ONCOLOGY

Associations of Polymorphic Variant of *MnSOD* Gene with Breast Cancer in Residents of the Altai Region

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The incidence of MnSOD genotypes in residents of the Altai Region suffering from breast cancer and individuals without a history of cancer corresponded to the Hardy-Weinberg equilibrium. No association of MnSOD with the incidence of sporadic breast cancer was detected. No association of MnSOD, tobacco smoking, or menopausal status, on the one hand, and breast cancer development, on the other, was detected.

Key Words: *MnSOD; SOD2; breast cancer; polymerase chain reaction*

Breast cancer (BC) is the most prevalent cause of mortality from cancer in women aged 40-69 years. It is assumed that the development of BC is a result of interactions between genetic factors, environmental factors, and life style.

Disorders in the work of enzymes involved in the metabolism of reactive oxygen derivatives potentially increase the risk of cancer cell appearance. Normally, reactive oxygen derivatives are formed as by-products of normal metabolism and are eliminated by antioxidant enzymes (SOD, catalase, glutathion peroxidases). Mitochondrial SOD-2 (MnSOD) transforms superoxide radicals into H_2O_2 and molecular oxygen. Then H_2O_2 degrades into water and molecular oxygen under the effect of catalase and glutathione peroxidase. Polymorphic variants of genes encoding enzymes involved in the metabolism of reactive oxygen derivatives attenuate or stimulate this process by directly modulating the content and quality of protein products of these genes. Hence, their genetic polymorphism can be a risk factor for tumor diseases. In addition, polymorphic variants of genes can modulate the risk of diseases associated with increased effects of reactive oxygen derivatives formed during alcohol abuse, tobacco smoking, exposure to ionizing radiation, or hormone therapy. High content of reactive oxygen derivatives and disorders in the work of these enzymes can lead to the development of oxidative stress [14].

Alcohol abuse, tobacco smoking, exposure to ionizing radiation, and hormone therapy leads to hyperproduction of reactive O_2 derivatives. Polymorphic variants of genes modulating activities of antioxidant enzymes can increase the risk of disease development under conditions of exposure to the above factors [2]. An association of polymorphic variants of *MPO*, *GPX*, *CAT*, and *MnSOD* genes with the risk of lymphoma and BC [6,13], prostatic cancer [10], and lung cancer was previously demonstrated.

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Single C for T nucleotide substitution (the second nucleotide in *MnSOD* gene codone 16, located in chromosome 6) leads to substitution of alanine (amino acid) with valine in position 9 of mature protein. This substitution reduces the efficiency of protein transport into mitochondria, which, in turn, is essential for the total enzyme activity. In humans the Ala *MnSOD* variant is 30-40% more active in mitochondria in comparison with Val *MnSOD* [2,14]. Association of *MnSOD* gene *Val**16**Ala* with lung cancer, prostatic cancer [10], and BC [6,13] was demonstrated.

We examined the residents of the Altai Region in order to clear out the impact of a single nucleotide substitution in the *MnSOD* gene (rs4880) for the risk of sporadic BC.

MATERIALS AND METHODS

The group consisted of 475 Caucasian women with sporadic BC aged 50-79 years (mean age 53±9 years). Control group consisted of 376 Caucasian women aged 19-84 years (mean age 54±13 years) without a history of cancer. The group was selected from patients of the Altai Affiliated Department of N. N. Blokhin Cancer Research Center and Altai Regional Oncological Center.

The following data were taken into consideration: age, menopausal status, diagnosis, date of BC diagnosis (clinical and pathomorphological), familial history of BC, tobacco smoking, body weight and length. The main criterion for inclusion in the studied group was histologically verified diagnosis of BC.

All women gave informed consent to participation in the study.

Venous blood samples were collected. DNA was isolated by the standard method (phenol-chloroform extraction).

Genotyping was carried out by real-time PCR using competitive TaqMan probes complementary to THE DNA polymorphic site.

Each sample was amplified using a pair of primers (R-sod2 5'-CGTTGATGTGAGGTTCCAG-3' and U-sod2 5'-CTGTGCTTTCTCGTCTTCAG-3'; PCR product size 127 b. p.) and 2 probes carrying the "quencher" on the 3' terminal and fluorescent stains (FAM or R6G) on 5' terminal. (FAM probe, 5'-FAM-CTGGCTCCGGCTTTGGGG-RTQ-3' and R6G probe, 5'-R6G-CTGGCTCCGGTTTTGGGG-FQ-3'). The reaction mixture (25 μl) contained 40-100 ng DNA, 300 nM of each primer, 100 nM TaqMan probe conjugated with FAM, 200 nM TaqMan probe conjugated with R6G, 200 μM dNTP, amplification buffer (65 mM Tris-HCl (pH 8.9), 0.05% Twin-20, 16 mM $(NH_4)_2SO_4$, and 2.4 mM $MgCl_2$), and Taq polymerase (0.5 U per reaction).

Polymerase chain reaction was carried out as follows: initial denaturation for 3 min at 96°C and 40 cycles of the following protocol: denaturation at 96°C for 8 sec, primer annealing, and subsequent elongation at 60°C for 35 sec. The fluorescent signal was recorded for the bands corresponding to the fluorescence bands of FAM and R6G fluorophores at each stage.

The incidence of alleles and genotypes of the polymorphic locus in the studied groups were compared using the χ^2 method. Correspondence of the samples to the Hardy—Weinberg equilibrium was verified using applied software.

RESULTS

During the first step of the study we optimized the method for genotyping of single-nucleotide substitution in the *MnSOD* gene by the real-time PCR method using competitive TaqMan probes complementary to the DNA polymorphic site. The structure of the probes differed by one nucleotide corresponding to SNP. In the reaction mixture the probes compete with each other for hybridization with the matrix. If the matrix and probe are fully complementary, hybridization is more effective than in case of incomplete complementariness.

Choosing the optimal conditions for amplification, we tried various proportions of probe concentrations, temperatures of primer annealing, and compositions of the amplification buffer. The main parameter taken into consideration for each reaction was the proportion of fluorescent values (in relative fluorescence units — RFU) for the FAM and R6G stains emission bands. For the C/C genotype, the fluorescence intensity increased mainly in the FAM band, for T/T genotype in the R6G band, and for heterozygotic genotype in both bands (Fig. 1).

The genotyping results were confirmed by sequencing. An important criterion of genotyping reliability was clusterization of genotypes into groups formed on the basis of fluorescence intensity values (Fig. 2).

The incidence of *MnSOD* gene *Val**16**Ala* polymorphic locus in control samples and in samples from patients with sporadic BC corresponded to the Hardy-Weinberg distribution in both groups (Table 1). No significant associations of *MnSOD* gene *Val**16**Ala* polymorphic locus and BC development were detected.

Analysis of the tobacco smoking status and the time of the disease onset (during the post- or premenopause) revealed no statistically significant differences in the incidence of *MnSOD* gene poly-

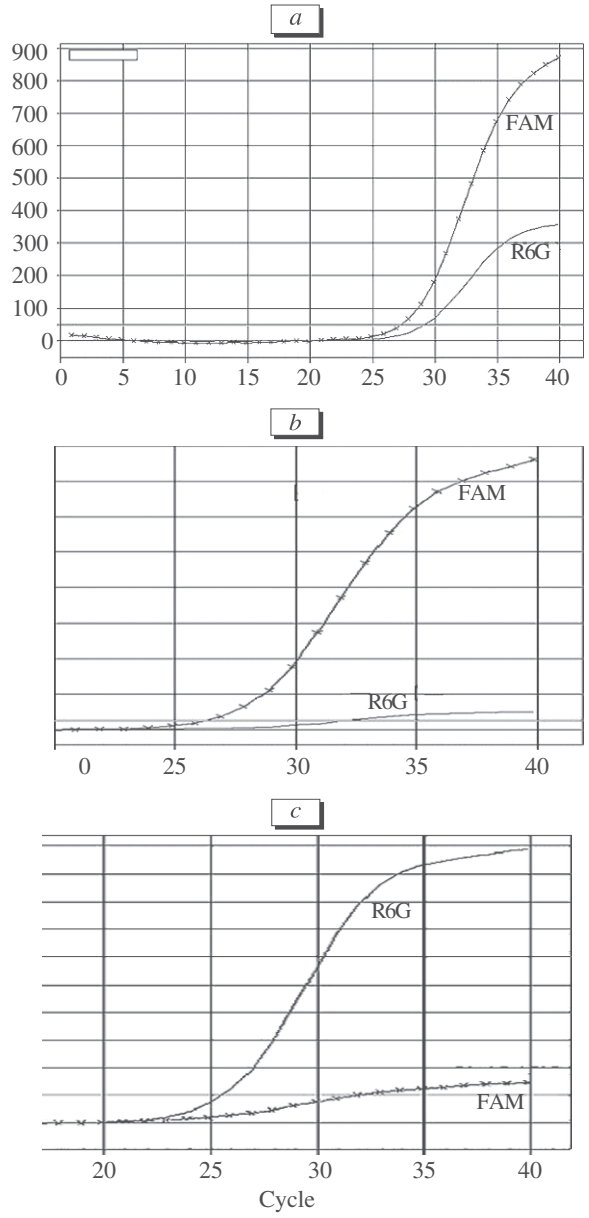

Fig. 1. Kinetic curves of *MnSOD* gene site amplification product accumulation for genotypes C/T (*a*), C/C (*b*), and T/T (*c*).

morphic variants in the studied groups (data not presented).

The incidence of genotypes in the control group determined in our study did not differ from the data published for Caucasian female residents of Germany [14], Australia [9], USA [10], and for male residents of the USA [6]. However, the incidence of *MnSOD* gene *Val**16**Ala* polymorphic locus genotypes differed significantly in female residents of the Altai Territory and Finland [13] (Table 2). The study carried out in Finland revealed a significant association of *MnSOD* gene *Val**16**Ala* polymorphic locus with the risk of BC development (OR=1.331 (1.006-1.762), p=0.045) [13].

The *MnSOD* polymorphic variant can be essential for the development of BC, though the values obtained in the analysis did not reach the level of statistical significance (RR=0.92 (0.82-1.02), p=0.056) [5]. This result can be explained as follows: carriership of a more prevalent *MnSOD* allele is associated with the formation of greater volume of H_2O_2, which, not neutralized to water and molecular oxygen, can potentiate further formation of reactive oxygen forms. Hence, carriership of a less prevalent allele is protective [14].

Our findings showed that the *MnSOD* gene polymorphic variant does not increase the risk of BC. Our results are in line with the previous data which did not confirm the relationship between *MnSOD* gene *Val**16**Ala* polymorphic variant (as an independent factor) and the risk of BC development [3,7,8,11,12,14,15]. Presumably, this polymorphic variant is essential for BC pathogenesis with consideration for such accessory factors as nutrition [15], tobacco smoking [8,12,14,15], alcohol consumption [13,14], and irradiation of the chest [12]. This polymorphic variant can be also essential for survival of patients after BC treatment.

Some authors demonstrated the modifying effect of *MnSOD* polymorphic locus on the risk of BC in tobacco-smoking women [8,13,15]. We failed to detect a correlation between BC and this polymorphic locus in tobacco-smoking women, but there were just few (<1%) tobacco smokers in the examined and control groups, and therefore adequate statistical analysis was impossible.

The effects of *MnSOD* polymorphic variant on the risk of BC development during the pre- and postmenopause have been studied. No association between this locus and BC development risk was

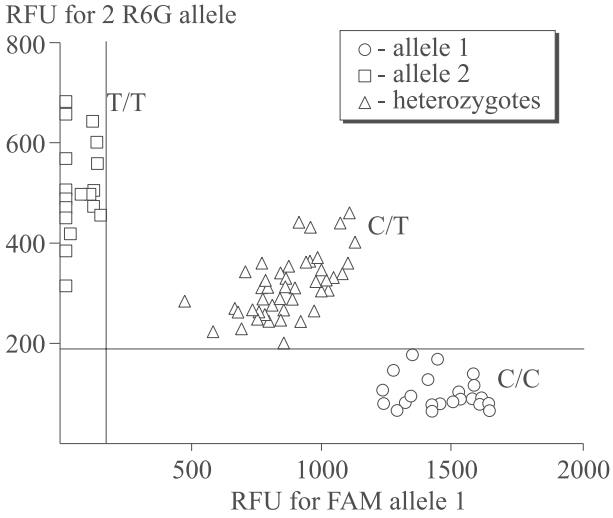

Fig. 2. Clusterization of samples by genotypes on the base of RFU values.

TABLE 1. Incidence of *MnSOD* Gene *Val**16**Ala* Polymorphic Locus in the Studied and Control Groups

Genotype	Percentage of women		Chance ratio (95% confidence interval)	*p*
	BC	control		
Val/Val (TT)	123 (25.8)	103 (27.4)		
Val/Ala (TC)	233 (49.0)	183 (48.7)	1.066 (0.770-1.477)	0.699
Ala/Ala (CC)	119 (25.0)	90 (23.4)	1.107 (0.758-1.617)	0.598
Val/Ala+Ala/Ala	352 (74.1)	273 (72.6)	1.080 (0.795-1.466)	0.623

TABLE 2. Incidence of *MnSOD* Gene *Val**16**Ala* Polymorphic Locus Genotypes in Different Populations

Region	Object of study	*n*	MnSOD population control (TT/CT/CC, %)	Statistical significance of differences in incidence	
				χ^2	*p*
Altai region, Russia	Women	376	27.4/48.6/24		
Germany	Women	24.4/48.9/26.8	1080	0.68	0.41
USA	Women	1098	24.4/49.7/25.9	0.35	0.55
Australia	Women	1130	27.3/48.3/24	0.46	0.50
Finland	Women	482	31.7/47.9/20.3	5.23	0.020
USA	Men	1382	27.2/49.6/25.2	0.11	0.73

detected in the majority of cases for the pre- and postmenopausal women. An association between *MnSOD* and high risk of BC during the postmenopausal period was detected in one study [13]. We detected no association between *MnSOD*, age of the menopause onset, and age of BC development [3,7, 8,11,12,15]. However, we do not rule out the possibility that the absence of associations in the present study was due to late diagnosis of BC and hence, incorrect formation of the groups (pre- and postmenopausal cancer).

In addition, the modifying effect of some accessory factors, such as vitamin C [15] or alcohol [13,14] consumption, on the association between the risk of BC development and *MnSOD* polymorphic variant have been shown. Presumably, the modifying effect of *MnSOD* on BC risk can manifest together with polymorphic variants of other genes encoding enzymes involved in the metabolism of reactive oxygen derivatives, such as catalase, glutathione peroxidase, and myeloperoxidase [4,6,11].

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