Role of Polymorphic Variants of *MTR* Gene A2756G and *SHMT1* Gene C1420T in the Development of Prostatic Cancer in Residents of the Western Siberian Region of Russia

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Allelic variants of folate cycle enzyme genes can contribute to predisposition to cancer. The impact of polymorphic loci A2756G of *MTR* gene and of C1420T of *SHMT1* gene for the risk of prostatic cancer was studied in residents of West Siberia. The frequency of alleles of these loci in patients (*N*=371) and controls (*N*=285) was determined and the data were statistically processed. No statistically significant association with prostatic cancer was detected for any of the studied loci.

Key Words: *prostatic cancer; folate cycle; polymorphic locus*

Abnormal DNA methylation plays an important role in the pathogenesis of prostatic cancer (PC) and other oncourological diseases [1,3,9]. Imbalance in folic acid metabolism can lead to reduced SAM (methyl group donor) production and modification of DNA methylation profile promoting stimulation of protooncogenes and inactivation of tumor growth suppressor genes. In addition, disorders in folate metabolism can reduce the efficiency of nucleotide synthesis and DNA reparation (Fig. 1). Therefore, allele variants of the folate cycle enzyme genes leading to changes in activities or amount of the corresponding enzymes can contribute to predisposition to cancer. Meta-analysis including studies of associations of single-nucleotide polymorphic substitutions (SNP) in the folate cycle genes and PC in Caucasian populations revealed a slight association of two loci A2756G of *MTR* gene (rs1805087) and C1420T of *SHMT1* gene (rs1979277) with PC risk [2]. The impact of these loci for PC risk in the West Siberian Region of Russia was studied.

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MATERIALS AND METHODS

DNA was isolated from venous blood by the standard method. Blood specimens were collected at the Altai Affiliated Department of N. N. Blokhin Cancer Research Center and A. I. Kryzhanovsky Regional Oncologic Dispensary, Krasnoyarsk. The group of patients included 371 men (mean age 69±8 years) with histologically verified PC. The control group consisted of 285 men (mean age 59±17 years) without history of cancer or benign prostatic hyperplasia. The examined men were residents of the Altai and Krasnoyarsk territories and belonged to the Russian ethnic group. All members of the groups gave informed written consent to participation in the study in accordance with regulations of the Ethic Committee.

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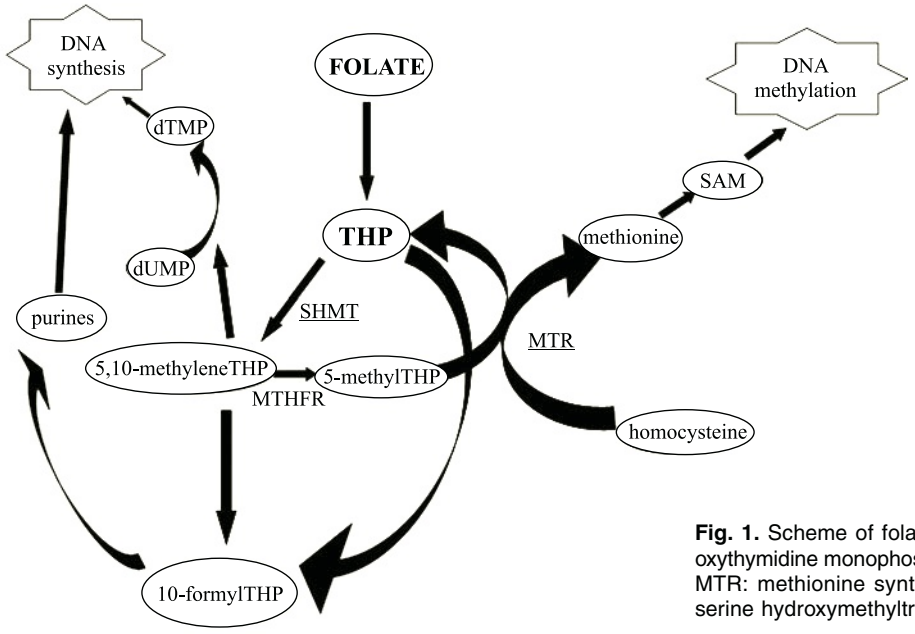

Fig. 1. Scheme of folate cycle . THP: tetrahydrofolate; dTMP: deoxythymidine monophosphate; dUMP: deoxyuridine monophosphate; MTR: methionine synthase; SAM: S-adenosylmethionine; SHMT: serine hydroxymethyltransferase.

Genotypes of the studied SNP were analyzed by real-time PCR using competitive TaqMan probes. PCR was carried out in a final volume of 25 μl containing 65 mM Tris-HCl (pH 8.9), 16 mM ammonium sulfate, 3.5 mM $MgCl_2$, 0.01% Twin-20, 0.2 mM deoxynucleoside triphosphates, 0.3 μM oligonucleotide primers, 0.1 μM TaqMan probes (Table 1), 20-100 ng DNA, and 1 U Taq-DNA polymerase.

Comparison of the frequencies of the studied SNP alleles and genotypes in the studied samples and testing of sample conformity to Hardy–Weinberg equilibrium were carried out using χ^2 test. The differences were considered significant at $p<0.05$. The estimations were carried out using on-line DeFinetti software (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl).

RESULTS

According to published data, A2756G substitution in *MTR* gene (D919G in the sequence of methionine synthase, the enzyme catalyzing methionine synthesis; Fig. 1) leads to impairment of the spatial structure and function of the protein [13], increase in intracellular level of homocysteine, and reduction of SAM level [6]. C1420T substitution in *SHMT1* gene leads to L425F substitution in serine hydroxymethyltransferase sequence, an enzyme catalyzing the synthesis of 5,10-methylene tetrahydrofolate, the key enzyme of folate cycle. The functional role of this polymorphic locus remains unclear. Meta-analysis has revealed an association of these loci with a slightly increased risk of PC (OR (odds ratio)=1.06, CI (confidence interval) 1.00-1.12 for A2756G *MTR*; OR=1.11, CI 1.00-1.22 for C1420T *SHMT1*) in the group of Caucasian subjects (control/experimental samples: 2689/4410 men for gene *SHMT1* locus and 7810/37543 for gene *MTR* locus) [2]. We studied the effect of these substitutions for PC risk in West Siberia.

We determined the frequency of the above SNP alleles in the group of PC patients and control group (Table 2). The frequency of genotypes of both loci corresponded to Hardy–Weinberg equilibrium in both samples. The frequency of *MTR* allele 2756G and *SHMT1* allele 1420T in the control group was compared to the values in Caucasian and Mongoloid populations. These values differed highly significantly from

TABLE 1. Primers and TaqMan Probes

Locus	Primers (*u*, *r*)	Probes
A2756G *MTR*	ctatcttgcattttcagtgttccatctgtttctaccacttaccttgag	R6G-ctcataatggcc<u>c</u>tgtctaa-BHQ
		FAM-ctcataatggtcc<u>t</u>gtctaa-BHQ
C1420T *SHMT1*	ccaggtgggtccagagtggagagactggcaggggataag	R6G-cttcgcctctct<u>c</u>ttccctct-BHQ
		FAM-cttcgcctct<u>t</u>tcttccctct-BHQ

TABLE 2. Frequencies of Alleles and Genotypes of *MTR* Locus A2756G and *SHMT1* Locus C1420T in the Studied Samples

Sample	Genotype			Allele	
	A2756G *MTR*				
	AA	AG	GG	A	G
Patients (370)	0.60 (221)	0.36 (134)	0.04 (15)	0.78	0.22
Control (285)	0.61 (173)	0.34 (96)	0.06 (16)	0.78	0.22
OR	1.00	1.09	0.73	1.00	0.98
CI		0.77-1.52	0.35-1.53		0.76-1.28
p		0.60	0.41		0.90
	C1420T *SHMT1*				
	CC	CT	TT	C	T
Patients (371)	0.44 (164)	0.42 (155)	0.14 (52)	0.65	0.35
Control (284)	0.47 (133)	0.44 (124)	0.10 (27)	0.69	0.31
OR	1.00	1.01	1.56	1.00	1.18
CI		0.73-1.41	0.93-2.62		0.93-1.48
p		0.94	0.09		0.18

TABLE 3. Frequency of the Studied SNP Alleles in Caucasian and Mongoloid Populations

Frequency of mutant allele		Sample size (population, reference)	*p*
2756G *MTR*			
Our data	0.22	285	
Mongoloids	0.14	910 (Japan [11])	0.008
Caucasians	0.20	2109 (Norway [12])	0.15
1420T *SHMT1*			
Our data	0.31	284	
Mongoloids	0.09	494 (Japan [4])	1.1×10^{-29}
Caucasians	0.34	2257 (Poland [7])	0.17

the values in the Mongoloid population and slightly from the frequency in the Caucasian sample (Table 3).

Comparison of the frequency of alleles and genotypes of these polymorphic substitutions in PC patients and controls failed to detect significant differences (Table 2). However, a trend to higher risk of PC was detected for homozygotes by the mutant allele *SHMT1* 1420T/T (OR=1.56, p=0.09). In a sample of our size, the OR values of at least 1.41 for *MTR* A2756G and at least 1.37 for *SHMT1* C1420T can be detected at an 80% level of significance. Hence, if these substitutions are really significant for PC risk, this impact can be detected only in studies on larger samples.

Hence, the absence of association suggests that the studied substitutions were either inessential for PC risk in the Russian West Siberia or their role in predisposition to this disease was negligible. Presumably, the effects of these SNP manifests in cooperation with environmental factors, for example, level of consumption of group B vitamins and methionine. Associations

of a high level of folate consumption with a lower risk of PC [8,10] and of high consumption of vitamin B_{12} with a higher risk of this disease [5,14] have been described. The effects of these factors should be taken into consideration in further studies.

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