ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Aug 15; 7(15):2488-2496.
https://doi.org/10.3889/oamjms.2019.667
eISSN: 1857-9655
Clinical Science

Polymorphisms in Phase I (CYP450) Genes *CYP1A1* (rs4646421), *CYP1B1* (rs1056836), *CYP19A1* (rs749292) and *CYP2C8* (rs1058930) and Their Relation to Risk of Breast Cancer: A Case-Control Study in Mazandaran Province in North of Iran

Golpar Golmohammadzadeh[1], Abbas Mohammadpour[2], Nematollah Ahangar[3], Mohammad Shokrzadeh[3*]

[1]Department of Toxicology and Pharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran; [2]Cell & Molecular Biology, Pharmaceutical Research Center, Department of Toxicology and Pharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran; [3]Pharmaceutical Research Center, Department of Toxicology and Pharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

Citation: Golmohammadzadeh G, Mohammadpour A, Ahangar N, Shokrzadeh M. Polymorphisms in Phase I (CYP450) Genes *CYP1A1* (rs4646421), *CYP1B1* (rs1056836), *CYP19A1* (rs749292) and *CYP2C8* (rs1058930) and Their Relation to Risk of Breast Cancer: A Case-Control Study in Mazandaran Province in North of Iran. Open Access Maced J Med Sci. 2019 Aug 15; 7(15):2488-2496.
https://doi.org/10.3889/oamjms.2019.667

Keywords: CYP1A1 (rs4646421); CYP1B1 (rs1056836); CYP19A1 (rs749292); CYP2C8 (rs1058930); Breastcancer; Polymorphism; Mazandaran province

***Correspondence:** Mohammad Shokrzadeh. Pharmaceutical Research Center, Department of Toxicology and Pharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran. E-mail: mslamuk@yahoo.com

Received: 16-Jun-2019; **Revised:** 13-Jul-2019; **Accepted:** 14-Jul-2019; **Online first:** 10-Aug-2019

Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: The second leading cause of cancer-related death in women is breast cancer. Xenobiotic Metabolizing Enzymes (XMEs) contribute to the detoxification of numerous cancer therapy-induced products. In the metabolism of xenobiotic, cytochrome P450s or monooxygenases perform an important function by catalysing the hydroxylation reaction. In this study, the susceptibility and genetic polymorphisms of CYP450 isoenzymes was investigated that may have an etiological role in breast cancer.

AIM: The main purpose of this study was to evaluate the association of *CYP1A1* (rs4646421), *CYP1B1* (rs1056836), *CYP2C8* (rs1058930), and *CYP19A1* (rs749292) polymorphisms with the risk of breast cancer in Mazandaran province.

MATERIAL AND METHODS: This cross-sectional case-control study were recruited 72 patients and 51 healthy individuals and was performed between March 2018 to May 2018 in the Oncology Department at Imam Hospital in Sari city, Iran. Peripheral blood samples were collected in EDTA tube, and DNA extraction was performed using the salting-out method and WizPrep extraction kits. Breast cancer patients with known clinicopathological characters and healthy women as control group were genotyped for genes polymorphisms by PCR-RFLP technique, using restriction enzymes. Chi-square, Fisher exact test and Logistic regression model, were applied for statistical analysis.

RESULTS: The results of the experiments showed that there was a significant relationship between two groups and the age of the patients is significantly higher than the control group (p = 0.044). According to the chi-square and Fisher exact test, education, pregnancy, menopause status and oppose were significant between the two groups. Based on using a logistic regression model in two normalized and age-adjusted models to finding relationship between the genotypes of each gene and breast cancer risk, it was determined that in the *CYP2C8* genotype, those who have the CG allele have a 7.74 degree increased risk of breast cancer (CI = 95% 0.95-62.5) and in the *CYP19A1* gene, individuals with GA genotype, increased risk of breast cancer (CI = %95 1.52-27.21), about the *CYP1B1* gene, people with two genotypes of CG + GG had higher risk of breast cancer (CI = %95 1.19-5.71) and allele G has decreased risk of breast cancer in this gene (P = 0.0271), also allele G in *CYP2C8* gene had the protective effect (P = 0.02). In the age-adjusted model, for the *CYP2C8* gene, GG genotype increased risk of breast cancer (CI = %95 1.11-75.84) as well as, the CG + GG genotype in *CYP1B1* gene (CI = %95 1.31-6.57).

CONCLUSION: Our results confirm the association between *CYP2C8* (rs1058930), *CYP19A1* (rs749292) and *CYP1B1* (rs1056836) gene polymorphisms and increased risk of breast cancer in women in Mazandaran province.

Introduction

Breast cancer is the most frequent malignancy in women in all races and a growing number in advanced and underdevelopment countries in Asia. According to a report of Iran's Ministry of Health and Medical Education, breast cancer is the most common primary cancer in Iranian women [1]. Today, this disease accounts for about one-third of all cancers in women, and it represents the second leading cause of cancer death among women (15% of cancer deaths) after lung cancer. According to WHO statistics, one in every 8 to 10 women have breast cancer [2]. According to the latest statistics from the Iranian Cancer Research Center, breast cancer cases

in Iran are one decade younger than their western counterparts [3]. About 8500 new cases of breast cancer are reported annually in the country that 1400 cases are reported died from breast cancer; it is also estimated in 2014, about 40,000 people were living with this disease [4].

Breast cancer disease is defined as uncontrolled growth of cells in breast tissue, which is caused by abnormal growth in the glands which have role of producing milk (lobules) or in the ducts that connect lobules to the nipple duct [5] and also is a heterogeneous disease [6] that is caused by environmental and genetic factors [7]. The genetic damage caused by endogenous and exogenous metabolites could be a cause of breast cancer. Clinical and epidemiological studies have demonstrated that estrogen and progesterone play a significant role in the growth and differentiation of normal tissue in the breast [8].

The aetiology of breast cancer is complex and still poorly understood. A small proportion of breast cancer cases can be attributed purely to genetic reasons whereas risk factors such as age, reproductive events (menarche, menopause, pregnancy, breastfeeding), estrogens, exogenous hormones (hormone replacement therapy and oral contraceptives), lifestyle and exposure to environmental carcinogens (pollution, alcohol, diet, obesity), ionizing radiation, chemopreventive agents, as well as genetic factors, breast cancer susceptibility genes high penetrance genes (*BRCA1, BRCA2, PTEN*) and low penetrance genes (*CYP450, GSH, UGTA*) that encodexenobiotic metabolizing enzymes in phase I and II of metabolism [9]. As mentioned above, one of the main risk factors for breast cancer is estrogen, and there are two forms of estrogen in our body: endogenous estrogen that biosynthesises from cholesterol in the body, then undergoes metabolic processes and exogenous form (hormonal drugs, etc.). The main effect of estrogens is stimulating of the breast cells and increases the chance of errors during multiple DNA divisions and the possibility of mutations [10]. Also, it has been identified that Xeno-estrogens, which include pesticides, paints, contaminants, plastics and food preservatives, would have similar effects with estrogen, and contributed to the causes of breast cancer. They have endocrine disruption effects [11].

Many carcinogenic compounds are oxidized by phase I enzymes, represented by cytochrome P450 family, into reactive metabolites that are detoxified by phase II enzymes. GSTs are a family of Phase II detoxification enzymes that catalyse the conjugation of glutathione (GSH) to a wide variety of xenobiotic. This detoxification ability plays an important role in cellular protection from environmental and oxidative stress. Hence, the toxic effects of exposure, absorption and detoxification of carcinogens depend on a delicate balance between the phase I and phase II enzymes [12], [13]. The cytochrome P450 and *GSTs, UGTA* and etc.that phase II enzymes increase its solubility in such a way as to facilitate its excretion and detoxification. These genes involved in the metabolism of these toxins (cytochrome p450), and also contribute to the pathway of biosynthesis and estrogen metabolism, and also contribute to the progression of breast cancer Polymorphisms in both phase I and phase II enzyme genes may result in alteration of their expression, function and activity. These genes are regulated at the transcriptional level, and their expression is influenced by genetic factors, polymorphism in the structural and regulatory gene and by environmental factors [14], [15]. Polymorphisms and expression pattern of these genes are believed to be key factors in determining cancer susceptibility to toxic or environmental chemicals [16]. Polymorphisms are including single nucleotide polymorphisms (SNPs). The most common form of human genetic differences is single Nucleoid Polymorphisms (SNPs) which may play an important role in individual allergies. Single-nucleotide polymorphism is a change in only one open DNA. These single-nucleotide variations cause different phenotypes, predisposing to or susceptibility to certain diseases.

SNPs in genes involved in xenobiotic metabolism and estrogen biosynthesis and metabolism might affect circulating estrogen levels and modulate the individual susceptibility to environmental carcinogens about developing breast cancer [10]. Candidate genes for this study are low-penetrance breast carcinoma susceptibility include those encoding for Xenobiotic Metabolizing Enzymes (XMEs) involved in carcinogen metabolism and detoxification [17]. These XMEs can be divided into phase I (Cytochrome P450 family) and phase II (*GST, UGTA*, etc.) enzymes that metabolically activate potentially carcinogenic forms. In this study, polymorphisms of phase I enzymes *CYP1A1 (rs4646421C/T*), *CYP2C8* (*rs1058930C/G*), *CYP1B1* (*rs1056836 C/G*) and *CYP19A1* (*rs749292A/G*) in breast cancer patients who referred to Mazandaran province clinics, during March 2018 to May 2018 were investigated. DNA extracted from peripheral blood leukocytes of patients with breast cancer and healthy patients. Identifying the genetic polymorphisms of breast cancer patients, in order to recognizing the mechanism of the disease is effective on diagnosis and screening patients who are prone to the disease and, therefore, preventing them. So, position, structure and function of studied genes were briefly explained, then polymorphisms of them were studied.

CYP1A1 is one of the “phase I” enzymes located on chromosome 15q22-q24 and is a 5987-bp long gene. It has 7 exons and 6 introns that encodes for a 512 amino acid protein. This is one of the major components of the detoxification pathway that is highly expressed in non-hepatic cells, such as breast tissue [18]. It is a polymorphic gene involved in the

metabolism of steroids and several potentially genotoxic chemicals. In estrogen metabolism, it plays a main role in producing of2-hydroxy estrogens. Four single nucleotide polymorphisms (SNP) were identified in*CYP1A1* gene including M1, T/C transition at nucleotide 3801; M2, A/G transition at position 2455 resulting in change of Ile to Val at codon 462; M3T/C transition at nucleotide 3205; and M4 C/A transition at position 2453 resulting in change of Thr to Asn at codon 461. The M1 polymorphism at 3'-flanking region (T3801C) was verified to be associated with increased activation of carcinogens [19], [20]. *CYP1B1* is located on chromosome 2 at position 2p22.2. The gene is 42930-fold-wide and 60846 Molecular weight. It has 3 exons and 2 introns and was considered as the key enzyme of P450, which is exogenous in metabolism and endogenous substrate.

CYP1B1 plays the main role in the metabolism of androgens and estrogen substrates. *CYP1B1* can catalyse 4-hydroxyl-estrogens, which is a key reaction to hormonal carcinogenesis. *CYP1B1*, 5 different SNPs have been identified that can play a vital role in replacing amino acids A119S, R48G, L432V, A443G and N453S. Val432Leu- polymorphism has recently been identified as the most influential agent on the catalytic properties of *CYP1B1* and will also affect the function of 4-hydroxy in the Val32 allele, which indicates a 3-fold increase in activity relative to the Leu432 allele, for the specific *CYP1B1* gene rs 163077, 163086 and 162556 who are involved with invasive breast cancer [21], [22]. *CYP19A*1 is located on chromosome 15, q21.2, consisting of 18 exons and 17 introns, and has a molecular weight of 57883 Da. The enzyme is stagnant, which plays an important role in biosynthesis and the final stages of estrogen biosynthesis. The aromatase is coded by the *CYP19A1* gene, a key estrogen biosynthesis enzyme and play a vital role in the development of breast cancer. The main effect of this enzyme is in the catalysing of the final stage of estrogen biosynthesis that converts androstenedione and testosterone into estrogen and estradiol. The direct effect of aromatase on cytotoxicity in the breast is completely reported [23]. A high level of aromatase expression has been reported in breast tumours, which is also visible in the normal breast. *CYP2C8* is located on chromosome 10 at position 10q23.33 consisting of 10 exons and 9 introns. This gene has 55825 Da molecular weight. *CYP2C8* is one of the first human cytochromes and plays an important role in drug metabolism in cytochrome P450, which can also be used in response to chemotherapy and survival chances. Patients with breast cancer are more closely related to the metabolism of *CYP2C8 * 2* and *CYP2C8 * 3* [24].

Material and Methods

Subjects

A total of 123 unrelated subjects (51 controls and 72 patients), living in Mazandaran province were enrolled in this study. The cases were all new incident breast cancer patients histologically diagnosed at the Oncology Department of Cancer Research Center at Imam Hospital in Sari city in Iran, during the period of March 2018 to May 2018. Controls were randomly selected from healthy women who visited patients admitted to the same hospitals and were healthy blood donors having no evidence of any personal or family history of cancer, or other illnesses patients' age ranged from 20 to 75 years. A detailed description of the clinical-pathological characteristics of this study was summarized in (Table1). Control subjects having years ranging from 23 to 66 years. Informed consent was obtained from all participants, and a structured questionnaire was administered by trained interviewers to collect information on demographic and anthropometric data, reproductive and medical history, residential history, and occupation as well, lifestyle, exposure parameters were reported in Table 1. Tobacco smoking and alcohol consumption also were asked from the subject, but there was no case. To investigate whether certain genotypes are a susceptible marker, 5 ml peripheral blood was collected in the EDTA tube from both patients and control group and stored at -20°C.

Table 1: Comparison of cases and controls by selected demographic factors and major risk factors for breast cancer

Clinic pathological Variables	Cases	Controls	P value
Age (mean ± SD)	48.08 ± 10.3	43.69 ± 13.5	0.044*
Age at menarche (mean ± SD)	1.2 ± 13.15	1.2 ± 13.31	0.485
Age at menopause (mean ± SD)	4.4 ± 21.52	5.0 ± 22.55	0.274
Age at 1 St pregnancy (mean ± SD)	4.4 ± 49.53	8.7 ± 47.13	0.21
Age < 45, n (%)	31 (43%.1)	29 (56%.9)	0.13
Age > 45, n (%)	41 (56%.9)	22 (43%.1)	
Pregnancy, n (%)			
NO	6 (8%.3)	-	0.036*
YES	66 (91%.7)	-	
Oral contraceptive use, n (%)			
NO	38 (52%.8)	36 (70%.6)	0.047*
YES	34 (47%.2)	15 (29%.4)	
Menopause Status, n (%)			
Premenopausal	29 (40%.3)	30 (58%.8)	0.043*
Past menopausal	43 (59%.7)	21 (41%.2)	
Body mass index, n (mean±SD kg/m^2)			
BMI < 20 kg/m2	3 (4%.2)	3 (5%.9)	
20 ≤ BMI < 25	19 (26%.4)	14 (27%.5)	0.9
BMI ≥ 25	50 (69%.4)	34 (66%.6)	
Family history of breast cancer in first-degree relatives n (%)			
No	53 (73%.6)	-	< 001*
Yes	19 (26%.4)	-	
Education, n (%)			
≤ 12 years	41 (56%.9)	12 (23%.5)	< 001*
> 12 years	31 (43%.1)	39 (76%.5)	
Occupational exposure to pesticides (Agriculturist), n (%)			
NO	52 (72%.2)	-	< 001*
YES	20 (27%.8)	-	
Grade, n (%)			
I	13 (18%.1)	-	
II	47 (65%.3)	-	< 001*
III	12 (16%.7)	-	
Stage, n (%)			
I	5 (6%.9)	-	< 001*
II	42 (58%.3)	-	
III	18 (25%.0)	-	
IV	7 (9%.7)	-	

DNA extraction

Blood samples were collected in EDTA-containing tubes, and genomic DNA was isolated from buffy coats using a WizPrep DNA blood kit and salting-out method [25]. In the method by using extraction kit, in the presence of strong anionic detergents, the white blood cells are lysed, then proteins are removed with dehydration and prophylaxis. Briefly, 200 µl of blood were mixed with 20 µl protein kinase K, then 200 µl GB buffer was added then the mixture was incubated (10 min 56°C), 200 µl EtOH %100 was added. Then, washing buffer 1 and 2 was added. In the final step, 50 µl Elution Buffer was added. Precipitated proteins were removed by centrifugation. The DNA in the supernatant fluid was precipitated with ethanol. In every step, we centrifuged the mixture based on the protocol of the kit. The DNA pellet was dissolved in 400 µl of sterile distilled water. After extraction, the quality and quantity of the extracted DNA were measured by the spectrophotometer. Then, DNA samples were stored at -20°C, and its purity was checked through agarose following the protocol of the manufacturer. SNPs were genotyped.

Genotyping

Polymorphic sites of genes were genotyped by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay. Amplification was performed using a specific primer. Pair of Primers were designed using Gene Runner software. The sequence of primers is listed in (Table 2) reaction contain. 25 µl Polymerase Chain Reaction (PCR) containing 2 µl genomic DNA (100 ng/µl), 12.5 µl Master mix PCR, and 1 µl (10 picomols) of each primer which ultimately reached 25 µl with distilled water. The PCR reaction program was set for each gen. Then the product of the enzyme was digested. Digestion was electrophoresed on 2% agarose gel and photos were taken by Gel Doc [26].

Table 2: Primers and restriction enzymes used for polymorphism genotyping

Genes	SNP	Polymorphisms	Primers	Restriction enzymes
CYP1A1	rs 4646421	Intron/splice mutation (m1) 3'uR T→C (T56392) (+303C>T)	Forward CCATTTATTCTCTG: CTCTCTGGTA Reverse CCCACCACACTTAGGA AAATCA	*RsaI*
CYP1B1	rs1056836	CYP1B1*3 Exon3/missensc C251G (Val432 Leu)	Forward CTGTGGTTTTTGTCAA CAAGTGGTC Reverse TGAGCCAGGATGGAG ATGAAGAGA	*BsrI*
*CYP19A1*1	rs749292	Intron / Exon1	Forward CCAAGGTCCCACAGCT AATTAGTGA Reverse TAAAAGGGCAAGAGCA GAGATGAGC	*TaqI*
CYP2C8	rs1058936	CYP2C8*4 Exon5/missense C792G (ILe 264 Met)	Forward AATCAGGGCTTGGTGT AAGATA Reverse CGATGAATCACAAAAT GGACAAG	*TaqI*

Polymorphisms analysis

Previously reported primers and restricted enzymes in RFLP-PCR are listed in (Table 2). All PCR reactions were performed in an independent blinded duplicate manner, and for each polymorphism, some samples were confirmed by sequencing the PCR products.

The polymorphic site of the *CYP1A1* (rs 4646421) (C—T intron) was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). A 519 bp fragment containing C/T allele was amplified. Using forward and reverse primers that the specifications of primers and size of product and restriction enzymes of genes have been explained in the (Table 2). *CYPP1B1* (rs1056836) (C-Gval432leucin) also simultaneously revealed by Restriction Fragment Length Polymorphism (PCR-RFLP) PCR reaction was performed in a total volume of 50 µl containing 100 ng of genomic DNA, 200 µmol dNTPs, 2 mM $MgCl_2$, 1 × Taq polymerase buffer, 100 pmol of *CYP1A1* and *CYP1B1* (primers and 1 U of Taq DNA polymerase). There action conditions used with the thermal cycler were as follows for *CYP1A1*: initial denaturation at 95°C for 5 min, 32 cycles of denaturation at 95°C for 30 sec, annealing at 62°C for 30 sec and extension at 72°C for 30 sec and a final extension at 72°C for 10 min for *CYP1B1* annealing set at 66.5°C for 30 sec. To verify proper amplification conditions, 10 µl of PCR product were analysed on a 2% agarose gel and stained with ethidium bromide, the amplification of was revealed by the presence of bands. To detect *CYP1A1* (C-T) and *CYP1B1* (C-G) polymorphisms, amplified DNA was digested with 10 U of Fast Digest *RsaI* (37°C, 5 min) and *BsrI* (65°C, 16 h) restriction enzymes. In *CYP1A1* the homozygote wild-type CC genotype produces two 183 and 36 bp fragments, homozygote mutated TT genotype results in single band of 183 bp length, and heterozygote TC genotype produces three fragments of 519, 183 and 336 bp fragments and in *CYP1B1* homozygote wild-type CC genotype produce single band of 44 bp length, homozygote mutated GG genotype results from two 44 and 171 bp fragments and heterozygote GC genotype produce three fragments of 215, 171, and 44 bp fragments.

To verify proper amplification conditions, 10 µl of PCR product were analysed on a 2% agarose gel and stained with ethidium bromide, the amplification of was revealed by the presence of bands. Homozygote wild-type CC genotype produce single band of 44 bp length, homozygote mutated GG genotype results from two 44 and 171 bp fragments and heterozygote GC genotype produces three fragments of 215, 171and 44 bp fragments. Digestion conditions were performed according to the manufacturer's instructions and summarized for each gen in (Table 3).

Digestion products were separated at the appropriate concentrations on a 2, 3 or 4% Low-melting point agarose gel and stained with ethidium bromide. The splice-site mutation of *CYP19A1* (rs749292) (A-G intron) and *CYP2C8*4* (rs1058930) (C-G exon5) was also analysed by PCR-RFLP. PCR amplification also was performed the same as the above genes. The cycling conditions for both of genes including one pre-treatment cycle denaturation at 94°C for 5 min, 32 cycles of denaturation at 94°C for 30 sec, followed by annealing at 66°C for 30 sec and elongation at 72°C for 25 sec and a final elongation at 72°C for 10 min. Products were analysed by electrophoresis at 2% agarose gel and visualised by ethidium bromide staining. This amplified fragment was digested *TaqI* restriction enzyme at 65°C overnight and was analysed on 2% agarose gel. *CYP19A1* when digested with *TaqI* the homozygote wild-type GG genotype results from two 144 and 214 bp fragments, homozygote mutated AA genotype produce single band of 144 bp and heterozygote GA genotype produce three fragments of 358, 144 and 214 bp fragments whereas Digestion of CYP2C8 yielded band of 84 bp, and heterozygote GC genotype produces three fragments of 303, 84 and 219 bp fragments (Table 3).

Table 3: Restriction enzymes conditions used for Genes polymorphism genotyping

SNPS	Restriction Enzymes	Temperature and Incubation time	Fragment size (bp)
rs4646421	FastDigestRsaI (10 µ)	37ºC-5 min	519, 183 + 336
rs1056836	BSrI (10 µ)	65ºC-1h16	215, 44 + 171
rs749292	TaqI (10 µ)	65ºC-1h16	358, 144 + 214
rs1058930	TaqI (10 µ)	65ºC-1h16	303, 84 + 219

Statistical analysis

The genotype and allele frequency of the genes were tested for both patient and control group using chi-square test, Fisher exact test, quantitative (numerical) parameters analyzed by t-student test Odds ratio (OR), confidence intervals (CI) and P-values were calculated using unconditional logistic regression and adjusted estimate the association between genotypes or some other clinicopathological data and the risk of breast cancer. In this research, statistical analyses were performed using SPSS software ver. 21.

Results

Demographic and clinicopathological data

This study was performed in 72 breast cancer patients and 51 healthy controls with known demographic and clinicopathological data in Mazandaran province in the north of Iran. Characteristics of the study population were compared by case-control status, as shown in Table 1. Student's t-test showed significant relationships between the two groups and the mean age of controls (43.69 ± 13.5 years) was significantly lower (P = 0.044) than that of breast cancer patients (48.08 ± 10.3 years). Chi-square and Fisher exact test showed that Pregnancy, menopausal status, family history of breast cancer and education, stage of cancer and grade of the tumour were significantly different between cases and controls. However, no significant differences were found between them regarding BMI, Age at menarche, age at menopause and Age at 1 St pregnancy. In the case group, the frequency of level education is lower than diploma and difference is significant ($p < 0.001$).

The majority of cases have a pregnancy in the patient group, and the difference between the two groups was significant ($p = 0.036$). Regarding the menopause status, the majority of cases were not at the menopause status (P = 0.044). In the cases groups, the majority of people have used LD tablets, and the difference between the two groups was significant ($p = 0.043$). About the stage of cancer, the majority of patients were in stage 2 ($p < 0.001$), and Grades of tumours were mostly reported in Grade 2 ($p < 0.001$). Regarding the agricultural occupation, few people were in cases group ($p < 0.001$). About the family history of cancer, the majority of patients in the first-degree subjects had no history of cancer in their family ($p < 0.001$).

Regarding the distribution of genotypes, it can be said that distribution of genotypes in two groups in *CYP2C8* (P = 0.11), *CYP19A1* (P = 0.019), *CYP1B* (P = 0.026) Had a significant difference, and only in *CYP1A1 (*p = 0.416) is homogeneous (Table 4).

Table 4: Genotype frequencies of genes among cases and controls, and risk of breast cancer

Gene name	Genotype	Groups		Chi-square test		
		Cases	Controls	Test statistic	Fisher's exact test	P value
	CC	62 (%86.1)	48 (%94.1)			
CYP2C8	CG	0 (%0.0)	2 (%3.9)	-	7.4	0.011*
	GG	10 (%13.9)	1 (%2.0)			
	AA	3 (%4.2)	10 (%19.6)			
CYP19A1	GA	42 (%58.3)	27 (%52.9)	7.7	-	0.019*
	GG	27 (%37.5)	14 (%27/5)			
	CC	56 (%77.8)	38 (%74.5)			
CYP1A1	TC	16 (%22.2)	13 (%25.5)	0.17	-	0.416
	TT	0 (%0.0)	0 (%0.0)			
	CC	38 (%52.8)	38 (%74.5)			
CYP1B1	GC	20 (%27.8)	5 (%9.8)	7.26	-	0.026*
	GG	14 (%19.4)	8 (%15.7)			

Also, there was no significant correlation between age and breast cancer risk (P = 0.13). But there was a significant relationship between the risk of cancer and menopause status, and the chance of having cancer is 0.466 times lower than those who did not have menopause status ($p = 0.048$). Besides, there was a significant relationship between the risk of cancer and LD Consumption, and these people have 0.47 times higher risk of breast cancer (P = 0.043) (Table 5).

Table 5: Relation between demographic characteristic and risk of breast cancer

		Group		Result		
				OR	P-value	CI
Age	< 45	31 (25%.2)	29 (22%.6)	0.57	0.13	(0.28,1.18)
	> 45	41 (33%.3)	22 (17%.9)			
Agriculture	NO	52 (72%.2)	-	-	-	-
	YES	20 (27%.8)	-			
Menopause status	NO	36 (29%.3)	36 (29%.3)	0.466	0.048*	(0.22,0.99)
	YES	15 (12%.2)	15 (12%.2)			
Family history in the first degree	NO	-	-	-	-	-
	YES	-	-			
OCP USE	NO	30 (24%.4)	30 (24%.4)	0.47	0.043*	(0.23,0.98)
	YES	21 (17%.1)	21 (17%.1)			
Grade	I, II	-	-	-	-	-
	III	-	-			
Stage	I, II	-	-	-	-	-
	III, IV	-	-			

Regarding the relationship between the genotypes of each gene and the demographic characteristics, it can be concluded that there is no significant difference between the genotype and the demographic characteristics such as the age of the patients, education, pregnancy history, menopause status, agricultural occupation, LD consumption, stage of disease, grade of tumours and BMI. There was only a significant relationship between genotypes of *CYP1A1* gene and education (p = 0.004), and the group with under diploma education had more CC genotype, and the group with diploma education and higher had the highest TC genotype.

Frequency of Genotypes and Alleles

In this case-control study, 72 breast cancer patients and 51 healthy controls were studied. The results of the experiments showed in (Table 6). The frequency of genotypes *CYP1B1* gene among of 72 patients was CC (52.8%), CG (27.8%) and GG (19.4%), and in healthy subjects was CC (74.5%) CG (9.8%) and GG (15.7%). The frequency of alleles in this genotype, allele C (66%) and G allele (34%) in patients and control subjects was C (80%) and G allele (20%). Regarding the *CYP1A1* gene, the frequency of genotypes in patients was CC (77.8%), CT (22.2%) and TT (0%) and in healthy subjects were CC (74.5%), CT (25.5%) and TT (0%), and the frequency of allele C was determined in patients (89%) and G allele (11%) and allele C (87%) and G (13%) in controls have frequency. About. *CYP19A1* gene, the frequency of genotypes was determined in patients with GG (37.5%), GA (58.3%) and AA (4.2%), and in healthy subjects GG (27.5%), GA (52.9%) and AA (19.6%). The prevalence of alleles in patients with allele Cis (66%) and T (34%) and the healthy subjects, allele C (54%) and T (46%). Regarding the *CYP2C8* gene, the frequency of CC (86.1%), CG (0%) and GG (13.9%) genotypes in patients and healthy subjects were CC (94.1%), CG (3.9%) and GG (2%), and frequency of alleles Patients were assigned C allele (86%) and T (14%), and in the control group, allele C (94%) and T (6%) were determined (Table 6). Regarding the relationship between the genotypes of each gene and breast cancer risk using a logistic regression model (Table 6) in two normalized and age-adjusted models, it was determined that in the *CYP2C8* genotype, those who have the CG allele have a 7.74 degree increased risk of breast cancer compared to those who have the CC genotype and in the *CYP19A1* gene of individuals with GA genotype, the risk of breast cancer was 6.42 compared to those of genotype AA (CI-%95 1.52—27.21) (Table 6), about the *CYP1B1* gene, people with two genotypes of CG + GG is associated with a higher risk of breast cancer 2.61 times higher compared to the CC genotype (CI = %95 1.19-5.71) and allele G has protective effect in breast cancer and decreased risk of breast cancer (P = 0.0271) and *CYP2C8* gene allele G decreased risk of breast cancer (P = 0.02). In the age-adjusted model, for the *CYP2C8* gene, GG genotype compared to CC has an increased risk of breast cancer about 9.17 compared to other genotypes (CL = %95 1.11-75.84). Regarding *CYP1B1*, the CG + GG genotype is 2.93 times higher than CC genotype increased risk of breast cancer (CL=%95 1.31-6.57).

Table 6: Relationship between the genotypes and breast cancer risk

Genes name	Genotype and Alleles	groups		Non-Adjusted		Adjusted	
		Cases (%)	controls(%)	P	OR (95% CI)	P	OR (95% CI)
CYP2C8	CC	62 (86%.1)	48 (94%.1)	0.016	-	0.12	-
	CG	0 (0%.0)	2 (3%.9)	0.06	7.74 (0.95,62.5)	0.04	9.17 (1.11,75.84)
	GG	10 (13%.9)	1 (2%.0)	0.99	1.615E + 10	0.99	1.560E + 10
	CC vs GG + CG	72 (58%.53)	5 1(41%.47)	0.167	2.58 (0.67,9.89)	0.112	3.027 (0.77,11.88)
	C (%)	86%	%96	-	Ref	-	-
	G (%)	14%	%4	P = 0.02	0.25 (0.08,0.81)	-	-
CYP19A1	AA	3 (4%.2)	10 (19%.6)	0.038	-	0.015	-
	GA	42 (58%.3)	27 (52%.9)	0.011	6.42 (1.52,27.21)	0.61	6.21 (1.43,26.86)
	GG	27 (37%.5)	14 (27%.5)	0.601	1.24 (0.55,2.77)	0.066	1.24 (0.54,2.8)
	AA + GA vs GG	72 (58%.53)	51 (41%.47)	0.246	1.58 (0.73,3.45)	0.263	1.57 (0.71,3.46)
	G (%)	%66	%54	-	Ref	-	-
	A (%)	%34	%46	P = 0.084	1.65 (0.93,2.92)	-	-
CYP1A1	CC	56 (77%.8)	38 (74%.5)	-	-	-	-
	TC	16 (22%.2)	13 (25%.5)	0.67	0.835 (0.36,1.93)	0.63	0.81 (0.34,1.9)
	TT	0 (0%.0)	0 (0%.0)	-	-	-	-
	CC vs GG + CG	72 (58%.53)	51 (41%.47)	0.67	0.835 (0.36,1.93)	0.63	0.81 (0.34,1.9)
	C (%)	%89	%87	-	Ref	-	-
	T (%)	%11	%13	P = 0.66	1.21 (0.51,2.84)	-	-
CYP1B1	CC	38 (52%.8)	38 (74%.5)	0.034	-	0.024	-
	GC	20 (27%.8)	5 (9%.8)	0.26	1.75 (0.66,4.65)	0.165	2.045 (0.74,5.6)
	GG	14 (19%.4)	8 (15%.7)	0.22	0.44 (0.118,1.62)	0.278	0.479 (0.127,1.8)
	CC vs CG+GG	72 (58%.53)	51 (41%.47)	0.016	2.61 (1.197,5.71)	0.009	2.93 (1.31,6.57)
	C (%)	%66	%80	-	Ref	-	-
	G (%)	%34	%20	P = 0.0271	0.48 (0.25,0.92)	-	-

The results of logistic regression model regarding the frequency of genotypes of each gene and the incidence of breast cancer indicated that there was a significant relationship between the frequency of genotypes and the risk of breast cancer only in *CYP1B1* gene (P-value = 0.015, OR = 0.38, CI = 0.18 0.84), and no significant results were obtained for other genes (Table 7).

It's noteworthy that CC genotype of CYP*1B1* decreases about 0.38-time risk of breast cancer in comparison the CG + GG genotype. In addition, for correlation between CC and CG + GG genotypes with demographic and clinical variables such as age (P-value = 0.25), agriculture (P-value = 0.2), menopause (P = 0.16) (P-value = 0.29), LDL consumption (P-value = 0.86), tumor grade (P-value = 0.83), and stage of cancer (P-value = 0.16), there was no significant relationship between two groups (Table 7).

Table 7: Relationship between the frequency of *CYP1B1* genotypes and the risk of breast cancer and demographic and clinical variables

		CYP1B1		Result		
		CC	GC + GG	OR	P-value	CI
Group	Case	38 (30%.9)	34 (27.6)	0.38	0.015*	(0.18,0.84)
	Control	38 (30%.9)	13 (10%.6)			
Age	< 45	34 (27%.6)	26 (21%.1)	0.65	0.25	(0.32,1.36)
	> 45	42 (34%.1)	21 (17%.1)			
Agriculture	NO	25 (34%.7)	27 (37%.5)	0.5	0.2	(0.17,1.45)
	YES	13 (18%.1)	7 (9%.7)			
Menopause status	NO	42 (34%.1)	32 (26%.0)	0.58	0.16	(0.27,1.24)
	YES	34 (27%.6)	15 (12%.2)			
Family history in first degree	NO	26 (36%.1)	27 (37%.5)	0.56	0.29	(0.19,1.65)
	YES	12 (16%.7)	7 (9%.7)			
OCP USE	NO	36 (29%.3)	23 (18%.7)	0.94	0.86	(0.45,1.94)
	YES	40 (32%.5)	24 (19%.5)			
Grade	I, II	32 (44%.4)	28 (38%.9)	1.14	0.83	(0.33,3.94)
	III	6 (8%.3)	6 (8%.3)			
Stage	I, II	22 (30%.6)	25 (34%.7)	0.49	0.16	(0.18,1.34)
	III, IV	16 (22%.2)	9 (12%.5)			

Discussion

Breast cancer is by far the most common female cancer and comprising about 21% of all new cancers in women. The highest age-adjusted incidence rate is reported for North America, is 87 per 100 thousand women per year, while the lowest rate reported in China. Breast cancer follows a steeply increasing age gradient up to 40 years of age, after which the rate of increase slows down. Even though there are three times as many new cases diagnosed annually as in the late 1980s, breast cancer mortality has remained largely unchanged. This may at least partly be explained by earlier detection of the disease due to effective screening programs and availability of improved therapies. The highest annual mortality rates for breast cancer are reported for the UK, The Netherlands and Denmark, being over 25 per 100 thousand in these countries. So far, conflicting results have been reported from association studies [27]. The aetiology of breast cancer could not be described by allelic variability at a single locus. Instead, the main burden of breast cancer in the population probably results from complex interactions between many genetic and environmental factors over time. An improved understanding of the interplay of xenobiotic exposures, endogenous physiology, and genetic variability at multiple loci may help to identify women who are at increased risk for breast cancer. The genetic polymorphisms that may be linked to breast cancer are multiple. Cumulative lifetime exposure to estrogen, estrogen metabolites, and other physiological factors, as well as environmental exposures, could play an important role in the aetiology of breast cancer in genetically predisposed women. Carcinogenesis, determining response to drugs and cell signalling. In the metabolism of xenobiotic (foreign chemicals), cytochrome P450s or monooxygenases perform an important function by catalysing the hydroxylation reaction [28]. *CYP450* enzymes associated with the development of breast cancer which involved in biosynthesis and metabolism of estrogens and other *CYP* enzymes can involve in the development of breast cancer risks like *CYP19, CYP21, CYP17, CYP1A2, CYP11A1, CYP2D6, CYP2C19, CYP3A4/5, CYP1A1, CYP1B1, CYP2C8/9.* In this study, distribution of *CYP450* isoenzymes *CYP1A1* (*rs4646421*), *CYP1B1* (*rs1056836*), *CYP19A1* (*rs749292*) and *CYP2C8* (*rs1058930*) and gene polymorphisms in patients with breast cancer in Mazandaran province was investigated by the PCR-RFLP method using restriction enzyme activity.

In a study by Mandana Gheysar et al., they determined that through PCR-RFLP reaction, polymorphisms in genes involved in xenobiotic metabolism and estrogen biosynthesis, like *CYP1A1* (*Ile462Val; rs1048943*), *CYP1B1* (*Leu432Val; rs1056836*) and *CYP19A1* (*C> T; rs10046*) and they found an independent association of *CYP1A1* (*Val*) with BC risk. *CYP1B1* and *CYP19A1* are not associated with breast cancer risk [29]. Joanna Trubicka et al. genotyped 597 cancer patients and 597 controls for three *CYP1B1* SNPs. They found that the three SNPs rs10012, rs1056827 and rs1056836 alone did not provide any significant evidence of association with colorectal cancer risk. Haplotypes of rs1056827 and rs10012 or rs1056827 and rs1056836 revealed an association with colorectal cancer which was significantly stronger in the homozygous carriers. Genetic variants within the *CYP1B1* that are associated with altered function appear to influence susceptibility to colorectal cancer in Poland (30). In another study, Marc T Goodman et al., determined genetic variation in two *CYP19A1* single-nucleotide polymorphisms (SNPs), rs749292 and rs727479, by PCR-RFLP method and association with the risk of ovarian cancer. Results showed that the A allele of rs749292 was positively associated with ovarian cancer risk in a codominant model for all races combined (AG versus AA genotype: odds ratio (OR), 1.48 and 95% confidence interval (CI, 1.07-2.04); GG versus AA: OR, 1.87 (CI, 1.24-2.82); P trend = 0.002). Similar significant associations of the rs749292 A allele on the risk of ovarian cancer were found among Caucasian and Japanese women. No relation of the rs727479 SNP to ovarian cancer risk was observed overall, although Caucasian women carrying the variant A allele compared with women with a CC genotype had an OR of 2.91 (CI, 1.15-7.37). These data suggest *CYP19A1* variants may influence susceptibility to ovarian cancer [31].

Jernstrometal. Investigated *CYP2C8* and *CYP2C9* polymorphisms about tumour characteristics and early breast cancer. In a prospective series of 652 breast cancer patients from southern Sweden was genotyped for *CYP2C8*3, CYP2C8*4, CYP2C9*2*, and *CYP2C9*3*. Frequencies of *CYP2C8/9* polymorphisms were similar to healthy European populations. Significantly less node involvement (P¼0.002) and fewer PR tumours (P¼0.012) were associated with *CYP2C8*4*. Median follow-up was 25 months, and 52 breast cancer-related events were reported. In a multivariate model,

*CYP2C8/9*3/*1*/*2/*1* was the only factor associated with increased risk for early events in 297 tamoxifen-treated, ER-positive patients, adjusted HR 2.54 (CI = 95% 1.11–5.79). The effect appeared to be driven by *CYP2C8*3*, adjusted HR 8.56 (95%CI 1.53-51.1). They found that polymorphic variants of *CYP2C8/9* may influence breast tumour characteristics and disease-free survival in tamoxifen-treated patients [32].

In conclusion, the results of this study indicate that *CYP2C8* (*rs1058930*), *CYP19A1* (*rs749292*) and *CYP1B1 (rs1056836)* gene polymorphisms are associated with breast cancer, and screening for these genes polymorphisms can be used to prognosticate disease, prevent disease progression, and to use appropriate therapeutic.

Acknowledgements

Acknowledgements from the Student Research Committee of Mazandaran University of Medical Sciences (Medical Ethics Code of the Committee Ethics: IR.MAZUMS.REC.1397.1435)

References

1. Mehrabani D, Almasi A, Farahmand M, Ahrari Z, Rezaianzadeh A, Mehrabani G, et al. Incidence of breast cancer in Fars province, southern Iran: A hospital-based study. World journal of plastic surgery. 2012; 1(1):16.

2. Stewart SL, King JB, Thompson TD, Friedman C, Wingo PA. Cancer mortality surveillance-United States, 1990-2000. MMWR Surveill summ. 2004; 53(3):1-108. https://doi.org/10.1037/e307142005-001

3. Khadivi R, Harrirchi I, Akbari ME. Ten year breast cancer screening and follow up in 52200 women in Shahre-Kord, Iran (1997-2006). Iranian Journal of Cancer Prevention. 2008; 1(2):73-7.

4. Nafissi N, Saghafinia M, Motamedi MHK, Akbari ME. A survey of breast cancer knowledge and attitude in Iranian women. Journal of cancer research and therapeutics. 2012; 8(1):46. https://doi.org/10.4103/0973-1482.95173 PMid:22531513

5. Allred DC. Ductal carcinoma in situ: terminology, classification, and natural history. Journal of the National Cancer Institute Monographs. 2010; 2010(41):134-8. https://doi.org/10.1093/jncimonographs/lgq035 PMid:20956817 PMCid:PMC5161057

6. Ladd AG-Z, Vásquez AA, Rivadeneira F, Siemes C, Hofman A, Stricker BC, et al. Estrogen receptor α polymorphisms and postmenopausal breast cancer risk. Breast cancer research and treatment. 2008; 107(3):415-9. https://doi.org/10.1007/s10549-007-9562-3 PMid:17453340 PMCid:PMC2217623

7. Wolff MS, Weston A. Breast cancer risk and environmental exposures. Environmental health perspectives. 1997; 105(suppl 4):891-6. https://doi.org/10.1289/ehp.97105s4891 PMid:9255576 PMCid:PMC1470027

8. Hankinson SE, Willett WC, Manson JE, Colditz GA, Hunter DJ, Spiegelman D, et al. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. JNCI: Journal of the National Cancer Institute. 1998; 90(17):1292-9. https://doi.org/10.1093/jnci/90.17.1292 PMid:9731736

9. Dumitrescu R, Cotarla I. Understanding breast cancer risk-where do we stand in 2005? Journal of cellular and molecular medicine. 2005; 9(1):208-21. https://doi.org/10.1111/j.1582-4934.2005.tb00350.x PMid:15784178 PMCid:PMC6741339

10. Kristensen VN, Børresen-Dale AL. Molecular epidemiology of breast cancer: genetic variation in steroid hormone metabolism. Mutation Research/Reviews in Mutation Research. 2000; 462(2-3):323-33. https://doi.org/10.1016/S1383-5742(00)00018-1

11. Gan C, Wang X, Cao Y, Ye W, Liu H, Sun Y. Association of CYP2C19* 3 gene polymorphism with breast cancer in Chinese women. Genet Mol Res. 2011; 10(4):3514-9. https://doi.org/10.4238/2011.December.5.3 PMid:22180071

12. Bartsch H, Nair U, Risch A, Rojas M, Wikman H, Alexandrov K. Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. Cancer Epidemiology and Prevention Biomarkers. 2000; 9(1):3-28.

13. Nebert DW, Dalton TP. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. Nature Reviews Cancer. 2006; 6(12):947. https://doi.org/10.1038/nrc2015 PMid:17128211

14. Pavek P, Dvorak Z. Xenobiotic-induced transcriptional regulation of xenobiotic metabolizing enzymes of the cytochrome P450 superfamily in human extrahepatic tissues. Current drug metabolism. 2008; 9(2):129-43. https://doi.org/10.2174/138920008783571774 PMid:18288955

15. Goth-Goldstein R, Stampfer MR, Erdmann CA, Russell M. Interindividual variation in CYP1A1 expression in breast tissue and the role of genetic polymorphism. Carcinogenesis. 2000; 21(11):2119-22. https://doi.org/10.1093/carcin/21.11.2119 PMid:11062177

16. Safarinejad MR, Dadkhah F, Asgari MA, Hosseini SY, Kolahi AA, Iran-Pour E. Glutathione Sâ€"Transferase Polymorphisms (GSTM1, GSTT1, GSTP1) and Male Factor Infertility Risk: A Pooled Analysis of Studies. Urology journal. 2012; 9(3):541-8.

17. Han W, Kang D, Park IA, Kim SW, Bae JY, Chung K-W, et al. Associations between breast cancer susceptibility gene polymorphisms and clinicopathological features. Clinical Cancer Research. 2004; 10(1):124-30. https://doi.org/10.1158/1078-0432.CCR-0834-3 PMid:14734460

18. Lu N, Wu B, Xia Y, Wang W, Gu A, Liang J, et al. Polymorphisms in CYP1A1 gene are associated with male infertility in a Chinese population. International journal of andrology. 2008; 31(5):527-33. https://doi.org/10.1111/j.1365-2605.2007.00804.x PMid:17651397

19. Srivastava DS, Mandhani A, Mittal RD. Genetic polymorphisms of cytochrome P450 CYP1A1 (* 2A) and microsomal epoxide hydrolase gene, interactions with tobacco-users, and susceptibility to bladder cancer: a study from North India. Archives of toxicology. 2008; 82(9):633-9. https://doi.org/10.1007/s00204-007-0276-4 PMid:18200441

20. Ding X, Kaminsky LS. Human extrahepatic cytochromes P450: function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. Annual review of pharmacology and toxicology. 2003; 43(1):149-73. https://doi.org/10.1146/annurev.pharmtox.43.100901.140251 PMid:12171978

21. Harth V, Schäfer M, Abel J, Maintz L, Neuhaus T, Besuden M, et al. Head and neck squamous-cell cancer and its association with polymorphic enzymes of xenobiotic metabolism and repair. Journal of Toxicology and Environmental Health, Part A. 2008; 71(13-14):887-97. https://doi.org/10.1080/15287390801988160 PMid:18569591

22. Ickstadt K, Schäfer M, Fritsch A, Schwender H, Abel J, Bolt HM, et al. Statistical methods for detecting genetic interactions: a head and neck squamous-cell cancer study. Journal of Toxicology and Environmental Health, Part A. 2008; 71(11-12):803-15.

https://doi.org/10.1080/15287390801985745 PMid:18569579

23. Haiman CA, Dossus L, Setiawan VW, Stram DO, Dunning AM, Thomas G, et al. Genetic variation at the CYP19A1 locus predicts circulating estrogen levels but not breast cancer risk in postmenopausal women. Cancer research. 2007; 67(5):1893-7. https://doi.org/10.1158/0008-5472.CAN-06-4123 PMid:17325027

24. Daily EB, Aquilante CL. Cytochrome P450 2C8 pharmacogenetics: a review of clinical studies. Pharmacogenomics. 2009; 10(9):1489-510. https://doi.org/10.2217/pgs.09.82 PMid:19761371 PMCid:PMC2778050

25. Shokrzadeh M, Fattahi I, Mohammadpour A, Mashhadban AH. Presence of CagA gene and its antibiotic resistance pattern in Helicobacter pylori isolates. Journal of Mazandaran University of Medical Sciences. 2017; 27(154):60-72.

26. Tafrihi M, Toosi S, Minaei T, Gohari AR, Niknam V, Arab Najafi SM. Anticancer properties of Teucrium persicum in PC-3 prostate cancer cells. Asia Pac J Cancer Prevent. 2014; 15(2):785-91. https://doi.org/10.7314/APJCP.2014.15.2.785 PMid:24568496

27. Economopoulos KP, Sergentanis TN. Does race modify the association between CYP1B1 Val432Leu polymorphism and breast cancer risk? A critical appraisal of a recent meta-analysis. Breast cancer research and treatment. 2010; 124(1):293-4. https://doi.org/10.1007/s10549-010-1097-3 PMid:20686834

28. PICADO-LEONARD J, MILLER WL. Cloning and sequence of the human gene for P450cl7 (steroid 17α-hydroxylase/17, 20 lyase): similarity with the gene for P450c21. Dna. 1987; 6(5):439-48. https://doi.org/10.1089/dna.1987.6.439 PMid:3500022

29. Ghisari M, Eiberg H, Long M, Bonefeld-Jørgensen EC. Polymorphisms in Phase I and Phase II genes and breast cancer risk and relations to persistent organic pollutant exposure: a case-control study in Inuit women. Environmental Health. 2014; 13(1):19. https://doi.org/10.1186/1476-069X-13-19 PMid:24629213 PMCid:PMC4234380

30. Trubicka J, Grabowska-Kłujszo E, Suchy J, Masojć B, Serrano-Fernandez P, Kurzawski G, et al. Variant alleles of the CYP1B1 gene are associated with colorectal cancer susceptibility. BMC cancer. 2010; 10(1):420. https://doi.org/10.1186/1471-2407-10-420 PMid:20701755 PMCid:PMC2929240

31. Goodman MT, Lurie G, Thompson PJ, McDuffie KE, Carney ME. Association of two common single-nucleotide polymorphisms in the CYP19A1 locus and ovarian cancer risk. Endocrine-related cancer. 2008; 15(4):1055-60. https://doi.org/10.1677/ERC-08-0104 PMid:18667686 PMCid:PMC2663409

32. Jernström H, Bågeman E, Rose C, Jönsson P-E, Ingvar C. CYP2C8 and CYP2C9 polymorphisms in relation to tumour characteristics and early breast cancer related events among 652 breast cancer patients. British Journal of Cancer. 2009; 101(11):1817. https://doi.org/10.1038/sj.bjc.6605428 PMid:19935798 PMCid:PMC2788256