EJGO European Journal of Gynaecological Oncology

The TP53 - R72P (rs1042522) polymorphism and risk factors in breast cancer patients

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Summary

The authors aimed to assess the incidence of *TP53*-R72P polymorphism correlating with risk factors and clinical-pathological features in breast cancer (BC). Genotypic distribution was higher for Arg/Pro (43.52%) comparing with Arg/Arg (38.36%) and Pro/Pro (18.12%), and the allele frequency was significantly higher for Arg (0.62%) in BC patients. Risk-factors such as age, menarche, pregnancy, hormonal therapy, ethnicity, and origin region showed relevance in case-control comparisons. Genotype distribution showed a high frequency of ER-positive and PR-positive in BC. HER-2 negative (87.8%) was significantly more frequently than positive, and the genotype classification was 40.5% Arg/Arg and 46.4% Arg/Pro. Almost all patients presented invasive ductal carcinoma (IDC) and underwent surgical treatment without neoadjuvant chemotherapy. *TP53* - R72P polymorphism seems to be associated with some risk factors related to reproductive life, hormonal treatments, ethnicity, and lifestyle. Genetic variation between Arg and Pro alleles seems not to be directly correlated with BC development.

Key words: Breast Cancer; R72P; TP53; Genetic polymorphism; p53 protein; SNP.

Introduction

Breast cancer (BC) is a heterogeneous tumor [1], and main cause of women mortality worldwide [2]. It has more than 12 histological variants [3], and present distinct biological features that lead to different patterns of treatments and clinical responses [4]. Histological appearance of tumor may not be sufficient to establish the underlying complex genetic alterations and the biological events involved in cancer development and progression. BC development might be related to several risk factors, as behavioral, environmental, reproductive, and endocrine history and genetic factors [5-7].

Behavioral and environmental factors are well defined and include alcohol consumption, feeding, physical exercise, exposure to radiation, and obesity especially in postmenopausal women. Endocrine factors are mainly related to exposure time to endogenous or exogenous estrogenic stimuli. Early menarche, late menopause, hormonal therapy, and nulliparity or first pregnancy after 30 years of age are considered decisive events for BC development [5-7]. Moreover, genetic factors have been frequently discussed due to global discrepancies found in the genetic alterations of relevant genes, such as *BRCA1*, *BRCA2,* and *TP53* [5, 8-10].

TP53 mutations are often found in approximately 20-30% of BC women [3, 4, 11-14]. Genetic alterations are notably associated with BC subtype. Some substitutions in luminal tumors can lead the p53 protein to have new functions, as the capacity of p63 and p73 inactivation, and greater assiduity of mutations by deletion or insertion in apocrine tumors and triple negative promoting the lack of p53 [15]. In addition, genetic polymorphism has a significant impact on the metabolic response. *TP53* - Arg72Pro (R72P) polymorphism is thoroughly investigated due to its high frequency of nucleotide change [16, 17], leading to functional alterations in its protein [14]. Proline (Pro) allele induces greater levels of G1 arrest in the cell cycle than Arginine (Arg), but Arg appears to be more effective in the apoptosis induction and in the cell protection against the stress of neoplastic development [18, 19]. Natural variants existence of p53 and other proteins that influence its activity are correlated with BC development in several populations. The p53 is potential predictive biomarker to prevention and treatment strategies. The aim of the present study was to fill the gap in the R72P polymorphism studies of *TP53* gene in gynecologic oncology by analyzing the occurrence of this polymorphism in BC women and to assess and correlate with risk factors and clinical-pathological features. Identification of this polymorphism may be useful for predicting the profile of clinical variables in BC women.

Revised manuscript accepted for publication October 19, 2017

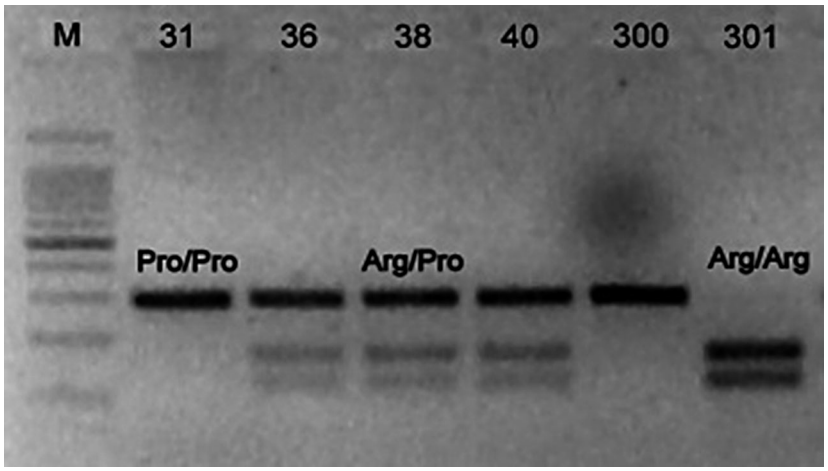

Figure 1. — Detection of R72P genetic polymorphism in BC samples by PCR-RFLP. Pro/Pro - homozygous Pro (279 base pairs), Arg/Pro - heterozygous (279, 160, and 119 base pairs), Arg/Arg - homozygous Arg (160 and 119 base pairs) and M = DNA ladders 100 base pairs.

Table 1. — *Restriction enzyme and primers specifications.*

***TP53* R72P (rs1042522)**			
Primers sequence (5'→ 3')	**SNP[a]**	**Amino acid change**	**Restriction enzyme**
F- 5' TCCCCCTTGCCGTCCCAA 3' R- 5' CGTGCAAGTCACAGACTT 3'	**C**C**C** → **C**G**C** (C ancestral)	Pro → Arg (P → R)	*BstU*I 60°C for 4h

Enzyme cut	**DNA fragments (base pairs)**	**Genotypes**	
5'...CG↓CG...3' 3'...GC↑GC...5'	279	Pro/Pro	Homozygous Pro
	279+160+119	Arg/Pro	Heterozygous Arg/Pro
	160+119	Arg/Arg	Homozygous Arg

[a]Single nucleotide polymorphism

Materials and Methods

The study was approved through the Research Ethics Committee of the Federal University of São Paulo UNIFESP/EPM, Cancer Hospital of Barretos and Dr. Ary Pinheiro Hospital under protocol number 0625/10, and all participants previously agreed with and signed an informed consent form. This cross-sectional study included 362 patients' samples from São Paulo (SP) - Southeast of Brazil that was collected in the São Paulo Hospital, and 297 patients' samples from Rondônia (RO) - North of Brazil that was collected at the Cancer Hospital of Barretos and Dr. Ary Pinheiro Hospital, the samples were distributed in case and control groups.

Clinical data were collected from patients' charts. All patients were submitted to anamneses and general physical examinations, patients diagnosed with BC underwent surgery and the biopsy was confirmed on histopathological examination.

Genomic DNA was extracted from the lymphocytes in peripheral blood samples using GenElute mammalian Genomic DNA according to the manufacturer's instructions. Samples were quantified in the NanoDrop 2000 and 50-100 ng of DNA were used to reactions.

Single nucleotide polymorphism (SNP) R72P (rs1042522) in the *TP53* gene was analyzed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Mix reaction for each sample contained 10 µl of Master Mix, 1 µl (10 pM) of primer forward, 1 µl (10 pM) of primer reverse, 50-100 ng of genomic DNA and nuclease-free water to complete the reaction in 25 µl final volume. Outlined oligonucleotides sequences were 5′-TCCCCCTTGCCGTCCCAA-3′ (forward) and 5′-CGTGCAAGTCACAGACTT-3′ (reverse) [14], and the conditions used to amplify the genetic fragment in the thermocycler were 94ºC/5 minutes - initial denaturation, 40 cycles of denaturing at 94ºC/30 seconds, annealing at 58ºC/45 seconds, polymerization at 72ºC/1 minute, and final extension at 72ºC/7 minutes. After PCR, the amplicons of each sample were submitted to enzymatic digestion, the mix reaction containing 8 µL of PCR product and 1 µL (10 units) of restriction enzyme *BstU*I and 1 µL of CutSmart Buffer 10X were incubated at 60ºC/4 hours. Fragments were analyzed in 3% agarose gel electrophoresis stained with 1 mg/mL ethidium bromide and observed under UV light, and the images were recorded using a Digital Science 1D system (Figure 1). Fragment size and amino acid changes was previously described (Table 1) [14].

TP53 sequencing assay was performed to confirm the results obtained from PCR-RFLP and some random samples were selected for each genotype (Figure 2). DNA samples were amplified and the R72P PCR fragments were purified to Sanger sequencing method. Sequencing products were analyzed by 3500 Genetic Analyzer, and the cycle sequencing was performed using BigDye and the extension products were purified with BigDye XTerminator Purification Kit according to the manufacturer's instructions [20]. Sequencing data were analyzed with Geneious 6.1.6 software and database NCBI (http://www.ncbi.nlm.nih.gov/).

Table 2. — *Comparisons of risk factors and genotypes distribution.*

Variables		Groups								*P*
		Control				Case				
		Arg/Arg n (%)	Arg/Pro n (%)	Pro/Pro n (%)	**Total n (%)**	Arg/Arg n (%)	Arg/Pro n (%)	Pro/Pro n (%)	**Total n (%)**	
Age[a]	≤40	41 (41)	37 (32.2)	20 (35.1)	98 (36)	12 (10.3)	16 (12.3)	3 (6.7)	31 (10.6)	**0.0001**
	>40 e ≤ 50	32 (32)	41 (35.7)	21 (36.8)	94 (34.6)	45 (38.8)	48 (36.9)	16 (35.6)	109 (37.5)	
	>50 e ≤60	20 (20)	30 (26.1)	12 (21.1)	62 (22.8)	36 (31)	36 (27.7)	17 (37.8)	89 (30.6)	
	>60	7 (7)	7 (6.1)	4 (7)	18 (6.6)	23 (19.8)	30 (23.1)	9 (20)	62 (21.3)	
Total		100 (36.8)	115 (42.3)	57 (20.9)	**272 (100)**	116 (39.9)	130 (34.7)	45 (15.4)	**291 (100)**	
Menarche[a]	≤12	20 (20)	24 (20.90)	14 (24.6)	58 (21.3)	40 (34.5)	54 (41.5)	24 (53.3)	118 (40.5)	**0.0001**
	>12	80 (80)	91 (79.1)	43 (75.4)	214 (78.7)	76 (65.5)	76 (58.5)	21 (46.7)	173 (59.5)	
Total		100 (36.8)	115 (42.3)	57 (20.9)	**272 (100)**	116 (39.9)	130 (34.7)	45 (15.4)	**291 (100)**	
Pregnancies	0	15 (15)	16 (13.9)	15 (26.3)	46 (16.9)	19 (16.4)	8 (6.2)	3 (6.7)	30 (10.3)	**0.004**
	1	12 (12)	16 (13.9)	7 (12.3)	35 (12.9)	7 (6)	9 (6.9)	3 (6.7)	19 (6.5)	
	>1	73 (73)	83 (72.2)	35 (61.4)	191 (70.2)	90 (77.6)	113 (86.9)	39 (86.7)	242 (83.2)	
Total		100 (36.8)	115 (42.3)	57 (20.9)	**272 (100)**	116 (39.9)	130 (34.7)	45 (15.4)	**291 (100)**	
Age at first full-term birth[a]	<20	19 (48.7)	18 (47.4)	17 (68)	54 (52.9)	45 (46.9)	53 (43.8)	20 (48.8)	118 (45.8)	0.629
	20-30	15 (38.5)	15 (39.5)	6 (24)	36 (35.3)	45 (46.9)	56 (46.3)	18 (43.9)	119 (46.1)	
	>30	5 (12.8)	5 (13.2)	2 (8)	12 (11.8)	6 (6.2)	12 (9.9)	3 (7.3)	21 (8.1)	
Total		39 (38.2)	38 (37.3)	25 (24.5)	**102 (100)**	96 (37.2)	121 (46.9)	41 (15.9)	**258 (100)**	
Miscarriage	No	78 (78)	89 (77.4)	47 (82.5)	214 (78.7)	45 (78.9)	47 (73.4)	16 (84.2)	108 (77.1)	0.860
	Yes	22 (22)	26 (22.6)	10 (17.5)	58 (21.3)	12 (21.1)	17 (26.6)	3 (15.8)	32 (22.9)	
Total		100 (36.8)	115 (42.3)	57 (20.9)	**272 (100)**	57 (40.7)	64 (45.7)	19 (13.6)	**140 (100)**	
Parity	0	15 (15)	17 (14.8)	16 (28.1)	48 (17.7)	19 (33.3)	9 (14.1)	4 (21.1)	32 (22.9)	0.100
	1	15 (15)	21 (18.3)	7 (12.3)	43 (15.8)	8 (14)	11 (17.2)	1 (5.3)	20 (14.2)	
	>1	70 (70)	77 (67)	34 (59.6)	181 (66.5)	30 (52.6)	44 (68.8)	14 (73.7)	88 (62.9)	
Total		100 (36.8)	115 (42.3)	57 (20.9)	**272 (100)**	57 (40.7)	64 (45.7)	19 (13.6)	**140 (100)**	
Hormonal therapy	No	26 (66.7)	29 (74.4)	21 (75)	76 (71.7)	98 (85.2)	114 (87.7)	39 (86.7)	251 (86.5)	**0.021**
	Yes	13 (33.3)	10 (25.6)	7 (25)	30 (28.3)	17 (14.8)	16 (12.3)	6 (13.3)	39 (13.5)	
Total		39 (36.8)	39 (36.8)	28 (26.4)	**106 (100)**	115 (39.7)	130 (44.8)	45 (15.5)	**290 (100)**	
Ethnicity	Non white	33 (71.7)	36 (69.2)	21 (70)	90 (70.3)	44 (37.9)	67 (51.5)	27 (60)	138 (47.4)	**0.0001**
	white	13 (28.3)	16 (30.8)	9 (30)	38 (29.7)	72 (62.1)	63 (48.5)	18 (40)	153 (52.6)	
Total		46 (36)	52 (41)	30 (23)	**128 (100)**	116 (39.9)	130 (44.7)	45 (15.4)	**291 (100)**	
Region[b]	North	15 (15)	24 (20.9)	12 (21.1)	51 (18.7)	9 (24.3)	13 (26)	10 (62.5)	32 (31.1)	**0.0001**
	Northeast	41 (41)	44 (38.3)	15 (26.3)	100 (36.8)	3 (8.1)	5 (10)	1 (6.2)	9 (8.7)	
	Southeast	30 (30)	33 (28.7)	19 (33.3)	82 (30.1)	11 (29.7)	14 (28)	2 (12.5)	27 (26.2)	
	Midwest	4 (4)	2 (1.7)	4 (7)	10 (3.7)	3 (8.1)	4 (8)	1 (6.2)	8 (7.8)	
	South	10 (10)	12 (10.4)	7 (12.3)	29 (10.7)	11 (29.7)	14 (28)	2 (12.5)	27 (26.2)	
Total		100 (36.8)	115 (42.3)	57 (20.9)	**272 (100)**	37 (36)	50 (49)	16 (15)	**103 (100)**	
Smoking	No	95 (95)	98 (85.2)	50 (87.7)	243 (89.3)	34 (91.9)	47 (94)	14 (87.5)	95 (92.2)	0.202
	Yes	5 (5)	17 (14.8)	7 (12.3)	29 (10.7)	3 (8.1)	3 (6)	2 (12.5)	8 (7.8)	
Total		100 (36.8)	115 (42.3)	57 (20.9)	**272 (100)**	37 (36)	50 (49)	16 (15)	**103 (100)**	

[a]Years; [b]Patients origin region; *P* value determined by chi-squared test.

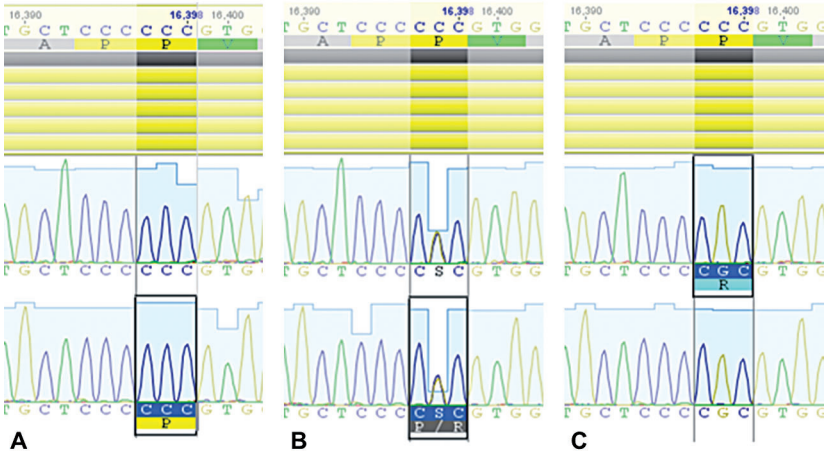

Figure 2. — TP53 gene sequencing for R72P polymorphism. A) Homozygous Pro, B) Heterozygous (Arg/Pro) and C) Homozygous Arg.

All statistical analyses were performed using SPSS 21.0. Continuous data were analyzed for normality. Student's *t*-test or ANOVA were used for between-group comparisons for parametric variables and Mann-Whitney U or Kruskal-Wallis for non-parametric variables. Pearson's chi-squared or Fisher's exact test were used for categorical variables. A logistic regression model was used to estimate the odds ratio (OR) and 95% confidence interval (CI) in the association between the *TP53* polymorphism and BC. Statistical significance was established as $p < 0.05$. Hardy-Weinberg equilibrium has assessed the distribution of the codon R72P genotypes.

Results

The study comprised 319 women in the control group (mean age 45±11.0 years), including 174 were from SP and 145 were from RO. In the case group, 340 women were selected (mean age 52±11.0) years, of which 188 were from SP and 152 from RO. Clinical variables age ($p < 0.0001$) and pregnancies ($p < 0.0001$) were significant, while parity ($p = 0.065$) showed a tendency to statistical significance. Menarche ($p = 0.539$) and miscarriage ($p = 0.249$) did not showed statistical significance in comparison to case and control groups. Risk factors were evaluated according to genotype distribution of case and control groups (Table 2).

Hardy-Weinberg equilibrium (HWE) test verified that control population deviated from equilibrium for R72P polymorphism, possibly, due to migration that leads to a heterogeneity of population in the North of the Country. Genotype of case population did not deviate from HWE. Allele frequency was assessed in the control group that showed 0.42% for Pro and 0.58% for Arg, and the case group showed 0.38% for Pro and 0.62% for Arg. Genotype distribution between groups did not show significance (Table 3).

BC patients showed high rate of ER-positive (67.3% Arg/Arg, 74.4% Arg/Pro and 60% Pro/Pro; $p = 0.162$) and PR-positive (60.2% Arg/Arg, 64.8% Arg/Pro, and 48.9% Pro/Pro; $p = 0.169$) in the genotype distribution compared to negative hormonal status. HER-2 status presented statistical relevance ($p = 0.011$), 87.8% (237) of BC patients exhibited HER-2 negative, and of these 40.5% (96) were Arg/Arg and 46.4% (110) were Arg/Pro. In the tumor stage, 45.04% of all BC group were TII, and 37.23% were T III ($p = 0.202$). The present authors found a greater incidence of Arg/Arg (48.03%) genotype with tumor Stage TII and Arg/Pro (48.6%) with TIII. IDC was most frequent in the three genotypes when compared with other breast histopathological tumors, Arg/Arg (91.4%), Arg/Pro (89.8%), and Pro/Pro (86.7%). The present authors evaluated the distribution of genotypes in IDC and the prevalence was Arg/Pro with 44.2%, followed by Arg/arg with 40.8% of patients ($p = 0.272$).

Case group with patients from SP and RO was assessed according to the origin region of each sample and tumor aggressiveness for differences determination among the profiles of BC (Table 4).

The present authors carried out a separate analysis of patients from RO for TP53 - R72P polymorphism and risk factors, due to heterogeneity of population in this region and the particularity of BC profile in these patients. A total of 152 women in the case group were evaluated with a mean age 51±11 years and 145 patients in the control group with mean age of 50±9.0 years. In a univariate analysis, the case and control groups were compared with risk factors; the results revealed that 55.6% of all women had less than 20 years at first full-term birth and only 7.3% had more than 30 years ($p = 0.171$). BC patients showed a greater body mass index (BMI) 30.9% when compared with control group 22.8% ($p = 0.113$). In addition, 21.2% of patients underwent hormonal therapy, and of this 26.9% were in the control group and 15.8% in the case group ($p = 0.019$).

The present authors assessed the ethnicity and smoke frequencies. Although there was a strong miscegenation in this population [21], the authors observed that 40.4% were considered white and 59.6% non-white, which included black persons and other. In the case group more than a half were non-white women (53.3%) and 46.7% were white ($p =$

Table 3. — *Genotype distribution of TP53 R72P in case and control groups (n=563).*

Groups	Pro/Pro n (%)	Arg/Pro n (%)	Arg/Arg n (%)	OR[a]	RR	95% CI	*P**
Control	57 (20.9)	115 (42.3)	100 (36.8)	1.449	1.281	0.941 to 2.231	0.091
Case	45 (15.4)	130 (44.7)	116 (39.9)				

[a]Adapted odds ratio, Pro/Pro vs Arg/Pro and Arg/Arg; RR - Relative risk; CI - Confidence interval; **P* value determined by chi-squared test.

Table 4. — *Comparisons of risk factors between BC patients from SP and RO.*

Variables		Total n (%)	Case RO n (%)	Case SP n (%)	*P*
Pregnancies	0	340 (100)	3 (2)	28 (14.9)	**0.001**[b]
	1		15 (9.9)	10 (5.3)	
	>1		134 (88.2)	150 (79.8)	
Age at first full-term birth[a]	≤20	306 (100)	81 (55.1)	60 (37.7)	**0.005**[b]
	>20 e ≤30		59 (40.1)	82 (51.6)	
	>30		7 (4.8)	17 (10.7)	
Neoadjuvant chemotherapy	No	340 (100)	92 (60.5)	138 (73.4)	**0.012**[b]
	Yes		60 (39.5)	50 (26.6)	
Surgical treatment	No	336 (100)	14 (9.3)	4 (2.2)	**0.004**[b]
	Yes		137 (90.7)	181 (97.8)	
Committed lymph node	≤5	323 (100)	128 (89.5)	142 (78.9)	**0.006**[c]
	> 5 e ≤25		14 (9.8)	38 (21.1)	
	> 25		1 (0.7)	0 (0)	
Positive lymph nodes	No	323 (100)	77 (53.8)	69 (38.3)	**0.005**[b]
	Yes		66 (46,2)	111 (61,7)	
HER-2	Negative	340 (100)	119 (83.2)	156 (90.7)	**0.047**[b]
	Positive		24 (16.8)	16 (9.3)	

[a]Years; [b]*P* value determined by chi-squared test; [c]*P* value determined by Fisher's exact.

0.023). Only 21.1% of patients with BC smoked and 73.7% never smoked; similar rates were observed in the control group (p = 0.041). Logistic regression was described for ethnicity, smoke, and hormonal therapy (Table 5).

Polymorphic analysis of TP53 - R72P in the case and control groups indicated that 79% were Arg/Arg or Arg/Pro genotypes and 21.1% Pro/Pro. Moreover, the authors assessed the genotype in the case group: 84.5% were Arg/Arg and Arg/Pro, and 15.5% were Pro/Pro. In comparisons between case and control, the authors observed that control group had more than a half (51.3%) patients with Arg/Arg genotype, and in the case group 56.2% were Arg/Pro (p = 0.098). Women with at least one allele Arg had a two-fold more susceptibility to cancer development (OR = 1.952;

Table 5. — *Logistic regression (n=297).*

Variables		df	OR	95% CI	*P**
Ethnicity		1	2.28	1.352 – 3.852	0.002
	No	2	-	-	0.049
Smoking	Stopped smoking	1	0.33	0.133 – 0.817	0.017
	Yes	1	0.74	0.398 – 1.404	0.366
Hormonal therapy		1	0.47	0.257 – 0.890	0.020

df - degrees of freedom; OR – Odds Ratio; CI - Confidence interval.

RR = 1.700; 95% CI = 0.982 – 3.877). The authors assessed the allele frequencies; the case group were 0.40% Pro and 0.60% Arg, and the control group was 0.45% and 0.55% respectively.

The authors also investigated the features consider risk factors with genotype distribution to BC development, almost all variables did not show significance, but the ethnicity (white *vs*. non-white women) showed a statistical relevance ($p = 0.032$).

Clinical and histological features were evaluated and some samples were excluded due to inconclusive results. Genotypes and tumor aggressiveness were assessed and the patients did not present differences in the tumor stage (Table 6). Hormonal receptors and molecular subtype were compared with genotype and the findings were relevant (Table 7).

Discussion

TP53 has a high incidence of genetic alterations in human cancer. Mutant p53 proteins constitute a complex family of several hundred proteins with heterogeneous properties [22]. Around 2% of all mutations occur at intron/exon boundaries and affect splicing donor or acceptor sites. Genome-wide splicing mutation analysis has identified *TP53* as the gene most commonly affected by a splicing mutation in BC [23]. Loss of heterozygosity by exclusion or methylation of 17p locus or the inactivation of p53 effectors can eliminate the *TP53* functions. Evidence suggests a role in the modulation of the frequency and mutagenesis mechanisms during the carcinogenesis [24]. The p53 inactivation in tumors highlights the relevance of its function as a tumor suppressor [25].

In the present study, it was demonstrated the polymorphic status of investigated women and it was analyzed the peripheral blood samples and not the tumoral corresponded samples. Thereby, the evaluation of genotypic profile of BC patients and risk factors showed that more than a half of case group were older than 50 years of age, and of this 39% had Arg/Arg genotype, 44% Arg/Pro, and 17% Pro/Pro. Women incidence who had menarche older than than 12 years of age was 80% in the control group and 65.5% in the case group with homozygous Arg. Early menarche was associated with the incidence of BC, mainly for patients with disease history in their family [26]. Decrease in the development BC risk may occur as a result of early pregnancy [27] that allows the breast development and influence the hormonal action [28, 29]. Moreover, early parity reduces the risk of BC development while nulliparity and late parity increase the risk. [30]. Studied patients with more than one pregnancy and with only one pregnancy were 70% in case and control groups, and the higher incidence was heterozygous (Arg/Pro). Hormonal therapy was few used by women in both groups.

The present authors performed the characterization exonic SNP in a non-silent substitution at codon R72P in exon 4, which showed genotype distribution between control and case groups and did not exhibit significant differences. Present findings corroborate with a study performed by Ma *et al.* [31]; it was observed that there was no association between R72P polymorphism with BC development, even when the studied population was grouped or carried out a stratified analysis according to ethnic group. Other studies emphasize that Arg and Pro alleles have no significant effects in patients with tumors and mutated TP53 with the loss of function [32].

A variation exists in the global population regarding codon R72P; the minor frequency allele Pro is present in 10% of Caucasians (Northern Europe) and 50% of Africans [18]. In Brazilian population, the Afro-Brazilians appear to have a high degree of European miscegenation, and the heterogeneity observed between Afro- and Euro-Brazilians is the same order of magnitude as detected in the comparison between European and African populations [33]. Allele incidence varies according to the region; Pro is most common in populations near the Equator line and Arg is most frequent in the population who live further [34]. In the present study, there was a greater incidence of Pro in the RO (North of Brazil) samples, which suggest some differences of the genotypic profile according to the region. The divergence between samples from RO and SP indicates that there are a miscegenation and differentiated migratory flow for each region of Brazil. Furthermore, the codon variations might influence the p53 functions; the p53 with Arg allele more effectively induces p53-mediated apoptosis than Pro, partially through targeting p53 to the mitochondria. Meanwhile, Pro allele when compared with Arg, more efficiently induces cell-cycle arrest and DNA repair [23, 35].

Table 6. — *Clinical and pathological feautures of patients from RO (n=103).*

Variables		Genotype			Total	*P*
		Arg/Arg	Arg/Pro	Pro/Pro	n (%)	
		n (%)	n (%)	n (%)		
Neoadjuvant chemotherapy	No	22 (30.99)	37 (52.11)	12 (16.90)	71 (100)	0.297[b]
	Yes	15 (46.88)	13 (40,62)	4 (12.50)	32 (100)	
Surgical treatment	No	1 (16.67)	4 (66.66)	1 (16.67)	6 (100)	0.534[c]
	Yes	36 (37.11)	46 (47.42)	15 (15.47)	97 (100)	
Tumor stage	0	0 (0)	3 (100)	0 (0)	3 (100)	0.607[c]
	I	5 (25)	11 (55)	4 (20)	20 (100)	
	II	21 (42.86)	21 (42.86)	7 (14.28)	49 (100)	
	III	10 (33.33)	15(50)	5(16.67)	30 (100)	
	IV	1 (100)	0 (0)	0 (0)	1 (100)	
Histological types	DCIS	0 (0)	3 (100)	0 (0)	3 (100)	0.379[c]
	IDC	32 (35.55)	44 (48.89)	14 (15.56)	90 (100)	
	ILC	2 (50)	2 (50)	0 (0)	4 (100)	
	Other	3 (50)	1 (16.67)	2 (33.33)	6 (100)	
Histological grade	I	6 (60)	4 (40)	0 (0)	10 (100)	0.365[c]
	II	20 (35.71)	28 (50)	8 (14.29)	56 (100)	
	III	11 (32.35)	15 (44.12)	8 (23.53)	34 (100)	
Adapted histological grade	I	6 (60)	4 (40)	0 (0)	10 (100)	0.201[c]
	II/III	31 (34.44)	43 (47.78)	16 (17.78)	90 (100)	
Positive lymph nodes	No	22 (41.50)	23 (43.40)	8 (15.10)	53 (100)	0.508[b]
	Yes	15 (30.61)	26 (53.06)	8 (16.33)	49 (100)	
Committed lymph node	≤5	34 (36.56)	43 (46.24)	16 (17.20)	93 (100)	0.702[c]
	5-25	3 (37.50)	5 (62.50)	0 (0)	8 (100)	
	>25	0 (0)	1 (100)	0 (0)	1 (100)	
ER	Negative	9 (27.30)	15 (45.50)	9 (27.30)	33 (100)	0.083[b]
	Positive	26 (38.20)	35 (51.50)	7 (10.30)	68 (100)	

[b]*P* value determined by chi-squared test; [c]*P* value determined by Fisher's exact; IDC – Invasive ductal carcinoma; ILC – Invasive lobular carcinoma.

Hormonal factors indicate the tumoral aggressiveness, and a better strategy for BC treatment are selected according to the tumor grade and tumor response. Hormonal receptor HER-2 is correlated with genotypic distribution:88% of cases was negative. Approximately 80% of BC women in the advanced stage are HER-2 negative, indicating that they have normal levels of HER-2 in the tumor and HER-2 therapy is not effective [36]. An accurate evaluation of histopathological factors may allow the best choice of adjuvant chemotherapy in patients with HER-2 negative BC with ER and PR positive [37].

Clinical and pathological variables, such as the pregnancy, age at first full-term birth, committed lymph node, as well as positive lymph nodes, neoadjuvant chemotherapy, and surgical treatment, are relevant to the assessment of patient profile and tumor feature. Neoadjuvant chemo-

Table 7. — *Genotype distribution and tumor aggressiveness.*

Variables		Genotype			Total	*P*
		Arg/Arg	Arg/Pro	Pro/Pro		
		n (%)	n (%)	n (%)	n (%)	
RP	Negative	10 (25.6)	18 (46.2)	11 (28.2)	39 (100)	**0.022**[b]
	Positive	25 (41.0)	31 (50.8)	5 (8.2)	61 (100)	
	Total	35 (35.0)	49 (49.0)	16 (16.0)	100 (100)	
HER-2	Negative	29 (35.8)	43 (53.1)	9 (11.1)	81 (100)	**0.008**[b]
	Positive	5 (29.4)	5 (29.4)	7 (41.2)	17 (100)	
	Total	34 (34.7)	48 (49)	16 (16.3)	98 (100)	
Molecular subtype	Luminal	22 (37.9)	30 (51.7)	6 (10.3)	58 (100)	**0.030**[c]
	Triple negative	5 (23.8)	12 (57.1)	4 (19)	21 (100)	
	HER-2 positive	2 (22.2)	2 (22.2)	5 (55.6)	9 (100)	
	Total	29 (33)	44 (50)	15 (17)	88 (100)	

[b]*P* value determined by chi-squared test; [c]*P* value determined by Fisher's exact.

therapy was not performed in 68% of patients, nevertheless, 95% of women were subjected to surgical treatment. This therapy is indicated for patients with early stage of tumor leading to an increase of conservative surgeries, improvement of surgical outcomes, and adequate evaluation of the prognosis [38, 39]. Late diagnosis is the main cause of high rates of women with BC. Almost all patients had tumor Stage II or III and IDC with positive lymph nodes, but only a few patients had more than five committed lymph nodes. A greater susceptibility to any type of metastasis was found in women with IDC who had Arg/Pro genotype [40]. Loss of p53 protein results in the pathway disruption that inhibits metastasis and defective transcriptionally *TP53* genes may acquire additional functions that promote metastasis [41].

Notably, risk factors as lifestyle, regionalization, reproductive aspects, and genetic inheritance are targets of unceasing studies between different populations, seeking to draw a demographic and genetic profile for BC development, therapeutic targets, diagnosis, and more specific and efficient treatments. Gonçalves *et al.* [42] observed that there exists an association of homozygous Arg and BC risk. There is a hypothesis that genetic factors might contribute to differences in BC among the Asian and Western populations [43]. Several studies highlight that the combination of genetic variations or *R72P* polymorphism can reduce the BC risk and show the importance of gene interaction by the means of p53 pathways [44- 46].

In contrast, in the present authors' previous study, the polymorphism in codon R72P showed possible associations between nuclear grade and adapted histologic grade in the BC. However, the present authors did not observe significance in the alleles and genotype comparisons with BC [14]. As the genetic nature of BC is complex, individual polymorphisms are likely to have a modest effect on risk. Evidences indicate that, besides environmental factors, genetic components and gene-gene and gene-environment interactions also play important roles in cancer development [22]. Population feature seems to be an important reason to discrepancies in the association of the polymorphism in the frequency of this disease [47, 48]. Although there is evidence that polymorphic variants in p53 may contribute to cancer susceptibility, the issue remains highly complicated [22]. Data presented and observed in some researches performed in different regions elucidate how it is necessary a cautious observation in polymorphism analysis and its risk factors considering the features of each studied population.

Conclusions

In summary, the present authors found that the R72P (rs1042522) polymorphism in *TP53* gene seems to be associated with some risk factors related to reproductive life, contraceptives or hormonal treatments, ethnicity, and lifestyle. Results presented here provide additional evidence that the genetic variation between the alleles Arg and Pro appears not to be directly correlated with BC development, and the R72P polymorphism may not be a potential marker for BC. However, more studies are necessary to further understand these genetic alterations in BC develop-

ment and metastasis, once there is a high mortality rate in BC.

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