



BRAZILIAN JOURNAL
OF MEDICAL AND BIOLOGICAL RESEARCH

www.bjournal.com.br

ISSN 1414-431X
Volume 45 (10) 875-994 October 2012

BIOMEDICAL SCIENCES

Braz J Med Biol Res, October 2012, Volume 45(10) 891-897

doi: 10.1590/S0100-879X2012007500081

Prevalence of *ERα-397 PvuII C/T*, *ERα-351 XbaI A/G* and *PGR PROGINS* polymorphisms in Brazilian breast cancer-unaffected women

J. Giacomazzi, E. Aguiar, E.I. Palmero, A.V. Schmidt, G. Skonieski, D.D. Filho, H. Bock, M.L. Saraiva-Pereira, I.P. Ewald, L. Schuler-Faccini, S.A. Camey, M. Caleffi, R. Giugliani and P. Ashton-Prolla

The Brazilian Journal of Medical and Biological Research is partially financed by



Ministério
da Ciência e Tecnologia



Ministério
da Educação



Institutional Sponsors



Explore High - Performance MS
Orbitrap Technology
In Proteomics & Metabolomics



All the contents of this journal, except where otherwise noted, is licensed under a [Creative Commons Attribution License](https://creativecommons.org/licenses/by-nc/4.0/)

Prevalence of *ERα-397 PvuII C/T*, *ERα-351 XbaI A/G* and *PGR PROGINS* polymorphisms in Brazilian breast cancer-unaffected women

J. Giacomazzi^{1,2,3}, E. Aguiar^{1,2,3}, E.I. Palmero⁴, A.V. Schmidt⁵, G. Skonieski³, D.D. Filho³, H. Bock^{6,7}, M.L. Saraiva-Pereira^{6,7,8,9,10}, I.P. Ewald^{1,2}, L. Schuler-Faccini^{9,10,11}, S.A. Camey¹², M. Caleffi³, R. Giugliani^{1,6,9,10,11} and P. Ashton-Prolla^{1,2,3,9,10,11}

¹Programa de Pós-Graduação em Medicina: Ciências Médicas; Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brasil

²Laboratório de Medicina Genômica, Centro de Pesquisa Experimental, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, RS, Brasil

³Núcleo Mama Porto Alegre, Associação Hospitalar Moinhos de Vento, Porto Alegre, RS, Brasil

⁴International Agency for Research on Cancer (IARC), Lyon, France

⁵Departamento de Estatística, UFRGS, Porto Alegre, RS, Brasil

⁶Laboratório de Identificação Genética, HCPA, Porto Alegre, RS, Brasil

⁷Programa de Pós-Graduação em Biologia Celular e Molecular, UFRGS, Porto Alegre, RS, Brasil

⁸Departamento de Bioquímica, UFRGS, Porto Alegre, RS, Brasil

⁹Serviço de Genética Médica, HCPA, Porto Alegre, RS, Brasil

¹⁰Programa de Pós-Graduação em Genética e Biologia Molecular, UFRGS, Porto Alegre, RS, Brasil

¹¹Departamento de Genética, UFRGS, Porto Alegre, RS, Brasil

¹²Departamento de Estatística, Instituto de Matemática, UFRGS, Porto Alegre, RS, Brasil

Abstract

Polymorphisms of hormone receptor genes have been linked to modifications in reproductive factors and to an increased risk of breast cancer (BC). In the present study, we have determined the allelic and genotypic frequencies of the *ERα-397 PvuII C/T*, *ERα-351 XbaI A/G* and *PGR PROGINS* polymorphisms and investigated their relationship with mammographic density, body mass index (BMI) and other risk factors for BC. A consecutive and unselected sample of 750 Brazilian BC-unaffected women enrolled in a mammography screening program was recruited. The distribution of *PGR PROGINS* genotypic frequencies was 72.5, 25.5 and 2.0% for *A1A1*, *A1A2* and *A2A2*, respectively, which was equivalent to that encountered in other studies with healthy women. The distribution of *ERα* genotypes was: *ERα-397 PvuII C/T*: 32.3% *TT*, 47.5% *TC*, and 20.2% *CC*; *ERα-351 XbaI A/G*: 46.3% *AA*, 41.7% *AG* and 12.0% *GG*. *ERα* haplotypes were 53.5% *PX*, 14.3% *Px*, 0.3% *pX*, and 32.0% *px*. These were significantly different from most previously published reports worldwide ($P < 0.05$). Overall, the *PGR PROGINS* genotypes *A2A2* and *A1A2* were associated with fatty and moderately fatty breast tissue. The same genotypes were also associated with a high BMI in postmenopausal women. In addition, the *ERα-351 XbaI GG* genotype was associated with menarche ≥ 12 years ($P = 0.02$). *ERα* and *PGR* polymorphisms have a phenotypic effect and may play an important role in BC risk determination. Finally, if confirmed in BC patients, these associations could have important implications for mammographic screening and strategies and may be helpful to identify women at higher risk for the disease.

Key words: Genetic polymorphisms; Estrogen receptor gene; Progesterone receptor gene; Breast cancer susceptibility

Introduction

Breast cancer (BC) is the most prevalent form of cancer in women worldwide. In Brazil, BC is a significant public health problem due to its morbidity, and high incidence and mortality rates. It is the first cause of cancer-related deaths

in women of all ages, about half of the affected women are diagnosed in advanced stages of the disease and, not surprisingly, mortality rates are still increasing. Porto Alegre, Brazil's southernmost capital, has one of the highest BC

Correspondence: P. Ashton-Prolla, Departamento de Genética, UFRGS e Serviços de Genética Médica e Centro de Pesquisa Experimental, HCPA, Rua Ramiro Barcelos, 2350, 90035-903 Porto Alegre, RS, Brasil. Fax: +55-51-3359-8010. E-mail: pprolla@hcpa.ufrgs.br

Received November 18, 2011. Accepted April 18, 2012. Available online May 18, 2012. Published September 3, 2012.

incidence rates in the country, estimated at 125 per 100,000 individuals for the year 2012 as compared to the average national rate of 52 per 100,000 individuals (1).

Polymorphisms in genes coding for hormone receptors have been linked to modifications in reproductive factors and to an increased BC risk in different populations. Among these, estrogen receptor (*ER*) and progesterone receptor (*PGR*) genes have been extensively studied in different populations.

Estrogen receptor

In the breast, estrogens bind to specific receptors with high affinity, triggering DNA synthesis, cell division, and proliferation of the breast epithelial cells. Two types of ERs have been identified, *ER α* and *ER β* , and the former, also named *ESR1*, is an important mediator of hormonal response in estrogen-sensitive tissues such as breast, endometrium, and bone (2,3). The *ER α* gene has been mapped to 6q25.1 and its two most commonly described single nucleotide polymorphisms are *ER α -397 PvuII C/T* (rs2234693) and *ER α -351 XbaI A/G* (rs9340799). Both are in strong linkage disequilibrium and have been associated with hormonal modifications and increase BC risk (3).

ER α -397 C/T polymorphism. Some studies have reported an increase BC risk associated with the *ER α -397 T* allele, and have suggested that this allele modulates the effect of hormone replacement therapy (HRT) on mammographic density and is independently associated with increased mammographic density (2-6). In addition, the *ER α -397 TT* genotype has been associated with an increased risk of BC diagnosis at a younger age, associated with a higher number of pregnancies, later age at menarche and use of oral contraceptives, and some studies have suggested that this genotype is more frequent in women with a positive family history of the disease (4,7).

ER α -351 XbaI A/G polymorphism. Some investigators have suggested that the *ER α -351 A* allele modulates the effect of HRT on mammographic density, and an independent study by the same authors demonstrated an association between the *ER α -351 A* allele and increased mammographic density regardless of HRT (4). The *ER α -351 AA* genotype was associated with a slight increase in body mass index (BMI), which could ultimately influence BC risk (4). Finally, a few haplotype studies involving both the *ER α -397 PvuII C/T* and *ER α -351 XbaI A/G* polymorphisms have indicated that the increase in BC risk may be particularly significant in the presence of certain haplotypes, such as the *ER α -397-ER α -351A-A-T-T* haplotype, that is associated with a relative risk for BC of 1.5 (3,8). There is no report on the genotypic and/or allelic frequencies of the *ER α -397 PvuII C/T* and *ER α -351 XbaI A/G* polymorphisms and their associated BC risk in Brazilian individuals.

Progesterone receptor

Progesterone participates in the regulation of most fe-

male reproductive processes, targeting the ovaries (release of mature oocytes), uterus (promotion of implantation and maintenance of pregnancy), and mammary glands (suppression of lactation before parturition). The biological actions of progesterone are mediated by the progesterone receptor (PR), which belongs to the steroid-retinoic acid receptor superfamily and is encoded by a single copy gene located on chromosome 11q22-23 (9).

PGR PROGINS polymorphism (rs1042838). One of the most widely studied polymorphisms in the *PGR* gene has been associated with abnormal gene expression and has a complex structure, consisting of a 306-bp *Alu* insertion in intron 7 and two sequence variations in exons 4 and 5 of the *PGR* gene, *Val660Leu* and *His770His* (10,11).

The polymorphic allele has been denominated A2 and its wild-type counterpart, A1. Theoretically, women carrying the A2 *PGR PROGINS* allele would have an increased risk of developing malignancies in organs where progesterone exposure has a protective effect, such as ovary and endometrium (12). In the breast, where progesterone exposure has no protective effect, but rather stimulates cell proliferation, *PGR PROGINS* theoretically would reduce the risk of BC (13).

Although some studies have reported an association of *PGR PROGINS* with increased lifetime risk for developing BC (14,15), data are conflicting and several other studies have shown an inverse relationship of the polymorphism with BC (13,16). An overt protective effect of the PR A2 allele was described by Wang-Gohrke et al. (17) in premenopausal women. The only Brazilian study on the association of *PGR PROGINS* with BC risk showed no statistically significant difference in its frequency between BC cases and controls (18).

Given the scarce data on the frequency and relevance of these polymorphisms in relation to BC risk in Brazilian women, the objective of the present study was to determine the genotypic and allelic frequencies of common *ER* and *PGR* gene polymorphisms and their relationship with BC risk factors in a sample of BC-unaaffected women undergoing mammographic screening in an area with high BC incidence and mortality rates.

Material and Methods

Study population

A consecutive and unselected sample of 750 BC-unaaffected women (of a total of 890; age 40-69 years) enrolled in a mammography screening program in the city of Porto Alegre (Núcleo Mama Porto Alegre, NMPOA Cohort) was recruited for this study during routine mammographic visits between November 2005 and March 2006 (19). Study approval was obtained from the Ethics Committees of the participating institutions and all individuals recruited for the study gave written informed consent. Demographic and clinical information as well as results from mammographic

screening were obtained from chart review.

Study variables

Study variables included age at recruitment, age at first childbirth, age at menarche and menopause, parity, BMI (classified into 3 categories: <18.5; 18.5-24.99; 25-29.99; ≥30), self-reported skin color - assessed by self-denomination (white or non-white), results of the mammographic examination and mammographic density (using the BIRADS and breast density categories of the American College of Radiology), previous breast biopsies, use of HRT and/or oral contraceptives. Five-year and lifetime risk of developing BC was estimated using the Gail model (20).

Polymorphism analysis

Genomic DNA was obtained from peripheral blood samples using a commercial DNA extraction kit (Illustra Blood genomicPrep Mini Spin Kit, GE Healthcare, UK). Genotyping was performed using the TaqMan PCR assay for *ERα-397 PvuII C/T* and *ERα-351 XbaI A/G* polymorphisms (rs2234693, C_3163590_10 and rs9340799, C_3163591_10, respectively; Applied Biosystems, USA), with fluorescent minor groove binding probes. Analyses were performed with an ABI 7500 real-time PCR system (Applied Biosystems), and results were analyzed with the Sequence Detection Software v 1.4 (Applied Biosystems). Genotyping of the *PGR PROGINS* polymorphism was based on the PCR amplification of a fragment encompassing the 306-bp insertion in intron 7. The A1 allele was defined as "absence of the insertion", according to previous citations (17). The PCR products were resolved by agarose gel electrophoresis and visualized under UV light. The A1 allele appeared as a 175-bp fragment and the A2 *PGR PROGINS* allele, as a 481-bp fragment.

Statistical analysis

SPSS version 14.0 was used for data handling and statistical analyses. For descriptive analysis, categorical variables were described by their absolute and/or relative frequencies and quantitative variables are reported as means ± SD. For analytical statistics, the *t*-test for independent variables and ANOVA were used to compare the mean values of the quantitative variables. The existence of an associa-

tion between categorical variables was examined by the chi-square test. The level of significance was set at 0.05 for all analyses. Comparative analysis of genotypic frequencies between this and other studies was done using WINPEPI (PEPI-for-Windows).

Results

Data on BC risk factors identified in the sample of cancer-affected women is presented in Table 1. The allelic and genotypic frequencies of the *ERα-397 PvuII C/T*, *ERα-351 XbaI A/G* and *PGR PROGINS* polymorphisms in the overall sample, and in both white and non-white women followed Hardy-Weinberg equilibrium and no significant differences in allelic or genotypic frequencies were observed between white and non-white women (Table 2).

The genotypic frequencies of all three polymorphism studied were significantly different from those de-

Table 1. Breast cancer risk factors in the sample studied (N = 750).

Variable	N (%)	Mean ± SD
Age at assessment (years)	-	51.0 ± 7.6
Age at menarche (years)	-	12.8 ± 1.8
Age at first childbirth (years)	-	22.0 ± 5.3
Nulliparous	31 (4.13)	-
Postmenopausal	421 (56.1)	-
Age at menopause (years)	-	46.9 ± 5.5
Use of hormone replacement	118 (28.1)	-
Body mass index (kg/m ²)	-	29.6 ± 5.8
<18.5	6 (0.8)	-
≥18.5-24.99	158 (21.1)	-
25-29.99	273 (36.4)	-
≥30	308 (41.1)	-
Family history of breast cancer*	100 (13.3)	-
Mammographic examination		
BIRADS 1 or BIRADS 2	732 (97.6)	
BIRADS 3	9 (1.2)	
BIRADS 4	5 (1.1)	
Mammographic density		
Fatty breast tissue or moderately fatty breast tissue	426 (56.8)	-
Moderately dense, dense or heterogeneously dense tissue	324 (43.2)	-
Estimated lifetime risk of developing breast cancer (using the Gail model)	-	7.8 ± 3.3
Previous breast biopsy	40 (5.3)	-

*Family history (FH) of breast cancer - if one of these questions was positive: 1) FH of breast or ovarian cancer in 1st degree relatives; 2) FH of bilateral breast cancer; 3) any man in the family with breast cancer; 4) any woman in the family with breast and ovarian cancer; 5) any woman in the family with breast cancer before the age of 50 years; 6) 2 or more relatives with breast and/or ovarian cancer; 7) 2 or more relatives with breast and/or bowel cancer.

Table 2. Genotypic and allelic frequencies of the *ERα-397 PvuII C/T*, *ERα-351 XbaI A/G* and *PGR PROGINS* polymorphisms in the overall sample (N = 750), and in white (N = 599) and non-white women (N = 151).

Name	Genotypic frequencies, N (%)			Allelic frequencies		P*	HWE**
<i>ERα-397 PvuII C/T</i>	<i>TT</i>	<i>TC</i>	<i>CC</i>	<i>T</i>	<i>C</i>		
Overall	242 (32.3)	356 (47.5)	152 (20.2)	0.56	0.44	-	1.00
						0.74	
White	195 (32.5)	286 (47.8)	118 (19.7)	0.56	0.44	-	0.50
Non-white	47 (31.1)	70 (46.4)	34 (22.5)	0.54	0.46	-	0.66
<i>ERα-351 XbaI A/G</i>	<i>AA</i>	<i>AG</i>	<i>GG</i>	<i>A</i>	<i>G</i>		
Overall	347 (46.3)	313 (41.7)	90 (12.0)	0.67	0.33	-	2.20
						0.42	
White	270 (45.1)	255 (42.6)	74 (12.3)	0.66	0.34	-	1.30
Non-white	77 (51.0)	58 (38.4)	16 (10.6)	0.70	0.30	-	1.00
<i>PGR PROGINS</i>	<i>A2A2</i>	<i>A1A2</i>	<i>A1A1</i>	<i>A2</i>	<i>A1</i>		
Overall	15 (2.0)	191 (25.5)	544 (72.5)	0.15	0.85	-	0.14
						0.39	
White	13 (2.2)	158 (26.4)	428 (71.4)	0.15	0.85	-	0.13
Non-white	2 (1.3)	33 (21.9)	116 (76.8)	0.12	0.88	-	0.04

*P = white versus non-white (chi-square test). **HWE = Hardy-Weinberg equilibrium (chi-square test).

scribed in other studies worldwide, but not different from frequencies published in a few studies with Brazilian BC-unaffected individuals (see Tables S1, S2, and S3).

Women with the *A2A2* and *A1A2* *PGR PROGINS* genotypes had a higher average BMI compared to women with the *A1A1* genotype (Table 3). In addition, the *A2A2* and *A1A2* *PR PROGINS* genotypes were encountered significantly more often in postmenopausal women with fatty and moderately fatty breast tissue. A significant association of the *GG* genotype with age at menarche (≥ 12 years) was found for the *ERα-351 XbaI A/G* polymorphism and its genotypes (*AA + AG* versus *GG*). No other significant associations of a particular genotype, or genotype combinations with the clinical characteristics of the women were found.

Finally, the haplotype frequencies of the *ERα-397 PvuII C/T* and *ERα-351 XbaI A/G* polymorphisms were assessed (Table 4). No significant association was observed between the breast cancer risk haplotype (*A-A-T-T*) versus other haplotypes in relation to breast density ($P = 0.47$), mean BMI ($P = 0.90$), mean estimated lifetime risk of developing BC ($P = 0.12$), and mean ages at menarche ($P = 0.31$) and menopause ($P = 0.75$).

Discussion

Normal breast tissue proliferation is very sensitive to the action of steroid hormones and their action is mediated

by specific receptors. The current literature shows that, in certain populations, specific polymorphisms in hormone receptor genes may be associated with an increased risk of developing BC. Knowledge about the allelic and genotypic frequencies of such polymorphisms and their interaction with other BC risk factors may be helpful to identify women at higher risk for the disease. In this study conducted on a population-based sample of breast cancer-unaffected women, we describe the allelic and genotypic frequencies of selected polymorphisms in the *ER* and *PR* genes that have been previously shown to modify BC risk in other populations and describe their relationship with established BC risk factors.

The genotypic frequencies observed for the *PGR PROGINS* polymorphism were comparable to those reported in other studies on Brazilian and non-Brazilian populations (12,17,18,29,30). On the other hand, the genotypic frequencies of the *ERα* gene polymorphisms were significantly different from those described in most studies from other populations, emphasizing the importance of the population-specific determination of such frequencies (2-5,7,31-33).

No significant reproductive risk factors for BC were identified in the sample of women studied; i.e., mean age at menarche was above 12 years, mean age at first childbirth was well below age 30 years, mean age at menopause was below the age of 50 years and only a small proportion of

women were nulliparous. However, mean BMI was above 25 and a significant proportion of women studied had a BMI ≥ 30 .

Considering this unexpected prevalence of overweight and obesity in this unselected population-based sample of women, and that the *ER α* gene has been implicated in adiposity, lipid metabolism and feeding behavior, we also investigated a possible relationship between these polymorphisms and BMI, but no significant correlation was identified.

The progesterone receptor, the product of the *PGR* gene, mediates interactions between the estrogen, insulin and insulin growth factor (IGF) hormonal pathways and hyperinsulinemia, increased free IGF and increased circulating estrogen concentrations have each been associated with obesity in postmenopausal women (34). However, a direct association of this polymorphism with increased body weight remains elusive and additional studies are necessary to clarify the relevance of this observation and ultimately its relationship with increased BC risk.

ER α -351 GG genotype and age at menarche ≥ 12 years are in contrast to a previous report where menarche tended to occur 6 months later in girls with the *ER α -351 AA* genotype (8). It is well known that *ER α* gene polymorphisms can impact the maturation of the hypothalamic-pituitary-gonadal axis, which determines the onset of menarche and thus, although the exact biological mechanism explaining the relationship between *ER α -351 XbaI A/G* and age at menarche remains unknown, one could hypothesize that the association between *ER α -351 AA* genotype and increased BC risk could be influenced at least in part by reproductive features such as earlier age at menarche.

The distribution of *ER α -351 XbaI* and *ER α -397 Pvull* haplotypes was significantly different from that previously observed in populations of European and Asian ethnicity and approached that observed in only one previous study with a small sample of African and African-American individuals. This is an unexpected finding because, although the contemporary Brazilian population is highly admixed, the African contribution to the genetic pool in Southern Brazil is quite low, as compared to other regions of the country, with a clear predominance of European alleles. Price et al. (35), for instance, have estimated that African and European ancestry corresponds to 11 and 71% of the alleles in Brazilian individuals. Alves-Silva et al. (36), studying mitochondrial and nuclear alleles, had already described a differential distribution of Amerindian, African and European alleles and found a surprisingly high African and Amerindian contribution in white Brazilian individuals. However, in Southern Brazil, the major contribution was still European, both in mitochondrial (66%) and nuclear

Table 3. Genotypes and breast cancer risk factors in the overall sample and in only postmenopausal women.

Variable	<i>PGR PROGINS</i>		P
	Genotypes		
	A1A2 + A2A2	A1A1	
Body mass index (mean \pm SD)			
Overall (N = 750)	30.3 \pm 6.0	29.3 \pm 5.7	0.041*
Postmenopausal (N = 421)	30.5 \pm 6.0	29.3 \pm 5.8	0.056*
Mammographic density, N (%)			
Overall (N = 750)			0.021#
A + B	131 (63.6)	295 (54.2)	
C + D + E	75 (36.4)	249 (45.8)	
Postmenopausal (N = 421)			0.044#
A + B	99 (74.4)	186 (64.6)	
C + D + E	34 (25.6)	102 (35.4)	
Variable	<i>ERα-351 XbaI A/G</i>		P
	Genotypes		
	AA + AG	GG	
Menarche, N (%)			
Overall (N = 750)			0.060#
<12 years	149 (22.6)	17 (18.9)	
12-13 years	306 (46.5)	34 (37.8)	
≥ 14 years	203 (30.9)	39 (43.3)	
Postmenopausal (N = 421)			0.021#
<12 years	81 (22.1)	8 (15.1)	
12-13 years	164 (44.7)	17 (32.1)	
≥ 14 years	122 (33.2)	28 (52.8)	

Mammographic density categories: A = fatty breast tissue; B = moderately fatty breast tissue; C = moderately dense breast tissue; D = dense; E = heterogeneously dense. *t-test. #Chi-square.

(79%) DNA. Thus, our observation of a haplotype distribution that is entirely different from all other reports in European populations is surprising and warrants further investigation. Finally, the observed frequency of the *px* haplotype, theoretically associated with an increased risk of BC, was the lowest among all studies published previously, and the inverse was observed for the *PX* haplotype.

Although a few significant associations between selected *ER* and *PR* polymorphisms and BC risk factors were identified in this population-based sample of women from a region with a high BC incidence rate, their exact role remains controversial and additional case-control studies are necessary to determine if they are indeed associated with an increased risk for the disease. Finally, if confirmed in other populations, the associations found in this study

Table 4. Frequencies of the *ERα-397 PvuII* and *ERα-351 XbaI* haplotypes.

Haplotypes	Origin	Country	P	<i>PX</i>		<i>Px</i>		<i>pX</i>		<i>px</i>	
				N	%	N	%	N	%	N	%
Present study*	Brazil	Brazil	0.13**	401	53.5	107	14.3	2	0.3	240	32.0
Kobayashi et al., 1996 (21)	Asia	Korea	<0.01	44	18.7	63	26.5	1	0.3	130	54.5
Han et al., 1999 (22)	Asia	Korea	<0.01	110	18.5	129	21.5	14	2.3	345	57.7
Patel et al., 2000 (23)	Europe	Canada	<0.01	236	35.6	63	9.5	0	0	363	54.9
Becherini et al., 2000 (24)	Europe	Italy	<0.01	249	40.9	35	5.7	8	1.3	318	52.1
Bagger et al., 2000 (25)	Europe	Denmark	<0.01	153	33.7	60	13.3	0	0	241	53.0
Albagha et al., 2001 (26)	Europe	UK	<0.01	69	33.5	19	9.2	2	12.0	116	56.1
Yamada et al., 2002 (27)	Asia	Korea	<0.01	410	18.3	499	22.3	0	0	1329	59.4
Van Meurs et al., 2003 (28)	Africa and USA	USA/Sub-Saharan Africa***	0.64	19	50.0	3	13.6	0	0	7	36.8

*Haplotype definitions and frequencies obtained in the present study: *PX* = C-C-G-G (N = 87, 11.6%); C-C-G-A (N = 57, 7.6%); C-T-G-G (N = 3, 0.4%); C-T-G-A (N = 254, 33.9%). *Px* = C-C-A-A (N = 8, 1.1%); C-T-A-A (N = 99, 13.2%). *pX* = T-T-G-G (N = 0, 0%); T-T-G-A (N = 2, 0.3%). *px* = T-T-A-A (N = 240, 32.0%). **P (chi-square test) between white and non-white women in the present study. ***Ten samples from African-American individuals and 9 samples from African individuals deposited in the Coriell Institute (Camden, NJ, USA).

could have important implications for mammographic screening strategies.

Supplementary Material

Table S1
Table S2
Table S3

Acknowledgments

The Núcleo Mama (NMPOA) Cohort, from which the patients derive, is maintained by Associação Hospitalar

Moinhos de Vento in partnership with Instituto da Mama do Rio Grande do Sul and the Municipal Health Agency from Porto Alegre. The authors are indebted to Giovana Skonieski, Bernardete Weber, Karen Barboza de Pereira, Ademar Bedin Júnior, Fávio Marcel Telis Gonzalez, Luciano Artico, and the NMPOA team for their help with the recruitment of the patients included in this study. Research supported in part by CNPq (Edital MCT-CNPq/MS-SCTIE-DECIT/CT-Saude #06/2005, protocol #400949/2005-9). S.G. Komen for the Cure (population specific grant #POP0403033) and Fundo de Incentivo à Pesquisa do Hospital de Clínicas de Porto Alegre (FIPE/HCPA, #05-182). P. Ashton-Prolla is supported by CNPq and J. Giacomazzi received a fellowship from CAPES.

References

- Instituto Nacional do Câncer (INCA). <http://www.inca.gov.br>. Accessed December 10, 2010.
- Shin A, Kang D, Nishio H, Lee MJ, Park SK, Kim SU, et al. Estrogen receptor alpha gene polymorphisms and breast cancer risk. *Breast Cancer Res Treat* 2003; 80: 127-131.
- Onland-Moret NC, van Gils CH, Roest M, Grobbee DE, Peeters PH. The estrogen receptor alpha gene and breast cancer risk (The Netherlands). *Cancer Causes Control* 2005; 16: 1195-1202.
- van Duijnhoven FJ, Bezemer ID, Peeters PH, Roest M, Uitterlinden AG, Grobbee DE, et al. Polymorphisms in the estrogen receptor alpha gene and mammographic density. *Cancer Epidemiol Biomarkers Prev* 2005; 14: 2655-2660.
- Hu Z, Song CG, Lu JS, Luo JM, Shen ZZ, Huang W, et al. A multigenic study on breast cancer risk associated with genetic polymorphisms of ER Alpha, COMT and CYP19 gene in BRCA1/BRCA2 negative Shanghai women with early onset breast cancer or affected relatives. *J Cancer Res Clin Oncol* 2007; 133: 969-978.
- van Duijnhoven FJ, Peeters PH, Warren RM, Bingham SA, Uitterlinden AG, van Noord PA, et al. Influence of estrogen receptor alpha and progesterone receptor polymorphisms on the effects of hormone therapy on mammographic density. *Cancer Epidemiol Biomarkers Prev* 2006; 15: 462-467.
- Shen Y, Li DK, Wu J, Zhang Z, Gao E. Joint effects of the CYP1A1 MspI, ERalpha PvuII, and ERalpha XbaI polymorphisms on the risk of breast cancer: results from a population-based case-control study in Shanghai, China. *Cancer Epidemiol Biomarkers Prev* 2006; 15: 342-347.
- Jakimiuk A, Nowicka M, Bogusiewicz M, Adamiak A, Skorupski P, Miotla P, et al. Prevalence of estrogen receptor alpha PvuII and XbaI polymorphism in population of Polish postmenopausal women. *Folia Histochem Cytobiol* 2007; 45: 331-338.
- Rousseau-Merck MF, Misrahi M, Loosfelt H, Milgrom E, Berger R. Localization of the human progesterone receptor

- gene to chromosome 11q22-q23. *Hum Genet* 1987; 77: 280-282.
10. De Vivo I, Hankinson SE, Colditz GA, Hunter DJ. A functional polymorphism in the progesterone receptor gene is associated with an increase in breast cancer risk. *Cancer Res* 2003; 63: 5236-5238.
 11. Fabjani G, Tong D, Czerwenka K, Schuster E, Speiser P, Leodolter S, et al. Human progesterone receptor gene polymorphism PROGINS and risk for breast cancer in Austrian women. *Breast Cancer Res Treat* 2002; 72: 131-137.
 12. Romano A, Lindsey PJ, Fischer DC, Delvoux B, Paulussen AD, Janssen RG, et al. Two functionally relevant polymorphisms in the human progesterone receptor gene (+331 G/A and progins) and the predisposition for breast and/or ovarian cancer. *Gynecol Oncol* 2006; 101: 287-295.
 13. Dunning AM, Healey CS, Pharoah PD, Teare MD, Ponder BA, Easton DF. A systematic review of genetic polymorphisms and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 1999; 8: 843-854.
 14. Garret E, Rowe SM, Coughlan SJ. Mendelian inheritance of a TaqI restriction fragment length polymorphism due to an insertion in the human progesterone receptor gene and its allelic imbalance in breast cancer. *Cancer Res, Ther Contr* 1995; 4: 217-222.
 15. Kieback DG, Tong XW, Weigel NL, Agoulnik IU. A genetic mutation in progesterone receptor (*PROGINS*) leads to an increased risk of non-familial breast and ovarian cancer causing inadequate control of estrogen receptor driven proliferation. *J Soc Gynecol Invest* 1998; 5: 40A.
 16. Pearce CL, Hirschhorn JN, Wu AH, Burt NP, Stram DO, Young S, et al. Clarifying the *PROGINS* allele association in ovarian and breast cancer risk: a haplotype-based analysis. *J Natl Cancer Inst* 2005; 97: 51-59.
 17. Wang-Gohrke S, Chang-Claude J, Becher H, Kieback DG, Runnebaum IB. Progesterone receptor gene polymorphism is associated with decreased risk for breast cancer by age 50. *Cancer Res* 2000; 60: 2348-2350.
 18. Linhares JJ, Silva IDCG, Souza NCN, Noronha EC, Coelho E, Ferraro O, et al. Polimorfismo do gene do receptor de progesterona (*PROGINS*) em mulheres com câncer de mama: estudo caso-controlado. *Rev Bras Ginecol Obstet* 2005; 27: 473-478.
 19. Caleffi M, Ashton-Prolla P, Weber B, Zignani JM, Dias EC, Antunes LP, et al. Breast cancer screening in 10.000 women of an underserved population in South Brazil: The NMAMAPOA cohort. *J Clin Oncol* 2005; 23: 1020.
 20. Gail MH, Brinton LA, Byar DP, Corle DK, Green SB, Schairer C, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. *J Natl Cancer Inst* 1989; 81: 1879-1886.
 21. Kobayashi S, Inoue S, Hosoi T, Ouchi Y, Shiraki M, Orimo H. Association of bone mineral density with polymorphism of the estrogen receptor gene. *J Bone Miner Res* 1996; 11: 306-311.
 22. Han K, Choi J, Moon I, Yoon H, Han I, Min H, et al. Non-association of estrogen receptor genotypes with bone mineral density and bone turnover in Korean pre-, peri-, and postmenopausal women. *Osteoporos Int* 1999; 9: 290-295.
 23. Patel MS, Cole DE, Smith JD, Hawker GA, Wong B, Trang H, et al. Alleles of the estrogen receptor alpha-gene and an estrogen receptor cotranscriptional activator gene, amplified in breast cancer-1 (AIB1), are associated with quantitative calcaneal ultrasound. *J Bone Miner Res* 2000; 15: 2231-2239.
 24. Becherini L, Gennari L, Masi L, Mansani R, Massart F, Morelli A, et al. Evidence of a linkage disequilibrium between polymorphisms in the human estrogen receptor alpha gene and their relationship to bone mass variation in postmenopausal Italian women. *Hum Mol Genet* 2000; 9: 2043-2050.
 25. Bagger YZ, Jorgensen HL, Heegaard AM, Bayer L, Hansen L, Hassager C. No major effect of estrogen receptor gene polymorphisms on bone mineral density or bone loss in postmenopausal Danish women. *Bone* 2000; 26: 111-116.
 26. Albagha OM, McGuigan FE, Reid DM, Ralston SH. Estrogen receptor alpha gene polymorphisms and bone mineral density: haplotype analysis in women from the United Kingdom. *J Bone Miner Res* 2001; 16: 128-134.
 27. Yamada Y, Ando F, Niino N, Ohta S, Shimokata H. Association of polymorphisms of the estrogen receptor alpha gene with bone mineral density of the femoral neck in elderly Japanese women. *J Mol Med* 2002; 80: 452-460.
 28. van Meurs JB, Schuit SC, Weel AE, van der Klift M, Bergink AP, Arp PP, et al. Association of 5' estrogen receptor alpha gene polymorphisms with bone mineral density, vertebral bone area and fracture risk. *Hum Mol Genet* 2003; 12: 1745-1754.
 29. Carvalho CV, D'Amora P, Sato H, Girão MJBC, Lima GR, da Silva IDCG, et al. Polimorfismo do gene do receptor de progesterona (*PROGINS*) em mulheres com endometriose pélvica. *Rev Bras Ginecol Obst* 2004; 26: 613-617.
 30. Gomes MTV, Castro RA, Villanova FE, Silva IDCG, Baracat ED, Lima GR. Relação entre polimorfismo do gene do receptor de progesterona, raça, paridade e ocorrência de leiomioma uterino. *Rev Bras Ginecol Obstet* 2006; 28: 278-284.
 31. Hsieh YY, Wang YK, Chang CC, Lin CS. Estrogen receptor alpha-351 XbaI*G and -397 PvuII*C-related genotypes and alleles are associated with higher susceptibilities of endometriosis and leiomyoma. *Mol Hum Reprod* 2007; 13: 117-122.
 32. Molvarec A, Ver A, Fekete A, Rosta K, Derzbach L, Derzsy Z, et al. Association between estrogen receptor alpha (*ESR1*) gene polymorphisms and severe preeclampsia. *Hypertens Res* 2007; 30: 205-211.
 33. Molvarec A, Szeplaki G, Kovacs M, Szeplaki Z, Fazakas A, Prohaszka Z, et al. Estrogen receptor alpha (*ESR1*) PvuII and XbaI gene polymorphisms in ischemic stroke in a Hungarian population. *Clin Chim Acta* 2007; 382: 100-105.
 34. Wasserman L, Flatt SW, Natarajan L, Laughlin G, Matusalem M, Faerber S, et al. Correlates of obesity in postmenopausal women with breast cancer: comparison of genetic, demographic, disease-related, life history and dietary factors. *Int J Obes Relat Metab Disord* 2004; 28: 49-56.
 35. Price AL, Patterson N, Yu F, Cox DR, Waliszewska A, McDonald GJ, et al. A genomewide admixture map for Latino populations. *Am J Hum Genet* 2007; 80: 1024-1036.
 36. Alves-Silva J, da Silva Santos M, Guimaraes PE, Ferreira AC, Bandelt HJ, Pena SD, et al. The ancestry of Brazilian mtDNA lineages. *Am J Hum Genet* 2000; 67: 444-461.