Breast cancer risk not only was not associated with CYP17/A2 allele but also was related to A1 allele

Mojgan Hosseini[1], Massoud Houshmand[2,3], Ahmad Ebrahimi[2]

[1]Islamshahr Branch, Islamic Azad University, Islamshahr, Tehran, Iran
[2]National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
[3]Special Medical Centre, Tehran, Iran

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Corresponding author:
Mojgan Hosseini (Ph.D)
Department of Science
Islamshahr Branch
Islamic Azad University
Sayad Shirazi St. Islamshahr
Tehran, Iran
Phone/fax: +98-21-66936779
E-mail: Mojgan-Hosseini@iiau.ac.ir

Abstract

Introduction: Breast cancer is the second most common cancer in the world and the most common cancer in Iranian women in terms of rate. The cytochrome P-450c17α (CYP17) gene, located on chromosome 10q24.3, encodes the enzyme cytochrome P-450c17α, which functions as a susceptibility factor in breast cancer.
Material and methods: Three common polymorphisms have been described in the CYP17 gene. The variant creates a recognition site for the MspAI restriction enzyme, the common allele as A1 and the variant alleles A2 (–34T → C). In total 53 Iranian sporadic breast cancer affected women compared to the control group were studied by PCR-RFLP for CYP17 variant.
Results: Even though the A2/A2 were reported as risk factors for breast cancer our results showed that the A1/A1 were a higher risk factor in our population. A2/A2 had an inhibitory effect in our patients [A1/A1 / A2/A2 odds ratio, 5.57 (95% confidence interval, 1.514-20.506) $p = 0.008$].
Conclusions: We conclude that not only was A2/A2 in our patients not associated with breast cancer risk but also there is a reverse relation between presence of A1/A1 and increase of breast cancer risk.

Key words: CYP17 gene, polymorphism, breast cancer, MSPA1, PCR-RFLP, susceptibility factor.

Introduction

Breast cancer is the second most common cancer in the world and the most common cancer in Iranian women in terms of rate [1-3].

A large population study in Iranian patients shows that breast cancer is the most common, with an incidence of about 22 per 100,000 [4].

Polymorphisms of the *CYP 17* genes were involved in oestrogens biosynthesis in modulating the susceptibility to breast cancer [5-8].

A single nucleotide polymorphism (SNP) creates a restriction recognition site, resulting in two alleles designated A1 and A2 [T (A1) to C (A2)] [6]. Ethnic differences of the variant allele frequencies were found by other groups between affected women [1, 2, 9-12].

The aim of our study was to show the allelic variants in the *CYP17* gene in Iranian sporadic breast cancer patients.

Material and methods

Patient data

Analyses were conducted for 53 patients and 53 controls genotyped for CYP17, including 14 patients and 21 controls of premenopausal women, and 39 patients and 32 controls of postmenopausal women, and ages were 35-55 years.

This study was ethically approved by the local Ethical Committee of Islamic Azad University from the point of view of patients' and also controls' rights.

All patients participated in the Special Medical Centre, part of chemotherapy, Tehran, Iran. A questionnaire including questions on breast cancer risk factors were completed and each patient completed a consent form. The blood samples were collected from patients and controls prior to the start of treatment. Subjects were genotyped for CYP17 SNP using genomic DNA extracted from peripheral blood lymphocytes. DNA was isolated from peripheral blood using the FelxiGene DNA extraction kit (Qiagen Germany).

Genotyping

Bio-Rad PCR System was used to amplify 414 bp fragments, which include the polymorphic site in the promoter region of CYP 17. The cycling condition for CYP17 A1/A2 gene polymorphism was set according to a previously published method [6], with some changes, such as: one cycle at 94°C for 5 min, 35 cycles at 94°C for 40 s, 55°C for 45 s, 72°C for 90 s, and one final extension cycle at 72°C for 7 min.

The genotypes and allelic frequencies of CYP17 A1/A2 polymorphisms in patient and control groups were analysed using χ^2 and Fisher's exact tests.

Genotyping of the MspA1 polymorphism in the 5'- untranslated region of the *CYP17* gene was determined by PCR restriction fragment length polymorphism, which includes the polymorphic site in the promoter region of CYP17, according to a previously published method [2], with slight modifications.

The PCR products were digested with restriction enzyme MspA1I (Biolab, USA), separated by 3% agarose gel electrophoresis and identified with ethidium bromide staining. This method is able to detect all three possible genotypes for the polymorphism: A1A1 (homozygous wild type), A1A2 (heterozygous variant type) and A2A2 (homozygous variant type) (Figure 1).

The primers used for PCR were designed as follows:

Forward 5'-CATTCGCACTCTGGAGTC-3'
Reverse 5'-AGGCTCTTGGGGTACTTG-3'

Results

Analyses of affected and controls show that A1/A2 genotype has the highest frequency in both groups (54.7 in patients and 62.3 in control group).

The A1 homozygote genotype has an increase in the patient group (34.0) compared with controls (13.2) and A2 homozygote genotype has a decrease in the patient group (11.3) compared to the control group (24.5) (Table I).

Comparison between genotypes, odds ratio and *p* value showed that *p* value of A1/A1 / A2/A2 was $p = 0.008$. Moreover, *p* value for A1/A1 / A1/A2 was $p = 0.033$ and for A2/A2 / A1/A2 was 0.242.

Odds ratio for A1/A1 / A2/A2 was 5.571 (95% confidence interval, 1.514-20.506), was 2.926 for

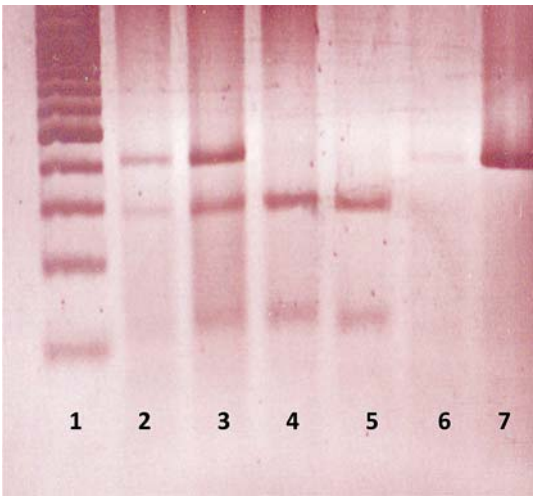

Figure 1. Amplification (PCR) and restriction digestion; the PCR products were digested with restriction enzyme MspA1I in groups: 1 – Ladder 100 bp, 2, 3 bands – A1/A2 heterozygote genotype, 4, 5 – A2A2 genotype (124 bp, 290 bp), 6 – negative control, 7 – A1/A1 genotype (414 bp)

Table 1. CYP17 genotype frequencies [*n* (%)] for patients and controls. Analyses of 53 affected women and 53 controls for CYP17 genotype frequency show that A1/A2 genotype has the highest frequency in both groups (54.7 in patient and 62.3 in control group). The A1 homozygote genotype has an increase in patient group (34.0) compared with controls (13.2) and A2 homozygote genotype has a decrease in patient group (11.3) compared to control group (24.5) ($p = 0.022$) (Table I)

Study group	*n*	Genotype [*n* (%)]		
		A1/A1	A1/A2	A2/A2
Patient	53	18 (34.0)	29 (54.7)	6 (11.3)
Control	53	7 (13.2)	33 (62.3)	13 (24.5)
Total*	106	25 (23.6)	62 (58.5)	19 (17.9)

**Breast cancer vs. control, p = 0.022*

A1/A1 / A1/A2 (95% confidence interval, 1.071-7.998) and was 1.904 for A2/A2 / A1/A2 (95% confidence interval, 641-5.654).

Discussion

The impact of CYP17 genetic polymorphism on the risk of breast cancer received a lot of interest after Feigelson and colleagues first reported an increase in risk of advanced breast cancer for carriers of the A2 allele [13].

Some population studies including young Indian, Korean, and Russian women suggest that CYP17 homozygote A2 allele gene polymorphism might play a significant role in breast cancer development [14-16]. The published data in American Caucasian women showed that women with homozygote A2 alleles were more likely to share characteristics associated with greater breast cancer susceptibility [2], although a few exceptions, in the study of Bergman *et al*. and Spurdle *et al*., were found [17, 18]. However, most subsequent studies did not find an overall increase in risk with the A2/A2 genotype [2, 19-23]. On the other hand, interestingly, some population-based studies showed no overall association between CYP17 gene polymorphism and breast cancer risk [5, 24, 25].

In agreement with the majority of the previous studies on CYP17 polymorphism and breast cancer risk, this study did not reveal any significant association between the CYP17 A2 allele and overall risk of breast cancer in Iranian women.

In the present study, we found that the A2 allele did not increase breast cancer risk. The difference between patient and control groups, regarding the genotype ratio of A1/A2 heterozygote, was non-significant. On the other hand, comparison of A1/A1 genotype and A2/A2 genotype ratio in the two groups was significant.

However, no association was found between the presence of A2 allele and breast cancer susceptibility. Nevertheless, there is a significant association between presence of A1 allele and breast cancer occurrence.

Our results showed that A1/A1 was significantly increased in the patient group and A2/A2 polymorphism was decreased in the control group.

In conclusion, that not only was A2/A2 in our patients not associated with breast cancer risk but also there is a relation between presence of A1/A1 and increase of breast cancer risk.

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