Journal of
Cancer Science & Therapy

Souza et al., J Cancer Sci Ther 2014, 6.5
DOI: 10.4172/1948-5956.1000262

Research Article Open Access

Clinical Factors Associated with High Mammographic Density in Postmenopausal Women and their Relationship with Dinucleotide *Gtn* Repeat Polymorphism in the Estrogen Receptor Alpha Gene

Marilene Alicia Souza[1]*, Angela Maggio da Fonseca[1], Vicente Renato Bagnoli[1], Nestor de Barros[1], Victor Hugo Souza Hortense[2], Kátia C Carvalho[3], José Maria Soares-Jr[1] and Edmund C Baracat[1]

[1]*Disciplina de Ginecologia da Faculdade de Medicina da Universidade de São Paulo – Brazil*
[2]*Pontifícia Universidade Católica – Curitiba (PR), Brazil*
[3]*Laboratório de Ginecologia Estrutural e Molecular (LIM-58), Disciplina de Ginecologia da Faculdade de Medicina da Universidade de São Paulo, Brazil*

Abstract

Introduction: Epidemiological evidence shows that variations in estrogen receptor (ER) genes cause alterations in the effect of estrogen in breast tissue, which may explain individual variations in mammographic density. High mammographic density (HMD) is an important risk factor for breast cancer.

Objective: To evaluate the association of clinical features and polymorphism of *ERα-(GT)n* gene and mammographic density in post-menopause women.

Casuistry and method: According to ACR-BIRADS criteria, 463 post-menopause women of ages between 45 and 60 have been prospectively analyzed through computer objective assessment, being 308 with HMD and 155 with non-dense breasts (Control group). The participants had not used hormone therapies 12 months prior to assessments and had no personal history of breast cancer. Risk factors for breast cancer considered by other studies also have been analyzed in this paper. Peripheral blood samples have been obtained to extract DNA and to analyze the presence of polymorphism in the *ERα-(GT)n* promoter region.

Results: From the risk factors considered for breast cancer, there was association with high mammographic density in: age (p=0.005); waist circumference (p=0.001); number of pregnancies (p=0.007); age at 1st birth (p=0.035); family history (p=0.035); time after menopause (p=0.007), and body mass index (p=0.022). Differences between HMD and controls for distribution of *tanden* repeats polymorphism genotype STRs-*(GT)n* (p=0.151) was verified as non-significant.

Conclusion: Our data showed that age, waist circumference, number of pregnancies, age at 1st birth, family history of breast cancer, time after menopause, and body mass index were associated to post-menopause HMD. However, *tanden* repeats polymorphism *(GT)n* may not be associated with HMD but it will be necessary studies with a larger number of cases as we have obtained few genotypes *(GT)n* higher than 17 repeats.

Keywords: Estrogen receptor; Breast cancer; Mammographic; Polymorphisms

Introduction

Breast cancer is the most common form of cancer among women, comprising approximately 23% of all tumors in women [1]. Although mortality has been decreasing in some countries, breast cancer is still the most frequent cause of death among women between ages 35 and 55. The broad understanding of risk factors for breast cancer results in better insight of biomolecular processes leading to the disease, allowing for health professionals to offer information, counseling, and objective answers to emerging patient questions. In terms of public health, the recognition of risk factors for a pathology and its adequate management towards tracing, treatment, and prevention are of essential importance in order to lower its incidence and, therefore, its prevalence. The study of polymorphisms related to the disease are tools that may have direct implications of great importance in the individual susceptibility to breast cancer on the study of response to several drugs, as well as prognostics. This study was motivated by a study of polymorphisms in lowly-penetrant genes previously associated to increased breast cancer risk and high mammographic density in post-menopause women.

Sexual steroids, the main regulators of breast lobule kinetics, are known to be liposoluble, penetrating passively into the cytoplasm and interacting with its receptors present in the nucleus of target cells, where they regulate genetic expression [2]. Estrogen Receptor (ER) is members of the super-family of nuclear receptors controlling genetic transcription. The α and β isoforms of ER are the main presenters of distribution and genetic expression patterns which are distinct in different tissue types.

ERα expression has been broadly studied in breast tumors due to its being an important measurement of humoral response, as well as for being involved in several estrogen actions onto target cells, directly inducing genes associated with the control of cell proliferation and apoptosis such as cyclin D1, TGFα, IGF1, and progesterone receptors

***Corresponding author:** Marilene A Souza, Rua Azarias Leite, 12-22, Bauru, SP, CEP 17010250, Brazil, Tel: 551432236538, Fax: 551432236538; E-mail: marilenealicia@hotmail.com

Received February 23, 2014; **Accepted** March 27, 2014; **Published** April 01, 2014

Citation: Souza MA, da Fonseca AM, Bagnoli VR, de Barros N, Souza Hortense VH, et al. (2014) Clinical Factors Associated with High Mammographic Density in Postmenopausal Women and their Relationship with Dinucleotide *Gtn* Repeat Polymorphism in the Estrogen Receptor Alpha Gene. J Cancer Sci Ther 6: 142-147. doi:10.4172/1948-5956.1000262

[3,4]. Therefore, ER are seen as prognostic factors for breast cancer and also predictors of hormonal therapy response with up to 70% for women with ER positive (ER+) tumors [5].

For the present study, the Sequential Tanden Repeats polymorphism of the ERα STRs-*(GT)n* gene has been chosen due to its association to increased death risk by breast cancer [6] (Figure 1). The ERα gene is located at chromosome 6 (6q25.1), being composed of 8 exons and over 140 kb [7].

Source: Modified of the Genari et al., 2004 [8]

Methods

The case-control study included 308 women with HMD (more than 50% mammographic density) and 155 control participants (50% density or less), aged 45-65, without menstrual periods or hormone therapy for at least 1 year, and without previous breast or ovarian cancer occurrences. Patients were initially selected subjectively through ACR-BIRADS® [9] standard, by a single reader (head of the Institute of Radiology, *Hospital das Clinicas*, *Faculdade de Medicina*, *Universidade de São Paulo* (HC-FMUSP), São Paulo, Brazil), from January 2010 to March 2013. Selected patients were evaluated a second time through computer objective method by another reader, as described by Boyd et al. [10]. The study was approved by the Ethics Committee for Analysis of Research Projects - CAPPesq at the HC-FMUSP, and all women have signed an informed consent form. Clinical history and physical examination has characterized: age at menarche and menopause, parity, age at first childbirth, family history of breast cancer (FHBC), smoking, alcohol intake, and body mass index (BMI). Peripheral blood samples were obtained for genomic DNA extraction and determination of polymorphisms.

Genomic DNA was extracted from peripheral blood using QIAamp DNA Blood Mini Kit (*Qiagen*), following manufacturer instructions. After DNA quality and integrity evaluation, tanden repeats polymorphism (*GT*)*n* has been confirmed by direct sequencing through automatic DNA sequence device *ABI PRISM 3700* (Life Technologies) as described by Cai et al. [11]. The laboratory was blind on subject identification.

Statistical analyses: Data was described using average, standard deviation (sd), absolute frequency (n), and relative frequency (%). In order to verify the association between qualitative variables with mammographic density, the chi-square test (X^2) was used. For comparison between the HMD and Control groups, the *Kolmogorov-Smirnov Test* was used as quantitative variable in order to verify data normality. Since there is no normal distribution in all groups, the nonparametric *Mann-Whitney Test* was used for comparison between groups. In order to verify the relation between the studied variables and the occurrence of high mammographic density, the Multivariate Logistic Regression model was used stepwise backward. The variables entered in the model were those presenting values p<0.20 in the bivariate analyses. A significance level of 5% (p<0.05) was adopted in all statistical tests.

Results

Distributions of selected demographic characteristics that are the main risk factors for breast cancer are presented in Table 1. Elevated risk of High Mammographic Density was observed for similar main risk factors to those reported in previous studies [12,13].

Significant differences have been observed between the HMD and Control groups in relation to Age (p=0.001); waist circumference (p<0.001); number of pregnancies (p<0.001); Age at first birth (p<0.001); Time after menopause (p=0.007); and BMI (p<0.022).

Regarding age, OR=0.93 e IC95%=0.88-0.99; p=0.005. It is observable that for each year added to age, the probability of being classified as dense mammography diminishes in 6.8%.We have verified that greater abdominal circumference has acted as a protective factor for the occurrence of dense mammography, with values of OR=0.96 e IC95%=0.94-0.98; p=0.001. Each unit added to abdominal

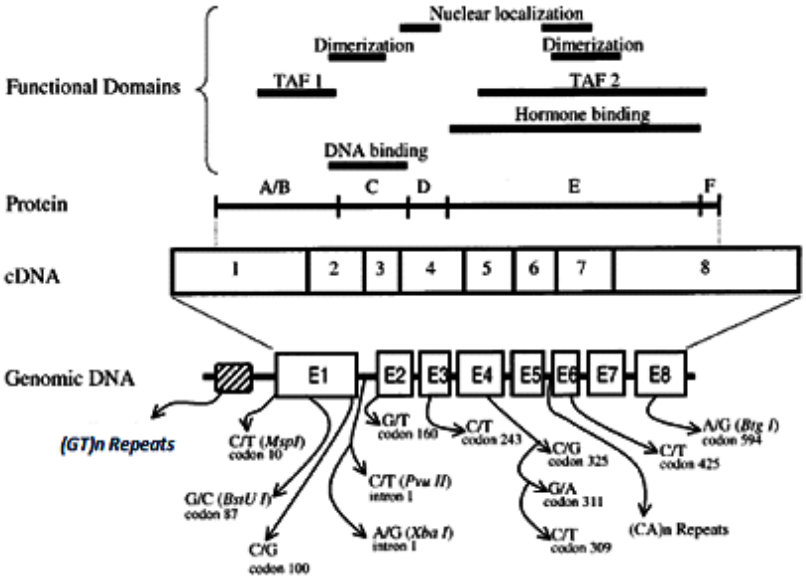

Figure 1: Structure of functional domains of and described polymorphisms in the human estrogen receptor α gene. Coding exons (E) are indicated with boxes. Estrogen receptor α divided into 6 functional regions (A-F). TAF, transcriptional activating function. Source: Modified of the Genari et al. [9].

		Control			HMD			
Quantitative variables		**Average**	**Sd**		**Average**	**Sd**	**Z**	**P**
Age		58.16	4.61		56.31	5.42	3.40	0.001*
Waist circumference		95.06	11.13		89.47	10.83	5.04	<0.001*
Number of Pregnancies		3.63	2.56		2.46	1.83	4.62	<0.001*
Number of births		2.84	1.98		1.99	1.54	4.37	<0.001*
Number of Abortions		0.79	1.20		0.48	0.87	2.93	0.003*
Menopause		46.83	6.26		46.45	6.33	0.49	0.621
Age at first birth		21.88	5.16		24.04	6.04	3.61	<0.001*
Menarche		12.87	1.78		13.17	1.78	1.66	0.096
Time after menopause		11.33	6.42		9.85	7.38	2.69	0.007*
Qualitative variables		**n**	**%**		**n**	**%**	**x^2**	**P**
BMI	Normal / overweight	81	52.3		195	63.3	5.23	0.022*
	Obese	74	47.7		113	36.7		
Smoking	No	140	90.3		264	85.7	1.97	0.161
	Yes	15	9.7		44	14.3		
Alcohol intake	No	137	88.4		258	83.8	1.76	0.185
	Yes	18	11.6		50	16.2		
Metabolic Syndrome	No	104	67.1		220	71.4	0.92	0.337
	Yes	51	32.9		88	28.6		

Quantitative variables*: statistically significant difference (p<0.05)
Nonparametric Mann-Whitney test
*Qualitative variables: statistically significant difference (p<0.05)
Chi-square test

Table 1: Comparison between the two groups of Mammographic Density: High Mammographic Density (HMD) and Control in quantitative and qualitative variables.

circumference lowers risk in 4.4%.Significant differences between the groups have been observed in relation to the number of pregnancies, with OR=0.83 e IC95%=0.72-0.95; p=0.007. Each pregnancy added lowers risk of dense mammography in 17%. The age upon having a first child has influenced positively on the occurrence of dense mammography. OR=1.05 e IC95%=1.004-1.106; p=0.035. For dense mammography, age average of 24.04 years was verified and, for non-dense mammography, 21.88 years of age, in average. Each year in delaying the first full pregnancy increases risk in 5.3%.

Values obtained in associating mammographic density and FHBC has shown the chance of having dense mammography being 2.02 times greater for people with such risk factor. OR=2.028 e IC95%=1.052-3.909; p=0.035.

Time since their menopause has influenced the patterns of mammographic density. The longer since the menopause, the greater chance they were classified as non-dense mammography. When correlating BMI and mammographic density patterns, an inverse association has been observed between obesity and high-density mammography.

Tanden repeats polymorphism *(GT)n*

Distribution of repeats polymorphism *(GT)n* between HMD and control groups can be seen in Table 2. Samples have been divided based on an average of 15 repetitions.

There was no significant difference between repeats polymorphism *(GT)n* and mammographic density patterns; however, we have observed that the greater the number of repetitions, the greater chance of having dense mammography (OR=1.34).

From the clinical features analyzed, only those presenting bivariate analyses values p<0.20 have been used in the model. Results presented are derived from a *stepwise backward* Multivariate Logistic Regression model (Table 3).

Considering variables which present association with HMD: (age, time after menopause, waist circumference, BMI, number of pregnancies, age at 1st birth, and FHBC), after multivariate logistic regression only age, waist circumference, number of pregnancies, age at 1st birth, and FHBC have been considered to be independent risk factors (Table 3).

Discussion

There is currently strong evidence that HMD is an independent risk factor for breast cancer [10]. It presents elevated risk (4-6 relative risk), being comparable to other risk factors such as: atypical epithelial hyperplasia, as well as mother and sister with breast cancer and proven genetic susceptibility – beating factors such as nulliparity, history of non-atypical hyperplasia, late menopause, and early menarche [14].

It is believed that mammographic patterns are multi-factorial and influenced by age, reproductive factors, age of menarche, menopausal status, family history, eating habits, sedentary lifestyle, hormone therapy, and excessive alcohol intake. However, such factors explain only 20-30% of mammographic density variation [15]. Studies in monozygotic twins have shown hereditary factors results in 60% of mammographic density [16]. Among potential genetic influences, it is observable that the combination of proliferating effects on (mitogenic) cells and (mutagenic) genetic damage may base the increased risk for breast cancer and HMD [14]. There is clear necessity in improving the understanding of specific factors involved in this process, and in the role of growth factors, as well as of hormonal intervention in the various components of breast tissue. It is particularly probable that gene identification, responsible for variations in percentages among the various tissues in the breast (and their biological functions), may shed light upon the biology of breasts while identifying potential markers for breast cancer prevention.

Clinical factors associated to breast cancer and high mammographic density

Age is still the main risk factor for breast cancer; occurrence rates

Dense Mammography	GT(n)		Total
	≤15	>15	
No	74	70	144
	51.4%	48.6%	100.0%
Yes	136	172	308
	44.2%	55.8%	100.0%
Total	210	242	452
	46.5%	53.5%	100.0%

χ^2 = 2.06; p = 0.151; OR = 1.34; IC95%=0.90 – 1.99

Table 2: Association between type of mammography and repeats polymorphisms *(GT)n.*

Independent Variables	B	Standard Deviation B	OR	OR (IC95%)		p
Constant	7.330	1.715				
Age	-0.070	0.025	0.932	0.888	0.979	0.005
Waist Circumference	-0.038	0.011	0.963	0.943	0.984	0.001
Number of Pregnancies	-0.186	0.069	0.830	0.725	0.950	0.007
Age at 1st birth	0.052	0.025	1.053	1.004	1.106	0.035
Family History	0.707	0.335	2.028	1.052	3.909	0.035

Table 3: Multivariate Logistic Regression using *stepwise backward* method, with dependent variable HMD and independent variables: Age, Menopause, Time after menopause, Ethnicity, Waist Circumference, BMI, Number of Pregnancies, Number of Birth, Number of A*bortions, Age at 1st childbirth, Menarche, Smoking, Alcohol Intake, Family* History.

increase rapidly up to age 50, increasing more slowly later. The opposite happens with mammographic density, which decreases with age. In young people, usually breasts are dense and progressively devolve with age. In our study, the sample comprised post-menopause women aged between 45 and 65. It is observable that for each year added to age, the probability of being classified as highly dense mammography diminishes in 6.8%. The group of HMD presented an age average of 56.31 and standard deviation of 5.42 while the Control group aged 58.16 ± 4.61. This relation was statistically significant (p<0.001), showing that the younger women are, the more likely they are to have HMD (for more than 50% of fibroglandular tissue). These findings are in agreement with the specialized literature, pointing towards a decrease in mammographic density upon aging [17,18].

With reduced rates of estrogen and progesterone after menopause, the cell proliferation cycle process acquiesces and the mammographic imaging quickly becomes radiolucent. Every 2 years in menopause diminishes mammography density in 9% [19]. In the present study, women have presented relatively precocious menopause, at an age average of 46.45 (sd 6.33) for the HMD group, and 46.83 (dp 6.26) for the Control group (p=0.540), not characterizing a risk factor for HMD. Such data also coincides with records by Matos et al. [20], who have also not found menopause age to be a risk factor for breast cancer (age average of 47). However, when mammography density is associated with time after menopause, we have verified on our data. Therefore, for every 2 additional years of menopause, there was a 25% lower risk in presenting HMD.

Menarche age is associated both to ovarian hormone exposition and to teenage nutritional factors. Women with menarche before age 12 have a 20% greater risk of developing breast cancer throughout their lifetime, in comparison to those whose menarche was after age 14. Late menarche (≥ 15 years old) lowers neoplasm risk in 28% when compared to those whose menarche was before age 12 [21]. However, other studies have not found any association between mammographic density and age of menarche, suggesting that the mechanism through which early menarche increases breast cancer risk is not through mammographic density [22,23]. In our study, we have verified that this factor has not influenced mammographic density (p=0,096), which coincides with previous studies performed on Brazilian populations [12,24,25].

During full pregnancy and breastfeeding, the breast reaches full development due to an initial growth phase followed by lobular differentiation marked by the shift from a type-1 breast to types 3 or 4, which results in the protection of the organ from carcinogenic factors. Such physiological and hormonal changes in breast tissue are a result of complex interactions between hormones and growth factors. The increase of dense mammography is associated with nulliparity and old age at having the first birth. Each birth reduces risk of breast cancer (ER+ and PR+) by 11%, and women with late 1st childbirth (≥ 35 years old) had 27% greater risk in developing breast cancer, in comparison with women whose first child was born before they reached age 20 [26]. MacMahon was the first to show the protective effects of women's age at first childbirth against breast cancer, concluding that mothers before age 20 had 50% reduction in the risk of developing breast cancer [27]. The protective effect of early age upon women's first childbirth was equally observed in our data (p<0.001).Among the 308 women with HMD, the age average upon their first childbirth was of 24.04 years; 19.58% women had their first child after age 28, 13.31% [28] after age 30, and 22.08% had no children. The average age was of 21.88 years old (sd=5.16) for women with liposubstituted breasts, and every year late in having their first child has increased risk in 5.3%. Meta-analysis of 9 cohort or case-control studies have also revealed risk reduction of ER+ breast cancer among women with first full pregnancy before age 20. Such protective factor against breast cancer has been observed particularly on post-menopause women [29]. Morphological and functional alterations in breast tissue related to childbirth have been studied extensively [30]. With successive pregnancies, epithelial cells become more differentiated and less proliferative, which contributes with lower mammographic density. Our data reveals statistically significant association between childbirth and mammographic density (p=0.001).The group of women with dense mammography had a parity average of 2.46 children, and women with liposubstituted breasts had an average of 3.63.Each extra child reduces risk of dense mammography in 17%, coinciding with data found by Lope et al. [31], which had detected 16% risk reduction per childbirth. The mechanisms through which these protective effects are mediated are unknown; however, early and complete maturation of the breast glands has been suggested as a protective factor against breast cancer [32]. Therefore, as the population ages, low fertility rates and delay in first childbirth favor an environment with increased risk for breast cancer.

As for ethnicity, breast cancer mortality varies considerably amongst different ethnic groups [33]. In the United States; there is greater occurrence among Caucasian and African-American women; intermediate occurrence among Hispanic and Native Americans; and smaller occurrence among Asians [1]. In Brazil, its miscegenated population influences on disease incidence [34]; however, several researchers have found greater breast cancer prevalence on Caucasian women (34).On the studied group, the greatest representation in HMD were Caucasians (58.44%) followed by Mulatto (21.75%); however, the difference presented no significance.

Mammographic density is a highly hereditary risk factor for breast cancer [35]. Women with first-degree relatives diagnosed with breast cancer are, in average, more likely to have high mammographic density

than women of the same age with no family history. Additionally, the average of dense mammography increases with the number of first-degree relatives with diagnosed breast cancer [36]. In Brazil, a review of literature performed by Pinho and Coutinho [24] has presented FHBC prevalence in first-degree relatives of 4% among the Brazilian female population. Our results have pointed towards 19.2% patients with HMD having mentioned breast cancer history in first-degree relatives (p=0.035).Chances of having HMD are 2.028 times greater for women with breast cancer family history, which shows greater prevalence of this risk factor in the population and suggests greater genetic influence of breast cancer, in accordance with data from the specialized literature.

High alcohol intake has been associated with increased mammographic density by Vanchon et al. [37]; Herrinton et al. [38]; and Matos et al. [20] studying prevalence of risk factors in women with breast cancer, which have observed that 13% were smokers and 21.2% drank occasionally. However, collaboration between 53 epidemiological studies has shown that smoking has little to no effect in the risk of developing breast cancer. Meta-analysis of data from 40 studies has estimated non-alcoholic women having 10% greater risk of breast cancer, when compared to women taking one daily dose (12 g) of alcohol (Ellison et al.) [39]. Our data has not presented significant association between HMD and smoking or drinking habits, with p values of 0.161 e 0.185, respectively.

Obesity is a known risk factor for breast cancer in post-menopause women, especially when the relation between BMI is examined in association with mammographic density and breast cancer [28,40]. Women with high BMI have presented lower probability in having dense mammography; however, the risk of developing breast cancer for women weighing more than 81Kg was of OR=1.7 when compared to those weighing less than 63 Kg. However, the OR increased to 2.1 after adjustments for HMD. Such increase indicates that density is an independent risk factor, and that obese women with HMD have increased risk of developing breast cancer. It is suggested still that the risk of developing breast cancer increases in 8% for every additional 5.00 Kg gained during adult life [28]. Our data points toward high prevalence of obesity (40.4%); however, women with liposubstituted breasts were predominant (47.7%). The greater the amount of body fat, the lower the mammographic density. Obese women comprised 36.7% of the HMD group, being a significant difference (p=0.022). The same has occurred for waist circumference measurements: the greater the circumference, the greater chance of being qualified as liposubstituted breasts. Therefore, for each unit added in waist circumference the risk lowers 4.7%. Such data is in accordance with the literature [10,31,41].

Mammographic density and polymorphism of the estrogen receptor gene

Estrogen receptors play a critical role in developing normal breast tissue, and are also involved in the pathogenesis of breast cancer. Actually, approximately 2/3 of breast cancers express the alpha estrogen receptor. Epidemiological evidence also correlates steroid hormones to chances in mammographic density, analyzing whether variations in biosynthesis-regulating genes and hormonal metabolism could explain individual differences in mammographic density. The ER gene, located in the long arm of chromosome 6q25, has been associated to mammographic density in several studies [42] due to its importance in breast cancer development, progression, and prognostics.

There are several known polymorphisms in the ERα gene, among which are SNPs *Pvull* and *Xbal* and, more recently, the repetition polymorphism *(GT)n*. Polymorphisms *Pvull* and *Xbal* are located at intron 1 of the REα gene, with 50 base pairs between them [13]. Tanden repeats polymorphism (GT)n [STRs (GT)n] is located 6627 bp before the onset region of the transcription site of exon 1 to 144 kb of exon 2. Recent evidence has shown that polymorphisms in promoter regions of cell cycle regulating genes can influence significantly in regulating transcription [43]. The dinucleotide repeat *GT of ERα gene* is highly polymorphic, and the number of *GT* repeats interferes with gene transcription. When seeking the relationship between polymorphism with risk of breast cancer, Cai et al. [11] showed that the genotyping containing $(GT)_{17}$ or $(GT)_{18}$ was associated with decreased breast cancer risk (OR=0.58), mainly among post-menopause women with negative progesterone receptor and more than 30 years of menstrual cycles [11]. When studying the TA repeat polymorphism of the promoter region of the *ERα* gene and risk of osteoporosis, Genari et al. [8] concluded that the smallest number of repeats was associated with lower bone mineral density and increased risk of fracture.

A total of 11 genotypes were observed in our sample, which varied from GT_{11} to GT_{22}. The most common repeats among women with HMD were $(GT)_{14}$, $(GT)_{15}$,$(GT)_{16}$ and $(GT)_{17,}$ with 12.01%, 25.97%; 38.31%; and 8.44%, respectively; and among the Control group *GT* common repeats were $(GT)_{14}$, $(GT)_{15}$,$(GT)_{16}$ and $(GT)_{17,}$ with 9.33%; 28.00%; 30.00%; and 8.66%, respectively. However, there was no significant difference between repetition polymorphism *(GT)n* and mammographic density patterns; however, we have observed that the greater the number of repetitions, the greater chance of having dense mammography (OR=1.34). Another group of researchers has found similar results, with greater frequency for genotype 16, also without statistical differences between the cases of breast cancer and control groups, with 41.5% and 37.6% respectively [11].

The molecular mechanisms through which these polymorphisms modify the receptor activity are not clear. Possible explanations include the existence of a functional combination between polymorphic alleles, in which both combining markers would alter genetic function as well as RNA stability [44]. Therefore, this study investigated whether the combination of repeat polymorphism *(GT)n* with *Pvull* and *Xbal* polymorphisms would modify dense mammography patterns. No interaction between them has been observed. Also observing whether polymorphisms *Pvull*, *Xbal,* and *GT* would modify survival of women with breast cancer, Boyapati et al. [7] have found that the genotype association has been modified by ER- or ER+ states. When comparing women with *pp* genotype, risk of death (RR) was of 3.30 and 0.54 for participants with, respectively, ER- and ER+. Similarly, women carrying repeat polymorphism GT_{23} have been strongly related to RE- breast tumors (RR=1.48 for ER- and RR=0.25 for ER+).

Finally, from the variables presenting association with high mammographic density (age, waist circumference, number of pregnancies, age at 1st childbirth, time after menopause, family history, BMI, and *Pvull* genotype), only clinical factors age, abdominal circumference, number of pregnancies, age at 1st childbirth, and family history have proven to be independent factors for HMD multiple logistical regression (p<0.05). However, *tanden* repeats polymorphism *(GT)n* may not be associated with HMD but it will be necessary studies with a larger number of cases as we have obtained a few genotypes *(GT) n* higher than 17 repeats.

References

1. American Cancer Society (2013).

2. Key TJ (2011) Endogenous oestrogens and breast cancer risk in premenopausal and postmenopausal women. Steroids 76: 812-815.

3. Souza MA, Fonseca AM, Bagnoli VR, Barros N, Hortense VHS, et al. (2013) Polymorphisms in the Estrogen Receptor Alpha Gene and Mammographic Density Result Study in Brazilian Women. J Cancer Sci Ther 5: 446-451.

4. Harvey JM, Clark GM, Osborne CK, Allred DC (1999) Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol 17: 1474-1481.

5. Bland KI, Copeland EM (2010) The breast comprehensive manegement of benign and malignant diseases. 4th edition, Saunders Editor.

6. Boyapati SM, Shu XO, Ruan ZX, Cai Q, Smith JR, et al. (2005) Polymorphisms in ER-alpha gene interact with estrogen receptor status in breast cancer survival. Clin Cancer Res 11: 1093-1098.

7. Greene GL, Gilna P, Waterfield M, Baker A, Hort Y, et al. (1986) Sequence and expression of human estrogen receptor complementary DNA. Science 231: 1150-1154.

8. Gennari L, Merlotti D, De Paola V, Calabrò A, Becherini L, et al. (2005) Estrogen receptor gene polymorphisms and the genetics of osteoporosis: a HuGE review. Am J Epidemiol 161: 307-320.

9. ACR BI-RADSÂ®. American College of Radiology (2003) Breast Imaging Reporting and Data System. Fourth ed. Reston VA.

10. Boyd NF (2013) Mammographic density and risk of breast cancer. Am Soc Clin Oncol Educ Book .

11. Cai Q1, Gao YT, Wen W, Shu XO, Jin F, et al. (2003) Association of breast cancer risk with a GT dinucleotide repeat polymorphism upstream of the estrogen receptor-alpha gene. Cancer Res 63: 5727-5730.

12. de Moura Ramos EH, Martinelli S, Silva I, Nazário A, Facina G, et al. (2009) Association between estrogen receptor gene polymorphisms and breast density in postmenopausal women. Climacteric 12: 490-501.

13. Souza MA, Fonseca AM, Bagnoli VR, de Barros N, Franzolin SO, et al. (2013) Polymorphisms of estrogen receptor-Î± gene in Brazilian women with high breast density after menopause. Gynecol Endocrinol 29: 771-774.

14. Martin LJ, Boyd NF (2008) Mammographic density. Potential mechanisms of breast cancer risk associated with mammographic density: hypotheses based on epidemiological evidence. Breast Cancer Res 10: 201.

15. Boyd NF, Martin LJ, Bronskill M, Yaffe MJ, Duric N, et al. (2010) Breast tissue composition and susceptibility to breast cancer. J Natl Cancer Inst 102: 1224-1237.

16. Martin KE, Helvie MA, Zhou C, Roubidoux MA, Bailey JE, et al. (2006) Mammographic density measured with quantitative computer-aided method: comparison with radiologists' estimates and BI-RADS categories. Radiology 240: 656-665.

17. Stomper PC, D'Souza DJ, DiNitto PA, Arredondo MA (1996) Analysis of parenchymal density on mammograms in 1353 women 25-79 years old. AJR Am J Roentgenol 167: 1261-1265.

18. Boyd NF, Guo H, Martin LJ, Sun L, Stone J, et al. (2007) Mammographic density and the risk and detection of breast cancer. N Engl J Med 356: 227-236.

19. Boyd NF, Greenberg C, Lockwood G, Little L, Martin L, et al. (1997) Effects at two years of a low-fat, high-carbohydrate diet on radiologic features of the breast: results from a randomized trial. Canadian Diet and Breast Cancer Prevention Study Group. J Natl Cancer Inst 89: 488-496.

20. Matos JC, Pelloso SM, Carvalho MDB (2010) PrevalÃªncia de fatores de risco para o cÃ¢ncer de mama no municÃpio de MaringÃ¡, ParanÃ¡. Rev Latino-Am Enfermagem 18: 57-64.

21. Pike MC, Krailo MD, Henderson BE, Casagrande JT, Hoel DG (1983) 'Hormonal' risk factors, 'breast tissue age' and the age-incidence of breast cancer. Nature 303: 767-770.

22. Jeon JH, Kang JH, Kim Y, Lee HY, Choi KS, et al. (2011) Reproductive and Hormonal Factors Associated with Fatty or Dense Breast Patterns among Korean Women. Cancer Res Treat 43: 42-48.

23. Butler LM, Gold EB, Greendale GA, Crandall CJ, Modugno F, et al. (2008) Menstrual and reproductive factors in relation to mammographic density: the Study of Women's Health Across the Nation (SWAN). Breast Cancer Res Treat 112: 165-174.

24. Pinho VF, Coutinho ES (2007) [Variables associated with breast cancer in clients of primary healthcare units]. Cad Saude Publica 23: 1061-1069.

25. Lamas JM & Pereira MG (1999) Fatores de risco para o cÃ¢ncer de mama e para lesÃµes prÃ©-malignas em mulheres assintomÃ¡ticas no Distrito Federal. Rev Bras Mastol 9: 108-114.

26. Ma H, Bernstein L, Pike MC, Ursin G (2006) Reproductive factors and breast cancer risk according to joint estrogen and progesterone receptor status: a meta-analysis of epidemiological studies. Breast Cancer Res 8: R43.

27. MacMahon B, Cole P, Lin TM, Lowe CR, Mirra AP, et al. (1970) Age at first birth and breast cancer risk. Bull World Health Organ 43: 209-221.

28. Lam PB, Vacek PM, Geller BM, Muss HB (2000) The association of increased weight, body mass index, and tissue density with the risk of breast carcinoma in Vermont. Cancer 89: 369-375.

29. Kobayashi S, Sugiura H, Ando Y, Shiraki N, Yanagi T, et al. (2012) Reproductive history and breast cancer risk. Breast Cancer 19: 302-308.

30. Russo J, Russo IH (2012) Molecular basis of pregnancy-induced breast cancer prevention. Horm Mol Biol Clin Invest 9: 3-10.

31. Lope V, Pérez-Gómez B, Sánchez-Contador C, Santamariña MC, Moreo P, et al. (2012) Obstetric history and mammographic density: a populacional-based cross-sectional study in Spain (DDM-Spain). Breast Cancer Res Treat 132: 1137-1146.

32. Toniolo P, Grankvist K, Wulff M, Chen T, Johansson R, et al. (2010) Human chorionic gonadotropin in pregnancy and maternal risk of breast cancer. Cancer Res 70: 6779-6786.

33. Ghafoor A, Jemal A, Ward E, Cokkinides V, Smith R, et al. (2003) Trends in breast cancer by race and ethnicity. CA Cancer J Clin 53: 342-355.

34. Hallal PC, Dumith Sde C, Bastos JP, Reichert FF, Siqueira FV, et al. (2007) [Evolution of the epidemiological research on physical activity in Brazil: a systematic review]. Rev Saude Publica 41: 453-460.

35. Sclowitz ML, Menezes AM, Gigante DP, Tessaro S (2005) [Breast cancer's secondary prevention and associated factors]. Rev Saude Publica 39: 340-349.

36. Linton L, Martin LJ, Li Q, Huszti E, Minkin S, et al. (2013) Mammographic density and breast cancer: a comparison of related and unrelated controls in the Breast Cancer Family Registry. Breast Cancer Res 15: R43.

37. Vachon CM, Sellers TA, Carlson EE, Cunningham JM, Hilker CA, et al. (2007) Strong evidence of a genetic determinant for mammographic density, a major risk factor for breast cancer. Cancer Res 67: 8412-8418.

38. Herrinton LJ, Saftlas AF, Stanford JL, Brinton LA, Wolfe JN (1993) Do alcohol intake and mammographic densities interact in regard to the risk of breast cancer? Cancer 71: 3029-3035.

39. Ellison RC, Zhang Y, McLennan CE, Rothman KJ (2001) Exploring the relation of alcohol consumption to risk of breast cancer. Am J Epidemiol 154: 740-747.

40. Trentham-Dietz A, Newcomb PA, Egan KM, Titus-Ernstoff L, Baron JA, et al. (2000) Weight change and risk of postmenopausal breast cancer (United States). Cancer Causes Control 11: 533-542.

41. Alvares BR, Freitas CHA, Jales RM, Almeida OJ, Marussi EF (2012) Densidade mamogrÃ¡fica em mulheres menopausadas assintomÃ¡ticas: correlaÃ§Ã£o de dados clÃnicos e exames ultrassonogrÃ¡ficos. Rev Radiologia 45: 149-154.

42. Souza MA, Fonseca AM, Bagnoli VR, Soares-Jr JM, Barros N, et al. (2012) Polimorfismo do gene do receptor estrogÃªnico como fator de risco do cÃ¢ncer de mama. Femina 40: 179-186.

43. Iwashita S, Koyama K, Nakamura Y (2001) VNTR sequence on human chromosome 11p15 that affects transcriptional activity. J Hum Genet 46: 717-721.

44. Wedrén S, Lovmar L, Humphreys K, Magnusson C, Melhus H, et al. (2004) Oestrogen receptor alpha gene haplotype and postmenopausal breast cancer risk: a case control study. Breast Cancer Res 6: R437-449.