	1.00	1.03(0.31 – 3.41)	4 (25)	0.04	0,98	0.86 (0.21 – 3.58)
CT/AA/LL	18 (72)	20 (71)			0.97(0.29 – 3.22)	12(75)			1.17 (0.28 – 4.87)
TT/AA/HH	0 (0)	0 (0)			1.00(0.02 –60.00)	0 (0)			3.67(0.05 – 274.53)
TT/AA/HL	0 (0)	0 (0)	0.00	1.00	1.00(0.02 –60.00)	0 (0)	0.00	1.00	3.67(0.05 – 274.53)
TT/AA/LL	5 (100)	5 (100)			1.00 (0.02 –60.00)	1(100)			3.67(0.05 – 274.53)
CC/AG/HH	2 (12)	1 (8)			0.68(0.05 – 96.38)	0(0)			0.27(0.01 – 6.17)
CC/AG/HL	12 (70)	3 (25)	7.36	0.03	0.14(0.03 – 0.74)	1(9)	14.45	7×10⁻⁴	0.04(0.00 – 0.42)
CC/AG/LL	3 (18)	8 (67)			9.33 (1.65 – 52.69)	10(91)			46.6 (4.22 –516.64)
CT/AG/HH	0 (0)	0 (0)			1.80(0.03 – 96.38)	1(25)			19.29(0.65–523.86)
CT/AG/HL	7(32)	1(8)	2.38	0.30	0.19(0.02 – 1.82)	0 (0)	6.80	0.03	0.23(0.01 – 4.84)
CT/AG/LL	15 (68)	11 (92)			5.13(0.55 – 47,98)	3 (75)			1.40 (0.12 – 15.97)
TT/AG/HH	0 (0)	0 (0)			1.89(0.03–112.07)	0(0)			2.43(0.04 – 148.44)
TT/AG/HL	3 (38)	1 (25)	0.19	0.91	0.56 (0.04 – 8.09)	1(33)	0.02	0.99	0.83(0.05 – 13.63)
TT/AG/LL	5 (62)	3 (75)			1.80 (0.12 –26.20)	2(67)			1.20 (0.07 – 19.63)
CC/GG/HH	0 (0)	0 (3)			0.71 (0.01 –49.71)	0 (2)			1.67 (0.02 –137.36)
CC/GG/HL	1 (50)	1 (33)	0.14	0.93	0.50(0.01 –19.56)	0 (0)	0.75	0.69	0.33 (0.01 – 16.80)
CC/GG/LL	2 (50)	1 (67)			2.00(0.05 – 78.25)	1(100)			3.00(0.06 – 151.20)
CT/GG/HH	0 (0)	0 (0)			2.20(0.03–146.04)	0 (0)			3.67(0.05 – 274.53)
CT/GG/HL	0 (0)	1 (50)	2.92	0.09	11.00(0.28–433.83)	0 (0)	0.00	1.00	3.67(0.05–274.53)
CT/GG/LL	5(100)	1 (50)			0.09 (0.00 – 3.59)	1(100)			3.67(0.05 – 274.53)
TT/GG/HH	0 (0)	0 (0)				0(0)			
TT/GG/HL	0 (0)	0 (0)	–	–		1(100)		–	
TT/GG/LL	0 (0)	0 (0)			–	0 (0)			–

Note: n – quantity of observation; **p <0.05** – statistically significant difference; **OR**– statistically significant association between genotype and alleles polymorphism and cancer susceptibility; High (H) and low (L) TS expression genotypes based on *TSER* and *TSER* 3R G>C polymorphisms (high: 3RG/3RG, 3RG/3RC, 3RG/2R; low: 3RC/3RC, 3RC/2R, 2R/2R); HH – 3RG3RG genotype; HL – 3RG2R, 3RG3RC genotypes; LL – 2R2R, 3RC2R, 3RC3RC genotypes

vs. 0.42; P<0.05). The frequency of the 2756 G allele among the patients with breast cancer (0.19) was statistically significant compared to the control (0.29; P<0.05). The possibility of breast cancer develop-

ment increases by 1.75 times (OR=1.75, CI – 95 %:1.08 – 2.84) in the presence of at least one copy of the 2756 A allele. The association with breast cancer risk was found for the Brazilian [14], Indian

[31] women carrying the *MTR* A2756G polymorphic allele. It was revealed by meta-analysis that the *MTR* A2756G polymorphism may contribute to the susceptibility to breast cancer among Europeans [32]. However, in many studies the scientists cannot find a significant association of the *MTR* A2756G polymorphisms with the breast cancer risk [33].

The distribution of the *MTR* 2756 AG genotypes and allele did not show significant difference in the control group and the group of patients with leukemia. The frequency of low enzyme activity 2756 GG genotype in the case group consists 5 % and in the control group 8 %. In the study on British population also was not found significant difference in the susceptibility to acute leukemia for the *MTR* 2756AG polymorphism [16]. The association of this polymorphism has been reinforced in a meta-analysis, being the 2756 AA genotype reduced risk of developing childhood acute lymphoblastic leukemia [34].

In our research we also analyzed the distribution of different genotypes combination in group of patient with breast cancer, leukemia and control group. In fact, we compared combination of two and three genotypes of *MTHFR* 677C>T, *MTR* 2756 A>G, DNMT3B -149C>T, DNMT3B -579 G>T and *TYMS* 3R2R, 3R G>C polymorphisms (Table 4, Table 5).

Our findings show statistically significant difference in distribution of *MTHFR* 677677C>T / *TYMS* 3R2R, 3R G>C polymorphic loci genotypes combination in control group and group of women with breast cancer. The frequency of *MTHFR* 677CT/ *TYMS* LL genotypes was significant higher in control group (71 %) compared to group of patient with breast cancer (21 %), therefore, the frequency of *MTHFR* 677CT/ *TYMS* HL genotypes was significant higher in observed group with breast cancer (79 %) compared to control (29 %). The OR calculation show that carriers of *MTHFR* 677CT/ *TYMS* LL genotypes has reduced risk of breast cancer (OR = 0.11, CI 95 %: 0.04 – 0.29), and the carriers of *MTHFR* 677CT/ *TYMS* HL genotypes, on the contrary, has increased breast cancer risk (OR = 9.04, CI 95 %: 3.50 – 23.39).

The analysis of three genotype combination shown that carriers of *MTHFR* 677CC/ *MTR*

2756AG/ *TYMS* LL genotypes were over represented in cases (breast cancer group – 67 %, group of leukemia – 91 %) compared to controls (18 %). The presence of the *MTHFR* 677CC/ *MTR* 2756AG/ *TYMS* LL genotypes was associated with 9.33-fold risk of breast cancer and 46.6-fold risk of leukemia. In group of patient with leukemia was established higher frequency of *MTHFR* 677CT/ *MTR* 2756AG/ *TYMS* HH (0 %) and *MTHFR* 677CT/ *MTR* 2756AG/ *TYMS* LL (68 %) genotypes combination compared to control group (25 % and 75 %, respectively). These differences between the cases and controls were statistically significant ($p \leq 0.05$).

In summary, we found that different genotypes combination of *MTHFR*, *MTR* and *TYMS* genes polymorphisms in the West Ukrainian population could mediate the risk of breast cancer and leukaemia via effects on pools of methyl groups needed for genes methylation. The reasons for contrary results obtained in our research and other studies remain ambiguous and might be attributed to the differences in ethnic backgrounds and the selection of the population studied, differences in sample sizes, and gene-environment interactions. It is reasonable to repeat a comprehensive research in the whole country for clarifying a role of the genes involved in the DNA methylation process in cancer susceptibility.

Conclusion

Our findings show that West Ukrainian residents carrying at least one *MTR* 2756A allele have a significantly increased risk of breast cancer. Also, we find out, that susceptibility to breast cancer and leukaemia in West Ukrainians relate to the combination of few genotypes: *MTHFR* 677CC/ *MTR* 2756AG/ *TYMS* LL – increased the risk of breast cancer and leukaemia, 677CT/ *TYMS* HL – raise the risk of breast cancer and the presence of *MTHFR* 677CT/ *TYMS* LL genotypes acts as a protector factor for breast cancer development.

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Поліморфізм генів задіяних в процесах метилювання ДНК у пацієнтів з онкологічними захворюваннями з західного регіону України

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Мета. Визначити потенційну роль поліморфізму генів *MTHFR*, *MTR* та *TYMS* в розвитку раку молочної залози і лейкемії серед жителів Західної України. **Методи.** Проведено генотипування поліморфних локусів *MTHFR 677C>T*, *MTR 2756A>G*, *TYMS 3R2R* та *TYMS 3RG>C* у 60 пацієнтів з лейкемією, 90 пацієнтів

з раком молочної залози та в 100 осіб контрольної групи. Молекулярно-генетичний аналіз проводили за допомогою полімеразної ланцюгової реакції та аналізу поліморфізму довжин рестрикційних фрагментів. Статистичний аналіз проведений шляхом розрахунку χ -квадрат та обчислення коефіцієнту відношення шансів (OR). **Результати.** Вірогідних відмінностей у розподілі генотипів та алелей щодо поліморфних локусів генів *MTHFR* та *TYMS* не встановлено. Встановлено вірогідно вищу частоту генотипу *MTR 2756AA* у групі осіб з раком молочної залози в порівнянні з контролем (0,67 та 0,50, $p=0,02$), у групі осіб з лейкемією показники не відрізнялися відносно контролю. Вищий ризик розвитку раку молочної залози асоційований з генотипом *AA* (OR=2,00, CI-95 %:1.11–3.60) та *A* алелю (OR = 1,75, CI-95 %:1,08–2,84) поліморфного локусу *MTR 2756A>G*. **Висновки.** Встановлено, що наявність однієї копії алеля *MTR 2756A* може підвищувати ризик виникнення раку молочної залози у жителів Західної України.

Ключові слова: метилювання, поліморфізм, ген, лейкемія, рак молочної залози

Поліморфізм генів, задіяваних в процесах метилювання ДНК у пацієнтів з онкологічними захворюваннями із західного регіону України

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Цель. Определить потенциальную роль полиморфизма генов *MTHFR*, *MTR* и *TYMS* в развитии рака молочной железы и лейкемии среди жителей Западной Украины. **Методы.** Проведено генотипирование полиморфных локусов *MTHFR 677 C>T*, *MTR 2756A>G*, *TYMS 3R2R* и *TYMS 3RG>C* у 60 пациентов с лейкемией, 90 пациентов с раком молочной железы и у 100 человек контрольной группы. Молекулярно-генетический анализ проводили с помощью полимеразной цепной реакции и анализа полиморфизма длин рестрикционных фрагментов. Статистический анализ проведен путем расчета χ -квадрат и вычисления коэффициента отношения шансов (OR). **Результаты.** Достоверных различий в распределении генотипов и аллелей полиморфных локусов генов *MTHFR* и *TYMS* не установлено. Установлено достоверно высокую частоту генотипа *MTR 2756AA* в группе лиц с раком молочной железы по сравнению с контролем (0,67 и 0,50, $p=0,02$), в группе лиц с лейкемией показатели не отличались относительно контроля. Риск развития рака молочной железы, ассоциированный с генотипом *AA* (OR = 2,00, CI-95 %:1.11–3.60) и *A* аллеля (OR = 1,75, CI-95 %:1,08–2,84) полиморфного локуса *MTR 2756 A>G*. **Выводы** установлено, что наличие одной копии аллеля *MTR 2756 A* может повышать риск возникновения рака молочной железы у жителей Западной Украины.

Ключевые слова: метилирование, полиморфизм, ген, лейкемия, рак молочной железы

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