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Original Article

Association of CYP1A1 IIe462Val (rs1048943) Polymorphism with Breast Cancer in Iranian Women

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Backgrounds and Aims: One member of the cytochrome P450 family, *CYP1A1*, is one of the genes involved in the metabolism of carcinogens and estrogen, which has been identified to be associated with breast cancer, as well. Considering the known effect of estrogen in different signaling pathways, disorders in these pathways will affect the risk of breast cancer. In this study, we evaluated the relationship between rs1048943 polymorphism of *CYP1A1* gene and the risk of breast cancer in a population of Iranian women.

Materials and Methods: This case-control study was conducted on 79 patients with breast cancer and 79 healthy women at shohad-e-tajrish hospital. After genomic DNA extraction from peripheral blood cells using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, the genotype of the participants was identified. Then, the relationship between A/G polymorphism and the disease risk was analyzed using SPSS version 23.

Results: The mean age of the patients and controls was 48±8 and 43±6 years, respectively. the results showed that the frequency of the G allele had a significant difference between cases and controls. Accordingly, the presence of the G allele, as a risk allele, increased the chance of breast cancer in the carriers of this allele by at least a factor of 2.33 in comparison with people without this allele (OR=2. 33, %95 CI:1.21-4.37, p=0. 006).

Conclusions: Our findings showed a significant correlation between the CYP1A1 gene polymorphism and increased risk of breast cancer in a population of Iranian women.

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Introduction

Breast cancer is a major health challenge in the world [1] and the leading cause of death due to cancer in Iranian women, as well [2]. Epidemiologic studies of breast cancer show that it is a multifactorial and polygenic disease, and a combination of environmental and genetic factors, as well as their interaction, plays a role in its development. Extensive genetic studies have been performed on this disease in recent years, leading to the identification of genetic variants related to breast cancer [3].

One of the known genes associated with breast cancer is *CYP1A1*, located at chromosome 15, which comprises 7 exons and 6 introns and spans 5810 base pairs [4]. Due to its pivotal role in the metabolism of carcinogens like polycyclic aromatic hydrocarbons (PAHs), it is considered as one of the gene candidates related to the risk of breast cancer. PAHs are known human carcinogens that cause breast cancer in rodents [5]. Accordingly, *CYP1A1* is involved in the activation of PAHs as reactive epoxide metabolites and hydroxylates to main estrogens named E1 and E2 at C2 position [6-8].

The first reported *CYP1A1* polymorphism was identified using the *MspI* restriction enzyme [9]. This polymorphism is located in the non coding region of *CYP1A1* and was named the *CYP1A1*2A* allele [10]. Altered alleles of *CYP1A1*2C* and *CYP1A1*2A* are also the most common alleles in Asians [11]. The most common allele identified on this gene is *CYP1A1*2C* (rs1048943) located at exon 7 of the *CYP1A1* gene and causes the substitution of isoleucine 462 with valine. Conducted studies have shown a relationship between this locus and increased risk of certain cancers [12, 13]. Therefore, this study was conducted to evaluate the relationship between *CYP1A1*2* (rs1048943) polymorphism, as a known polymorphism in the estrogen metabolism pathway, and the risk of breast cancer in an Iranian population.

Materials and Methods

Study samples

This case-control study was conducted on 79 women with breast cancer whose diagnosis was confirmed through medical examinations and clinical tests performed in Shohadaye Tajrish Hospital and 79 women who were confirmed to not have special diseases such as diabetes and hypertension and a history of diseases such as breast cancer, particularly among their first degree relatives. Ethical clearance was taken from institutional Ethical committee. Informed consent was obtained from all cases and controls before participation. Three to five milliliters peripheral blood was taken from each participant and collected into tubes coated with Ethylenediaminetetraacetic acid. Then, the tubes were transferred to the laboratory for genetic tests considering biosafety precautions and cold chain requirements.

DNA extraction and genotyping

The genomic DNA was extracted using the standard salting-out method. Then, the quality

and quantity of the extracted DNA were evaluated. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect the G/A single-nucleotide polymorphism of the *CYP1A1* gene, in which a primer pair and a restriction enzyme named *BseMI (BsrDI)* (Thermo Fisher Scientific, America), were used. Table 1 shows the sequence of the primers.

Table 1. View primers designed to amplify *CYP1A1* gene rs1048943 polymorphism using PCR-RFLP technique

Primer	Sequence 5′→3′	Tm	Restriction Enzyme	Product Size
Forward	5′-CTGTCTCCCTCTGGTTACAGGAAGC-3′	64°C	*BseMI(BsrDI)*	**204bp**
Reverse	5′-TTCCACCCGTTGCAGCAGGATAGCC-3′			

The PCR reaction was done with a final volume of 23 µl, including 1 µl genomic DNA, 10 µl commercial master mix (Amplicon, Denmark), 10 µl distilled water, 1 µl forward primer, and 1 µl reverse primer (Genefanavaran, Iran). Amplification reaction according to the employed program was primary denaturation at 95ºC for 5 minutes, secondary denaturation at 95ºC for 1 minute, annealing at 64ºC for 30 seconds, amplification at 72ºC for 40 seconds, and final amplification at 72ºC for 7 minutes. All PCR products were subjected to electrophoresis (Bio-Rad, America) on a 3% agarose gel at 100 volts for 40 minutes. After determining the optimal annealing temperature, all samples were amplified using specific primers to yield a 204 bp fragment. Finally, after ensuring the amplification of the specific fragment, all samples were digested using *BseMI (BsrDI)* restriction enzyme. The digestion reaction included 9 µl nuclease-free water, 1 µl 10x buffer, 5U *BseMI* enzyme, and 5 µl PCR product, and the microtubes were incubated at 37ºC for 24 hours. Then, the digested PCR products were separated and visualized on a 3% agarose gel for genotype determination. The definition of a single 204 bp band indicated lack of enzyme digestion and showed the presence of the G allele. In case of digestion, two 150 bp and 54 bp segments were produced indicating the presence of the A allele. Accordingly, visualization of three bands (204, 150, and 54 bp) indicated the presence of an AG heterozygous genotype (Fig. 1).

Statistical analysis

Chi square test was used to compare genetic and allele frequency between cases and controls and odds ratios were measured in both groups. P values less than 0.05 were considered significant. SPSS version 23 was used for data analysis.

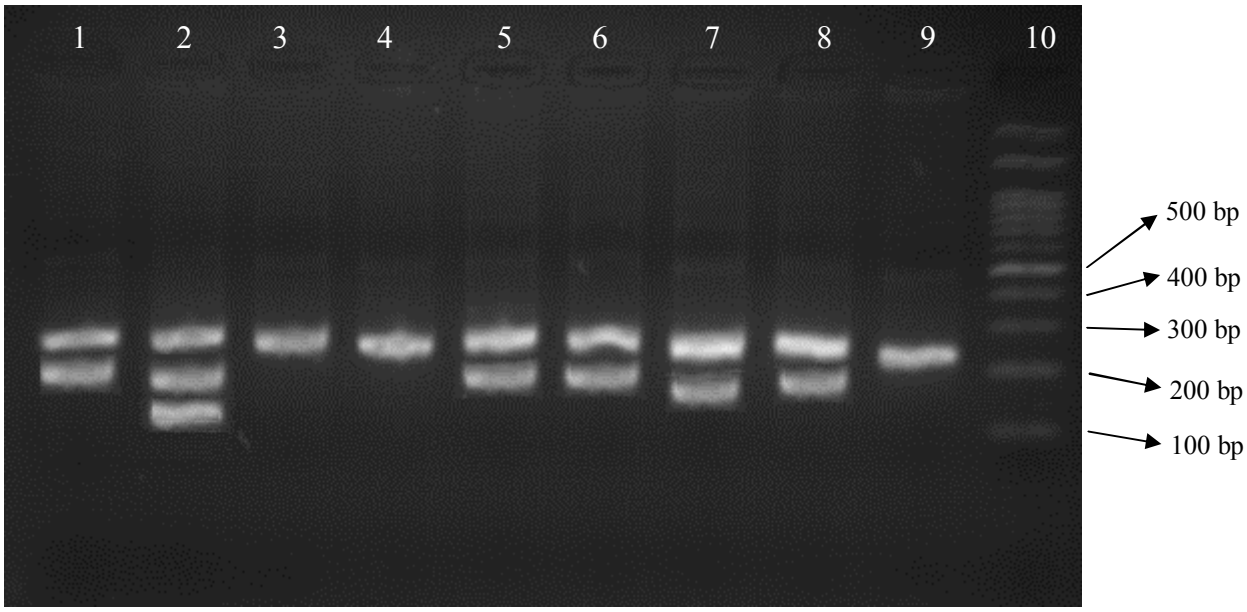

Fig. 1. Pictures of electrophoresis products by restriction enzyme digestion of PCR-RFLP after BsrDI on agarose gel 3 %. 1 to 5 wells cancer samples selected at random: Wells 1 and 5 = genotype homozygous wild-type genotype AA and sink 2 = mutant heterozygous AG, Wells 3 and 4 =Homozygous mutant genotype GG and wells 6 to 8 control samples selected at random, Wells 6, 7 and 8=Homozygous wild-type genotype AA, Wells 9= PCR product as a negative control

Results

The cases were 32-70 years old (mean age 49.68 years old) and controls were the same age range with a mean age of 47.34 years old. In the case group, 26 patients (32.91%) had invasive lobular carcinoma, 45 patients (56.96%) had invasive ductal carcinoma, 5 patients (6.33%) had ductal carcinoma in situ, and 3 patients (3.80%) had lobular carcinoma in situ. Moreover, 38 persons were in the metastatic stage, 16 were in grade III, 13 were in grade II/III, and 12 were in grade II.

Twenty-three patients (29.11%) and 40 controls (50.63%) had the AA homozygous genotype (p=0.01), and 13 patients (16.46%) as well as 12 controls (15.19%) had the AG/GA heterozygous genotype (p=0.827). The GG homozygous genotype was observed in 43 patients (54.43%) and 27 controls (34.18%) (p=0.006) (Table 2).

Table 2. The odds ratio and genotype frequency in the cancer & control groups

Genotype	Cancer Group	Control Group	P-Value	Odds Ratio	CI(95%)
AA	23 (29.11%)	40 (50.63%)	0.01	0.43	**0.21-0.77**
GG	43 (54.43%)	27 (34.18%)	0.006	2.3	**1.21-4.37**
AG	13 (16.46%)	12 (15.19%)	0.827	1.1	**0.47-2.59**

The frequency of the A allele was 37.40 and 58.23, and the frequency of the G allele was 62.60 and 41.77 in the patients and controls, respectively. In general, evaluation of the allele frequency distribution in cases and controls showed that the G allele, as the risk

allele, had the highest frequency in the case group (62.60) while the A allele had the highest frequency (58.23) in the control group. Moreover, the results showed that the calculated odds ratio for the GG genotype was 2.3, indicating the very great effect of this genotype on developing breast cancer. On the contrary, the odds ratio of the AA genotype was 0.4, indicating its lack of effect on disease development. The results showed that the frequency of the G allele had a significant difference between cases and controls. Accordingly, the presence of the G allele as the risk allele increases the risk of breast cancer in carriers by a factor of 2.33 as compared to those who lack this allele (OR=2. 33 p=0.006 CI 95%: 1.21-4.37).

Discussion

Breast cancer is one of the most common cancers among women in different countries, including Iran and each year about 1 million women are diagnosed worldwide. In developed countries, it comprises about 10% of all cancers in 23% of the cancers in women [14, 15]. Breast cancer starts with the breast tissue and invades other organs through the blood or lymph as the disease progresses. Therefore, its evaluation is of extreme importance [16]. Considering the known effect of estrogen in different cell signaling pathways and the fact that the level of endogenous estrogen is much higher in the patients with breast cancer than healthy controls, and these disorders in the estrogen metabolism pathway increase the risk of breast cancer, it can be concluded that estrogen has an important role in the development of breast cancer in different populations [17]. Moreover, the *CYP1A1* gene is regarded as one of the candidate genes in susceptibility to breast cancer due to its role in the metabolism of estrogen and polycyclic aromatic hydrocarbons [5]. Several polymorphic sites have been reported for this gene. The most common allele identified on this gene is *CYP1A1*2C*. Studies have shown a relationship between this locus and increased risk of certain cancers. Therefore, it is one of the most important *CYP1A1* polymorphisms [10, 12, 13].

The results of this study, similar to the other studies, confirmed a significant relationship between the GG genotype, as a mutant homozygous genotype, and the risk of breast cancer as compared to individuals with the AA homozygous genotype. Accordingly, there is significant correlation between the presence of the G risk allele and increased susceptibility to breast cancer; the risk allele can increase the risk of breast cancer by a factor of 2.33 in its carriers as compared to the carriers of the normal allele (CI 95%: 1.21-4.37 OR: 2.3, p=0.006). Moreover, the results obtained from the patients' records revealed no significant relationship between the genotypes of the evaluated polymorphism (GG, AG, AA) and other demographic and clinical variables like age, sex, estrogen, and progesterone as the only significant relationship was observed between the GG genotype and cancer grade (p=0.037). We evaluated the association between genotypes and stage of diseases. But no significant relationships were observed between AA (p=0.570), GG (p=0.143) and AG

(0.080) genotypes and the stage of disease in the case groups.

In 2004, Hefler et al. evaluated 10 polymorphisms out of the most commonly known *CYP1A1* polymorphisms, including the Ile462Val (rs1048943) polymorphism, in a case-control study using the Multiplex PCR method. The results showed that the odds ratio of breast cancer increased by 1.5 times in women with a mutant homozygous genotype in comparison with other women (OR: 1.5, CI 95%: 1.0-2.2, p=0.03) [18]. In 2008, Diergaarde et al. conducted a case-control study to investigate the relationship between common polymorphisms in the genes involved in estrogen and progesterone metabolism and the risk of breast cancer in menopausal women undergoing hormone therapy. The results showed that the risk of breast cancer increased in menopausal women receiving esterogen and progesterone who had a mutant allele in *CYP1A1* Ile462VaL as compared to women homozygous for the wild allele (OR: 1.9; CI 95%: 1.3-2.8) [19]. In another study in 2013, Martinez-Ramirez et al. evaluated the prevalence of the Ile462Val (rs1048943) polymorphism in 150 healthy Mexican women and 150 Mexican women with breast cancer and it was found the positive effect of this polymorphism on the development of breast cancer (OR: 1.95, CI 95%=1.13-3.36, p=0.04) [20]. Ghisari et al. evaluated the polymorphisms of the genes effective in the metabolism of xenobiotics and estrogen biosynthesis, including *CYP1A1* rs 1048943 polymorphism in association with the risk of breast cancer. Their results showed that the presence of this polymorphism increased the risk of breast cancer in the women (OR: 4.35; CI 95%: 1.08-17.4; p=0.038) [21]. A meta-analysis by Sergentani et al. on 29 published papers related to the effect of *CYP1A1* (rs1048943) polymorphism and the risk of breast cancer showed that homozygous people carrying the *CYP1A1* (rs1048943) mutant allele, especially the Caucasians, had an increased risk of breast cancer (OR: 2.185 CI 95%: 1.253-3.808) [22]. Wu B et al. conducted a meta-analysis on 198 articles published on the relationship of *CYP1A1 Msp1* (rs 4646903) and *CYP1A1* Ile462Val (rs1048943) polymorphisms and the risk of different cancers in different populations. The results showed that these polymorphisms were associated with an increased susceptibility to different cancers, including breast cancer, in Asians [23].

The results of several investigations on the effect of *CYP1A1* (rs1048943) polymorphism on cancers, including bile duct [24], lung [25, 26], cervix [27], and colorectal cancer [28], indicate the effect of the G allele of this polymorphism on increasing the risk of cancer in mutant carriers.

Conclusion

The results indicate that carriers of the G allele of *CYP1A1* (rs1048943) polymorphism have an increased odds of developing breast and other cancer when compared with wild allele carriers. Therefore, this genetic change is a determinant of cancer development and can be used to detect the susceptible individuals.

Conflict of Interest

The authors have no conflicts of interest to declare.

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