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Association between Cytokine Gene Polymorphisms and Breast Cancer in Postmenopausal Women

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Abstract—The goal of this study was to investigate the association between the polymorphic loci of genes *IL*1*B* (rs16944), *IL*1*RN* (rs4251961), *IL*6 (rs1800795, rs1800796, rs1554606), *IL*8 (rs4073), *IL*10 (rs1800896), and *TNFA* (rs1800629) and breast cancer in women. The DNA samples of 521 women with breast cancer and 267 women without oncological pathology were studied. The study showed that age, concomitant chronic morbidity, and polymorphism of the *IL*6 gene are risk factors in postmenopausal women. Allele –572C (OR = 5.68; 95% CI = [2.70–11.97], $p_{cor} < 0.001$), and haplotype rs1800796[C]–rs1800795[G]–rs1554606[G] (OR = 5.31, 95% CI = [1.87 – 15.10], $p_{cor} = 0.002$) of the *IL*6 gene were associated with breast cancer. No association of loci in *IL*1*B* (rs16944), *IL*1*RN* (rs4251961), *IL*8 (rs4073), *IL*10 (rs1800896), or *TNFA* (rs1800629) was associated with the risk of breast cancer. The obtained data indicate that the –572C allele of the *IL*6 gene is associated with susceptibility in postmenopausal women to breast cancer. Our results can be useful for an understanding of the molecular mechanisms of breast cancer.

Keywords: breast cancer, postmenopause, cytokine genes

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INTRODUCTION

Breast cancer ranks at the top of the structure of female cancer incidence in Russia and the world. According to epidemiological data, breast cancer is most often diagnosed in women aged 50–65 years [5].

Aging of the body is a universal biological process that is associated with a progressive loss of cell functions and tissue renewal due to the action of complex heterogeneous and dynamic mechanisms. The aging process is influenced by genetic, epigenetic, environmental, and random factors [22]. The molecular mechanisms of cell aging have become better understood due to their relationship to carcinogenesis and inflammation. It is believed that the cellular aging syndrome determines the life span and aging of the organism as a whole [1].

Inflammatory mediators, cytokines, and chemokines, as well as reactive oxygen and nitrogen species, are capable of modulating cell malignancy in tissues [15]. An imbalance in pro- and anti-inflammatory cytokines can lead to an inadequate immune response to a tumor. Long-term and sluggish inflammation can cause changes in the expression of certain oncogenes and tumor suppressor genes and trigger the mechanisms of cancer progression [29]. Clinical studies have shown that cytokines of the IL-1, TNF-α, IL-6, IL-8, and IL-10 families are promising markers of tumor growth and prognostic factors in malignant neoplasms [36]. For breast cancer, it was found that the serum concentration of these cytokines is associated with the treatment aggressiveness, metastasis risk, and disease recurrence [21].

The interindividual differences in the cytokine profile depend on the polymorphism of the regulatory regions of cytokine genes. Individual alleles of cytokine genes affect the transcription rate, the stability or quality of mRNA, and the activity of protein products of their expression [3]. Therefore, it is hypothesized that functional sites in cytokine genes can indirectly affect the pathogenesis of breast cancer.

The goal of this work was to study the relationship between polymorphism in cytokine genes and the risk of breast cancer in postmenopausal women.

Table 1. Descriptive characteristics of women with breast cancer (BC) and without oncological pathology

Parameter	BC (n = 521), n (%)	Control group (n = 299), n (%)	P
Age, years: Me (Q25–Q75)	63 (57–69)	58 (53–61)	<0.001*
Concomitant chronic systemic diseases**			
Yes	446 (85.6)	215 (71.9)	<0.001***
No	–	9 (3)	
No data	75 (14.4)	75 (25.1)	
Unilateral BC	500 (96)		
Bilateral BC	11 (2.1)	–	–
No data	10 (1.9)		
Tumor stages (TNM)			
T1	186 (35.7)		
T2	218 (41.8)		
T3	97 (18.6)	–	–
T4	6 (1.2)		
No data	14 (2.7)		
Regional metastasis			
N_0	304 (58.3)	–	–
$N_{1–3}$	208 (39.9)		
No data	9 (1.8)		
Distant metastases			
M_0	505 (96.9)	–	–
M_1	7 (1.3)		
No data	9 (1.8)		

* Mann–Whitney U-test was used.
** The most frequent were obesity of varying severity, type 2 diabetes mellitus, arterial hypertension, ischemic heart disease, etc.
*** Two-tailed Fisher test was used.

EXPERIMENTAL

We studied 820 DNA samples of women with sporadic breast cancer and women without oncological pathology. All women were postmenopausal and belonged to the Russian ethnic group.

The study group (breast cancer) consisted of 521 women (mean age of 63.6 ± 8.7 years) with histologically verified breast cancer. The women were being treated at the Regional Clinical Oncological Dispensary (city of Kemerovo, Russia). Table 1 presents the clinical anamnestic characteristics of the surveyed women. The control group included 299 women without cancer (mean age of 57.4 ± 6.7 years).

Genomic DNA was isolated from blood lymphocytes via phenol-chloroform extraction followed by ethanol precipitation; the DNA samples were stored at –20°C.

Single nucleotide substitution (SNP) rs1800795 in the *IL*6 gene was typed via asymmetric real-time polymerase chain reaction (PCR) with a fluorescently labeled oligonucleotide probe, followed by analysis of the melting curves. The amplification reaction was carried out under the following conditions: initial denaturation at 96°C for 3 min; then 54 cycles, including denaturation at 96°C for 6 s, primer annealing at 56°C for 6 s, and elongation at 72°C for 6 s. The melting curves were determined in a temperature range of 35–85°C, with a 0.5°C increase in temperature in each cycle from the initial temperature; at each step, a fluorescent signal was detected within the range corresponding to the fluorophore interval. The total volume of the reaction mixture was 20 μL: 10 mM Tris-HCl (pH 8.9), 55 mM KCl, 2.5 mM $MgCl_2$, 0.05% Tween 20, 0.2 mM dNTP, 20–100 ng DNA, 1 unit Klentaq-DNA polymerase, and solutions of oligonucleotide primers and the probe with limiting primer, 0.1 mM (5'-TGGGGCTGATTGGAAACCT-3'); redundant primer, 1 mM (5'-AGGAAGAGTGGTTCTGCTTCT-3'), and the probe, 0.1 mM (5'-R6GCTTTAGCATCGCAAGACA-BHQ-3').

Genotyping of the remaining SNPs, *IL*1*B* rs16944, *IL*1*RN* rs4251961, *IL*6 rs1800796 and rs1554606, *IL*8

Table 2. Sequence of primers and probes

Polymorphism	Primers	Primer sequences	Probe sequences
IL1B	Forward	5'-ccccagccaagaaaggtca-3'	5'-R6G-ctctgcctcGggagctctc-BHQ-3'
rs16944	Reverse	5'-ttgagggtgtgggtctctac-3'	5'-Fam-ctctgcctcAggagctctc-BHQ-3'
IL1RN	Forward	5'-cggtgagccctaagtctaag-3'	5'-R6G-atggacctgGtgctatctgc-BHQ-3'
rs4251961	Reverse	5'-cttcagacctcattttgacagc-3'	5'-Fam-atggacctgAtgctatctgc-BHQ-3'
IL6	Forward	5'-catctgagttcttctgtgttctg-3'	5'-R6G-caacagccCctcacagg-BHQ-3'
rs1800796	Reverse	5'-cgagacgccttgaagtaactg-3'	5'-Fam-caacagccGctcacagg-BHQ-3'
IL6	Forward	5'-caactgtcaaatgtttaaaactcc-3'	5'-Fam-ccctgAgagtacctttccc-BHQ-3'
rs1554606	Reverse	5'-ccaggggcagccagagag-3'	5'-Hex-ccctgCgagtacctttccc-BHQ-3'
IL8	Forward	5'-tgttctaacacctgccactct-3'	5'-Fam-aagcataca Attgataattc-BHQ-3'
rs4073	Reverse	5'-acatttaaaatactgaagctccaca-3'	5'-Hex-aagcataca Ttgataattc-BHQ-3'
IL10	Forward	5'-cacaaatccaagacaacactact-3'	5'-R6G-cttcccc Ctcccaaagaagc-BHQ-3'
(rs1800896)	Reverse	5'-gataggaggtcccttactttcc-3'	5'-Fam-cttcccc Ttcccaaagaagc-BHQ-3'
TNFA	Forward	5'-gtcctacacacaaatcagtcagt-3'	5'-Fam-tcctccctgctc Tgattc-BHQ-3'
(rs1800629)	Reverse	5'-ttggggacacacaagcatca-3'	5'-R6G-tcctccctgctc Cgattc-BHQ-3'

rs4073, *IL10* rs1800896, and *TNFA* rs1800629, was carried out by real-time PCR with competing TaqMan probes. The amplification reaction was carried out under the following conditions: initial denaturation at 96°C for 3 min, followed by 50 cycles, including denaturation at 96°C for 8 s, primer annealing at 58°C for 40 s, and subsequent elongation at 72°C for 8 s. The total volume of the reaction mixture was 20 μL. The mixture contained 65 mM Tris-HCl (pH 8.9), 24 mM $(NH_4)_2SO_4$, 3.0 mM $MgCl_2$, 0.05% Tween 20, 0.2 mM dNTP, 20–100 ng DNA, 300 mM of each primer (Table 2), 100–200 nM TaqMan probes (see Table 2), and 0.5 units of thermostable Taq polymerase. Amplification was carried out with a CFX-96 thermal cycler (Bio-Rad, United States).

The results were analyzed with the Statistica software for Windows v.8.0 (StatSoft, Inc., United States) and the R library GenABEL (www.r-project.org). The Hardy–Weinberg genotype frequencies for the studied cytokine genes were estimated with the Pearson χ^2 test. In this case and with other tests, the null hypothesis was rejected at $p \le 0.05$. The odds ratio (*OR*) and its confidence interval (95% CI) were estimated via logistic regression analysis (glm function in R). An additive model that coded the homozygote for the risk allele as "2," the heterozygote as "1," and the homozygote for the reference allele as "0" was employed as the basic trait inheritance model. The haplotype frequency was calculated in the HapStat software with the expectation-maximization algorithm for haplotype reconstruction (http://www.bios.unc.edu/~lin/hapstat). The frequency of haplotypes between groups was compared with the χ^2 test and Fisher's exact test if $n \le 5$. Linkage disequilibrium was analyzed based on *D*' values calculated with the CubeX software (http://www.oege.org/software/cubex/). Bonferroni correction was applied to all experimentally determined *p* values in order to exclude statistical errors in multiple comparisons.

RESULTS AND DISCUSSION

The effects of the following factors on the risk of breast cancer were studied: age, concomitant chronic pathology, and polymorphism in cytokine genes. Significant differences were revealed between women with breast cancer and the control group in terms of age and concomitant chronic morbidity ($p < 0.001$, see Table 1).

To analyze the associations with breast cancer risk, we examined polymorphisms in genes *IL1B*, *IL1RN*, *IL6*, *IL10*, and *TNFA* according to the literature data on their functional significance (Table 3).

The frequency distribution of genotypes for polymorphic loci *IL1B* rs16944, *IL1RN* rs4251961, *IL6* rs1800795, rs1800796, and rs1554606, *IL8* rs4073, *IL10* rs1800896, and *TNFA* rs1800629 in women with breast cancer and in the control group corresponded to the Hardy–Weinberg equilibrium ($p > 0.05$, Table 4). In most cases, there was no association between the polymorphic loci of the studied cytokine genes and the risk of breast cancer, with the exception of polymorphism rs1800796 of gene *IL6*. According to the results of logistic regression analysis, *IL6* SNP –572G>C was associated with breast cancer. A significant association of the –572C allele of the *IL6* gene with breast cancer risk was found (*OR* = 5.68; 95% CI = [2.70–11.97], $p_{cor} < 0.001$; see Table 4).

Table 3. Functional significance of polymorphic loci of cytokine genes

Gene		Polymorphism			MAF 1000 genomes*	Functional effect
symbol	gene product	RefSNP	nucleotide substitution	chromosome		
IL1B	IL-1β	rs16944	−511T>C	2(q13)	32–38%	Regulation of gene expression and IL-1β protein induction [11]
IL1RN	IL1-Ra	rs4251961	+1018T>C	2(q14.2)	29–42%	Development of acute inflammation in healthy people [12]
IL6	IL-6	rs1800795 rs1800796 rs1554606	−174G>C −572G>C *g*.6942T>G	7(p21–14)	35–52% 3–5% 36–53%	Upregulated transcription [8]; association of allele C with upregulated C-RP and IL-6 production in blood serum [33, 37]; upregulated production of IL-6 protein [23]
IL8	IL-8	rs4073	−251A>T	4(q13–21)	39–46%	Regulation of gene transcriptional activity [30]
IL10	IL-10	rs1800896	−1082A>G	1(q31—32)	40–54%	Upregulated transcription and IL-10 protein production [8]
TNFA	TNF-α	rs1800629	−308G>A	6(p21.3)	9–18%	Upregulated gene expression and TNF-α production [10]

MAF is the frequency of the minor allele in European populations.

The linkage disequilibrium between the functional sites rs1800796, rs1800795, and rs1554606 of the *IL6* gene was analyzed. It was found that all the three sites are characterized by linkage disequilibrium (Table 5). The most frequently detected haplotype in the studied groups was rs1800796[G]–rs1800795[G]–rs1554606[G] (0.492 and 0.533, respectively). Comparison of the *IL6* haplotype frequency in the two groups showed significant differences only for the rs1800796[C]–rs1800795[G]–rs1554606[G] haplotype (0.068 versus 0.013, respectively). It was found to be associated with breast cancer risk ($OR = 5.31$; 95% CI = [1.87–15.10], $p_{cor} = 0.002$).

Thus, in our study, age, concomitant chronic morbidity, and the genetic factor affected the development of breast cancer in women. Old age is an independent risk factor for cancer in humans [6]. Chronic pathology associated with systemic inflammation is another distinctive feature of older breast cancer patients [35].

Allele −572C (rs1800796) and the haplotype that includes this *IL6* gene variant (rs1800796[C]–rs1800795[G]–rs1554606[G]) were found to be significantly associated with the risk of breast cancer. No associations with breast cancer risk were found for the other genetic polymorphisms (*IL1B* rs16944, *IL1RN* rs4251961, *IL8* rs4073, *IL10* rs1800896, and *TNFA* rs1800629).

It is known that IL-6 is an important component of the cytokine cascade that triggers and regulates inflammation. Under physiological conditions, IL-6 functions are associated with the cessation of inflammation via inhibition of the synthesis of proinflammatory cytokines such as TNF-α and IL-1β. Chronic IL-6 upregulation is typical of old age, severe inflammation, and neoplastic growth [24]. Activation of the IL-6 signaling pathway is a link between chronic inflammation and cancer [27]. The IL-6 functions are associated with apoptosis inhibition and activation of the critical transcription factors NF-kB, JAC/STAT3, and PI3K/AKT, which regulate the expression of genes that trigger the mechanisms of tumor promotion, angiogenesis, and metastasis [16, 17, 26]. It is assumed that in the etiology of breast cancer, the proinflammatory effects of IL-6 can be implemented via the mechanisms of regulation of insulin, steroid hormones, and lipid metabolism [2, 32].

According to meta-analysis data, *IL6* gene polymorphism affects human susceptibility to cancer, with three SNPs in the promoter region of this gene (rs1800797, rs1800796, and rs1800795) playing a special role. However, the main contributor to the development of breast cancer in women is rs1800795 of the *IL6* gene. Associations of the −174G allele (rs1800795) of the *IL6* gene were found to be associated with breast cancer predisposition [28], a worse prognosis in overall survival, and breast cancer metastasis [14], although some European studies reported different results [18, 19]. Interestingly, the effects of this allele can vary depending on the ER phenotype of the tumor [15, 25].

Table 4. Associations of polymorphism in cytokine genes with the risk of breast cancer (additive inheritance model)

Polymorphic locus	Breast cancer, *n*	Control group, *n*	*p**
−511T>C *IL*1*B*, rs16944			
CC	242 (0.464)	151 (0.505)	0.22
CT	224 (0.430)	124 (0.415)	
TT	55 (0.106)	24 (0.080)	
Risk allele T	334 (0.321)	172 (0.288)	
HWE	0.77	0.84	
+1018T>C *IL*1*RN*, rs4251961			
TT	261 (0.501)	164 (0.548)	0.53
CT	216 (0.415)	112 (0.375)	
CC	44 (0.084)	23 (0.077)	
Risk allele C	304 (0.292)	158 (0.264)	
HWE	0.94	0.53	
−174G>C *IL*6, rs1800795			
GG	178 (0.342)	95 (0.318)	0.76
GC	247 (0.474)	150 (0.502)	
CC	96 (0.184)	54 (0.181)	
Risk allele G	603 (0.579)	340 (0.568)	
HWE	0.53	0.70	
−572G>C *IL*6, rs1800796			
GG	450 (0.864)	291 (0.973)	<0.001** *OR* = 5.68 [2.70–11.97]***
CG	70 (0.134)	8 (0.027)	
CC	1 (0.002)	–	
Risk allele C	72 (0.069)	8 (0.013)	
HWE	0.31	0.81	
g.6942T>G *IL*6, rs1554606			
GG	166 (0.318)	89 (0.298)	0.87
GT	251 (0.482)	151 (0.505)	
TT	104 (0.200)	59 (0.197)	
Risk allele T	459 (0.440)	269 (0.450)	
HWE	0.61	0.73	
−251A>T *IL*8, rs4073			
TT	153 (0.294)	85 (0.284)	0.95
AT	249 (0.478)	154 (0.475)	
AA	119 (0.228)	60 (0.201)	
Risk allele A	487 (0.467)	274 (0.458)	
HWE	0.36	0.52	
−1082A>G *IL*10 (rs1800896)			
AA	172 (0.330)	104 (0.348)	0.28
GA	253 (0.486)	150 (0.502)	
GG	96 (0.184)	45 (0.151)	
Risk allele G	445 (0.427)	240 (0.401)	
HWE	0.86	0.45	

Table 4. (Contd.)

Polymorphic locus	Breast cancer, *n*	Control group, *n*	*p**
−308G>A *TNFA* (rs1800629)			
GG	402 (0.772)	235 (0.786)	
GA	112 (0.215)	60 (0.201)	0.57
AA	7 (0.013)	4 (0.013)	
Risk allele A	126 (0.121)	68 (0.114)	
HWE	0.80	0.94	

* *p* was estimated with allowance for age correction.
** $p < 0.005$ after Bonferroni correction (the number of statistical tests is five).
*** *OR* is the odds ratio [95% CI]; HWE, Hardy–Weinberg genotype frequency equilibrium.

Table 5. Linkage-disequilibrium matrix and the *IL6*-gene haplotype frequency distribution in the studied groups

Polymorphism	rs1800796	rs1800795	rs1554606
rs1800796	–	−1.0	0.99
rs1800795	<0.0001	–	−1
rs1554606	<0.0001	<0.0001	–
Haplotype*	Breast cancer, *n* = 521	Control group, *n* = 299	*p*
GGG	0.492 (256)	0.533 (159)	Ref.
GCT	0.421 (220)	0.428 (128)	0.71
GGT	0.018 (9)	0.022 (7)	0.79
CGG	0.068 (35)	0.013 (4)	0.002**
			OR = 5.31 [1.87–15.10]
CGT	0.001 (1)	–	0.99
GCG	–	0.004 (1)	0.99

In the first part of the table, the *D*' values are above the diagonal, and the *p* values are below the diagonal; in the second part of the table, parentheses show the number of observations.
* The gene variant is indicated in accordance with the location of SNP on the chromosome: rs1800796, rs1800795, and rs1554606; Ref. is the reference haplotype.
** *p* values of Fisher's exact test after Bonferroni correction (the number of statistical tests is five); *OR* is the odds ratio [95% CI].

Contrary to expectations, this study revealed that it is the rs1800796 polymorphism (allele −572C) but not the rs1800795 polymorphism (allele −174G) that is associated with the risk of breast cancer. Another polymorphism, rs1554606 of the *IL6* gene, also did not have an independent significant effect on the risk of breast cancer. At the same time, all three studied SNPs of the *IL6* gene (rs1800796, rs1800795, and rs1554606) were characterized by linked inheritance, and both the functional alleles −572C and −174G in the promoter region of the gene were in strong linkage disequilibrium ($D' = -1$). Our results are consistent with the previously published data from a large-scale Russian study [31].

Analysis of association studies showed that the rs1800796 polymorphism of the *IL6* gene in European women is not associated with the risk of breast cancer in postmenopausal women [18, 19], but its contribution to the development of this pathology in Asian women may be quite significant [37]. At the same time, the combination of the *IL6* rs1800796 polymorphism with other additional factors, such as type 2 diabetes [37], an increased waist-to-hip ratio [33], and the use of aspirin to suppress aromatase activity and prevent breast cancer [32] can affect breast cancer risk. It was previously revealed that the *IL6* −572C allele is associated with human susceptibility to lung, prostate, and intestinal cancers (meta-analysis including 80361 cancer patients and 78712 healthy people from 16 countries) [28].

According to the 1000 Genomes database, the *IL6* −572C allele is rare in people of European descent (3–5%) but is widespread in Asian populations (39–79%). In the studied groups of women, the frequency of the *IL6* −572C allele was 6.9% for breast cancer and 1.3% for the control group. The homozygous C/C genotype was detected in only one woman in the breast cancer group. Despite the high *OR* values and the level of sta-

tistical significance of the effect of the *IL*6 –572C allele, it cannot serve as a marker for mass or professional screening of breast cancer. A statistical test [9] showed that the *IL*6 –572C allele as a marker has low sensitivity (7%, $SE = 0.07$) and is a poor classifier ($AUC = 0.53$). At the same time, it has a high diagnostic value, since 98% of its carriers were breast cancer patients ($SP = 0.98$, test specificity). Due to the prevalence of breast cancer in the structure of oncological morbidity in women in the world [5], there is reason to believe that the *IL*6 –572C allele may be a diagnostic marker. In this case, its use in clinical practice is possible when patients have additional symptoms and indications [9]. Apparently, the age of the women examined in our study may be such an additional factor. Russian women (23–95 years old), as compared to men, have a low G/C genotype frequency as they age and, conversely, long-livers have a high G/G genotype frequency for *IL*6 rs1800796 [7].

The molecular mechanisms by which inflammatory stimuli controlled by the *IL*6 gene contribute to the early stages of oncogenesis remain unclear. It became known that SNP rs1800796 may be associated with differentiated methylation of CpG islands within the –666 to –426 position relative to the *IL*6 gene transcription start site and repression of the *IL*6 gene in pancreatic adenocarcinoma cell lines and chorioamnionitis placentas [13, 20]. Repression of the *IL*6 gene in pancreatic adenocarcinoma cell lines occurred due to the interaction of methyl-CpG-binding proteins with modified CpG dinucleotides in this region of the gene [13]. As it turned out, carriers of the C/C genotype had a higher methylation level at five CpG sites (cg01770232, cg02335517, cg07998387, cg13104385, and cg0526589) of the placental DNA *IL*6 promoter than carriers of the G/G genotype. It is assumed that SNP rs1800796 can change the interaction of methyl-CpG-binding proteins with the cg01770232 site and thereby affect the expression and methylation of the *IL*6 gene and susceptibility to infection in humans [20].

It is known that the phenomenon of total DNA hypomethylation with hypermethylation of individual genes during the aging process can lead to genome instability and the risk of pathology, especially cancer. There is an assumption that the methylation of individual genes in normal aging cells and transforming cells leads to the repression of those genes that are directly related to the ontogeny of the cell [4]. It was found that the *IL*6 gene polymorphism rs1800796 is associated with *TP*53 gene mutations in rectal tumor cells. Carriers of the *IL*6 –572C allele had *TP*53 gene mutations in tumor cells significantly more often than carriers of the –572G allele [34].

In this work, we did not consider in detail the issues of the relationship between polymorphism in cytokine genes, concomitant chronic morbidity, and breast cancer in postmenopausal women. Meanwhile, a number of clinical studies have shown that metabolic disorders associated with obesity, hyperinsulinemia, and metabolic syndrome are the cause of the risk of breast cancer occurrence and progression [2]. We will study these issues in detail in further works and on a larger sample of women.

CONCLUSIONS

Our study showed that age, concomitant chronic morbidity, and *IL*6-gene polymorphism are risk factors for breast cancer in postmenopausal women. Associations of the –572C allele (rs1800796) and the rs1800796[C]–rs1800795[G]–rs1554606[G] haplotype of the *IL*6 gene with the risk of breast cancer were revealed. Individually, polymorphic loci of genes *IL*1*B* (rs16944), *IL*1*RN* (rs4251961), *IL*8 (rs4073), *IL*10 (rs1800896), and *TNFA* (rs1800629) did not significantly affect the susceptibility to breast cancer. However, the complex interactions of the cytokine network in the implementation of pro- and anti-inflammatory functions do not rule out their participation in the pathogenesis of breast cancer, which may be revealed in research on the relationship of their genes. The obtained results may be useful for an understanding of the molecular mechanisms of breast cancer development in elderly women.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. Before sampling the material (peripheral whole blood), the women gave their voluntary, written, informed consent to participate in the study. The study was conducted confidentially and in accordance with the ethical standards of the 1975 Declaration of Helsinki.

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