Association of CYP1A1 M2 (A2455G) Polymorphism with Susceptibility to Breast Cancer in Mazandaran Province, Northern Iran: A Case–control Study

Abstract

Background: Breast cancer is one of the most frequent women malignancies in the world. The cytochrome P450 1A1 (CYP1A1) is a key enzyme in xenobiotics metabolism. Moreover, CYP1A1 plays a critical role in the etiology of breast cancer by involving in 2-hydroxylation of estrogen. Therefore, single-nucleotide polymorphisms (SNPs) of its coding gene have been verified to be important in cancer susceptibility. The aim of the study was to evaluate the association of CYP1A1 M2 (A2455G) with the risk of breast cancer in Mazandaran province.

Methods: Ninety-six breast cancer patients with known clinicopathological characters and 110 healthy women as control were genotyped for CYP1A1 M2 polymorphisms by the restriction fragment length polymorphism technique.

Results: The analysis of CYP1A1 gene (polymorphism M2) showed that the frequency of homozygous wild genotypes (AA), heterozygous (AG), and mutant genotype (GG) in the patient group, respectively, 78%, 22%, and 0%, and also the frequency of genotypes AA, AG, and GG in healthy included 82%, 16%, and 2%, respectively. Statistical analysis by Logistic regression model at P < 0.05 showed no significant correlation between polymorphisms in CYP1A1M2 and breast cancer risk (odds ratio = 0.84, confidence interval = 0.33–2.17).

Conclusions: The results indicated that the M2 allelic genotypes were significantly associated neither with breast cancer risk nor with clinicopathological characteristics in Mazandaran province.

Keywords: Breast neoplasms, cytochrome P-450, Iran, polymorphism, restriction fragment length

Introduction

Breast cancer is one of the main causes of cancer death among women. In 2012, it was responsible for the deaths of 522,000 women worldwide.1 The results of the 10-year national cancer registry of Iran show that the breast cancer was the most common type of cancer in Iranian females, accounting for 24.6% of all cancers.2 Much work on risk factor determination as well as risk factor evaluation on breast cancer has been carried out worldwide.3–6 Existing studies have demonstrated that more than 80 genetic variants or single nucleotide polymorphisms (SNPs) are associated with breast cancer risk.3–6 On the other hand, a growing body of literature has shown that racial-ethnic identity is responsible for breast cancer risk and outcome.7

There are racial disparities in Iranian population. The largest of the population of Iran consists of Persians and Kurds, with smaller communities including Gilakis, Mazandaranis, Lurs, Tats, Talys, and Baloch.8 The Mazandarani population number is around three million people that are currently one of the main ethnic groups residing in the northern parts of Iran.9 Mazandaran province is one of the major agricultural areas in Iran. Therefore, pesticides have widely been overused in this province. Following that the entering pesticides into the environment are the reasons for which the rate of cancer is increasing in this province. Despite the fact that these prevalence and modifiable risk factors related to breast cancer have been evaluated in Mazandaran province, no one to the best of our knowledge assessed genetic factors among Mazandaran breast cancer patients.10,11

CYP1A1, an enzyme of the cytochrome P450 superfamily, plays an important role in the metabolism of numerous endobiotics and xenobiotics. They profoundly

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DNA extraction

Five ml of peripheral blood was collected in ethylenediaminetetraacetic acid-containing tubes from both patients and control group, and DNA was extracted from blood lymphocytes by proteinase-K/SDS digestion and phenol-chloroform extraction as described elsewhere.[13] DNA concentration and purity of each sample were measured by ultraviolet spectrophotometer, and its purity was checked through agarose gel electrophoresis, then extraction was routinely stored at −20°C.

**CYP1A1 (NcoI) genotyping (CYP1A1 m2)**

An isoleucine 462 valine (rs1048943) substitution in exon7, which results in a loss of NcoI restriction site at the heme binding region, was determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism. A 333 bp fragments containing T/C allele was amplified using forward: 5′-GAAAGGCTGGGTCCACCCTCTTCT-3′ and reverse: 5′-CCAGGAAGAGAAGACCTTCCACCGGCGC-3′ primers. PCR amplification was performed in a 25 μl reaction containing 1X PCR buffer, 100 ng genomic DNA, 1.5 mM MgCl₂, 0.3 mM each forward and reverse primers, 0.2 μM dNTPs, and 2.5 U *Taq* DNA polymerase (10 u/μl). The cycling conditions including an initial denaturation at 94°C for 3 min, 31 cycles of denaturation at 94°C for 40 s, annealing at 65°C for 30 s, extension at 72°C for 40 s, and a final extension at 72°C for 7 min. Products were analyzed by electrophoresis at 1.5% agarose gel and visualized by ethidium bromide staining. The amplified fragment (333 bp) was digested by 1 U *NcoI* restriction enzyme (ThermoFisher, USA) at 37°C for 2 h and analyzed on 2.5% agarose gel [Figure 1]. Wild-type DNA is cut by enzyme *NcoI* resulting in fragments 232 and 30 bp. The DNA carrying the variant is not cut resulting in 263 bp band.

**Statistical analysis**

All of the data were analyzed by SAS 9.1 statistics software (The SAS Institute, NC, USA). *P* < 0.05 was considered as statistical significance. The genotype and allele frequency of CYP1A1 M2 genotype were tested for Hardy–Weinberg equilibrium for both patient and control group using Chi-square test. Odds ratio (OR), confidence intervals (CIs), and *P* value were calculated to estimate the association between risk of breast cancer or clinicoopathological data and genotypes, using unconditional logistic regression. The OR was adjusted for potential confounding factor including age.

**Results**

**Demographic and clinicopathological data of patients**

The study performed on 96 patients and 110 control with known demographic and clinicopathological data which are shown in Table 1. The Student’s *t*-test showed...
no significant difference between two groups (patients and control) in some demographic data such as smoking and menopause ($P > 0.05$). Despite the importance role of family history in disease occurrence, only nine patients (10%) had a positive history.

### The CYP1A1 genotype distribution

Analysis of the results of polymorphism M2 showed that the frequency of wild homozygous (AA), heterozygous (AG) and homozygous mutant (GG) for polymorphism M2 in the patient group, was, respectively, 78%, 22%, and 0% in the control group, respectively, 82%, 16%, and 2%. The frequency of allele A and G were 89% and 11% in patients group, and 90% and 10% in control group. The Chi-square test showed that there is no association between genotypes in healthy and patients group ($P = 0.31$). The logistic regression model yielded no significant correlation between M2 polymorphism and breast cancer risk ($OR = 0.74$, CI = 0.33–2.17 and $P = 0.72$) [Table 2].

### Table 1: The demographic and clinicopathological characteristics of patients

<table>
<thead>
<tr>
<th>Clinicopathological variables</th>
<th>Number of the patient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>≤45</td>
<td>42 (45.6)</td>
</tr>
<tr>
<td>&gt;45</td>
<td>50 (54.4)</td>
</tr>
<tr>
<td>Menopause status</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>38 (42)</td>
</tr>
<tr>
<td>Negative</td>
<td>53 (58)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>3 (5)</td>
</tr>
<tr>
<td>II</td>
<td>53 (60)</td>
</tr>
<tr>
<td>III</td>
<td>31 (35)</td>
</tr>
<tr>
<td>TNM staging</td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>38 (45)</td>
</tr>
<tr>
<td>III-IV</td>
<td>47 (55)</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Negative</td>
<td>76 (90)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Negative</td>
<td>80 (93)</td>
</tr>
<tr>
<td>Cancer type</td>
<td></td>
</tr>
<tr>
<td>Ductal carcinoma</td>
<td>77 (83)</td>
</tr>
<tr>
<td>Lobular carcinoma</td>
<td>15 (16)</td>
</tr>
</tbody>
</table>

TNM=Tumor/node/metastasis

### Table 2: Distribution of CYP1A1 gene polymorphisms and breast cancer risk - as mentioned, compare the distribution by chi test

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genotype</th>
<th>Number of subjects (%)</th>
<th>Nonadjusteda</th>
<th>Adjustedb</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Case</td>
<td>Control</td>
<td>$P$</td>
<td>$OR$</td>
</tr>
<tr>
<td>M2</td>
<td>AA</td>
<td>75 (78)</td>
<td>91 (82)</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>21 (22)</td>
<td>17 (16)</td>
<td>0.53</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>0 (0)</td>
<td>2 (2)</td>
<td>0.98</td>
<td>&gt;999.999</td>
</tr>
<tr>
<td></td>
<td>AG + GG</td>
<td>21 (0)</td>
<td>19 (17)</td>
<td>0.41</td>
<td>0.80</td>
</tr>
</tbody>
</table>

*Logistic regression model, nonadjusted, bLogistic regression model, adjusted for diagnostic age. OR=Odds ratio, CI=Confidence interval

Association between CYP1A1 M2 polymorphism and known clinicopathological variables

The association between different genotypes of CYP1A1 gene and clinicopathological features is listed in Table 3. Results showed that there is no significant association between the mutant genotypes and these characteristics including age at diagnosis ($P = 0.56$), type of cancer ($P = 0.69$), menopause ($P = 0.20$), grade ($P = 0.72$), stage ($P = 0.65$), smoking ($P = 0.95$), and family history ($P = 0.90$).

### Discussion

In the present molecular epidemiological study, we attempted to find the association between CYP1A1 M2 polymorphism and the risk of breast cancer and clinicopathological features in Mazandaran Province, Iranian population. Our results showed the mean age of females with breast cancer was 48.2 years. This finding is agreement with previous surveys by Sadjadi et al. in 2009 and Harirchi et al. in 2011.16,17 Their results revealed that the mean age of breast cancer on the Iranian population is between 47 and 49 years. Furthermore, in a meta-analysis study, in which 52 studies with 332,999 breast cancer patients were included, the average age of Iranian patients estimated 48.59 years.19 The major of the epidemiological finding confirmed that mean age of breast cancer in Iranian population is one decade lower than Western countries.19,20 Moreover, pathological features showed that most of the cases were diagnosed with an advanced stage (Stage III and IV) (55%). Our experiments were in lined with previous findings by Harirchi et al.21 Apparently, the leading cause of this issue is lack of systematic screening programs for early detection of breast cancer in developing country including Iran.

Cancer investigations have been progressing toward the toxicogenomics studies examining the dynamic interactions between a specific individual genotype and different carcinogenic, teratogenic, and other xenobiotics. Cytochrome P450 superfamily is the major part of PhaseI biotransformation of xenobiotics. CYP1A1 is a member of this family expressed in extrahepatic organs, especially in breast tissues.22,23 The CYP1A1 Ile/Val polymorphism (M2) is a result of an A/G change in exon 7, causing the amino acid exchange (462 Ile/Val) in the heme-binding region of the
The Val allele variant demonstrates an approximately 2-fold higher catalytic enzyme activity than Ile form. Many studies have shown the association between CYP1A1 M2 polymorphism and risk of lung cancer, ovarian cancer, colorectal cancer, esophageal cancer, and cervical cancer. Statistical analysis by logistic regression model showed no statistical relationship between M2 genotype and breast cancer risk (P = 0.72 and OR = 0.84). Our results are in line with some previous reports in different population like Indian, Danish, and American, and different Iranian ethnic like Gilaki and Fars. Their finding revealed that there is no correlation between polymorphism M2 of CYP1A1 gene and breast cancer risk. Contrary to our results, some reports pointed out that there is a positive association between this polymorphism and risk of breast cancer. Noticeably, Sergentanis and Economopoulo conducted a meta-analysis on Caucasian, Chinese, and African populations, as well as on premenopausal and postmenopausal women to examine the correlation of CYP1A1 M2 polymorphism with breast cancer. The results demonstrated mutant genotype (GG) elevate risk of breast cancer and their results suggested that this polymorphism would be a good marker for prediction of breast cancer in these populations. On the other hands, some lectures showed that GG genotype is associated with a trend of reduced breast cancer risk. Furthermore, there was no significant association between clinicopathological features such as age, histological type, grade, and stage of tumors with M2 genotype. There are numerous conflicting epidemiological studies addressing correlations between the polymorphism and breast cancer development. The answer of these disagreements is in interaction between difference intrinsic and extrinsic factors. Intrinsic factors such as genetic variation and extrinsic factors are included ethnic difference, diet, geographical variation, and environmental exposures. The difference in genomic structure in ethnic groups can play a significant role on the rate of incidence and mortality among various cancers in populations. Therefore, a complex investigation in different ethnic groups is needed to achieve a validation answer for the association of genomic variation and breast cancer risk.

Conclusions

In summary, the results of present study suggest that CYP1A1 M2 polymorphism alone does not play a critical role in the breast cancer risk in Mazandaran province. Further studies, which take larger group from different ethnicities, will need to clarify this issue.

Acknowledgments

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Conflicts of interest

There are no conflicts of interest.

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