The Breast 22 (2013) 335–343

Contents lists available at SciVerse ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Original article

# Polymorphisms of catechol estrogens metabolism pathway genes and breast cancer risk in Mexican women

O.C. Martínez-Ramírez [a], R. Pérez-Morales [b], C. Castro [a], A. Flores-Díaz [a], K.E. Soto-Cruz [a], A. Astorga-Ramos [c], M.E. Gonsebatt [a], L. Casas [d], M. Valdés-Flores [d], J. Rubio [a,*]

[a] Departamento de Medicina Genómica y Toxicología Ambiental, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Apartado Postal 70228, C.P. 04510, México D.F., Mexico
[b] Departamento de Biología Molecular, Facultad de Ciencias Químicas, Universidad Juárez del Estado de Durango, C.P. 35010, Durango, Mexico
[c] Instituto Nacional de Cancerología, C.P. 14080, México D.F., Mexico
[d] Departamento de Genética, Instituto Nacional de Rehabilitación, C.P. 14389, México D.F., Mexico

ARTICLE INFO

*Article history:*
Received 18 January 2012
Received in revised form
7 August 2012
Accepted 20 August 2012

*Keywords:*
Breast cancer
Genetic polymorphism
Estrogens
Catechol estrogens
Mexican women

ABSTRACT

Breast cancer is associated to estrogen exposure. Allelic variants involved in estrogen metabolism might change the risk of developing this neoplasia. We examined the potential association of breast cancer risk in Mexican women with the polymorphisms CYP1A1 rs1048943, CYP1B1 rs1056836, COMT rs4680, GSTP1 rs1695, GSTT1 null and GSTM1 null which are involved in estrogen metabolism pathway. This study included 150 cases and 150 controls. A significant association was observed between, CYP1A1 rs1048943 (OR = 1.95, C.I. 1.13–3.36) and GSTP1 rs1695 (OR = 2.39, C.I. 1.24–4.24) polymorphisms with the risk of breast cancer. This risk was increased when the women were stratified according to their menopausal status. The results show that breast cancer risk significantly increases in women with 3–6 risk polymorphisms (OR = 3.75, C.I. 1.44–9.74).



## Introduction

Breast cancer (BC) is the most common cancer worldwide among women and is also the leading cause of death associated with neoplasias in women.[1,2] Hereditary BC is linked to mutations in high penetrance genes such as BRCA1, BRCA2 and p53, and occurs in about 5% or 10% of total cases of BC. Other 90–95% of other cases are sporadic BC and are more related with low penetrance genes and interactions between carcinogenic agents such as estrogens.[3–6]

The association between exposure to estrogens and BC risk is well established and attributed to the ability of estrogens to induce the proliferation of breast tissue cells, formation of reactive oxygen species (ROS) and the release of carcinogenic metabolites.[7–10]

Most of the known risk factors for BC are linked to an increased lifetime exposure to estrogens.[11] Exposure to endogenous estrogens principally arises from woman's ovulatory cycles. If menarche begins under the age of 12 and/or if menopause starts over the age of 55, these women may be considered at high risk to develop BC.[10,12,13] An increased risk for BC has also been associated with nulliparity, tardy first childbirth, the use of hormonal contraceptives and hormone replacement therapy.[14–16]

Estrogens are mainly metabolized by the catechol estrogens metabolism pathway (CEMP), which produces ROS and adducts that may cause mutations and DNA damage that may initiate the development of the neoplasia.[17–19]

In the CEMP, estrogens are hydroxylated by either cytochrome CYP1A1 producing 2-hydroxycatechol estrogen (2-OH CE) or by CYP1B1 producing 4-hydroxycatechol estrogen (4-OH CE). These two catechol estrogens (CEs) can be inactivated by O-methylation catalyzed by COMT; however, if this reaction does not occur, the CEs are oxidized to catechol estrogen quinones (CE-Qs). These CE-Qs can then be conjugated with glutathione by the action of glutathione-S-transferases (GSTs), GSTT1, GSTM1 and GSTP1, to

*Abbreviations:* BC, breast cancer; CEMP, catechol estrogens metabolism pathway; CYP1A1, cytochrome P450 1A1; CYP1B1, cytochrome P450 1B1; GSTP1, glutathione-S-transferase P1; GSTM1, glutathione-S-transferase M1; GSTT1, glutathione-S-transferase T1; COMT, catechol-O-methyl-tranferase; OR, odds ratio; ROS, reactive oxygen species; 2-OH CE, 2-hydroxycatechol estrogen; 4-OH CE, 4-hydroxycatechol estrogen; CEs, catechol estrogens; CE-Qs, catechol estrogen quinones; MLR, multivariable logistic regression; MDR, multifactor-dimensionality reduction; HWE, Hardy–Weinberg equilibrium.

* Corresponding author. Tel.: +52 55 56 22 91 78; fax: +52 55 56 22 91 82.
*E-mail address:* juruli@unam.mx (J. Rubio).

0960-9776/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.breast.2012.08.004

prevent DNA damage, or they may react with DNA to form adducts that ultimately generate apurinic sites, which may lead to mutations and may potentially initiate BC.[17,18,20–22]

The genes involved in the CEMP have allelic variants with modified enzymatic activities (Table 1). These alleles have been widely studied in different populations providing evidence of their contribution to BC risk,[23–26] often with inconsistent or contradictory results, even within the same ethnic group.[23,27–29]

In this study, we analyzed the association of polymorphisms in six genes (individually and in cluster) of the CEMP with the risk of developing BC in a cohort of Mexican women.

## Materials and methods

### Subjects

#### Mexican Mestizo cohort

In order to determine the allelic frequency of polymorphisms in a Mexican population, as previously reported for four of the studied polymorphisms,[40] we selected 382 (420 for CYP1B1) unrelated, healthy Mexican Mestizo men and women who attended to the Hospital "20 de Noviembre" in Mexico City from October 2001 to November 2004 as blood donors. Subjects filled out a questionnaire, which included data about sex, age, birthplace, parents' and grandparents' birthplace, and lifestyle. All participants, their parents and grandparents were born in Mexico. With these figures we calculated the sample size needed for the cases–controls study.

#### Retrospective study of cases and controls

We included 150 cases: unrelated women who attended from 2006 to 2007 to the Instituto Nacional de Cancerología (Mexico City). These patients were diagnosed with primary breast carcinoma (stages IIA–IV) and their histopathologycal analysis demonstrated the presence of invasive or in situ ductal carcinoma in 105 cases, and invasive or in situ lobular carcinoma in 45 cases. All of the patients were over 30 years old (mean age 48.09 ± 9.6 years old) and had no history of hereditary BC syndrome, according to the specifications of the National Cancer Institute of United States[3,41,42] and the National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology, which reduces the possibility of including women with mutations in high penetrance genes such as BRCA1 and BRCA2. The population controls were healthy age-paired women without diagnostic of BC (mean age 48.27 ± 10.75 years old). Control samples were also collected between 2006 and 2007.

Cases and controls were born in Mexico, their parents and grandparents were born in Mexico. Informed consent was obtained from all participants, who also answered a questionnaire about risk factors for BC.

The research protocol was approved by the Bioethics Committees of the Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México and the Instituto Nacional de Cancerología (Mexico City). The Hospital "20 de Noviembre" gave permission to use the buffy coat of blood bank samples.

### DNA samples

DNA preparations used for the genotyping analyses were extracted from blood samples collected and stored at −20 °C until used. Mononuclear white cells and the genomic DNA were isolated as described in Ref..[43]

### Genotyping

Restriction fragment length polymorphism (RFLP) analyses were used to assess the CYP1A1 rs1048943, CYP1B1 rs1056836, COMT rs4680 and GSTP1 rs1695 polymorphisms. A multiplex polymerase chain reaction (PCR) assay was used to simultaneously determine the presence of the GSTM1 and GSTT1 null alleles. The restriction endonucleases were acquired at Fermentas and New England Biolabs. GSTM1 null, GSTT1 null, CYP1A1 rs1048943 and GSTP1 rs1695 polymorphisms were previously reported in Ref..[40]

The PCR products and restriction fragments were separated by electrophoresis in 2% agarose gels (4% agarose for COMT), and visualized after staining with ethidium bromide on a UV transilluminator (Bio-Rad Hercules, CA, USA). Primers were obtained from Invitrogen.

The primers and PCR conditions used, as well RFLP features are summarized in Table 2.

### Case–control study design

Due to frequencies of polymorphisms for CYP1B1 rs1056836 and COMT rs4680 have not been previously determined in Mexican population, we estimated these allelic frequencies in a sample of unrelated healthy Mexican individuals (Table 3). Based on the obtained frequencies, the minimal necessary sample size for the case–control study was estimated for each polymorphism according to the formula

$$n = \left[\frac{z\left(\frac{\alpha}{2}\right)\sqrt{2p(1-p)} + z_{1-\beta}\sqrt{p_1(1-p_1)+p_2(1-p_2)}}{p_1 - p_2}\right]^2,$$

where $z_{\alpha/2} = 1.96$ for a confidence level of 95%, $z_{1-\beta} = 0.80$ for a statistical power of 78.8%, $p_2$ is the exposure frequency of controls, $p_1$ is an estimate of the exposure frequency of cases derived from previously reported OR's[47–52] and $p = (p_1 + p_2)/2$.

**Table 1**
Genetic polymorphisms involved in catechol estrogens metabolism pathway.

| Gene (rs) | Role in estrogen metabolism | Genotype | Functional effect | References |
|---|---|---|---|---|
| CYP1B1 (rs1056836) | 4-hydroxylase generates 4-OH CEs | C → G<br>Leu → Val | Increased activity | [30,31] |
| CYP1A1 (rs1048943) | 2-hydroxylase generates 2-OH CEs | A → G<br>Ile → Val | Increased activity/inducibility | [32,33] |
| COMT (rs4680) | Methyltransferase inactivation of CEMP | G → A<br>Val → Metl | Four-times reduced methylation activity | [34–36] |
| GSTT1 (deletion) | Conjugation of estrogen quinones to glutathione | Null | Lack of enzyme | [37,38] |
| GSTM1 (deletion) | | Null | Lack of enzyme | |
| GSTP1 (rs1695) | | A → G<br>Ile → Val | Reduced activity | [39] |

**Table 2**
Primers and conditions of PCR-RFLP and multiplex PCR.

| Gene (rs) | Primer sequence 5′–3′ | Fragment length (bp) | PCR conditions | Cycles | Restriction endonuclease | Reference |
|---|---|---|---|---|---|---|
| CYP1A1 (rs1048943) | Forward CTGTCTCCCTCTGGTTACAGGAAGC<br>Reverse TTCCACCCGTTGCAGCAGGATAGCC | 204 | 30 s 94 °C<br>30 s 63 °C<br>30 s 72 °C | 35 | BsrDI | [40] |
| CYP1B1 (rs1056836) | Forward TCACTTGCTTTTCTCTCTCC<br>Reverse AATTTCAGCTTGCCTCTTG | 650 | 30 s 94 °C<br>25 s 60 °C<br>40 s 72 °C | 35 | Eco57I | [44] |
| COMT (rs4680) | Forward GGGCCTACTGTGGCTACTCA<br>Reverse GGCCCTTTTTCCAGGTCTGACA | 165 | 30 s 94 °C<br>30 s 60 °C<br>40 s 72 °C | 40 | NlaIII | [45] |
| GSTP1 (rs1695) | Forward ACCCCAGGGCTCTATGGGAA<br>Reverse TGAGGGCACAAGAAGCCCCT | 176 | 20 s 94 °C<br>20 s 60 °C<br>20 s 72 °C | 30 | BsmA1 | [40] |
| GSTM null | Forward GAACTCCCTGAAAAGCTAAAGC<br>Reverse GTTGGGCTCAAATATACGGTGG | 215 | 60 s 94 °C<br>45 s 59 °C | 35 | NA[a] | [40,46] |
| GSTT1 null | Forward TTCCTTACTGGTCCTCACATCTC<br>Reverse TCACCGGATCATGGCCAGCA | 480 | 45 s 72 °C | | NA[a] | [40] |

[a] NA – Not applicable.

*Statistical analyses*

Hardy–Weinberg Equilibrium (HWE) was tested separately in cases and controls for each polymorphism, when applicable, with statistical package GenePop version 4.0.10 (http://genepop.curtin.edu.au).

Epidemiological risk factors and risk of developing BC were examined using Person's $X^2$ tests, Fisher's exact tests, and the Freeman–Halton extension of the Fisher's exact test. The former two tests were carried out using GraphPad Prism 5 software. Freeman–Halton extension was computed using the test calculator (http://in-silico.net/statistics/fisher_exact_test/2x3).

To explore a possible association between BC risk and individual polymorphisms, an unconditional logistic regression analysis to calculate the crudes odds ratios (ORs) with 95% confidence intervals (CIs), $X^2$ tests and Fisher's exact tests were performed in GraphPad Prism 5 for CYP1A1, CYP1B1, COMT, GSTT1, GSTM1 and GSTP1. The heterozygous and homozygous variant genotypes of these genes were compared to the homozygous wild-type genotype, when applicable.[53–56] Groups having the lowest risk were used as reference value. Assuming a biological activity gradient (codominant model), a linear regression Cochran–Armitage trend test was performed in XLSTAT 2012 for individual polymorphisms in CYP1A1, CYP1B1, COMT, and GSTP1.[57,58] The associations in unstratified and stratified women according to menopausal status were studied.

Multivariate logistic regression (MLR) models were performed with the Statistical Package for Social Sciences (SPSS-PC; SPSS Inc. Chicago, Illinois, USA) to assess the effect of covariates in the BC risk.

Under the hypothesis of additivity of BC risk alleles in women, we determined the gene–gene interactive effects of the six studied polymorphisms using Multifactor-Dimensionality Reduction (MDR; Version 1.1.0), which reduces dimensionality by pooling multilocus genotypes into two groups.[59] This program calculates the OR's of different models of interaction among genes and is proposed as a quantitative measure of disease risk.[60]

Statistical differences were considered significant at $p \leq 0.05$. All $p$-values reported are two tailed.

## Results

The women included in this study were from 30 to 74 years. The evaluation of additional risk factors for BC is shown in Table 4. There was no significant difference in the age distribution between patients and controls. In fact, we only found that the age at menopause was statistically different between them ($p = 0.003$).

The genotypes tested were under HWE. It was not possible to analyze GSTT1 and GSTM1 HWE.

Table 5 shows the allelic distribution and the association of their polymorphisms with BC risk. A statistically significant association was found between the CYP1A1$^{Val/Val}$ ($p = 0.01$) and GSTP1$^{Val/Val}$

**Table 3**
Frequency of breast cancer risk polymorphisms among Mexican Mestizo cohort and sample required for a case–control study.

| Gene (rs) | Genotype frequency, *n* (%) | | | | | Allelic frequency | | Reference | Sample required for a case–control study[d] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mutant allele | *n* | Wild-type homozygous | Heterozygous | Mutant homozygous | $p$[b] | $q$[c] | | Case | Control |
| CYP1A1 (1048943) | Val | 382 | 86 (22.5) | 176 (46.1) | 120 (31.4) | 0.45 | 0.55 | [40] | 143 | 143 |
| CYP1B1 (1056836) | Val | 420 | 60 (14.28) | 230 (54.7) | 130 (30.95) | 0.41 | 0.59 | This study | 150 | 150 |
| COMT (4680) | Met | 382 | 161 (42.1) | 156 (40.8) | 65 (17.1) | 0.62 | 0.38 | This study | 133 | 133 |
| GSTP1 (947894) | Val | 382 | 102 (27.7) | 192 (50.3) | 88 (23) | 0.52 | 0.48 | [40] | 135 | 135 |
| GSTM1 (deletion) | Null | 382 | 239 (62.6) | N.D.[a] | 143 (37.4) | N.D.[a] | N.D.[a] | [40] | 133 | 133 |
| GSTT1 (deletion) | Null | 382 | 324 (84.8) | N.D.[a] | 58 (15.2) | N.D.[a] | N.D.[a] | [40] | 206 | 206 |

Table modified with permission from Pérez-Morales et al., 2011.
[a] Not determined.
[b] $p$ wild-type allele.
[c] $q$ mutant allele.
[d] According to the calculation described in Materials and methods with a power of 78.8% and a confidence interval 95%.

**Table 4**
Evaluation of risk factors for breast cancer.

| Risk factor | Cases *n* (%) | Controls *n* (%) | *p*-value |
|---|---|---|---|
| Age (years) | | | |
| <40 | 36 (24.0) | 38 (25.3) | 0.51[a] |
| 40–50 | 50 (33.3) | 45 (30.0) | |
| 50–60 | 49 (32.6) | 44 (29.3) | |
| >60 | 15 (10.0) | 23 (15.3) | |
| Body mass index (BMI) | | | |
| Healthy weight (from 18.5 to 25) | 62 (41.4) | 69 (46) | 0.72[b] |
| Overweight and obese (>25) | 88 (58.6) | 81 (54) | |
| Age at menarche (years) | | | |
| <12 | 29 (19.3) | 34 (22.6) | 0.23[a] |
| 12–14 | 95 (63.3) | 100 (66.6) | |
| >14 | 26 (17.3) | 16 (10.6) | |
| Menopausal status | | | |
| Pre | 82 (58.0) | 81 (54.0) | 0.9[b] |
| Post | 68 (42.0) | 69 (46) | |
| Age at menopause (years) | | | |
| <45 | 4 (4.70) | 16 (23.1) | 0.003[c,*] |
| 45–55 | 60 (90.4) | 52 (75.3) | |
| >55 | 4 (4.70) | 1 (1.44) | |
| Parity | | | |
| >5 | 13 (8.60) | 19 (12.6) | 0.48[a] |
| 1–5 | 110 (73.3) | 108 (72.0) | |
| 0 | 27 (18.0) | 23 (15.3) | |
| Use of contraceptives (years) | | | |
| Never | 104 (69.3) | 92 (61.3) | 0.28[a] |
| <5 | 34 (22.6) | 46 (30.6) | |
| >5 | 12 (8.0) | 12 (8.0) | |
| Use of hormone replacement therapy | | | |
| No | 133 (88.6) | 136 (90.7) | 0.70[b] |
| Yes | 17 (11.3) | 14 (9.3) | |

[*] Significant values.
[a] Chi-square.
[b] Fisher's exact test.
[c] Freeman–Halton–Fisher's exact test.

($p = 0.009$) genotypes and BC risk, these Fisher's exact test results were consistent with the ones of the Cochran–Armitage's test. Also, CYP1B1 and COMT polymorphisms showed a marginal significance ($p = 0.057$ and $p = 0.085$, respectively).

The association with BC risk was also observed in premenopausal and postmenopausal women (Table 6). The statistical analysis with Fisher's exact test showed that the association of both genotypes with BC risk was higher in the premenopausal group ($CYP1A1^{Val/Val}$ $p = 0.009$, $GSTP1^{Val/Val}$ $p = 0.005$) than in the postmenopausal women ($CYP1A1^{Val/Val}$ $p = 0.06$, $GSTP1^{Val/Val}$ $p = 0.09$).

The significance observed in premenopausal women is consistent with Cochran–Armitage's test results, but the significance observed in postmenopausal women is not present within the trend test.

Using MLR we adjusted the model to the only risk factor that was significantly different in our study (age at menopause). We could not make the adjustment to age at menopause and THR for premenopausal women because is not possible to have these variables in this group. However, when we adjusted for age at menopause in postmenopausal women, the significance of $CYP1A1^{Val/Val}$ was lost, but in contrast, a significant association between $CYP1B1^{Leu/Val}$ $CYP1B1^{Val/Val}$, $COMT^{Met/Met}$ and BC risk emerged and the significance of $GSTPI^{Val/Val}$ was preserved (Table 6).

We examined whether there was an additive effect of the risk alleles (homozygous mutants) of the CEMP genes associated with BC risk. This analysis (Table 7) showed a tendency of BC risk increment with increased numbers of risk polymorphisms, and a significant escalation of cancer risk in women with more than 3 risk alleles ($p = 0.008$).

Contrary to postmenopausal women, the additive effect in premenopausal women showed a clear association to BC risk (Table 8).

To determine the relative risk of combinations of risk polymorphisms, and their additive effect we carried out an MDR analysis. Table 9 shows a significant increased risk of developing BC (OR-MDR) associated with an increasing number of interactions of risk genes.

## Discussion

Prior to the case–control study we determined the genotypic frequencies of CYP1B1 and COMT in Mexican population (Table 3) and obtained the sample size for a case–control study required (Table 3) by using the formula and the model for sample size estimation described in Materials and methods; it was considered

**Table 5**
Association of genetic polymorphisms with breast cancer risk.

| Genotype | Cases *n* (%) | Controls *n* (%) | OR (95% CI) | *p*-value | Cochran–Armitage's trend test *p*-value |
|---|---|---|---|---|---|
| CYP1A1 | | | | | |
| Ile/Ile | 39 (27.3) | 57 (37.3) | 1.0[a] | | *0.01*[*] |
| Ile/Val | 37 (26.0) | 43 (30.0) | 1.18 (0.65–2.1) | 0.65 | |
| Val/Val | 74 (46.6) | 50 (32.6) | 1.95 (1.13–3.36) | 0.01[*] | |
| CYP1B1 | | | | | |
| Leu/Leu | 27 (18) | 33 (20.6) | 1.0[a] | | 0.057 |
| Leu/Val | 46 (30.6) | 62 (42) | 0.83 (0.44–1.59) | 0.62 | |
| Val/Val | 77 (51.3) | 55 (37.3) | 1.57 (0.84–2.93) | 0.15 | |
| COMT | | | | | |
| Val/Val | 52 (34.6) | 68 (44.6) | 1.0[a] | | 0.085 |
| Val/Met | 66 (44.0) | 59 (38.6) | 1.46 (0.88–2.43) | 0.15 | |
| Met/Met | 32 (21.3) | 23 (16.6) | 1.64 (0.87–3.11) | 0.14 | |
| GSTT1 | | | | | |
| Wild type | 103 (68.6) | 108 (72) | 1.0[a] | | |
| Null | 47 (31.3) | 42 (28) | 1.17 (0.71–1.92) | 0.61 | |
| GSTM1 | | | | | |
| Wild type | 85 (56.6) | 89 (58.6) | 1.0[a] | | |
| Null | 65 (43.3) | 61 (41.3) | 1.08 (0.68–1.71) | 0.81 | |
| GSTP1 | | | | | |
| Ile/Ile | 40 (27.3) | 43 (32) | 1.0[a] | | *0.008*[*] |
| Ile/Val | 39 (34.6) | 66 (48.6) | 0.83 (0.48–1.44) | 0.57 | |
| Val/Val | 71 (38.0) | 41 (19.3) | 2.39 (1.24–4.24) | *0.009*[*] | |

[*] Significant values.
[a] Reference value; OR, odds ratio; CI, confidence interval.

**Table 6**
Association of genetic polymorphisms with breast cancer risk according to menopausal status.

| Genotype | Premenopausal women | | | | | Postmenopausal women | | | | | Adjusted by age at menopause | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unadjusted | | | | | Unadjusted | | | | | | |
| | Cases *n* (%) | Controls *n* (%) | OR (95% CI) | *p*-value | Cochran–Armitage's trend test *p*-value | Cases *n* (%) | Controls *n* (%) | OR (95% CI) | *p*-value | Cochran–Armitage's trend test *p*-value | OR (95% CI) | *p*-value |
| CYP1A1 | | | | | | | | | | | | |
| Ile/Ile | 20 (24.3) | 31 (38.2) | 1.0[a] | | | 19 (27.9) | 26 (37.6) | 1.0[a] | | | | |
| Ile/Val | 19 (23.1) | 26 (32.0) | 1.13 (0.50–2.56) | 0.83 | | 18 (26.4) | 17 (24.6) | 0.53(0.21–1.31) | 0.25 | | 1.02 (0.46–2.24) | *0.96* |
| Val/Val | 43 (52.4) | 24 (29.6) | 2.77 (1.30–5.89) | *0.009** | *0.006** | 31 (45.5) | 26 (37.6) | 2.33 (1.01–5.35) | *0.06* | 0.229 | 1.46 (0.72–2.94) | *0.28* |
| CYP1B1 | | | | | | | | | | | | |
| Leu/Leu | 16 (19.5) | 20 (24.6) | 1.0[a] | | | 11 (16.1) | 13 (18.8) | 1.0[a] | | | | |
| Leu/Val | 25 (30.4) | 28 (34.5) | 1.11 (0.47–2.61) | 0.83 | | 21 (30.8) | 34 (49.2) | 0.72 (0.27–1.92) | 0.62 | | 0.49 (0.24–1.00) | 0.05* |
| Val/Val | 41 (50.0) | 33 (40.7) | 1.55 (0.69–3.46) | 0.31 | 0.242 | 36 (52.9) | 22 (31.8) | 1.85 (0.70–4.82) | 0.23 | 0.058* | 2.22 (1.09–4.49) | 0.02* |
| COMT | | | | | | | | | | | | |
| Val/Val | 30 (36.5) | 39 (48.1) | 1.0[a] | | | 22 (32.3) | 29 (42.0) | 1.0[a] | | | | |
| Val/Met | 38 (46.3) | 29 (35.8) | 1.70 (0.86–3.35) | 0.16 | | 28 (41.1) | 30 (43.4) | 1.19 (0.56–2.53) | 0.70 | | 0.77 (0.38–1.57) | *0.48* |
| Met/Met | 14 (17.0) | 13 (16.0) | 1.40 (0.57–3.41) | 0.50 | 0.266 | 18 (26.4) | 10 (14.4) | 2.37 (0.91–6.14) | 0.10 | 0.102 | 2.37 (0.98–5.70) | *0.05** |
| GSTT1 | | | | | | | | | | | | |
| Wild type | 57 (69.5) | 60 (74.0) | 1.0[a] | | | 46 (67.6) | 48 (69.5) | 1.0[a] | | | | |
| Null | 25 (30.4) | 21 (25.9) | 1.25 (0.63–2.48) | 0.60 | | 22 (32.3) | 21 (30.4) | 1.04 (0.5–2.1) | 1.0 | | 0.99 (0.49–2.01) | 0.98 |
| GSTM1 | | | | | | | | | | | | |
| Wild type | 44 (53.6) | 46 (56.7) | 1.0[a] | | | 41 (60.2) | 43 (62.3) | 1.0[a] | | | | |
| Null | 38 (46.3) | 35 (43.2) | 1.13 (0.61–2.10) | 0.75 | | 27 (39.7) | 26 (37.6) | 1.11 (0.56–2.21) | 0.86 | | 1.11 (0.53–2.32) | 0.77 |
| GSTP1 | | | | | | | | | | | | |
| Ile/Ile | 21 (25.6) | 28 (34.5) | 1.0[a] | | | 19 (27.9) | 15 (21.7) | 1.0[a] | | | | |
| Ile/Val | 24 (29.2) | 38 (46.9) | 0.84 (0.39–1.81) | 0.70 | | 15 (22.0) | 28 (40.5) | 1.16 (0.50–2.64) | 0.83 | | 0.36 (0.16–0.79) | *0.01** |
| Val/Val | 37 (45.1) | 15 (18.5) | 3.28 (1.44–7.50) | *0.005** | *0.004** | 34 (50.0) | 26 (37.6) | 2.40 (0.94–6.11) | *0.09* | 0.657 | 1.87 (0.92–3.77) | *0.07* |

* Significant values.
[a] Reference value; OR, odds ratio; CI, confidence interval.

**Table 7**
Estimated odds ratio of breast cancer risk associated with the number of high-risk genotypes.

| No. of high-risk genotypes | Cases *n* (%) | Controls *n* (%) | OR (95% CI) | *p*-value |
|---|---|---|---|---|
| Zero | 8 (5.3) | 15 (10.4) | 1.0[a] | |
| One | 33 (22.0) | 51 (33.9) | 1.21 (0.46–3.10) | 0.81 |
| Two | 43 (28.6) | 51 (33.3) | 1.58 (0.61–4.08) | 0.48 |
| Three-six | 66 (40) | 33 (55) | 3.75 (1.44–9.74) | *0.008** |

* Significant values. *p*-value calculated by use of Fisher's exact test.
[a] Reference value; OR, odds ratio not adjusted; CI, confidence interval.

to have a first approximation of the importance of these polymorphisms for BC risk in Mexican women.

Although we determined that for GSTT1 a greater sample size was necessary, a preliminary estimation of the effect of GSTT1 on BC risk was also included in our study due to the essential role that this enzyme has in the detoxification of carcinogenic metabolites of steroid hormones.

In our univariated analyses, if multiple testing corrections were performed, our associations would not be detected. Although, without these corrections, the probability of false positive is incremented, like this is a preliminary study, is important to label all possible interactions.

Our sample included a high percentage of premenopausal women (58%: Table 4). This is consistent with findings of previous studies in Mexican women, in which the most frequently affected age group for BC was the 40–49 years old group with a median age one decade younger than that of Caucasic women (60–64-year old).[61,62] Mean age for menopause in Mexican women is 45–47-year old,[63,64] hence, these facts explain the elevated percentage of premenopausal women with BC compared to other reported populations. This difference in the age to develop BC in Mexican women might explain the smaller impact of conventional risk factors associated with exposure to endogenous and exogenous estrogens, which are more strongly related to BC in women over 55 years old.[24,65–70] Nevertheless, as this is an exploratory study, confirmation of these data are needed.

A difference between the age at menopause between cases and controls was found (Table 3). An explanation might be the decreased exposure time to endogenous estrogen.[65,70,71]

On the other hand, there is evidence that wild-type homozygous individuals have less risk than the heterozygous ones and the latter, have less risk than mutant homozygous individuals. The polymorphism CYP1B1 (rs1056836) has recently been found to have the most profound impact on its catalytic properties, with 4-hydroxylase activity of the Val432 allele displaying three-fold higher activity compared to Leu432 allele.[72] In CYP1A1, the polymorphism rs1048943 has been suggested to lead to higher enzyme activity and mRNA expression and therefore higher rates of carcinogen activation[73]; the COMT rs4680 polymorphism has been associated with three- to four-fold decreased activity of COMT activity compared with the wild-type and the polymorphism GSTP1 (rs1695) also results in a decreased enzymatic activity.[74] These works show that heterozygous variants of these four polymorphisms have an intermediate risk falling between the wild-type homozygous and mutant homozygous genotypes. Based on these evidences we applied a codominant model where the associations among BC risk, $CYP1A1^{Val/Val}$ and $GSTP1^{Val/Val}$ may be explained by the increased enzymatic activity of $CYP1A1^{Val/Val}$ and the decreased enzymatic activity of $GSTP1^{Val/Val}$. In both cases, the polymorphic genotypes may still increase the overall levels of mutagenic metabolites.

In the trend test, associations of polymorphisms CYP1A1 rs1048943 and GSTP1 rs1695 with BC risk were found in premenopausal women, but not in postmenopausal women. Associations found only in premenopausal women highlight the potential role of these polymorphisms in the development of BC in younger women who have been less exposed to endogenous estrogens and extrinsic related risk factors emphasizing the role of genes in the development of BC in this group.

However, it is interesting that when we introduced the variable age at menopause variable to our model of postmenopausal women we found a significant association with $CYP1B1^{Leu/Val}$, $CYP1B1^{Val/Val}$, $COMT^{Met/Met}$, $GSTPI^{Ile}/^{Val}$, $GSTPI^{Val/Val}$ and the BC risk. This points to the importance of these polymorphisms in older women, that might be exposed to estrogen by more number of ovulatory cycles.

In a previous study in Mexican women, an increased BC risk associated with $CYP1A1^{Val/Val}$ variant was only found in premenopausal women, and no association with $COMT^{Met/Met}$ was found neither in premenopausal women nor in postmenopausal women.[27]

Our results on the association of $CYP1A1^{Val/Val}$ and the BC risk are in agreement with previous reports.[23–26] The association of

**Table 8**
Estimated OR of breast cancer associated with the number of high-risk genotypes according to menopausal status.

| No. of high-risk genotypes | Premenopausal women | | | | Postmenopausal women | | | |
|---|---|---|---|---|---|---|---|---|
| | Cases *n* (%) | Controls *n* (%) | OR (95% CI) | *p*-value | Cases *n* (%) | Controls *n* (%) | OR (95% CI) | *p*-value |
| Zero | 2 (2.43) | 11 (13.2) | 1.0[a] | | 5 (7.3) | 5 (7.1) | 1.0[a] | |
| One | 18 (21.9) | 26 (31.3) | 3.8 (0.75–19.2) | 0.11 | 15 (22.0) | 26 (37.14) | 0.57 (0.14–2.3) | 0.48 |
| Two | 27 (32.9) | 28 (33.7) | 5.3 (1.07–6.19) | 0.03* | 24 (27.9) | 23 (32.85) | 1.04 (0.26–4.08) | 1 |
| Three-six | 35 (42.6) | 16 (19.75) | 12.3 (2.38–60.7) | 0.001* | 24 (35.29) | 15 (21.7) | 1.60 (0.39–6.47) | 0.71 |

* Significant values *p*-value calculated by use of Fisher's exact test.
[a] Reference value; OR, odds ratio not adjusted; CI, confidence interval.

**Table 9**
Estimated multivariate-OR of genetic interaction of the homozygous mutants of genes with risk for breast cancer.

| No. of risk genes | Models | Testing balanced accuracy | CVC | OR-MDR | 95% CI | *p*-value of $X^2$ test |
|---|---|---|---|---|---|---|
| 2 | CYP1B1–GSTP1 | 0.667 | 7/10 | 2.88 | (1.78–4.65) | <0.0001* |
| 3 | COMT–CYP1A1–GSTP1 | 0.634 | 8/10 | 3.91 | (2.42–6.31) | <0.0001* |
| 4 | COMT–CYP1A1–GSTP1–GSTT1 | 0.662 | 8/10 | 6.43 | (3.87–10.6) | <0.0001* |
| 5 | COMT–CYP1A1–GSTP1–GSTT1–CYP1B1 | 0.572 | 9/10 | 13.0 | (7.24–23.3) | <0.0001* |
| 6 | COMT–CYP1A1–GSTP1–GSTT1–CYP1B1–GSTM1 | 0.591 | 10/10 | 37.6 | (17.11–82.9) | <0.0001* |

Abbreviations: CVC, cross-validation consistency; OR-MDR, odds ratio-multifactor-dimensionality reduction; 95% CI, confidence interval 95%.
Risk estimate was based on the combination and dichotomization of the distribution of genetic factors according to the MDR software.
* Significant values.

**Table 10**
Reports showing association of breast cancer with polymorphisms of catechol estrogens metabolism pathway genes.

| First author | Year | Gene | Genotype | Population | Results OR (95% CI) | Number of participants | | References |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Cases | Controls | |
| Miyushi | 2002 | CYP1A1 | Val/Val | Japanese | 0.66 (0.45–0.96) | 195 | 272 | [23] |
| Shin | 2007 | | | Korean | 0.98 (0.56–1.71) | 513 | 447 | [26] |
| Torresan | 2008 | | | Brazilian | 1.46 (0.76–2.79) | 102 | 102 | [25] |
| Moreno-Galván | 2010 | | | Mexican | 0.86 (0.38–1.95) | 91 | 94 | [27] |
| This study | 2012 | | | Mexican | 1.94 (1.13–3.34) | 150 | 150 | |
| Kocabas | 2002 | CYP1B1 | Val/Val | Turkish | 1.27 (0.47–3.43) | 84 | 103 | [75] |
| Jiao | 2010 | | | Asian | 0.85 (0.67–1.08) | 152 | 156 | [76] |
| Okobia | 2009 | | | Nigerian | 1.09 (0.61–1.95) | 250 | 250 | [77] |
| This study | 2012 | | | Mexican | 1.17 (0.68–2.01) | 150 | 150 | |
| Wu | 2003 | COMT | Met/Met | Asian | 0.84 (0.54–1.30 | 589 | 563 | [78] |
| Sazci | 2004 | | | Turkish | 1.0 (0.93–1.29) | 130 | 233 | [79] |
| Wen | 2005 | | | Chinese | 0.92 (0.67–1.26) | 1135 | 1235 | [29] |
| Moreno-Galván | 2010 | | | Mexican | 0.89 (0.39–2.25) | 91 | 94 | [27] |
| This study | 2012 | | | Mexican | 1.74 (0.91–3.0) | 150 | 150 | |
| Mitrunen | 2001 | GSTP1 | Val/Val | Finnish | 0.57 (0.31–1.04) | 483 | 482 | [65] |
| Torresan | 2008 | | | Brazilian | 1.81 (1.04–3.16) | 102 | 102 | [25] |
| Kaushal | 2010 | | | Indian | 1.43 (0.96–2.11) | 117 | 174 | [56] |
| This study | 2012 | | | Mexican | 2.39 (1.30–4.40) | 150 | 150 | |
| Charrier | 1999 | GSTM1 | Null | French | 1.99 (1.19–3.32) | 361 | 437 | [49] |
| Helzlsouer | 1998 | | | Caucasian | 2.10 (1.22–3.64) | 110 | 113 | [50] |
| Mitrunen | 2001 | | | Finnish | 1.49 (1.03–2.15) | 483 | 482 | [65] |
| Kaushal | 2010 | | | Indian | 0.57 (0.32–1.00) | 117 | 174 | [56] |
| This study | 2012 | | | Mexican | 1.09 (0.69–1.84) | 150 | 150 | |
| Mitrunen | 2001 | GSTT1 | Null | Finnish | 1.18 (0.80–1.76) | 483 | 482 | [65] |
| Torresan | 2008 | | | Brazilian | 0.91 (0.51–1.65) | 102 | 102 | [25] |
| Kaushal | 2010 | | | Indian | 0.59 (0.36–0.99) | 117 | 174 | [56] |
| This study | 2012 | | | Mexican | 1.13 (0.69–1.84) | 150 | 150 | |

OR, odds ratio; 95% CI, 95% confidence interval. All studies were case–control.

$GSTP1^{Val/Val}$ with an increased BC risk has been described.[69] Interestingly, in the Brazilian women, although the mentioned genotypes were also found to increase the risk of developing BC, no such association was observed for $CYP1B1^{Val/Val}$ and GSTM1 null. Some reports have demonstrated the association of those polymorphisms of the CEMP with BC risk (Table 10).

The additive effect of risk polymorphisms shows a more significant contribution in premenopausal women than in postmenopausal women (Table 7) underlining the importance of the additive risk of polymorphisms in young women, less exposed to susceptibility factors.

The results of multivariate MDR model (Table 8) show that the BC risk increases proportionally to the number of risk alleles in the individuals.

Although our results need to be confirmed in larger studies, this study provides, for the first time, evidence of the participation of some CEMP polymorphisms in BC risk in Mexican women.

## Conclusions

We found that CYP1A1 rs1048943 and GSTP1 rs1695 polymorphisms are associated with a significantly increased BC risk.

We determined a significant association between menopausal status and the BC risk due to the participations of the polymorphisms involved in the CEMP in Mexican women.

A greater number of risk polymorphisms present in a woman may generate a higher risk to develop for BC.

Further studies are needed to confirm our results and to explore the relationship between risk allelic variants and BC, in Mexican women.

## Ethical approval

This work was performed under the approval of the Ethics Committees of Instituto de Investigaciones Biomédicas, UNAM and of Instituto Nacional de Cancerología, Mexico.

## Conflict of interest statement

The authors have declared that they do not have conflict of interest.

## Acknowledgements

This work was supported in part by the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT, IN219807-3 and IN210111 to JR and KESC received a scholarship through this grant), Universidad Nacional Autónoma de México (UNAM) and Consejo Nacional de Ciencia y Tecnología (CONACYT, 46311-M, to MEG). OCMR received a scholar fellowship from CONACYT (210363). We thank all our blood donors for their valuable contribution to this project. To Pavel Petrosyan for careful and critical reading of the manuscript and to Tzipe Govezensky for supervision in statistical analyses.

## References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN. *Int J Cancer* 2008;**127**(12):2893–917.
2. Knaul FM, Nigenda G, Lozano R, Arreola-Ornelas H, Langer A, Frenk J. Breast cancer in Mexico: an urgent priority. *Salud Pública Mex* 2009;**51**(Suppl. 2):s335–44.
3. Hemel D, Domchek SM. Breast cancer predisposition syndromes. *Hematol Oncol Clin North Am* 2010;**24**(5):799–814.
4. Palacios J, Robles-Frías MJ, Castilla MA, López-García MA, Benítez J. The molecular pathology of hereditary breast cancer. *Pathobiology* 2008;**75**(2):85–94.
5. Lynch HT, Silva E, Snyder C, Lynch JF. Hereditary breast cancer: part I. Diagnosing hereditary breast cancer syndromes. *Breast J* 2008;**14**(1):3–13.
6. Lynch MA, Jackson J, Kim JA, Leeming RA. Optimal number of radioactive sentinel lymph nodes to remove for accurate axillary staging of breast cancer. *Surgery* 2008;**144**(4):525–31. discussion 531-2.
7. Henderson BE, Ross R, Bernstein L. Estrogens as a cause of human cancer: the Richard and Hinda Rosenthal Foundation award lecture. *Cancer Res* 1988;**48**(2):246–53.
8. Banerjee MR. Responses of mammary cells to hormones. *Int Rev Cytol* 1976;**47**:1–97.

9. Nandi S, Guzman RC, Yang J. Hormones and mammary carcinogenesis in mice, rats, and humans: a unifying hypothesis. *Proc Natl Acad Sci U S A* 1995;**92**(9): 3650–7.
10. Pike MC, Spicer DV, Dahmoush L, Press MF. Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. *Epidemiol Rev* 1993;**15**(1):17–35.
11. Davis DL, Bradlow HL, Wolff M, Woodruff T, Hoel DG, Anton-Culver H. Medical hypothesis: xenoestrogens as preventable causes of breast cancer. *Environ Health Perspect* 1993;**101**(5):372–7.
12. Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. *Epidemiol Rev* 1993;**15**(1):36–47.
13. Bernstein L, Ross RK. Endogenous hormones and breast cancer risk. *Epidemiol Rev* 1993;**15**(1):48–65.
14. Russo J, Hasan Lareef M, Balogh G, Guo S, Russo IH. Estrogen and its metabolites are carcinogenic agents in human breast epithelial cells. *J Steroid Biochem Mol Biol* 2003;**87**(1):1–25.
15. Mitrunen K, Hirvonen A. Molecular epidemiology of sporadic breast cancer. The role of polymorphic genes involved in oestrogen biosynthesis and metabolism. *Mutat Res* 2003;**544**(1):9–41.
16. Beral V. Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet* 2003;**362**(9382):419–27.
17. Russo J, Russo IH. The role of estrogen in the initiation of breast cancer. *J Steroid Biochem Mol Biol* 2006;**102**(1–5):89–96.
18. Cavalieri E, Chakravarti D, Guttenplan J, Hart E, Ingle J, Jankowiak R, et al. Catechol estrogen quinones as initiators of breast and other human cancers: implications for biomarkers of susceptibility and cancer prevention. *Biochim Biophys Acta* 2006;**1766**(1):63–78.
19. Ball P, Knuppen R. Catecholoestrogens (2-and 4-hydroxyoestrogens): chemistry, biogenesis, metabolism, occurrence and physiological significance. *Acta Endocrinol Suppl (Copenh)* 1980;**232**:1–127.
20. Cavalieri E, Frenkel K, Liehr G, Rogan E, Roy D. Estrogens as endogenous genotoxic agents–DNA adducts and mutations. *J Natl Cancer Inst Monogr* 2000;**27**: 75–93.
21. Cavalieri E, Rogan E, Chakravarti D. The role of endogenous catechol quinones in the initiation of cancer and neurodegenerative diseases. *Meth Enzymol* 2004;**382**:293–319.
22. Yager JD, Davidson NE. Estrogen carcinogenesis in breast cancer. *N Engl J Med* 2006;**354**(3):270–82.
23. Miyoshi Y, Takahashi Y, Egawa C, Noguchi S. Breast cancer risk associated with CYP1A1 genetic polymorphisms in Japanese women. *Breast J* 2002;**8**(4): 209–15.
24. Surekha D, Sailaja K, Rao DN, Padma T, Raghunadharao D, Vishnupriya S. Association of CYP1A1*2 polymorphisms with breast cancer risk: a case control study. *Indian J Med Sci* 2009;**63**(1):13–20.
25. Torresan C, Torrezan GT, Oliveira MM, Abuázar CS, Losi-Guembarovski R, Lima RS, et al. Genetic polymorphisms in oestrogen metabolic pathway and breast cancer: a positive association with combined CYP/GST genotypes. *Clin Exp Med* 2008;**8**(2):65–71.
26. Shin A, Kang D, Choi JY, Lee KM, Park SK, Noh DY, et al. Cytochrome P450 1A1 (CYP1A1) polymorphisms and breast cancer risk in Korean women. *Exp Mol Med* 2007;**39**(3):361–6.
27. Moreno-Galván M, Herrera-González NE, Robles-Pérez V, Velasco-Rodríguez JC, Tapia-Conyer R, Sarti E. Impact of CYP1A1 and COMT genotypes on breast cancer risk in Mexican women: a pilot study. *Int J Biol Markers* 2011;**25**(3):157–63.
28. Dunning AM, Dowsett M, Healey CS, Tee L, Luben RN, Folkerd E, et al. Polymorphisms associated with circulating sex hormone levels in postmenopausal women. *J Natl Cancer Inst* 2004;**96**(12):936–45.
29. Wen W, Cai Q, Shu X, Cheng J, Parl F, Pierce L, et al. Cytochrome P450 1B1 and catechol-O-methyltransferase genetic polymorphisms and breast cancer risk in Chinese women: results from the shanghai breast cancer study and a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2005;**14**(2):329–35.
30. Hanna IH, Dawling S, Roodi N, Guengerich P, Parl F. Cytochrome P450 1B1 (CYP1B1) pharmacogenetics: association of polymorphisms with functional differences in estrogen hydroxylation activity. *Cancer Res* 2000;**60**(13):3440–4.
31. Shimada T, Watanabe J, Kwajiri K, Sutter TR, Guengerich FP, Gillam EM, et al. Catalytic properties of polymorphic human cytochrome P450 1B1 variants. *Carcinogenesis* 1999;**20**(8):1607–13.
32. Evens AM, Gartenhaus RB. Molecular etiology of mature T-cell non-Hodgkin's lymphomas. *Front Biosci* 2003;**8**:d156–75.
33. Crofts F, Taioli E, Trachman J, Cosma GN, Currie D, Toniolo P, et al. Functional significance of different human CYP1A1 genotypes. *Carcinogenesis* 1994;**15**(12):2961–3.
34. Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melén K, Julkunen I, et al. Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry* 1995;**34**(13):4202–10.
35. Weinshilboum RM, Otterness DM, Szumlanski CL. Methylation pharmacogenetics: catechol O-methyltransferase, thiopurine methyltransferase, and histamine N-methyltransferase. *Annu Rev Pharmacol Toxicol* 1999;**39**:19–52.
36. Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics* 1996;**6**(3):243–50.
37. Jefferies H, Coster J, Khalil A, Bot J, McCauley RD, Hall JC. Glutathione. *ANZ J Surg* 2003;**73**(7):517–22.
38. Bruhn C, Brockmoller J, Kerb R, Roots I, Borchert HH. Concordance between enzyme activity and genotype of glutathione S-transferase theta (GSTT1). *Biochem Pharmacol* 1998;**56**(9):1189–93.
39. Pandya U, Srivastava SK, Singhal SS, Pal A, Awasthi S, Zimniak P, et al. Activity of allelic variants of Pi class human glutathione S-transferase toward chlorambucil. *Biochem Biophys Res Commun* 2000;**278**(1):258–62.
40. Pérez-Morales R, Méndez-Ramírez I, Castro-Hernández C, Martínez-Ramírez OC, Gonsebatt ME, et al. Polymorphisms associated with the risk of lung cancer in a healthy Mexican Mestizo population: application of the additive model for cancer. *Genet Mol Biol* 2011;**34**(4):546–52.
41. Eccles DM, Evans DG, Mackay J. Guidelines for a genetic risk based approach to advising women with a family history of breast cancer. UK Cancer Family Study Group (UKCFSG). *J Med Genet* 2000;**37**(3):203–9.
42. Negri E, Braga C, La Vecchia C, Silvia F, Parazzini F. Family history of cancer and risk of breast cancer. *Int J Cancer* 1997;**72**(5):735–8.
43. Lahiri DK, Nurnberger Jr JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991;**19**(19):5444.
44. Zheng W, Xie D, Jin F, Cheng J, Dai Q, Wen W, et al. Genetic polymorphism of cytochrome P450-1B1 and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* 2000;**9**(2):147–50.
45. Ho BC, Wassink TH, O'Leary DS, Sheffield VC, Andreasen NC. Catechol-O-methyl transferase Val158Met gene polymorphism in schizophrenia: working memory, frontal lobe MRI morphology and frontal cerebral blood flow. *Mol Psychiatry* 2005;**10**(3):229. 287–298.
46. Abdel-Rahman SZ, El-Zein RA, Anwar WA, Au WW. A multiplex PCR procedure for polymorphic analysis of GSTM1 and GSTT1 genes in population studies. *Cancer Lett* 1996;**107**(2):229–33.
47. Sergentanis TN, Economopoulos KP. Four polymorphisms in cytochrome P450 1A1 (CYP1A1) gene and breast cancer risk: a meta-analysis. *Breast Cancer Res Treat* 2010;**122**(2):459–69.
48. Ambrosone CB, Freudenheim JL, Graham S, Marshall JR, Vena JE, Brasure JR, et al. Cytochrome P450 1A1 and glutathione S-transferase (M1) genetic polymorphisms and postmenopausal breast cancer risk. *Cancer Res* 1995;**55**(16): 3483–5.
49. Charrier J, Maugard CM, Le Mevel B, Bignon YJ. Allelotype influence at glutathione S-transferase M1 locus on breast cancer susceptibility. *Br J Cancer* 1999;**79**(2):346–53.
50. Helzlsouer KJ, Selmin O, Huang HY, Stricklnd PT, Hoffman S, Alberg AJ, et al. Association between glutathione S-transferase M1, P1, and T1 genetic polymorphisms and development of breast cancer. *J Natl Cancer Inst* 1998;**90**(7): 512–8.
51. Lavigne JA, Helzlsuer KJ, Huang HY, Strickland PT, Bell DA, Selmin O, et al. An association between the allele coding for a low activity variant of catechol-O-methyltransferase and the risk for breast cancer. *Cancer Res* 1997;**57**(24): 5493–7.
52. Thompson PA, Shield PG, Freudenheim JL, Stone A, Vena JE, Marshall JR, et al. Genetic polymorphisms in catechol-O-methyltransferase, menopausal status, and breast cancer risk. *Cancer Res* 1998;**58**(10):2107–10.
53. Hatagima A. Genetic polymorphisms and metabolism of endocrine disruptors in cancer susceptibility. *Cad Saude Publica* 2002;**18**(2):357–77.
54. Zhong SL, Zhoy S, Chen X, Huang M. Rapid determination of common mutations in glutathione S-transferase gene by PCR-based methods in healthy Chinese. *Clin Chim Acta* 2006;**364**(1–2):205–8.
55. Schindler J, Li Y, Marion MJ, Paroly A, Brandt-Rauf PW. The effect of genetic polymorphisms in the vinyl chloride metabolic pathway on mutagenic risk. *J Hum Genet* 2007;**52**(5):448–55.
56. Kaushal M, Mishra AK, Raju BS, Ihsan R, Chakraborty A, Sharma J, et al. Betel quid chewing as an environmental risk factor for breast cancer. *Mutat Res* 2010;**703**(2):143–8.
57. Sasieni PD. From genotypes to genes: doubling the sample size. *Biometrics* 1997;**53**(4):1253–61.
58. Armitage P. Tests for linear trends in proportions and frequencies. *Biometrics* 1955;**11**:375–86.
59. Ritchie MD, Hahn LW, Roodi N, Bailey LR, Dupont WD, Parl FF, et al. Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. *Am J Hum Genet* 2001;**69**(1):138–47.
60. Chung Y, Lee SY, Elston RC, Park T. Odds ratio based multifactor-dimensionality reduction method for detecting gene-gene interactions. *Bioinformatics* 2007;**23**(1):71–6.
61. Rodríguez-Cuevas S, Macías Martínez CG, Labastida Almendaro S. Breast cancer in Mexico. Is it a young women disease?. *Ginecol Obstet Mex* 2000;**68**: 185–90.
62. Rodríguez-Cuevas S, Macías CG, Franceschi D, Labastida S. Breast carcinoma presents a decade earlier in Mexican women than in women in the United States or European countries. *Cancer* 2001;**91**(4):863–8.
63. Ortega-Ceballos PA, Morán C, Blanco-Muñoz J, Yunes-Díaz E, Castañeda-Iñiguez MS, Salmerón J, et al. Reproductive and lifestyle factors associated with early menopause in Mexican women. *Salud Publica Mex* 2006;**48**(4):300–7.
64. Garrido-Latorre F, Lazcano-Ponce EC, López-Carrillo L, Hernández-Avila M. Age of natural menopause among women in Mexico City. *Int J Gynaecol Obstet* 1996;**53**(2):159–66.
65. Mitrunen K, Jourenkova N, Kataja V, Eskelinen M, Kosma VM, Benhamou S, et al. Polymorphic catechol-O-methyltransferase gene and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2001;**10**(6):635–40.

66. Huang CS, Chern HD, Chang KJ, Cheng CW, Hsu SM, Shen CY. Breast cancer risk associated with genotype polymorphism of the estrogen-metabolizing genes CYP17, CYP1A1, and COMT: a multigenic study on cancer susceptibility. *Cancer Res* 1999;**59**(19):4870–5.
67. Sangrajrang S, Sato Y, Sakamoto H, Ohnami S, Laird NM, Khuhaprema T, et al. Genetic polymorphisms of estrogen metabolizing enzyme and breast cancer risk in Thai women. *Int J Cancer* 2009;**125**(4):837–43.
68. Linhares JJ, Da Silva ID, De Souza NC, Noronha EC, Ferraro O, DeCarvalho CV, et al. Genetic polymorphism of GSTM1 in women with breast cancer and interact with reproductive history and several clinical pathologies. *Biol Res* 2005;**38**(2–3):273–81.
69. Egan KM, Cai Q, Shu XO, Jin F, Zhu TL, Dai Q, et al. Genetic polymorphisms in GSTM1, GSTP1, and GSTT1 and the risk for breast cancer: results from the Shanghai Breast Cancer Study and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2004;**13**(2):197–204.
70. Opdahl S, Nilsen TI, Romundstad PR, Vanky E, Carlsen SM, Vatten LJ. Association of size at birth with adolescent hormone levels, body size and age at menarche: relevance for breast cancer risk. *Br J Cancer* 2008;**99**(1):201–6.
71. Clemons M, Goss P. Estrogen and the risk of breast cancer. *N Engl J Med* 2001;**344**(4):276–85.
72. Li DN, Seidel A, Pritchard MP, Wolf CR, Friedberg T. Polymorphisms in P450 CYP1B1 affect the conversion of estradiol to the potentially carcinogenic metabolite 4-hydroxyestradiol. *Pharmacogenetics* 2000;**10**(4):343–53.
73. Landi MT, Bertazzi PA, Shields PG, Clark G, Lucier GW, Garte SJ, et al. Association between CYP1A1 genotype, mRNA expression and enzymatic activity in humans. *Pharmacogenetics* 1994;**4**(5):242–6.
74. Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA. Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. *Carcinogenesis* 1998;**19**(2):275–80.
75. Kocabas NA, Sardas S, Cholerton S, Daly AK, Karakaya AE. Cytochrome P450 CYP1B1 and catechol O-methyltransferase (COMT) genetic polymorphisms and breast cancer susceptibility in a Turkish population. *Arch Toxicol* 2002;**76**(11):643–9.
76. Jiao H, Liu C, Guo W, Peng L, Chen Y, Martin FL. Association of CYP1B1 polymorphisms with breast cancer: a case-control study in the Han population in Ningxia Hui Autonomous Region, P. R. China. *Biomark Insights* 2010;**5**:21–7.
77. Okobia MN, Bunkar CH, Garte SJ, Zmuda JM, Ezeome ER, Anyanwu NC, et al. Cytochrome P450 1B1 Val432Leu polymorphism and breast cancer risk in Nigerian women: a case control study. *Infect Agent Cancer* 2009;**4**(Suppl. 1):S12.
78. Wu AH, Tseng CC, Van Den Band Yu MC. Tea intake, COMT genotype, and breast cancer in Asian-American women. *Cancer Res* 2003;**63**(21):7526–9.
79. Sazci A, Erqul E, Utkan NZ, Cantuik NZ, Ky G. Catechol-O-methyltransferase Val 108/158 Met polymorphism in premenopausal breast cancer patients. *Toxicology* 2004;**204**(2–3):197–202.