Polymorphisms in the Estrogen Receptor Alpha Gene and Mammographic Density Result Study in Brazilian Women

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Abstract

The estrogen receptor (ER) is a ligand-activated transcription factor that mediates the actions of the estrogen in target tissues. Several ERα gene polymorphisms are associated with changes in the receptor expression and function. The aim of this study is to verify the hypothesis that the ERα gene polymorphisms could be associated with high mammographic density (HMD), a well-known independent risk factor for breast cancer (BC) in a case-control study carried out in the city of São Paulo (SP, Brazil) from 2010 to 2013. Two ERα gene polymorphisms named PvuII and XbaI was examined in 308 cases and 155 controls. The PvuII polymorphism was associated with an increased risk of having high mammographic density (HMD) post menopause, after adjustment for other risk factors, the odds ratio for PP genotypes was 1.75 (confidence interval of 95% CI 95%=1.10-2.79) compared with the genotypes PP and Pp. The XbaI polymorphism was also associated with a high risk of HMD, but not statistically significant, odds ratio for xx genotype was 1.31 (95% CI=0.7 to 1.9). No apparent synergistic effects of these two polymorphisms were identified. It was concluded that the PvuII polymorphism in the gene ERα was associated with an increasing chance of having HMD, a strong risk factor for BC. Thus recognizing these risk factors will be of great importance in the analyses of individual susceptibility to BC, in both the study of the response to various drugs and the prognosis.

Keywords: Breast neoplasms; Estrogen receptors; Genetic polymorphism; Mammography; Risk factors

Introduction

Estrogens affect the growth, differentiation and function of many target tissues including breast, uterus, vagina, ovary, testicles, epididymis, and prostate [1]. The biological effect of the estrogens such as growth and differentiation of normal breast tissue is mediated primarily through high affinity binding to its ER. The ERs are intranuclear proteins that possess a binding domain to the estrogen and a binding domain with the DNA [2]. There are two types of ERs (α and β). The ERα gene is located [3] on chromosome 6q25.1, and the ERβ gene is located on chromosome 14q22-24. Among the steroid receptors the ERα and the progesterone receptors (PR) are of special interest because their protein levels are elevated in malignant breast cells [4-6]. Both ER and PR prove to be significant prognostic factors for BC [7]. Therefore the inhibition of ERα has become a major strategy for the prevention and treatment of BC [8].

The combination of ERα gene polymorphisms and the risk of diseases, including BC have become subject of growing interest. Thus, several studies have indicated that variations in the DNA sequence of the ERα gene increase the risk of developing BC and HMD after menopause. Some studies are summarized in Tables 1 and 2.

The studies of polymorphisms related to diseases are tools that may have direct implication of great importance in the analysis of individual susceptibility to BC in the study of response to various drugs and prognosis. The ultimate goal of these strategies is to reduce the anxiety of the patients and greatly improve the approach and management of a woman with or without risk, facilitating the implementation, planning and the adoption of preventive strategies.

This article presents the results of the case-control study in the city of São Paulo, that examined the association of the polymorphisms in the genes ERα-PvuII and ERα-XbaI with the risk of high mammographic density after menopause. The associations of these polymorphisms with other risk factors for breast cancer were also evaluated.

Methods

Case-control study that included 308 women with HMD (for more than 50% density) and 155 controls (to 50% density or less) evaluated by computerized objective method [25], aged 45-65, without menstruation or hormone therapy for at least 1 year, without previous BC and ovarian cancer. Initially, the patients were selected in a subjective way by the standard ACR-BIRADS®, by a unique reader (the head) from the Institute of Radiology, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo (HC-FMUSP), São Paulo, Brazil, from January 2010 to March 2013. The selected patients were evaluated again, now objectively, by another reader as described by Boyd et al. 2013 [25]. The study was approved by the Ethics Committee for Analysis of Research Projects - CAPPESQ the HC-FMUSP, and all women signed informed consent. It was characterized in the clinical
Tests were adopted a significance level of 5% (p<0.05). Those presented in the bivariate analyzes values p<0.20. In all statistical model stepwise backward. The variables that entered in the model were data, as these are not normally distributed in all groups, was used the Kolmogorov-Smirnov Test to verify the normality of the was used the for comparison between groups.

| Genotype | Allele | Country | Type and size of the study | Association | OR/RR | References |
|----------|--------|---------|----------------------------|-------------|-------|------------|
| ERα-397 T/C | PvuII | TT USA pre- and postmenopausal | Only Cases/Case Control 257/140 | Diagnosis in younger women | OR: 1.4 (IC 95%: 1.1-1.8) p=0.042 | Cai et al. [10] |
| TT Shanghai, China | Case/control 1069/1166 | † risk for breast cancer | OR: 3.04 (IC 95%: 0.73-12.67) | Shen et al. [12] |
| TT Netherlands | Case/control 380/422 | † risk for breast cancer | RR: 1.5 (IC 95%: 0.94-2.42) | Onland-Moret et al. [11] |
| TT China 25 a 55 years-old | Case/control 259/278 | † risk of breast cancer in women with a positive family history | OR: 1.4 (IC 95%: 1.25-11.6) | Gonzalez-Mancha et al. [13] |
| TT Spain 26 to 86 years-old | Case/control 444/704 | † risk of breast cancer in women after menopause | OR: 3.81 (IC 95%: 0.8-2.2) | Ladd et al. 2007 [14] |
| TT Netherlands 55 years more | Case/control 190/3513 | † risk of breast cancer in women after menopause | OR: 1.4 (IC 95%: 1.25-11.6) | Onland-Moret et al. [11] |
| TT USA peri-menopause 42 to 52 years-old | Longitudinal cohort 451 | † Increased mammographic density in white women | 7.0%; p=0.01 | Crandall et al. [15] |
| TT Brazil After-menopause | Only cases 308 women | Mammographic density>50% | TT=32.14% greater than CC=20.13% | Souza et al. [16] |
| TT/TC Netherlands and England After-menopause | Population Cohort prospective case/ control (795 with HT/781 with no HT) | † Increased mammographic density only in HT users | 2.24% p<0.01 | van Duijnhoven et al. [17] |
| TC Netherlands | Case/control 380/422 | † risk for BC | OR: 1.14 (IC 95%: 1.00-1.32) | Onland-Moret et al. [11] |
| TC China 25-55 anos | Case/control 259/278 | † risk for BC (with family history) | OR: 2.46 (IC 95%: 0.61-9.88) | Shen et al. [12] |
| CC Shanghai, China 22-64 years-old | Prospective Population Cohort 1459 cases | RE negative expression; worse prognosis for breast cancer | OR: 3.30 (IC 95%: 1.42-7.69) | Boyapati et al. [5] |
| CC Netherlands and England After-menopause | Population Cohort prospective case/ control (795/781) | Mammographic density without changes in user HT | 0.90%; p=0.47 | van Duijnhoven et al. [17] |

Table 1: Association studies of the ERα-397 gene polymorphism PvuII C/T with BC and/or risk factors for disease.

Results

The distributions of selected demographic characteristics that are the main risk factors for breast cancer are presented in Table 3. It was observed elevated risk of HMD for the main risk factors that have been reported in other previous studies [20,16].

The risk of having HMD was also elevated for younger women, lower WC, fewer pregnancies, higher age at having first birth, high number of women with a FHBC. It was not observed any apparent modification effect for other indicators of exposure to endogenous estrogens and lifestyle factors. In our study, the sample consisted of postmenopausal women, aged 45-65 years.

The allele frequencies of ERα-PvuII and ERα-XbaI in both groups were similar to those reported in previous studies [16,17,20,27-29].

Regarding the genotype, 21.5% of controls and 32.5% of cases were wild homozygous (PP), with statistically significant difference (X²=7.42, p=0.024). OR adjusted for pp genotype was 1.75 (95% CI=1.10 to 2.79) compared with PP and Pp genotype. There were not any significant difference in allele frequency or genotype polymorphism between controls and cases (Table 4).

When the two ERα polymorphisms were analyzed together, no synergistic effect was consistently noted.

Additional analyzes (Multivariate Logistic Regression) were performed to evaluate the independent risk factors for HMD. From all clinical characteristics analyzed, entered only those presented in the bivariate analyzes values p<0.20 were included in the model: the PvuII polymorphism of the ERα gene; indicators of exposure to endogenous estrogen: age at menarche, menopause, time after menopause, smoking, alcohol intake and body mass index (BMI). Peripheral blood samples were obtained for genomic DNA extraction and determination of polymorphisms in question.

The genomic DNA was extracted from peripheral blood using QIAamp DNA Blood Mini Kit (Qiagen), following manufacturer instructions. After DNA quality and integrity evaluation were performed PCR-RFLP assay for Estrogen Receptor PvuII and XbaI polymorphism analysis as described by Herrington et al. 2002 [26]. The laboratory was blinded on the subject identification.

Hardy-Weinberg Equilibrium (H₁W) was used to verify if the genotype frequencies of PvuII and XbaI polymorphisms in our population were in genetic equilibrium. Simple mathematical model [(p+q)^2=p^2 +2 pq+q^2] used to calculate genotype frequencies from allele frequencies.

Statistical analysis: The data were described using average, standard deviation (sd), absolute frequency (n) and relative frequency (%). To verify the association between qualitative variables with the mammographic density was used the chi-square (χ²). For comparison between the groups of HMDand controls as quantitative variables was used the Kolmogorov-Smirnov Test to verify the normality of the data, as these are not normally distributed in all groups, was used the nonparametric Mann-Whitney Test for comparison between groups. To verify the relationship of the variables with the occurrence of high mammographic density was used Multivariate Logistic Regression model stepwise backward. The variables that entered in the model were those presented in the bivariate analyzes values p<0.20. In all statistical tests were adopted a significance level of 5% (p<0.05).
### Table 2: Association studies of the ERα-351 XbaI A/G gene polymorphism with BC and/or risk factors for disease.

| Genotype | Allele | Population | Type and size of the study | Association | OR/RR | References |
|----------|--------|------------|----------------------------|-------------|-------|------------|
| ERα351 XbaI A/G | AA | Norway 27 to 94 years-old | Case/control 360/672 | ↑ risk for breast cancer after menopause | OR: 2.02 IC 95%: 0.96-4.31 | Andersen et al. [18] |
| | AA | Korea | Case/control 205/205 | ↑ risk for breast cancer | OR: 2.38 IC 95%: 1.58-3.58 | Shin et al. [6] |
| | AA | Korea | Case/control 205/205 | ↑ risk for breast cancer in nulliparous | RR: 4.0 IC 95%: 1.9-8.8 | Shin et al. [6] |
| | AA | Netherlands and England After-menopause | Cohort prospective 791 with HT/781 no HT | ↑mammographic density in HT users | 2.20% p<0.01 | van Duijnhoven et al. [17] |
| | AA | China | ↑ risk for breast cancer | OR: 6.88 IC 95%: 0.80-59.15 p=0.079 | Hu et al. [19] |
| | AA | Korea 25-55 years-old | Case/control | ↑ risk for breast cancer, with positive family history | OR: 4.20 IC 95%: 0.65-27.28 | Ladd et al. [14] |
| | AA | Netherlands 55 years or more | Case/control 190/3513 | ↑ risk for breast cancer in women after menopause | OR: 1.3 IC 95%: 0.7-2.2 | Shin et al. [6] |
| | AA | Brazil After-menopause | Prospective 120 | ↑ higher breast density | OR: 2.34 IC 95%: 1.06-5.16 p<0.03 | Ramos et al. [20] |
| | | England | Systematic review of case-control studies | Nonsignificant difference | P=0.06 | Dunning et al. [21] |
| | AA | Pakistan, 15-65 years-old | Case/control 100/100 | ↑ of the risk of BC post menopause | AA 45% greater than GG p<0.01 | Javed et al. [22] |
| | AA | Brazil After-menopause | Only cases 308 women | Mammographic density>50% | AA 33.44% greater than GG 16.56% p=0.079 | Souza et al. [16] |
| HaplotypXbaI- C975→G | Sweden after-menopause | Case/control 1556/1512 | ↑ risk of breast cancer in postmenopausal and obese women | OR=1.48 IC 95%: 1.17-1.88 | Wedren et al. [23] |
| AG | Norway 27-94 years-old | Case/control 360/672 | ↑ risk for breast cancer | OR: 2.00 IC 95%: 0.92-4.37 | Andersen et al. [18] |
| GG | USA, Caucasian greater than 65 years-old | Case/control 393/790 | ↓ risk for breast cancer | OR: 0.82 IC 95%: 0.68-1.00 p=0.04 | Wang et al. [24] |
| GG | Netherlands and England After-menopause | Prospective Cohort 795 with HT/781 no HT | Mammographic density without changes in users of HT | 0.65% p=0.70 | van Duijnhoven et al. [17] |
| A | China | Case/control 114/121 | ↑ risk for breast cancer | OR: 1.4 IC 95%: 1.0-1.9 | Hu et al. [19] |
| G | Korea | Case/control 205/205 | ↓ risk for breast cancer in postmenopause | RR: 0.3 IC 95%: 0.1-0.5 | Shin et al. [6] |

### Table 3: Comparison between the two groups of Mammographic Density: HMD and controls in quantitative and qualitative variables.

| Quantitative variables | Average | HMD | Controls | Z | P |
|------------------------|---------|-----|----------|----|----|
| Age                    | 58.16   | 4.61| 56.31    | 5.42| 3.40| 0.001* |
| Wast cirunference      | 95.06   | 11.13| 10.83    | 5.42| 0.001* |
| Number of Pregnancies  | 2.56    | 2.46| 1.83     | 0.001* |
| Number of Abortions    | 2.84    | 1.99| 1.54     | 4.37| 0.001* |
| Date of last menstruation | 46.83  | 46.45| 4.33     | 0.001* |
| Age at first birth     | 21.88   | 5.16| 6.04     | 0.001* |
| Time after menopause   | 12.81   | 13.17| 1.78     | 0.001* |
| Time after menopause   | 11.33   | 9.62| 7.38     | 2.69| 0.007* |
| Qualitative variables  | n %     | n % | x² | P |
| BMI                    | Normal/overweight| 81| 52.3| 195| 63.3| 5.23| 0.022* |
| Obese                  | 74      | 113 | 36.7 | 14.3 | 0.161 | 0.016 |
| Alcohol intake         | No      | 137 | 88.4 | 258| 83.8| 1.76| 0.185 |
| Metabolic syndrome     | No      | 104 | 67.1 | 220| 71.4| 0.92| 0.337 |
number of pregnancies, age at first birth, number of births, number of abortions, FHBC, BMI, waist circumference (WC), and the variables that characterize the sample (age, race, alcohol intake, smoking). The risk associated with the p allele of the PvuII was high in all strata. It was observed an increased risk of having HMD for women with younger age, smaller WC, fewer pregnancies, old age at having the first birth, the higher number of women with a FHBC. There was no apparent modification effect for the other indicators of exposure to endogenous estrogens.

Likewise, it was performed the association between the x allele of the Xbal and the same independent variables that characterize the sample, but no results confirmed the association.

Discussion

The association of ERα gene polymorphisms with the risk of BC draws attention because the ER functions as a regulator of the hormone dependent transcription, which plays a significant role in the development of BC [10]. Indeed, approximately two thirds of BC expresses the estrogen receptor alpha. Epidemiologic studies also correlate steroid hormones to changes the MD, and have examined whether variations in genes that regulate the biosynthesis and hormonal metabolism could explain individual differences in MD. The ER gene, located in the long arm of chromosome 6q25, has been associated to HMD in many studies, due to its importance in the development, progression and prognosis of BC. Several polymorphisms of ERα gene have been reported, with the PvuII and XbaI – located in intron 1 of the ERα gene with 50 base pairs between them – being the most studied. Several diseases, including BC [6,9,10,18,30], endometrial cancer [31], Alzheimer’s disease [32], obesity [33,34], endometriosis [35], leiomyomas [36] and bone mineral density [37], have been evaluated for a possible connection with ERα gene polymorphisms.

Our findings revealed a statistically significant difference between the two groups of mammographic density as also with respect to the distribution of the PvuII polymorphism genotype (p=0.024). It was found that women with two mutated alleles (homzygous mutant) had 76% greater chances of being diagnosed with HMD, compared to those with one or two normal alleles for this polymorphism (Table 4). The risk associated with the p allele of PvuII was shown to be an independent risk for other indicators of high exposure to endogenous estrogens. It was not observed any apparent effect modification for other indicators of exposure to endogenous estrogens and lifestyle factors. Similarly, van Duijnhoven et al. [17] studied the influence of ERα and HT on the MD, reporting an association between increased MD and the presence of the mutated genotype ERα-397 (OR=2.24) in women using HT when compared to those with wild genotype and not HT users. Parl et al. [30] found that the pp genotype of the PvuII polymorphisms was higher in women with a diagnosis of BC at a younger age. Yaich et al. [9] examined the PvuII polymorphism in tumor tissue of 257 women with primary breast cancer and compared it to peripheral blood collected from 140 controls not affected by the disease. Women with BC and pp genotype had a diagnosis of BC at an earlier age compared with those with PP or Pp genotypes of the group that had cancer.

| High mammographic density | PvuII      |
|---------------------------|------------|
|                           | Pp | Pp | PP | Total |
| No                        | 72 | 31 | 41 | 144   |
|                           | 50.0% | 21.5% | 28.5% | 100.0% |
|                           | OR=1.75 | p=0.024* |
| Yes                       | 147 | 100 | 61 | 308   |
|                           | 47.7% | 32.5% | 19.8% | 100.0% |
|                           | IC 95%=1.10-2.79 |
| Total                     | 219 | 131 | 102 | 452   |
|                           | 48.5% | 29.0% | 22.6% | 100.0% |

| Xbal                      |
|---------------------------|
|                           | Xx | Xx | Xx | Xx |
| No                        | 74 | 40 | 30 | 144   |
|                           | 51.4% | 27.8% | 20.8% | 100.0% |
|                           | p=0.362 |
| Yes                       | 154 | 103 | 51 | 308   |
|                           | 50.0% | 33.4% | 16.6% | 100.0% |
|                           | IC 95%=0.85-2.02 |
| Total                     | 228 | 143 | 81 | 452   |
|                           | 50.4% | 31.6% | 17.9% | 100.0% |

Table 4: Association between mammographic densities and polymorphisms PvuII and Xbal.
the authors found that women with two mutated alleles (homozygous mutant) were 30% more likely (OR=1.31) of having HMD, though this difference was not significant (p=0.36). On the other hand, Andersen et al. [18], while investigating the allelic frequency of the PvuII and XbaI SNPs in 360 cases of the BC and 672 controls, found significant differences only for the XbaI polymorphism. The x allele frequency among women with BC was 40% higher compared to that of the controls. In a case-control study conducted in South Korea by Shin et al. [6], the OR associated with the xx genotype of XbaI was 2.38 compared to the XX genotype. In that same vein, Cai et al. [10], in a large population study, found a significant association between the PvuII polymorphism and BC (OR=1.4). For XbaISNPs, the association was risk of 1.3 and only for women after menopause.

The molecular mechanisms through which these polymorphisms alter the receptor activity are not clear because PvuII and XbaI are located in an intronic, and apparently nonfunctional, region of the gene. Possible explanations include: (a) the existence of a functional combination between polymorphic alleles, where the two markers in combination would alter the gene function, as well as the mRNA stability [23]; (b) the existence of a functional combination between polymorphic alleles, where the two markers in combination would alter the gene function, as well as the mRNA stability [23]; (c) another explanation would be that polymorphisms in intron could have a Linkage Disequilibrium (LD) with the exon 1 and to 144 kb after the beginning of the transcription site of the exon 1 and to 144 kb before the exon 2; (c) Growth factors and their signaling molecules are important for cancer growth and its progression. There is considerable cross-talk between ER and growth factors such as insulin and IGFI (growth factors like insulin), and the family of epidermal growth factors [43].

The allelic and genotypic frequencies obtained for the ERα-397 PvuII polymorphism (P=46.8%, p=53.2%) were similar to those found in other studies in which this polymorphism was correlated with MD or BC [11,13,17,19,27,29]. The genotype distribution was in Hardy-Weinberg equilibrium with the following frequencies: pp genotype 29.0%, Pp 48.5% and PP 22.6% (Table 4).

The allele frequencies for the XbaI polymorphism (x=56.9%, X=43.1%) were lower when compared to two other studies conducted in Brazil [20,27], but they were in accordance with the frequency found by van Duijnhoeven et al. [17], Molvarec et al. [28] and Hsieh et al. [29]. The genotype distribution for ERα-351 XbaI was also in Hardy-Weinberg equilibrium with the following frequencies: xx genotype 31.6%, Xx 50.4% and XX 17.9% (Table 4).

In relation to the independent variables that showed associations with MD (age, race, WC, menarche, menopause, time after menopause, number of pregnancies, age at the first birth, number of births, number of abortions, FH, BMI, alcohol intake, smoking and PvuII and XbaI genotypes), after performing a multiple logistic regression, only the clinical factors (age, WC, number of pregnancies, age at the 1st birth, FH and PvuII polymorphism in the ERα) showed to be independent risk factors for HMD (p<0.05).

In summary, the present case-control study concluded that the PvuII polymorphism in the gene ERα was associated with an increased chance of having HMD, a factor of high risk for BC. Thus, recognizing these risk factors will be of great importance in the analyses of individual susceptibility to BC; in both the study of the response to various drugs (for example HT) and the prognosis (Table 5).

Footnote

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