RESEARCH COMMUNICATION

CYP2W1, CYP4F11 and CYP8A1 Polymorphisms and Interaction of CYP2W1 Genotypes with Risk Factors in Mexican Women with Breast Cancer

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Abstract

Breast cancer (BCa) is the leading type of cancer in Mexican women. Genetic factors, such as single nucleotide polymorphisms (SNP) of P450 system, have been reported in BCa. In this report, and for the first time in the literature, we analyzed the rs3735684 (7021 G>A), rs11553651 (15016 G>T) and rs56195291 (60020 C>G) polymorphisms in the CYP2W1, 4F11 and 8A1 genes in patients with BCa and in healthy Mexican women to identify a potential association between these polymorphisms and BCa risk. Patients and controls were used for polymorphism analysis using an allelic discrimination assay with TaqMan probes and confirmed by DNA sequencing. Links with clinic-pathological characteristics were also analyzed. Statistical analysis was performed using the standard χ^2 or Fisher exact test statistic. No significant differences were observed in the distributions of CYP2W1 (OR 8.6, 95%CI 0.43-172.5 P>0.05; OR 2.0, 95%CI 0.76-5.4, P>0.05) and CYP4F11 (OR 0.3, 95%CI 0.01-8.4 P>0.05) genotypes between the patients and controls. Only the CYP8A1 CC genotype was detected in patients with BCa and the controls. All polymorphism frequencies were in Hardy-Weinberg Equilibrium (HWE) in the controls (P>0.05). We found a significant association between BCa risk and smoking, use of oral contraceptives or hormonal replacement therapy (HRT), obesity, hyperglycemia, chronic diseases, family history of cancer and menopausal status in the population studied (P<0.05). Tobacco, oral contraceptive or HRT, chronic diseases and obesity or overweight were strongly associated with almost eight, thirty-five, nine and five-fold increased risk for BCa. Tobaco, obesity and hyperglycemia significantly increased the risk of BCa in the patients carrying variant genotypes of CYP2W1 (P<0.05). These results indicate that the CYP2W1 rs3735684, CYP4F11 rs11553651 and CYP8A1 rs56195291 SNPs are not a key risk factor for BCa in Mexican women. This study did not detect an association between the CYP2W1, 4F11 and 8A1 genes polymorphisms and BCa risk in a Mexican population. However, some clinico-pathological risk factors interact with CYP2W1 genotypes and modifies susceptibility to BCa

Keywords: P450 2W1/4F11/8A1 - polymorphisms - breast cancer - Mexican women - gene-environment interaction

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Introduction

Breast cancer (BCa) is the major cause of cancer death in women worldwide and most of the cases are in industrialized countries (WHO, 2008). BCa is the leading type of cancer in Mexican females (SINAIS, 2008). The etiology of most BCas is obscure. However, there are known risk factors for BCa including exposure to genotoxic agents during breast development, nulliparity, exposure to ionizing radiation, alcohol, tobacco and other lifestyle factors (Hsieh et al., 1990; Henderson, 1993). Several classes of environmental chemicals have also been implicated in BCa development and the putative agents are involved in the initiation of BCa. These agents include polycyclic aromatic hydrocarbons, nitropolycyclic aromatic hydrocarbons, aromatic amines, heterocyclic amines and polycyclic aromatic hydrocarbons produced the by incomplete combustion of carbon-containing compounds that are present in the atmosphere, tobacco smoke, and food (El-Bayoumi, 1992 Windmill, 1997) A majority of these potential human mammary carcinogens require multiple enzyme-catalyzed steps to transform the carcinogenic agent into DNA-reactive metabolites. This process may proceed via a primary metabolic step in the liver which is followed by secondary conjugation with a suitable leaving group or it may proceed entirely via

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metabolic activation in the breast in situ. Aditionally it may proceed through a combination of both processes (Malfatti et al., 1999). The cytochromes P450 (CYPs) family is a multi-gene family of constitutive and inducible heme-containing oxidative enzymes that have an important role in the metabolism of a diverse range of xenobiotics (Nelson et al., 1996). The primary role attributed to CYPs is the deactivation of a wide variety of environmental chemicals (Gonzalez & Gelboin, 1994). The CYPs are believed to play central role in tumor development and progression and are involved in tumor initiation and promotion because they can activate or deactivate most carcinogens (Gonzalez & Gelboin, 1994). Furthermore, the CYPs can influence the response of established tumors to anti-cancer drugs by metabolizing the drugs, in normal tissues and tumor cells (Kivistoé et al., 1995). Many of the CYPs identified to date that catalyze the metabolism of potential BCa-associated carcinogens are considered to be activating and result in C- or N-hydroxylation to the potential carcinogen (Hellmold et al., 1998).

Recent studies have provided important new insights into the molecular epidemiology and genetics of BCa (Newman et al., 1997). The search for other genetic markers of BCa susceptibility has led to an increasing number of epidemiological studies of relatively common genetic polymorphisms that may have a role in the metabolism of estrogen or in the activation or detoxification of drugs and environmental carcinogens (Smith et al., 1995). Polymorphisms in the CYP2W1, 4F11 and 8A1 genes may modify the risk for BCa .

The human CYP can also be divided based on their ability to metabolize xenobiotic compounds: sterols, xenobiotics, fatty acids, eicosanoids, and vitamins, and the orphans (Guengerich et al., 2005). The CYP2W1 and 4F11 monooxygenases are considered to be "orphans" because functional information, expression patterns, and regulation are still unknown (Guengerich et al., 2005).

CYP2W1 First cloned by Ingelman-Sundberg (2005). CYP2W1 is one of the potentially most interesting orphans in connection to targeted drug therapy. Of note, CYP2W1 have been identified as having tumor-specific expression. mRNA expression was detected in tumor tissues from colon, adrenal, and lung, while no expression was seen in a number of normal tissues: brain, heart, colon, kidney, and placenta (Karlgren et al., 2005). CYP2W1 has been shown to participate in the oxidation of arachidonic acid and catalysts the reductive activation of AQ4N to AQ4 (an anticancer drug) and in the bioactivation of several pro-carcinogens including polycyclic aromatic hydrocarbons and aflatoxin B1 (Wu et al., 2006; Nishida et al., 2010). On the other hand, since mouse CYP2W1 showed fetal-specific expression (Choudhary et al., 2005). CYP2W1 activity might be involved in development and differentiation (Yoshioka et al., 2006).

CYP4F11 was originally cloned from a human brain and liver cDNA library, mRNA is mostly found in liver and kidney; minor expression was noted in skeletal muscle, placenta, and heart (Cui et al., 2000). CYP4F11 showed catalytic activity against endogenous eicosanoids (leukotriene B4) arachidonic acid, prostaglandins and lipoxins, and hydroxyeicosatetraenoic acids and commonly used drugs (erythromycin, benzphetamine, and chlorpromazine) (Kalsotra et al., 2004). CYP4F11 is thought to be primarily involved in the metabolism of fatty acids and arachidonic acid metabolites (Dhar et al., 2008; Uno et al., 2011). Currently, none polymorphism of this CYP has been associated with some type of cancer.

The CYP8A1 (Prostacyclin I2 synthase; PGIS) gene encodes for an enzyme that acts as an isomerase and rearranges PGH2 to PGI2 and PGIS is considered to be an atypical CYP because it does not possess oxygenase activity (Ulrich et al., 1981; DeWitt & Smith, 1983). A number of studies have demonstrated that the prostanoid biosynthesis profile of malignant cells is different compared to normal tissues (Yokoyama et al., 1996. Wang et al.,1996). CYP8A1 signaling through arachidonic acid metabolism affects a number of tumor cell survival pathways including cell proliferation and apoptosis as well as, tumor cell invasion, metastasis and angiogenesis. PGI2 is a potent antimitogenic and anti-metastatic cancer agent (Hohn et al., 1981). The association between CYP8A1 SNP and cancer (lung, colorectal, thyroid and breast) has been well-documented (Keith et al., 2004; Kajita et al., 2005; Gustafsson et al., 2007; Abraham et al., 2009). Six polymorphisms in CYP8A1 have been reported in BCa. Homozygotes for the minor alleles of rs5602 (67730 T>C), rs477627 (9650 T>C) and rs6125671 (14110 G>A) were associated with an increased BCa risk compared with common alleles, and a protective effect was observed for minor allele homozygotes with the rs477627 (9650 T>C) polymorphism in a Caucasian population (Abraham et al., 2009). For rs6095541 (48110188 C>T) and rs6095543 (48113300 T>C) in women with progesterone positive BCa an association of BCa with this allele in Caucasian individuals has been shown (Mavaddat et al., 2009).

There are currently no data for Mexico, on the genetic susceptibility to BCa and its association with CYP2W1, 4F11 and 8A1 variants. Our study is the first to evaluate the possible association between CYP2W1, 4F11 and 8A1 genotypes and BCa. We studied the rs3735684 (7021 G>A), rs11553651 (15016 G>T) and rs56195291 (60020 C>G) polymorphisms in the CYP2W1, 4F11 and 8A1 genes in a sample of Mexican women with and without BCa to identify a potential link. To further identify the involvement of gene–environment interactions in BCa, the interaction of CYP2W1 genotypes and susceptibility to BCa was also studied in some clinico-pathological risk factors.

Materials and Methods

Biological samples

All samples were collected from the Service of Pathology, Clínica de Especialidades de la Mujer and Clinical laboratory, Hospital Central Militar, Secretaría de la Defensa Nacional (SEDENA) in Mexico City. A total of 109 samples from women were collected. Samples of peripheral blood in EDTA, which was used for leukocyte isolation, from 54 healthy women and samples from radical mastectomy surgeries of 55 patients with BCa were obtained for polymorphism analysis. Informed written consent was obtained from the patients and healthy

100.0
75.0
50.0
25.0
0
6.3
56.3
31.3
Newly diagnosed without treatment

women (without BCa). Ethical approval was provided by the Bioethics and Research committees of the Clínica de Especialidades de la Mujer and Hospital Central Militar, SEDENA with registration number SI-378. Collection information of demographic status, tumor characteristics, as well as anthropometric measures, reproductive and medical history and lifestyle behavior in patients and controls was used. The human experimentation guidelines of these committees were followed.

Four different pathologists confirmed the histological analysis. The sample collection was conducted from October 2010 to November 2011 and was considered for inclusion, exclusion and elimination criteria. The age of the patients and tumor types were obtained from the histopathological study. None of the healthy women had cancer or a family history of cancer.

DNA extraction

Isolation of genomic DNA from leucocytes and tissues was performed using Wizard™ genomic DNA Purification kit (Promega; CA, USA). To verify nucleic acid integrity, the DNA was resolved by electrophoresis in a 1% agarose gel stained with ethidium bromide (500 mg/L in water) and visualized under a UV transilluminator (EDAS 290 analysis system). The DNA was quantified using a Nanodrop Spectrophotometer (Delaware, USA). All extracted DNAs were stored at -20°C.

Allelic discrimination assay

The primer and probe sequences used are presented in Table 1. The synthesized primers and probes (Applied Biosystems; CA, USA) were optimized at 61, 62 and 63ºC (Table 1). The RT-PCR conditions were optimized with a thermal cycler (gradient Px2 Thermal Cycler Hybaid; Franklin, MA) using TaqMan 2X Genotyping Master Mix (Applied Biosystems; CA, USA). The primer and probes sequences correspond to GenBank™ sequence under accession number NG_007934.1 (CYP2W1), NG_008335.1 (CYP4F11) and NG_007940.1 (CYP8A1). The primer sequences were examined by BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) to confirm their specificity. PCR was performed in a final volume of 20 μL. The reaction mixture contained 10 μL of TaqMan 2X Genotyping Master Mix (Applied Biosystems; CA, USA), 500 nM FAM-labeled (6-carboxyfluorescein) probe, 500 nM VIC-labeled probe, 500 nM forward primer, 500 nM reverse primer and 100 ng of genomic DNA for CYP2W1 and 4F11 polymorphisms and 300 nM FAM-labeled probe, 300 nM VIC-labeled probe, 0.4 μM forward primer, 0.4 μM reverse primer and 80 ng of genomic DNA for CYP8A1 polymorphism. Reporter is quenched by TAMRA (6-carboxy-N,N,N,N´-tetramethylrhodamine) attached via a linker arm located at the 3´ end of each probe. The allele 1 FAM dye-labeled probe corresponded to wild-type. The allele 2 VIC dye-labeled probe corresponded to mutant type. Table 2 shows the fluorescence signals for allele 1 and 2 in the samples. The thermal cycler program included one cycle at 95°C for 10 min to activate the AmpliTaq Gold Polymerase, followed by 40 cycles of 95°C for 15 s for DNA denaturation and 61°C for 1 min for annealing/extension. The real time PCR amplification products were analized by electrophoresis on a 2% agarose gel with the electrophoresis EDAS 290 analysis system. All experiments were performed in triplicate.

DNA sequencing

To confirm the results of DNA genotyping, 10 randomly selected PCR products from each primer pair were cleaned with ExoSAP-IT (Affymetrix, USB-Products, CA USA). Presence of the polymorphism and its allelic status were determined by bi-directional PCR sequencing using the BigDye® Terminator v.3.1 Sequencing Kit (Applied Biosystems; CA, USA). Each 5 μL sequencing reaction contained 2 μL of PCR product, 2 μM of primer and 2μL of Sequencing RR-100 Buffer. Cycling conditions were 96°C for 1 min and 25 cycles at 96°C for 30 s, 50°C for 15 s and 60°C for 3 min. The extension products obtained were diluted with 5 μL of nuclease- free water and purified with CENTRI-SEP™ Spin Columns (Applied Biosystems; CA, USA). Finally, was added 10 μL of Hi-Di™ Formamide (Applied Biosystems; CA, USA). Analysis was performed on a capillary automated sequencer ABI PRISM® 3100 Avant Genetic Analyzer (Applied Biosystems; CA, USA).

Statistical analysis

The Hardy-Weinberg (HWE) equilibrium test (in the controls) was used as quality control measures for genotyping using the standard $\chi 2$ statistic. Allele and genotype frequencies were calculated. $\chi 2$ or Fisher exact test was used to estimate odds ratio (OR) and the corresponding 95% confidence intervals (CI) for BCa in relation to the CYP2W1 and 4F11 polymorphisms. $\chi 2$ or Fisher exact test was used to estimate the association between individual clinic-pathological factors and risk of BCa. All statistical analysis was performed using GraphPad Prism version 3.0 software. A P-value less than 0.05 was considered statistically significant.

Table 1. Probe, Primer Sequences and Annealing Temperature for the CYP SNPs rs3735684, rs11553651 and rs56195291 Genotyping Reactions.

CSNP	Annealing (°C)	Forward primer (5´to 3´)	Reverse primer (5´to 3´)	FAM-allele probe (5´to 3´)	VIC-allele probe (5´to 3´)
rs3735684 (7021 G>A)	62°C	CCTGAGGC CCGTCTCC	GACCCAGCA GGGACACAA	TCACCTTCGC GCTCCTCTTCG	TCACCTTCACG CTCCTCTTCGG
rs11553651 (15016 G>T)	63°C	GCTTTGGCTC TGTTTTTTCTC	AAGCTGAAGA CACATTTCTGC	CCTCAGAGGGC AGCGCCAGAC	TCAGAGGGCAT CGCCAGACTGG
rs56195291 (60020 C>G)	61°C	TACAGCTGC CCCCTTCATC	GCCCCTGCC ATTTACCTCT	ATTCAACCTGCG ACGTGGTGACC	ATTCAACCTGCG AGGTGGTGACC

* 'Colon, unspecified' excluded from correlation analysis; UICC, Union for International Cancer Control

Results

Patients and controls characteristics

DNA from 55 patients with BCa and 54 healthy women were included in the study. The mean age was 70 (SD=4.0) and 56.9 years (SD =11.6) for the control and test samples, respectively; the age range for the BCa patients was 31–83 years and the age range for the healthy women was 60-84 years. A total of 40 women were diagnosed with invasive ductal BCa, 8 with invasive lobular and 7 with invasive mixed BCa. The characteristics of the patients with BCa are presented in Table 3.

In terms of chronic diseases, 4 (7.3%), 11 (20%), 17 (30.9%), 3 (5.5%), 4 (7.3%), 2 (3.6%) and 14 (25.5%) patients presented BCa previously, diabetes, systemic arterial hypertension, gastrointestinal diseases, hyper or hypothyroidism, respiratory diseases or others diseases, respectively.

A total of 6 (10.9%), 6 (10.9%) and 4 (7.3%) patients had a family history of BCa, cervical cancer or others cancers, respectively.

A total of 17 (30.9%), 1 (1.8%) and 1 (1.8%) patients had metastatic disease with cancer growth in the axillary lymph nodes, liver or lung, respectively.

A total of 8 (14.5%) and 4 (7.3%) patients underwent chemotherapy or chemotherapy/radiotherapy, respectively. Tobacco, oral contraceptives or HRT, chronic diseases, family history of cancer, obesity or overweight, menopausal status and serum glucose were different between patients with BCa and controls. However, no significant differences were found between them in regard to age, alcohol, exposure to biomass, drugs, pregnancies, menarche, place of residence and birthplace. The use of tobacco, oral contraceptives or HRT, chronic diseases and obesity/overweight significantly increased the BCa risk (P<0.05).

Tobacco, oral contraceptive or HRT, chronic diseases and obesity or overweight were associated with almost eight, thirty-five, nine and five-fold increased risk for BCa (Table 4).

Table 2. Fluorescence Signals for Allele 1 and Allele 2

Type (Homozygote)	Allele 1 (FAM dye)	Allele 2 (VIC-dye)
Wild-type	+	-
SNP	-	+
Wild type/SNP	+	+

Table 3. Clinicopathological Characterization of the Patients

Characteristics		Total (n=55)	Characteristics		Total (n=55)
Average age (years)		56.9 ±11.6	HER2 status	ve+	33 (60%)
Cancer Type:	Invasive ductal	40 (72.7%)		ve-	22 (40%)
	Invasive lobular	8 (14.5%)	Ki67 status:	ve+	55 (100%)
	Invasive ductal & lobular	7 (12.7%)		ve-	0 (0%)
Alcohol:	Yes	9 (16.4%)	p53 status:	ve+	34 (61.8%)
	No	46 (83.6%)		ve-	20 (36.4%)
Tobacco:	Yes	13 (23.6%)		Unknown	1 (1.8%)
	No	42 (76.4%)	CD34 status:	< 10-15 vessels	17 (30.9%)
Exposure to biomass:	Yes	7 (12.7%)		≥10-15 vessels	38 (69.1%)
	No	48 (87.3%)	Histological grade:	Grade I	7 (12.7%)
Drugs:	Yes	0 (0%)		Grade II	22 (40%)
	No	55 (100%)		Grade III	26 (47.3%)
Pregnancies:	0-2	9 (16.4%)	BIRADS:	< 3	2 (3.6%)
	3-6	33 (60%)		≥ 3	52 (94.5%)
				Unknown	1 (1.8%)
	≥ 7	12 (21.8%)	BMI (kg/m2):	12-18.4	0 (0%)
	None	1 (1.8%)		18.5-24.9	14 (25.5%)
Menarche:	<12 years	15 (27.3%)		25-29.9	18 (32.7%)
	≥ 12 years	40 (72.7%)		≥30	23 (41.8%)
Chronic diseases:	Yes	31 (56.4%)	Menopausal status:	<52 years	33 (60%)
	No	24 (43.6%)		≥52	8 (14.5%)
Family history of cancer:	Yes	16 (29.1%)		Not	14 (25.5%)
	No	37 (67.3%)	Metastasis:	Yes	19 (34.5%)
	Unknown	2 (3.6%)		No	36 (65.5%)
Place of residence:	North	4 (7.3%)	Therapy:	Yes	12 (21.8%)
	Center	50 (90.9%)		No	43 (78.2%)
	South	1 (1.8%)	Glucose (mg/dL):	< 70	2 (3.6%)
Birthplace:	North	3 (5.5%)		70-105	36 (65.5%)
	Center	51 (92.7%)		≥ 105	17 (30.9%)
	South	1 (1.8%)	Average age of menarche (years):		12.6 ± 1.7
ER status:	ve+	33 (60%)	Average age of menopausal (years):		48.1 ± 4.6
	ve-	22 (40%)	Average number of pregnancies:		5 ± 2
PR status:	ve+	29 (52.7%)	Average BMI (kg/m2):		28.9 ± 5.2
	ve-	26 (47.3%)	Average glucose (mg/dL):		106.3±32

* 'HRT: Hormone replacement therapy; ER: Estrogen receptor, PR: Progesterone receptor, BMI: Body Mass Index. The values of average age, average age of menarche, average age of menopause, average number of pregnancies, average BMI and average glucose represent the mean ± SD

Table 4. Frequency Distribution of Demographic Variables and Putative Risk Factors of BCa

Characteristics	BCa	Controls	P value	OR(95% CI)	Characteristics	BCa	Controls	P value	OR(95% CI)
Age	(57 ±12)	(70 ±4)			Family history of cancer				
<40	4 (7)	0 (0)	0.12	10(0.5-182)	Yes	16 (29)	0 (0)	<0.0001a	
≥40	51 (93)	54 (100)			No	37 (67)	54 (100)		
Alcohol					Unknown	2 (4)	0 (0)		
Yes	9 (16)	2 (4)	0.05	5(1.0-25)	Place of residence				
No	46 (84)	52 (96)			North-center	54 (98)	54 (100)	1	0.3(0.01-8)
Tobacco					South	1 (2)	0 (0)		
Yes	13 (24)	2 (4)	0.00	8(1.7-38)	Birthplace				
No	42 (76)	52 (96)			North-center	54 (98.2)	54 (100)	1	0.3(0.01-8)
Exposure to biomass					South	1 (1.8)	0 (0)		
Yes	7 (13)	8 (15)	0.75	0.8(0.3-3)	BMI (kg/m2)	(28.9 ±5.2)	(23.4 ± 1.9)		
No	48 (87)	46 (85)			12-18.4	0 (0)	0 (0)		
Drugs					18.5-24.9	14 (26)	34 (63)	-	
Yes	0 (0)	0 (0)	-		25-29.9	18 (33)	20 (37)		
No	55 (100)	54 (100)			≥30	23 (42)	0 (0)		
Pregnancies					Obesity or overweight				
Yes	54 (98)	53 (98)	1	1(0.06-17)	Yes	41 (75)	20 (37)	<0.0001	5(2.2-0.1)
No	1 (2)	1 (2)			No	14 (26)	34 (63)		
Menarche	(13 ± 1.7)	(13.5 ± 0.7)			Menopausal status	(48.1 ± 4.6)	(43.6 ± 4.0)		
<12	15 (27)	20 (37)	0.28	0.6(0.3-1)	< 52	33 (60)	40 (74)	0.0003a	
≥12	40 (73)	34 (63)			≥52	8 (15)	14 (26)		
Oral contraceptives or HRT					Not	14 (26)	0 (0)		
Yes	13 (24)	0 (0)	0.0001	35(2.0-600)	Glucose (mg/dL)				
No	42 (76)	54 (100)			Normal	38 (69)	48 (89)	0.011	0.3(0.1-1)
Chronic diseases					Hyperglycemia	17 (31)	6 (11.1)		
Yes	31 (56)	7 (13)	<0.0001	9(3.3-23)					
No	24 (44)	47 (87)							

[a]The p-value for the family history of cancer and menopausal status was calculated by Fisher's exact test on the 2 x 3 contingency table

Table 5. Genotype and Allele Frequencies of CYP2W1 rs3735684 (7021 G>A), CYP4F11 rs11553651 (15016 G>T) and CYP8A1 rs56195291 (60020 C>G) in BCa and Control Patients

Genotype	BCa (N=55) n(%)	Gen-Fre[1]	Al[2]	Al-Fre[3]	Cont[4] (N=54) n(%)	Gen-fre[1]	Al-fre[3]
rs3735684 CYP2W1 (7021 G>A)							
GG	47 (86)	0.9	G	0.9	38 (70)	0.7	0.7
GA	0 (0)	0			3 (6)	0.1	
AA	8 (15)	0.1	A	0.2	13 (24)	0.2	0.3
rs11553651 CYP4F11 (15016 G>T)							
GG	54 (98)	0.9	G	0.9	54 (100)	1	1
GT	1 (1.8)	0			0 (0)	0	
TT	0 (0)	0	T	0.01	0 (0)	0	0
GG	0 (0)	0	G	0	0 (0)	0	0
rs56195291 (60020 C>G) CYP8A1							
GC	0 (0)	0	G	0	0 (0)	0	0
GC	0 (0)	0			0 (0)	0	
CC	55 (100)	1	C	1	54 (100)	1	1

[1]Genotype Frequency, [2]Alleles, [3]Alleles Frequency, [4]Controls

Distribution of alleles and genotypes

A 137-bp PCR amplicon (6953-7090 nt; reference sequence: GenBank NG_007934.1) was obtained for the CYP2W1 7021 G>A (rs3735684), 142-bp PCR amplicon (14946-15088 nt; reference sequence: GenBanK NG_008335.1) for the CYP4F11 15016 G>T (rs11553651) and 169-bp PCR amplicon (59,928–60,097; nt reference sequence: GenBank NG_007940.1) was obtained for the CYP8A1 60020 C>G (rs56195291) SNPs. The correct amplification was verified by agarose gel electrophoresis

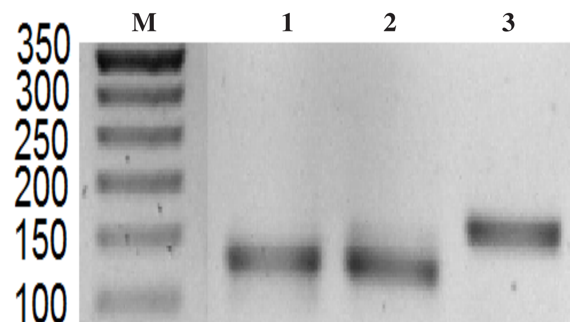

Figure 1. PCR Amplification of rs3735684 of CYP2W1, rs11553651 of CYP4F11 and rs56195291 of CYP8A1. The amplicon was resolved using an agarose gel stained with ethidium bromide, which resulted in 137, 142 and 169 bp products. M=molecular marker of 50 bp, 1=142-bp product, 2=137-bp product, 3=169 bp product.

(Figure 1). The DNA genotyping results were consistent with the sequencing results (Figure 2).

The allele and genotype frequencies for the CYP2W1 polymorphisms (GG, GA and AA), CYP4F11 polymorphisms (GG, GT, TT) and CYP8A1 polymorphisms (GG, GC and CC) detected in the patients and controls are summarized in Table 5. Only the CYP8A1 CC genotype was detected in patients with BCa and the controls All polymorphism frequencies were in HWE in the controls (P>0.05).

Overall, we found 85.5 and 98.2% wild type sequences and 0 and 1.8% polymorphic sequences in patients with BCa, while in control samples the frequency was 70.4 and 100% wild type sequences and 5.6 and 0% polymorphic

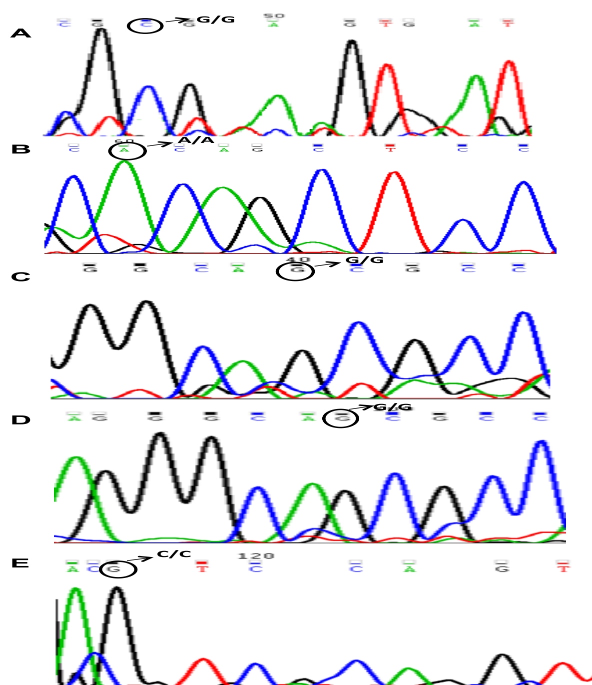

Figure 2. Results of DNASequencing Which Confirms the DNA Genotyping. (A). Homozygote (WT) in CYP2W1 for control, (B) Homozygote (Variant) in CYP2W1 for patient, (C) Homozygote (WT) in CYP4F11, for control (D) Homozygote (WT) in CYP4F11 for patient and finally (E) Homozygote (WT) in CYP8A1 for patient.

Table 6. Association of CYP2W1 rs3735684 (7021 G>A) and CYP4F11 rs11553651 (15016 G>T) with the Risk of BCa

Genotypes	BCa (%) (N=55)	Controls (%) (N=54)	OR	95%CI
CYP2W1				
GG	47 (85.5)	38 (70.4)	1.0 (reference)	
GA	0 (0)	3 (5.6)	8.6[a]	0.43-172.5
AA	8 (14.5)	13 (24)	2.0[b]	0.76-5.4
CYP4F11				
GG	54 (98.2)	54 (100)	1.0 (reference)	
GT	1 (1.8)	0 (0)	0.3[c]	0.01-8.4
TT	0 (0)	0 (0)	-	

[a]P=0.0971 compared with CYP2W1 GG genotype, [b]P=0.1578 compared with CYP2W1 GG genotype, [c]P=1.0, compared with CYP4F11 GG genotype

sequences for CYP2W1 and 4F11, respectively.

No significant differences were observed in the distributions of CYP2W1 (OR 8.6, 95%CI 0.43-172.5 P>0.05; OR 2.0, 95%CI 0.76-5.4, P>0.05) and CYP4F11 (OR 0.3, 95%CI 0.01-8.4 P>0.05) genotypes between the patients and controls (Table 6).

The effect of the interaction of the risk modifiers, tobacco, use of oral contraceptives or HRT, obesity, hyperglycemia and chronic diseases and the genotypes distribution of CYPW1 in controls and patients is summarized in Table 7.

The tobacco in patients with either heterozygous or homozygous variant genotype of CYP2W1 resulted in fourteen fold increase in the BCa risk (OR 14.3, 95%CI 0.8-260.9) which was also found to be statistically significant (P<0.05). Similarly nine fold increase in the risk was associated with obesity and hyperglycemia in the patients carrying variant genotypes of CYP2W1 (OR 9.0, 95%CI 1.1-74.9, P<0.05) when compared with non obesity and non hyperglycemic patients.

Table 7. Interaction of CYP2W1 Genotypes and Tobacco, use of Oral Contraceptives or HRT, Obesity, Hyperglycemia and Chronic Diseases

Genotypes	BCa	Controls	OR	95%CI	P value
Non Tobacco:					
Variant[a]	2 (4)	16 (30)	0.009	0.02-0.4	0.0002
Wild type[b]	53 (96.4)	38 (70.4)	1.0 (Ref.)		
Tobacco:					
Variant	6 (11)	0 (0)	14.3	0.8-261	0.03
Wild type	49 (89.1)	54 (100)	1.0 (Ref.)		
Non Oral contraceptives or HRT:					
Variant	7 (13)	16 (30)	0.3	0.1-0.9	0.031
Wild type	48 (87.3)	38 (70.4)	1.0 (Ref.)		
Oral contraceptives or HRT:					
Variant	1 (2)	0 (0)	3.0	0.1-75	1.0
Wild type	54 (98.2)	54 (100)	1.0 (Ref.)		
Non obesity:					
Variant	0 (0)	15 (28)	0.023	0.001-0.4	<0.0001
Wild type	55 (100)	39 (72.2)	1.0 (Ref.)		
Obesity:					
Variant	8 (15)	1 (2)	9.0	1.1-75	0.03
Wild type	47 (86)	53 (98)	1.0 (Ref.)		
Non hyperglycemia:					
Variant	0 (0)	15 (28)	0.023	0.001-0.4	<0.0001
Wild type	55 (100)	39 (72.2)	1.0 (Ref.)		
Hyperglycemia:					
Variant	8 (14.5)	1 (2)	9.0	1.1-75	0.03
Wild type	47 (85.5)	53 (98.1)	1.0 (Ref.)		
Non chronic diseases:					
Variant	4 (7.3)	15 (28)	0.2	0.06-0.7	0.006
Wild type	51 (92.7)	39 (72.2)	1.0 (Ref.)		
Chronic diseases:					
Variant	4 (7)	1 (2)	4.2	0.4-38.5	0.36
Wild type	51 (93)	53 (98)	1.0 (Ref.)		

[a]Heterozygous and homozygous variant genotype, [b]Wild type genotype

Discussion

The CYP have important roles in tumor development and progression and the presence of specific CYP enzymes in individual tumors may have diagnostic and therapeutic applications. This CYP provides a therapeutic target which can be exploited with a variety of approaches (Windmill et al., 1997). The CYP enzymes are capable of metabolizing a wide variety of carcinogens of different chemical classes including polycyclic aromatic hydrocarbons, heterocyclic amines, nitrosamines, azo dyes, and alkylating agents (Gonzalez & Gelboin, 1994). For this reason, many P450 substrates are carcinogenic while others are anti-cancer drugs. Thus, the CYPs have various important roles in tumor biology. In humans, CYPs enzyme expression has been studied in a variety of human tumors, particularly in BCa, colon cancer, and lung cancer (Nebert et al., 1999).

CYPW1 and 4F11 are located at the long arm of chromosome 7p22.3 and 19p13.1-2, respectively. Human CYP2W1 and 4F11 genes contain 9 and 12 exons and encoded 490 and 524 amino acids long polypeptides,

respectively (Hellmold et al., 1998; Cui et al., 2000).

In a study was shown that CYP2W1 transcript is abundant in the genital organs such as ovaries, placenta, prostate, and testes suggesting that CYP2W1 is involved in the metabolism of sex hormones (Yoshioka et al., 2006).

At present, have been demonstrated that 5F-203 and GW-610 (chemotherapeutic fluorobenzothiazoles in current clinical use against cancer) are capable of inducing CYP2S1 and CYP2W1 in BCa cells (Tan et al., 2011). A recent study suggests that the extent of CYP2W1 expression in colorectal cancers might be a prognostic marker for malignancy and survival (Edler et al., 2009).

CYP4F11 has been reported to be located in a cluster with the other five members of the P450 4F subfamily and recently the ω-oxidation of two β-hydroxy fatty acids (3-hydroxystearate and 3-hydroxypalmitate) has been demonstrated with human P450 4F11 expressed in Spodoptera frugiperda insect cells (Cui et al., 2000; Choudhary et al., 2005).

The non-synonymous CYP2W1 SNP rs3735684 (7021 G>A) in exon 4 encodes for an aminoacid substitution of A to T at position 181 and CYP4F11 SNP rs11553651 (15016 G>T) in exon 5 encodes for an aminoacid substitution of S to I at position 186. To date, the CYP2W1 and 4F11 crystal structure remains unclear.

PGIS or CYP8A1 is considered to be an atypical CYP because it does not possess oxygenase activity. CYP8A1 acts as an isomerase that rearranges PGH2 to form PGI2. PGI2 is a product of arachidonic acid metabolism.

CYP8A1 is constitutively expressed in vascular cells such as endothelial and smooth muscle cells and is encoded by a single gene copy located at the long arm of chromosome 20q13. The human CYP8A1 gene spans 60 kb and contains 10 exons (McLemore et al., 1988). CYP8A1 promoter activity is inducible by IL-6 and IL-1, and the mRNA levels of CYP8A1 are increased by tumor necrosis factor α (Fleisher & Danon, 1999).

CYP8A1 expression may be inducible by diverse factors including cytokines, mitogenic growth factors, and reproductive hormones. CYP8A1 upregulation is crucial in the physiological functions and pathophysiological processes of reproduction, neuroprotection, and cancer growth (Gupta et al., 2000).

CYP8A1 colocalizes with cyclooxygenase-1 (COX-1) and COX-2 to the nuclear envelope and endoplasmic reticulum in resting cells. Following cell activation, cPLA2 is translocated to the nuclear membrane and endoplasmic reticulum, which is where it is functionally coupled with COX-2 and CYP8A1 (Gupta et al., 2000; Tong et al., 2000). It has been hypothesized that tumor development and progression may be modulated by the balance of the expression and activity of CYP8A1, thromboxane synthase and COX-2, and their downstream prostanoid products. CYP8A1 has been shown to be an anti-tumor regulator in a variety of cancer types (lung, colorectal and bladder) and a pro-tumor role for thromboxane synthase and COX-2 has been frequently identified (Cathcart et al., 2010).

The non-synonymous CYP8A1 SNP rs56195291 (60020 C>G) in exon 8 encodes for an amino acid substitution of R to G at position 379. The CYP8A1 structure contains three β-sheets and 12 α-helices in residues R23–P500, and a structural comparison revealed that the CYP8A1 has the most structural homology to CYP51 (Chiang et al., 2006). Although the overall folds of CYP8A1 are similar to those of other CYP enzymes, its heme environment, meander region and location of several helices differ. The significant differences include the B', F, G and I helices, which are important for substrate binding (Chiang et al., 2006).

The CYP8A1 structure reveals a well-defined distal heme pocket that is surrounded by residues from the regions commonly used by other P450s for substrate binding: the I helix (Q280, L281, W282, A283, T284, Q285 and N287), the loop between the K helix and β1-4 strand (A353, A354, P355 and F356), and the loop between two β3 strands at the C terminus (G482, F483, G484 and L485). Residue R379 is part of the heme pocket (Chiang et al., 2001; Ruan et al., 2002; Li et al., 2008).

In our study we determined the allele and genotype frequencies for rs3735684, rs11553651 and rs56195291 in CYP2W1, 4F11 and 8A1, respectively in Mexican women with and without BCa. The CYP4F11 SNP was only found in 1.8 % of the patients with BCa representing little relevance for this disease. The CYP2W1 and 8A1 SNPs were not found in the patients with BCa. Therefore, these SNPs do not represent an association with increased BCa risk in Mexican women. Our results contrasted with those reported by Gervasini and coworkers for a Caucasian population, who reported a positive association between rs3735684 genotypes and colorectal cancer (Gervasini et al., 2010).

With respect to the studied clinico-pathological factors in the patients, the average age, age of menarche and menopause and place of residence and birthplace in Mexico were consistent with the literature. In Mexican women, the age range of the risk for BCa is between 40-69 years, the median age of menarche is 12 years and the median age of menopause has been reported to be between 47 and 48.2 years (Garrido et al., 1996; Gold et al., 2001; Torres et al., 2005). BCa is more frequent in the northern and central regions of México (SINAIS, 2008)

The lifestyle behavior such as smoking, oral contraceptives or HRT, obesity/overweight and hyperglycemia were found to be associated with BCa risk as report in other studies (Pan et al., 2004; Zeng et al., 2010; Lai et al., 2011; Pronk et al., 2011). We found a significant association between BCa risk and chronic diseases, family history of cancer and menopausal status.

These results suggest that pregnancy does not protect against BCa, and the presence of chronic diseases such as obesity and diabetes are associated with BCa development. Some evidence suggests that women with chronic conditions such as hypertension or diabetes undergo more cancer than women without these diseases (Bostick et al., 1994). However, much more research is needed on the relation between chronic disease and cancer screening. In this study, the hormonal changes associated with pregnancy appear to have little influence on BCa prognosis (Von et al., 1995). However, menopausal status was related with the BCa development, probably by the circulating concentrations of estrogens in not menopausal women.

In this study, BCa seems to be strongly dependent on prolonged oral hormones use. Previous studies have showed an increase in risk of BCa associated with current or recent use of oral contraceptives (Hunter et al., 2010). The hormones increase BCa risk through their effects on differentiation of breast tissue. The undifferentiated breast tissue is exposed to mitogenic effects of estrogen and progesterone (Li et al., 2008).

As indicated in this and other studies family history, especially having a first degree relative with BCa, has been consistently reported as an important risk factor by BCa (Lorenzo & Hermminki, 2004; Li et al., 2006). Speiser (1996) reported that syndrome familial cancer was inherited in the dominant gene autosome (Speiser, 1996).

The role of obesity and cancer has been previously associated in several studies (Calle et al., 2003; Vigneri et al., 2006). Although, the effect of diabetes or diabetes-treatment drugs on cancer is not conclusive (Suh and Kim, 2011). in this study hyperglycemia was found to be a risk factor for BCa. Hyperglycemia might provide energy substrate promoting tumor growth (Scheen et al., 2011).

Finally, the major burden of BCa in the population likely results from complex interactions between many genetic and environmental factors over time. Cumulative lifetime exposure to estrogen, estrogen metabolites, and other physical and physiological factors as well as exposure to environmental factors could play an important role in the etiology of BCa in genetically predisposed women (Newman et al., 1997). Tobaco, obesity and hyperglycemia were also found to interact with CYP2W1 genotypes in increasing the risk to BCa further demonstrating the role of gene–environment interaction in development of BCa.

However, we hypothesize that residue R379 which is in the CYP8A1 heme-binding pocket, could be important for cancer growth regulation by CYP8A1 because this residue is conserved in the studied female population.

In conclusion, the frequencies of CYP2W1, 4F11 and 8A1 polymorphisms are unknown in the world population and our results indicate that the CYP2W1, 4F11 and 8A1 SNPs rs3735684, rs11553651 and rs56195291 do not play a role in the susceptibility to develop BCa in Mexican women. This study did not find an association between these CYP2W1, 4F11 and 8A1 genes polymorphisms and BCa risk in a Mexican population. Likewise significant interactions of CYP2W1 genotypes with tobacco, obesity and hyperglycemia have demonstrated the important of gene–environment interactions in modifying the susceptibility to BCa.

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