Breast cancer risk and polymorphisms in genes involved in metabolism of estrogens (*CYP17*, *HSD17β1*, *COMT* and *MnSOD*): Possible protective role of *MnSOD* gene polymorphism Val/Ala and Ala/Ala in women that never breast fed

SUSANA N. SILVA[1], MARISA N. CABRAL[1], GUILHERME BEZERRA DE CASTRO[5], MARCELO PIRES[5], ANA PAULA AZEVEDO[3], ISABEL MANITA[4], JULIETA ESPERANÇA PINA[2,3], JOSÉ RUEFF[1] and JORGE GASPAR[1]

Faculty of Medical Sciences, [1]Department of Genetics, [2]Department of Laboratorial Medicine, New University of Lisbon; [3]Department of Clinical Pathology, Hospital de S. Francisco Xavier, Lisboa; [4]Hospital Garcia da Horta, Unit of Endocrinology, Av. Torrado da Silva, Almada, Portugal; [5]Molecular Cancer Research Center, Av. Isaac Póvoas 1251, Sala 103, Ed. Nacional Palácius, Cuiabá MT 78045-200, Brazil

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Abstract. Polymorphisms in genes encoding enzymes involved in estrogen metabolism are held to be candidates for associations with breast disease, since there is evidence that circulating estrogens are associated with breast cancer risk. In this study, we evaluated the frequency of different polymorphisms related with estrogen metabolism [*COMT* Val158Met, *CYP17* (5'UTR, T27C); *HSD17β1* Gly313Ser and *MnSOD* Val16Ala] in a breast cancer resistant population, the Xavante Indians, and the frequencies were compared with the ones reported in other populations where breast cancer case-control studies dealing with these polymorphisms have been carried out. The data obtained showed that, apart from the *MnSOD* Val16Ala polymorphism where the frequency of the variant allele was much higher than that reported in other populations, all the others were within the range reported in other populations. Considering these data we carried out a case-control study in the Portuguese population (241 cases and 457 controls) in order to evaluate the potential role of this polymorphism in breast cancer susceptibility. The results obtained did not reveal a significant association between individual genotypes and breast cancer risk. However, when the population was stratified for breast feeding, it was observed that for the patients that never breast fed the presence of the variant allele (Ala) was marginally associated with a decreased risk for this pathology (adjusted OR: 0.575 (0.327-1.011). These data seem to suggest that individuals who never breast fed with *MnSOD* Val16Ala variant allele are at a lower risk for breast cancer, but larger studies are required to confirm these results.

Correspondence to: Dr José Rueff, Faculty of Medical Sciences, Department of Genetics, New University of Lisbon, Rua da Junqueira 96, P-1349-008 Lisboa, Portugal
E-mail: rueff.gene@fcm.unl.pt

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Introduction

Breast cancer is the most common form of cancer among women. Despite the enormous number of cases, the exact causes of breast cancer remain elusive. Progress in molecular genetics has identified key genes that account for a hereditary predisposition to breast cancer. However, about 90-95% of breast cancer cases are sporadic and occur in women in the absence of mutations in susceptibility genes such as *BRCA-1* and *BRCA-2* (1,2). The data from epidemiological studies indicates that, apart from a family history of breast cancer, most of the risk factors for this pathology are related to reproductive and hormonal factors. In fact, an increased risk of breast cancer due to prolonged exposure to estrogen has been well documented by epidemiological observations showing that estrogen-related risk factors, including age at menarche, age at menopause, parity and age at first full-term pregnancy, are significantly associated with breast cancer risk (3), but the explanation to this remains unanswered.

Several genetic polymorphisms have been identified in genes involved in estrogen biosynthesis (e.g. *CYP17* and *CYP19*) and estrogen metabolism (e.g. *CYP1B1* and *COMT*) that may influence estrogen concentrations. Additionally, catechol estrogens produce Reactive Oxygen Species (ROS) suggesting a potential involvement of the polymorphic forms of genes coding to ROS detoxifying enzymes (e.g. *MnSOD*).

The *CYP17* gene codes for a protein involved in the conversion of 17-hydroxypregnenolone and 17-hydroxy-

progesterone to dehydroepiandrosterone (DHEA) and androstenedione, respectively. A polymorphism identified in the 5' untranslated region consists of a T→C substitution at position 27, which results in an additional Sp1-type promoter site (4). The role of this polymorphism in breast cancer susceptibility has been extensively studied giving rise to contradictory results (5-7).

The *HSD17β1* (17ß-hydroxysteroid dehydrogenase type 1) gene produces the enzyme that catalyzes the final step of estradiol biosynthesis, estrone to estradiol. Several single nucleotide polymorphisms (SNPs) in the *HSD17β1* gene have been described, although the function of these polymorphisms remains unclear. A non-synonymous polymorphism in exon 6 (Gly313Ser) that leads to an amino acid change from serine to glycine at position 313 has been studied in relation to breast cancer but the results obtained do not seem to support an association between this polymorphism and breast cancer (8,9).

COMT catalyzes the methylation of various endobiotic and xenobiotic substances preventing quinone formation and redox cycling, and therefore might protect DNA from oxidative damage. A G to A transition, leads to an amino acid change from valine to methionine at codon 108, resulting in a lower *COMT* activity. The enzyme activity of the Met/Met genotype is a quarter of that obtained with wild genotype, and subjects heterozygous exhibit intermediate enzyme activity, but the data available concerning its involvement in breast cancer are also contradictory (3,10-12).

Antioxidant enzymes such as superoxide dismutase (SOD) protect cells from oxidative stress, and generation of ROS has been implicated in the etiology of a diversity of human diseases. SOD catalyzes the dismutation of superoxide radical to H_2O_2 and O_2. A T to C substitution, in the *MnSOD* gene leads to a Val to Ala change at the codon 16 (Val16Ala), which alters the secondary structure of the protein, has been noted to affect the transport of *MnSOD* into the mitochondria (13), but the data obtained concerning the role of this polymorphism on breast cancer susceptibility has also been contradictory (14-17).

Since the data available concerning the role of these polymorphisms on breast cancer risk has been contradictory and taking advantage of the use of a breast cancer resistant population, the Xavante Indians from Sangradouro (Mato Grosso, Brazil), identified by Dr Guilherme Bezerra de Castro (18), the frequency of the polymorphisms described above was evaluated on that population in order to have insights into their potential role in breast cancer susceptibility. For the polymorphisms that showed a major frequency difference, compared with the values reported in different populations (*MnSOD*), we carried out a hospital based case-control study in a Caucasian Portuguese population in order to evaluate the potential modifying role of the *MnSOD* polymorphism (Val16Ala) in breast cancer susceptibility.

Materials and methods

Study subjects. Healthcare services in Portugal are mainly public and generally assist the whole population, and breast cancer treatment units are located in all the major hospitals.

This study includes 241 Caucasian breast cancer female patients, recruited at São Francisco Xavier Hospital (Department of Laboratorial Medicine), without previous history of neoplasic disease, thyroid pathology and blood transfusions. Histological diagnosis was confirmed in all the cases and includes 213 ductal type carcinomas (88.4%), 11 lobular type carcinomas (4.6%) and 17 cases classified as other types of breast tumours (7.1%). The control population (n=457), matched for sex and ethnicity, with no previous or concurrent malignant disease, was recruited at the same hospital where they were observed for non-malignant pathology. The anonymity of the patients and the control population was guaranteed, and all studies were conducted with the written informed consent of all the individuals involved, obtained prior to blood withdrawal. Information on demographic characteristics, family history of cancer, lifestyle habits (e.g. smoking, alcohol drinking) was collected using a questionnaire administered by trained interviewers. Former smokers were defined as those who gave up smoking two years before cancer diagnosis or two years before the inclusion date as corresponding matched case. The response rate was higher than 95% for both the cases and controls.

A second control population (n=179) characterized by an absolute absence of breast cancer were also included in this study. This population was identified by Dr Guilherme Bezerra de Castro in the Indian reserve of Sangradouro. In this reserve the predominant ethnic group are Xavante, and since 1906, very close to the reserve is a Salesian Mission with a medical ambulatory structure having a reliable event registry of the epidemiological events. This medical unit is available for the Xavante population residing around Sangradouro (around 10.000 individuals). The epidemiological data available show that this population is characterized by an absolute absence of breast cancer. The Indians adopt the Iroques crossbreeding model and they do not mix with other tribes living nearby. Interestingly, all the non-mixed Indian races resident in the state of Mato Grosso has a 0 Rh+ blood group type (18,19).

DNA extraction. Blood samples of the patients and controls were collected into 10 ml heparinized tubes and stored at -20˚C until use. Genomic DNA was obtained from 250 μl of whole blood using a commercially available kit according to the manufacturer's instructions (QIAamp DNA extraction kit; Qiagen, Hilden, Germany). Each DNA sample was stored at -20˚C until analysis.

Genotyping CYP17 (5'UTR, T27C), HSD17β1 Gly313Ser, COMT Val158Met and MnSOD Val16Ala gene polymorphisms. The genotyping of *CYP17* (5'UTR, T27C), HSD17ß1 (Gly313Ser), *COMT* (Val158Met) and *MnSOD* (Val16Ala), was determined by PCR-RFLP. The primers and PCR conditions for the polymorphic sites of these genes are shown in Table I. For all of them (*CYP17*, *HSD17β1*, *COMT* and *MnSOD*) the nucleotide polymorphisms resulted in either a gain or a loss of restriction site, which therefore allowed the wild-type and variant alleles to be discriminated by RFLP after appropriate restriction enzyme digestion.

For *CYP17* the PCR was carried out as described by Ambrosone *et al* (20); for *HSD17β1* as described by Feigelson *et al* (21); for the amplification of *COMT* fragment the reaction was performed as described by Garner *et al* (22)

Table I. PCR-RFLP for *CYP17* (5'UTR, T27C), *HSD17β1* Gly313Ser, *COMT* Val158Met and *MnSOD* Val16Ala polymorphisms.

Gene	Primer sequences	Temperature (˚C)	PCR product (bp)	Polymorphism; effect on restriction enzyme site	Patterns after restriction enzyme digestion
CYP17	Forward: CAT TCG CAC TCT GGA GTC Reverse: AGG CTC TTG GGG TAC TTG	56	414	5'-UTR, T27C T→C, an additional *MspA1I* site.	T/T: 414 bp; T/C: 414, 291, 123 bp; C/C: 291, 123 bp
HSD17β1	Forward 1: CGG GAG CCG CTC TGG GGC GAT CT Reverse 1: GTG CCA CTG TGC TGA TTT TTA AAT TTT CT Forward 2: AAG CCG ACC CTG CGC TAC TTC AC Reverse 2: TCT ATC TTA ATT AGC CAC CCA CAG C	60	349	Gly313Ser G→A, destroyed one *BstUI* site.	G/G: 192,77, 80 bp; G/A: 269, 192, 77, 80 bp; A/A: 269, 80 bp
COMT	Forward: TAC TGT GGC TAC TCA GCT GTG C Reverse: GTG AAC GTG GTG TGA ACA CC	60	236	Val158Met G→A, an additional *NlaIII* site.	G/G: 114, 54, 40, 28 bp; G/A: 114, 96, 54, 40, 28, 18 bp; A/A: 96, 54, 40, 28, 18 bp
MnSOD	Forward: TAG ACG GTC CCG CGG CGC TGA Reverse: CCG TAG TCG TAG GGC AGG TCG GGG A	66	134	Val16Ala T→C, destroyed one *BsaWI* site.	T/T: 71, 63 bp; T/C: 134, 71, 63 bp; C/C: 134 bp

with minor modifications. Concerning the *MnSOD* polymorphism the primers used were described by Egan *et al* (14). The PCR performed for this gene was carried out with 50 ng of DNA in 50 μl reaction volume, containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.8 mM dNTP, 1.0 μM of each primer and 1.25 U of AmpliTaq Gold (Applied Biosystems). The amplification started with an initial denaturation step at 95˚C for 7 min, cycling parameters were 35 cycles of 95˚C for 30 sec, specific annealing temperature for 30 sec, 72˚C for 30 sec and a final extension at 72˚C for 10 min. After amplification 10 μl of the PCR products was digested with the appropriate restriction enzymes and eletrophoresed in 4% or 2% (*HSD17β1*, *CYP17*) agarose gel with ethidium bromide (0.5 μg/ml) for visualization under ultraviolet light. The expected products for each genotype of the tested genes are shown in Table I.

All the genotype determinations were carried out twice in independent experiments and inconclusive samples were reanalysed.

Statistical analysis. The analysis of Hardy-Weinberg frequencies for *CYP17*, *HSD17β1*, *COMT* and *MnSOD* alleles in the control, patients and Xavante populations were carried out using exact probability tests available in Mendel (V5.7.2) software (23).

The χ^2 test was used to evaluate the differences in genotype frequency, smoking status and alcohol consumption distributions between the cases and controls.

The Kolmogorov-Smirnov test were used in order to verify the normality of the continuous variables (e.g. age) and the Levene test was used to analyse the homogeneity of variances. The statistical analysis of the homogeneity of age distributions between the cases and controls was carried out using the t-test.

The crude and adjusted odds ratio (OR) and the corresponding 95% confidence intervals (CI) were calculated using unconditional multiple logistic regression. The model for adjusted OR included terms for age at diagnosis (30-49, 50-69 and ≥70 years), the lower age group being the referent class; alcohol consumption (never, social and regular drinkers) never drinkers being the referent group, and smoking habits (smokers/non-smokers), non-smokers being the referent group. All analyses were performed with an SPSS statistical package (version 10.5) (SPSS Inc., Chicago, IL).

Results

The frequency of the polymorphisms *CYP17* in the 5' untranslated region, *HSD17β1* in exon 6 (Gly313Ser) that leads to an amino acid change from serine to glycine at position 313, *COMT* with a G→A transition, leading to an amino acid change from valine to methionine at codon 108, and *MnSOD*, leading to a Val→Ala change at codon 16 (Val16Ala), were evaluated in the Xavante Indian population (Table II). The distributions of the different genotypes are in agreement with Hardy-Weinberg equilibrium except for the *COMT* Val→Met polymorphism (P<0.05).

Table II. Allelic and genotypic frequencies for the four polymorphisms under study in the Xavante Indian population (n=179).

Gene/polymorphism	Genotypic frequency n	(%)	Allelic frequency ± standard error
CYP17 (5'-UTR T27C)	T/T=58	(34.4%)	T=0.5503 (±0.0263)
	T/C=81	(45.3%)	
	C/C=40	(22.3%)	C=0.4497 (±0.0263)
HSD17β1 (Gly313Ser)	Gly/Gly=22	(12.3%)	Gly=0.6955 (±0.0243)
	Gly/Ser=65	(36.3%)	
	Ser/Ser=92	(51.4%)	Ser=0.3045 (±0.0243)
COMT (Val158Met)	Val/Val=71	(39.7%)	Val=0.6620 (±0.0250)
	Val/Met=95	(53.1%)	
	Met/Met=13	(7.3%)	Met=0.3380 (±0.0250)
MnSOD (Val16Ala)	Val/Val=10	(5.6%)	Val=0.2318 (±0.0223)
	Val/Ala=63	(35.2%)	
	Ala/Ala=106	(59.2%)	Ala=0.7682 (±0.0223)

When the allelic and genotypic frequencies of the *CYP17* polymorphism in the Xavante population were compared with the data reported for other populations (Caucasians, Chinese, Korean, Japanese, Afro-Americans) it was observed that the frequencies of the *CYP17* (5'-UTR) polymorphism are similar to the values reported for these populations (Table III). Interestingly in these studies the role of this polymorphism in breast cancer susceptibility was only reported in a small study conducted in a Swedish population (5), but not in other studies conducted in Caucasian (6) Chinese (7,9), Korean (24), Japanese (25) and Afro-American (25) populations.

Concerning the *HSD17β1* polymorphism (Gly313Ser) the data reported until now, in Caucasian and Chinese populations, do not suggest an involvement of this polymorphism in breast cancer risk (8,9). However, the allelic and genotypic frequencies observed in the Xavante population (Table I) show that the frequency of the wild-type allele is higher than that reported in Caucasian and Chinese populations (8,9) (Table III).

The allelic and genotypic frequencies of the *COMT* valine to methionine polymorphism at codon 108 observed in the Xavante population are in the range described for the Chinese and Japanese (Table III), but the breast cancer case-control studies conducted on Asian populations show contradictory results (3,10-12).

Interestingly, concerning the *MnSOD* polymorphisms the allelic and genotypic frequencies observed in the Xavante population (Table II) are very different when compared with the frequencies observed in other populations (Table III). Taking into account the data available concerning the role of this polymorphism in breast cancer susceptibility, albeit contradictary, and since the frequency of this polymorphism in a population characterized by an absolute absence of breast cancer is very different from the frequencies observed in other populations we carried out a hospital based case-control study in order to evaluate the potential role of this polymorphism in breast cancer susceptibility.

Table IV shows the main characteristics of the Portuguese cancer and control populations. The results show that there are no significant differences in the age groups and *MnSOD* polymorphism frequencies between the populations. However, regular drinkers and current smokers are over represented in the cases when compared with the control population (Table IV).

The results obtained concerning the *MnSOD* polymorphism showed that the allelic frequencies of the wild-type alleles are 0.5477±0.0277 and 0.5186±0.0165 for both the cases and controls respectively, and the genotypic frequencies (Table IV) observed in the control population are in agreement with the results previously reported in other Caucasian populations (14,16,26).

The frequencies of *MnSOD* genotypes in the control and cancer populations were not in agreement with the Hardy-Weinberg expectations ($P<0.001$, exact probability test).

The results obtained for the *MnSOD* polymorphism do not support an association between the presence of one genotype of this polymorphism and individual susceptibility towards breast cancer, since the genotypic frequencies in the Portuguese cases and the control populations are not significantly different (Table IV) and after logistic regression analysis a significant OR was not observed concerning the effect of the different genotypes after adjustments for age, tobacco smoking and alcohol consumption (Table V). However, after stratification by breast feeding it was observed that in women that never breast fed the presence of a variant allele is associated with a reduced risk for this pathology, and this effect almost reached significance, adjusted OR=0.575 (C.I: 0.327-1.011) (P=0.054) (Table V).

Discussion

Since the polymorphic genes *CYP17*, *HSD17β1*, *COMT* and *MnSOD* are involved in the metabolism of estrogens, we selected different polymorphisms in these genes, previously evaluated in several case-control studies on breast cancer, to

Table III. Allelic and genotypic frequencies of the polymorphisms evaluated in the Xavante and in different populations.

Population	Reference	*CYP17* 5'UTR, T27C	
		Allelic frequency (T; C) %	Genotypic frequency (TT; TC) %
Caucasian	Jungestrom *et al* (5)	T=68.80%; C=31.20%	T/T=45.00%; T/C=47.00%;
	Haiman *et al* (6)	T=60.00%; C=40.00%	T/T=35.10%; T/C=49.70%;
	Miyoshi and Noguchi (27)	T=55.00%; C=45.00%	T/T=34.00%; T/C=42.00%;
	Miyoshi *et al* (25)	T=65.80%; C=34.20%	T/T=49.00%; T/C=33.00%;
	Miyoshi *et al* (25)	T=62.20%; C=37.80%	T/T=39.00%; T/C=47.00%;
	Miyoshi *et al* (25)	T=61.90%; C=38.10%	T/T=37.00%; T/C=50.00%;
Asian	Huang *et al* (7)	T=47.20%; C=52.80%	T/T=22.20%; T/C=50.00%;
	Shin *et al* (24)	T=43.30%; C=56.70%	T/T=20.80%; T/C=45.10%;
	Wu *et al* (9)	T=41.10%; C=58.90%	T/T=16.20%; T/C=49.60%;
	Miyoshi *et al* (25)	T=51.80%; C=48.20%	T/T=25.00%; T/C=54.00%;
Others	Miyoshi *et al* (25)	T=62.80%; C=37.20%	T/T=39.00%; T/C=48.00%;
	Miyoshi and Noguchi (27)	T=65.00%: C=35.00%	T/T=42.00%; T/C=46.00%;
		HSD17β1 Gly313Ser	
Population	Reference	Allelic frequency (Gly; Ser) %	Genotypic frequency (Gly/Gly; Gly/Ser) %
Caucasian	Setiawan *et al* (8)	Gly=46.40%; Ser=53.60%	Gly/Gly=20.70%; Gly/Ser=51.40%;
Asian	Wu *et al* (9)	Gly=56.60%; Ser=43.40%	Gly/Gly=32.40%; Gly/Ser=48.30%;
		COMT Val158Met	
Population	Reference	Allelic frequency (Val; Met) %	Genotypic frequency (Val/Val; Val/Met) %
Caucasian	Wedrén *et al* (33)	Val=44.00%; Met=57.00%	Val/Val=18.30%; Val/Met=49.40%;
	Lavigne *et al* (12)	Val=48.20%; Met=51.80%	Val/Val=23.70%; Val/Met=49.10%;
	Sazci *et al* (28)	Val=60.30%; Met=39.70%	Val/Val=26.60%; Val/Met=62.70%;
	Kocabas *et al* (11)	Val=60.70%; Met=39.30%	Val/Val=33.98%; Val/Met=53.40%;
Asian	Yim *et al* (10)	Val=76.40%; Met=23.90%	Val/Val=61.96%; Val/Met=28.20%;
	Cheng *et al* (3)	Val=74.50%; Met=25.50%	Val/Val=61.96%; Val/Met=28.20%;
	Huang *et al* (7)	Val=74.80%; Met=25.20%	Val/Val=52.80%; Val/Met=44.00%;
	Wu *et al* (9)	Val=72.90%; Met=27.10%	Val/Val=53.30%; Val/Met=39.20%;
	Wu *et al* (9)	Val=65.70%; Met=34.30%	Val/Val=43.70%; Val/Met=44.20%;
	Wu *et al* (9)	Val=73.50%; Met=26.50%	Val/Val=54.20%; Val/Met=38.60%;
		MnSOD Val16Ala	
Caucasian	Egan *et al* (14)	Val=50.30%; Ala=49.70%	Val/Val=26.20%; Val/Ala=48.30%;
	Mitrunen *et al* (26)	Val=55.70%; Ala=44.30%	Val/Val=31.70%; Val/Ala=47.90%;
	Millikan *et al* (16)	Val=49.30%; Ala=50.70%	Val/Val=23.40%; Val/Ala=51.60%;
	Kocabas *et al* (29)	Val=44.00%; Ala=56.00%	Val/Val=27.00%; Val/Ala=39.00%;
Asian	Cai *et al* (17)	Val=86.00%; Ala=14.00%	Val/Val=73.90%; Val/Ala=24.20%;
	Cheng *et al* (3)	Val=85.40%; Ala=14.60%	Val/Val=73.10%; Val/Ala=24.50%;
Others	Millikan *et al* (16)	Val=55.30%; Ala=44.70%	Val/Val=28.95%; Val/Ala=52.70%;

Table IV. General characteristics of breast cancer cases and control population studied concerning the role of *MnSOD* polymorphism on breast cancer risk.

Characteristics	Cases n (%)	Controls n (%)	P-value[a]
Age			
30-39	9 (3.7%)	20 (4.4%)	
40-49	42 (17.4%)	78 (17.1%)	
50-59	56 (23.2%)	109 (23.9%)	0.994
60-69	75 (31.1%)	141 (30.9%)	
≥70	59 (24.5%)	109 (23.9%)	
Smoking habits			
Never and former	208 (86.7%)	417 (91.6%)[b]	
Current	32 (13.3%)	38 (8.4%)	0.038
Missing	1	2	
Alcohol consumption			
Never	186 (77.2%)	376 (83.0%)	
Occasional	19 (7.9%)	52 (11.5%)	<0.001
Daily	36 (14.9%)	25 (5.5%)	
Missing	4	0	
MnSOD Val16Ala			
CC	59 (24.5%)	99 (21.7%)	
CG	146 (60.6%)	276 (60.4%)	0.497
GG	36 (14.9%)	82 (17.9%)	

[a]See Materials and methods, [b]includes 6 former smokers.

Table V. ORs (95% CI) for breast cancer in relation to the *MnSOD* (Val16Ala) genotypes.

Val16Ala *MnSOD* genotypes; n (number of cases)	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
	All cases	
Val/Val; (n=59)	1 (Reference)	1 (Reference)
Val/Ala; (n=146)	0.888 (0.607-1.298)	0.860 (0.583-1.268)
Ala/Ala; (n=36)	0.737 (0.444-1.224)	0.733 (0.436-1.232)
Val/Ala + Ala/Ala; (n=182)	0.853 (0.590-1.533)	0.837 (0.574-1.219)
	Cases that breast fed	
Val/Val; (n=35)	1 (Reference)	1 (Reference)
Val/Ala; (n=106)	1.090 (0.698-1.703)	0.973 (0.612-1.547)
Ala/Ala; (n=27)	0.931 (0.521-1.665)	0.905 (0.495-1.653)
Val/Ala + Ala/Ala; (n=133)	1.054 (0.683-1.626)	0.958 (0.611-1.504)
	Cases that never breast fed	
Val/Val; (n=22)	1 (Reference)	1 (Reference)
Val/Ala; (n=39)	0.636 (0.359-1.125)	0.607 (0.339-1.088)
Ala/Ala; (n=9)	0.494 (0.216-1.131)	0.468 (0.201-1.088)
Val/Ala + Ala/Ala; (n=48)	0.566 (0.321-0.999)	0.575 (0.327-1.011)

[a]ORs were adjusted for age (30-39[b], 40-49, 50-59, 60-69 and ≥70 years) and smoking status (never[b], former and current smokers) and alcohol consumption (never[b], social and regular drinkers), [b]depicts referent classes.

determine their frequency in a breast cancer resistant population (Xavante Indians). The results obtained (Table II) for *CYP17* T27C polymorphism showed that the allelic frequency of this polymorphism in the Xavante population is within the range reported in other populations (Table III). Since the frequency of this polymorphism in the Xavante Indians is similar to the frequencies reported in other populations, and only one (5) in six (6,7,9,24,27) studies report a weak association (OR=2.0 for the presence of the variant allele) it is reasonable to consider that this polymorphism does not seem to have a major role in breast cancer susceptibility.

Concerning the allelic frequency of the *HSD17β1* polymorphism in the Xavante population it was observed that wild-type alleles are over-represented when compared with Caucasian and Chinese populations (Tables II and III), and also two large studies dealing with its potential role in breast cancer consistently reported an absence of this association (8,9). Taken together these data do not support a major role of this polymorphism in breast cancer susceptibility.

For the *COMT* polymorphism the frequency observed in Xavante population is also in the range described in other populations (Table II) and very close to the frequencies reported in Asian populations (Table III). In the Xavante population the genotypic frequency observed is not in agreement with the Hardy-Weinberg equilibrium suggesting that this polymorphism is under selective pressure. The data reported on the potential role of this polymorphism in breast cancer have been contradictory (5-7), but the studies showing a positive association, between this polymorphism and breast cancer risk, are mainly observed in Asian populations (Table III), and only one study conducted in the Turkish population revealed a positive association between the presence of the Met/Met genotype and increased breast cancer risk (28). The studies on a positive association between *COMT* variant alleles and breast cancer risk reported OR values between 1.3 (n=740) (3) and 3.6 (n=125) (7). Considering these data, and since the number of cases evaluated in the different studies is small and in eight of ten studies the number of cases evaluated are less than 250, we can not exclude a potential role of the variant allele on breast cancer susceptibility.

The frequency of the variant allele of *MnSOD* in the Xavante population is higher than the frequencies reported in other populations (Tables II and III). This polymorphic gene codes for a protein that catalyzes the dismutation of two superoxide radicals in the mitochondrion, producing H_2O_2 and oxygen. When there is an excessive production of superoxide radicals as a consequence of endogenous metabolism (e.g. the metabolism of estradiol, in which superoxide anions are produced via redox cycling of quinones and semiquinones, and other intermediates), by exposure to toxic agents, or due to pathological processes, and/or when there are insufficient *in vivo* defence mechanisms, oxidative stress may occur, leading to DNA damage, lipid peroxidation, protein modification, membrane disruption, and mitochondrial damage. The function of the *MnSOD* polymorphism is not fully understood. Recent studies show that the variant form of *MnSOD* is more efficiently transported through the mitochondrial membrane (13), suggesting that, individuals with at least one variant allele might have higher *MnSOD* activity.

The frequency of the *MnSOD* polymorphism in the Portuguese control population is similar to the values reported in other Caucasian populations (Tables III and IV), and the deviation of the Hardy-Weinberg equilibrium suggests that this polymorphism might be under selective pressure. The results obtained concerning the role of this polymorphism in breast cancer in the Portuguese population did not reveal a major role of this polymorphism in breast cancer susceptibility (Table V), which is in agreement with the data reported by other groups (3,14,16,17,29) except with that by Mitrunen *et al* (26), who found that carriers of at least one variant allele are at an increased risk for this pathology. The inconsistency of results might be explained by different genetic backgrounds, or by different lifestyles (e.g. different exposure levels to chemical carcinogens, differences in the intake of antioxidants). Several studies reported a gene-environment interaction between this polymorphism and the levels of endogenous antioxidants (30), but these levels are strongly influenced by cultural dietary habits, possibly explaining the inconsistent results observed in the different studies.

When the population was stratified according to breast feeding (woman that breast fed and woman that never breast fed) it was observed that, in woman that never breast fed, the presence of a variant allele is associated with a decreased risk for breast cancer (adjusted OR=0.575), and this value almost reached statistical significance (P=0.054). A review of 47 studies carried out in 30 countries, involving about 50,000 women with breast cancer, and 97,000 controls, suggested that breastfeeding may be responsible for 2/3 of the estimated reduction in breast cancer. The longer the duration of breastfeeding the lower the potential risk of breast cancer. It was estimated that the incidence of breast cancer in developed countries could be reduced to less than half (from 6.3 to 2.7%) if breastfeeding duration was longer (31). It is well known that milk contains exfoliated ductal cells, and in these cells obtained from milk samples it is possible to detect several types of aromatic DNA damage (32). Thus, considering these results it is reasonable to assume that the exfoliation of ductal cells as a consequence of breast feeding might remove a significant number of cells with genetic damage, preventing their transformation into neoplastic cells. In this case the potential protective role of the variant allele of *MnSOD* (Ala), in women who never breast fed, might be related with a more efficient transportation to mitochondria resulting in a higher activity as reported by Sutton *et al* (13), leading to a more efficient detoxification of superoxide arising from exposure to environmental and endogenous genotoxicants (e.g. Catechol estrogens). However, since other polymorphisms have been identified in this gene (http://snp500cancer.nci.nih.gov/) and other polymorphic enzymes are also involved in the detoxification of ROS or products arising from cellular ROS reactions (e.g. GSTM1, catalase), we can not exclude that other putative polymorphisms in this gene and/or in other genes associated with the detoxification of ROS might alone or in association be involved in the susceptibility to breast cancer. Additionally, by comparing genotypic and allelic frequencies in isolated breast cancer resistant population, and in other populations with significant incidences of breast cancer, might also provide information

on the role of the different genetic polymorphisms in breast cancer risk, allowing a rapid identification of the most relevant polymorphisms associated with breast cancer risk.

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