European Review for Medical and Pharmacological Sciences 2020; 24: 9990-10002

# The rs1008562, rs2234671, and rs3138060 polymorphisms of the CXCR1 gene are associated with breast cancer risk in a Mexican population

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**Abstract. – OBJECTIVE: The rs1008562, rs2234671 and rs3138060 polymorphisms of the *CXCR1* gene have been shown to be associated with many diseases, but in breast cancer (BC) their association has not been detected. The purpose of this study was to determine the frequency and association of the rs1008562, rs2234671 and rs3138060 polymorphisms of *CXCR1* gene in BC patients in the Mexican population.**

**PATIENTS AND METHODS: The *CXCR1* polymorphisms were determined by Polymerase Chain Reaction (PCR) and real time-PCR in healthy Mexican subjects and BC patients.**

**RESULTS: The prevalent patron in BC patients was observed, the majority were overweight and obesity (72%) with metastatic lymph nodes (48%), luminal A/B subtypes (63%), and advanced stages (60%). Triple negative breast cancer (TNBC) patients: they were younger (58%) than 43 years old, overweight (33%), obesity (42%), ductal type histological (98%), metastasis to lymph nodes (47%), advanced stages III-IV (61%) and metastasis (33%). The rs2234671 polymorphism was associated with BC susceptibility when BC patients and the control group were compared for the CC genotype ($p$=0.037), *CG* (heterozygous model: $p$=0.018), *GC/CC* (dominant model: $p$=0.004), and the C allele ($p$=0.001), as well as the *GC/CC* genotype with hormone replace therapy (HRT, $p$=0.016). The rs3138060 polymorphism was associated with BC susceptibility for *CG/GG* genotype (dominant model: $p$=0.032) and G allele ($p$=0.018). Although the association between the dominant model of rs1008562, rs2234671, rs3138060 polymorphisms and BC patients and control was evident for tobacco and alcohol consumption ($p$<0.05). The rs1008562, rs2234671, and rs3138060 polymorphisms of the *CXCR1* gene classified by molecular subtype and stage were also associated with BC patients, indicating that these factors may significantly contribute to BC risk. The CCC (OR 1.75, 95% CI 1.03- 2.97, $p$=0.046), GGG (OR 3.73, 95% CI 1.61- 8.65, $p$=0.0018) haplotypes were also associated with BC susceptibility.**

**CONCLUSIONS: Rs2234671 and rs3138060 polymorphisms in the *CXCR1* gene were associated with BC susceptibility in the Mexican population. The dominant model of the rs1008562, rs2234671 and rs3138060 polymorphisms could significantly contribute to BC risk in tobacco and alcohol consumption, molecular subtype and stage. The rs1008562, rs2234671 and rs3138060 polymorphisms, and the haplotypes CCC and GGG could significantly contribute to BC risk in the Mexican population analyzed.**

*Key Words:*
Breast cancer, *CXCR1*, Rs1008562, Rs2234671, Rs3138060.



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### Abbreviations

BC: breast cancer; CI: confidence intervals; CXCR1: C-X-C chemokine receptor type 1; CXCR1: C-X-C chemokine receptor type 2; HER2/neu: human epidermal growth factor receptor; IL-8: interleukin-8; OR: odds ratio; PCR: polymerase chain reaction; SAH: systemic arterial hypertension; SD: standard deviation; TNBC: triple negative breast cancer.

## Introduction

Breast cancer (BC) is the most frequent type of cancer in women and its incidence varies between different types of ethnic groups[1]. In Mexico, BC is the main cause of mortality in adult women[1,2]. The gradual accumulation of genetic and epigenetic events might influence the development of BC[3]. It has been shown an association between C-X-C chemokine receptor type 1 (CXCR1, IL8-RA), which is a specific receptor to interleukin-8 (IL-8 or CXCL-8). CXCL-8 is a member of the chemokine family that is released in response to an inflammatory stimulus. It is also involved in neutrophil activation and has a high affinity for CXCR1 and the CXC type 2 chemokine receptor (CXCR2). In particular, CXCR2 has affinity for post chemokines, while CXCR1 is more selective. Both CXCR1 and CXCR2 induce migration of neutrophils through similar pathways, but phospholipase D activation is exclusively mediated by CXCR1, which induces superoxide anion production, being essential for ROS generation and oxidative response in neutrophils, mediated by IL-8. There is evidence that IL-8 receptors identify pathogen clearance, although they also promote the disease process in terms of tissue damage, fibrosis, angiogenesis, tumorigenesis, and metastasis[4]. CXCR1 participates in the inflammation process, and it is activated by neutrophils, T cells and basophils. CXCR1 has a dual function. It can defend the cell against cancer by regulating the inflammation mechanism, or it can stimulate the growth and progression of the tumor by activating metalloproteinases[5]. In response to acute inflammation mechanism after activation of neutrophils, the CXCR1 binds to IL-8, through G-proteins. G-proteins are phosphorylated by multi-stage processes which participate in the transduction of signals required in motility, growth and gene expression. CXCR1 also participates in the angiogenesis process and tumor formation, through RAS, RHO families and vascular growth factors[4-6]. The importance of the participation of IL-8 and its CXCR1 and CXCR2 receptors has been showed by the findings *in vitro* and *in vivo* studies[5-7]. It has also been observed[5,6] in high expression of CXCR1 and CXCR2 verified *in vitro* in human epidermal growth factor receptor (HER2/neu) from positive BC cell lines. Acharyya et al[8] observed that in BC the increased copy numbers of CXCL1/2 genes contributed to a higher expression of CXCL1/2 in invasive breast tumors. CXCL1/2 participated in a paracrine loop involving tumor microenvironment and cancer cells to enhance chemoresistance and metastasis in breast tumors.

It has also been suggested that, through the mechanism of cyclooxygenase-2 mediated production of IL-8 might contribute for bone metastasis in estrogen receptor negative tissues[6]. IL-8 and vascular endothelial growth factor participate in the expansion of tumor neovasculature[7]. Thrombin stimulated CXCL1 expression and secretion in tumor and endothelial cells. Antibodies against CXCL1 inhibited thrombin-induced angiogenesis (endothelial tube formation). Depletion of CXCL1 *via* shRNA in 4T1 cells reduced tumor growth, angiogenesis, and metastasis[4]. Elevated serum level of IL-8 is associated with poor prognosis in BC[9]. Advances in the area of pharmacology have also shown that parixin (the antibody-CXCR1) can inhibit the growth and metastasis of tumors. The administered reparixin in conjunction with placlitaxel enhances its effect, arresting the cell cycle and inhibit tumor formation (*in vitro*) and metastasis reduction (*in vivo*)[9]. *CXCR1* gene located in chromosomal region 2q35, together with its homolog *CXCR2* and its pseudogene (2q34-35). *CXCR1* its structured by 2 exons and more than 125 polymorphisms were identified in the gene. However, few studies[10-12] associated with cancer development are available. Polymorphism were the rs1008562 is located in the promotor (*C/G*, physical position 219,026,972 base pair), and their function has been associated with over expression caused by the *G* allele[13]. The variation in the reported frequency also depends on the population analyzed, and the *G* allele showed a frequency in controls of 30-70% among European, African, American and Asian populations[11]. One of the most studied is the rs2234671 located in the exon 2 (+860 *G/C*, S276T, G+2607C, physical position 219,029,108 base pair), but its function is unknown[14]. The variation in the reported frequency also depends on the population

analyzed, and the *C* allele showed a frequency in controls of 6-22% among European, African and Asian populations[14]. Another polymorphism is the rs3138060 (-1566 *C/G*, physical position 219,031,500 base pair) polymorphism, located in the intron 1 and it has been associated with the over expression of gene caused by the *G* allele[15]. The reported frequency of the rs3138060 polymorphism depends on the population studied, and the *G* allele showed a frequency in controls of 9-13% among European, Asiatic, African and American, population[15].

The participation of CXCR1 as a modulator in the immune response, angiogenesis, migration and invasion, in tumor cells, suggests that polymorphisms in the *CXCR1* gene could have an important role in the risk to the development of BC[5-12]. However, the association studies that examined the rs1008562, rs2234671 and rs3138060 polymorphisms and BC have not been determine[10,11]. In the Mexican population, the association of the rs1008562, rs2234671 and rs3138060 polymorphisms of *CXCR1* gene in BC remains unknown. Therefore, the aim of this investigation was to determine the frequency and association of *CXCR1* gene polymorphisms in Mexican women with BC.

## Patients and Methods

### Study Groups Analysis

Blood samples were collected from 328 patients with clinically and histologically confirmation of BC followed by international guidelines for breast cancer[16]. DNA was extracted from peripheral blood lymphocytes using standard protocols[17], and 243 healthy women who donated blood. The patient and control groups were not age-matched, and no familial samples were included. All patients were residents of the metropolitan area of Guadalajara and were recruited from June 2014 to February 2020. All samples were obtained after patients and controls provided a written informed consent, as approved by the Local Ethics Committee (1305). All procedures performed in studies involving human participants were in accordance with the 1964 Declaration of Helsinki. Clinical and demographic data were obtained using written questionnaires. All patients were interviewed to determine occupational exposure and current drug regimens. The BC patient database and patient DNA samples were examined for other polymorphisms[3,18].

### PCR Polymorphisms Analysis

Amplification of the *CXCR*1 exon 2 region for rs2234671 was performed by PCR using the following primers: 5'-CTCATGAGGACCCAGGTGAT-3' and 5'-GGTTGAGGCAGCTATGGAGA-3', as previously described by Lurje et al[19]. The PCR amplifications were performed in a total of 15 µl containing 0.2 mM dNTPs (Invitrogen, Carlsbad, CA, USA), 5 pmol of primers, 1.5 mM $MgCl_2$, 2.5 U of *Taq* polymerase (Invitrogen, Carlsbad, CA, USA), and 50 ng of genomic DNA. The annealing temperature was 59°C. The PCR product was digested with *Alu* I restriction enzyme. In the previous electrophoretic procedure amplified products were separated on 8% polyacrylamide gels (19:1), followed by silver staining[20]. The fragments of 29 bp, 66 bp and 11 bp were identified as *GG* genotype, the fragments of 95 bp, 66 bp, 29 bp and 11 bp as *GC* genotype and the fragments 95 bp, 11 bp as *CC* genotype.

The rs1008562, and rs3138060 polymorphisms were identified by real time PCR using the following probes: VIC-5'-GCCTTATAGCTACTAAGCCTTCCCT[C/G]-3' and FAM-5'-ATATTCATTCCAGGCCTGACACCCC-3'(C_8841222_10) and VIC-5'-CACCTGCTAACTCCATGTATGAGTG[C/G]-3' and FAM- 5'TGGTCACAAGAGAGGAAGCGGGCAG-3' (C_27466344_20) respectively, as validated and designed by Applied Biosystem (Thermo Fisher Scientific, Waltham MA, USA). The reaction included a volume of 5 µl (~10 µg) genomic DNA, 6.25 µl of TaqMan universal buffer, 0.32 µl of VIC and FAM TaqMan labeled probe and 3.43 µl of water per sample. They were always place in 96 well plates in a light covered system and were read in the ABI 7300 Real Time PCR System (Applied Biosystem/Thermo Fisher Scientific, Waltham, MA, USA). The amplification conditions were as follows 40 cycles: 95°C for 10 minutes, 92°C for 10 seconds, and 60°C for 1 minute. As an internal control, 10% of the reactions were analyzed in duplicate to observe concordance in all of the samples analyzed.

### Statistical Analysis

Allele frequencies were obtained by direct counting. The Hardy-Weinberg equilibrium was tested by a goodness-of-fit Chi-square test to compare the observed genotype frequencies with the expected frequencies among control subjects. Odds ratios (OR) and 95% confidence

intervals (CI) were also calculated. A two-tailed $p<0.05$ was considered statistically significant. The association analysis by the Odds ratio and binary logistic regression analysis between the studied groups were performed using the PASW Statistic Base 18 software, 2009 (Chicago, IL, USA). Haplotype analysis was performed by to https://www.snpstats.net/start.htm, program.

## Results

The demographic and clinical characteristics of BC patients and controls are shown in Table I. The mean age, tobacco, and alcohol consumption were statistically different in BC patients compared with the control group ($p<0.05$). The main characteristics of BC patients were hormonal (oral/injection; 46%) and

**Table I.** Demographic characteristics and clinical data of participants the study groups.

| | | BC patients (n = 328) | | Controls (n = 243) | | *p*-value[1] |
|---|---|---|---|---|---|---|
| **Age (years), media ± SD** | | **52.48 +/- 11.38** | | **49.1 +/- 12.59** | | **0.001** |
| **Tobacco consumption*** | | **(n)** | **%** | **(n)** | **%** | |
| | Yes | (91) | 28.0 | (47) | 19.0 | |
| | No | (237) | 72.0 | (196) | 20.0 | 0.026 |
| Alcohol consumption** | | | | | | |
| | Yes | (88) | 27.0 | (38) | 16.0 | |
| | No | (240) | 73.0 | (205) | 94.0 | 0.002 |
| Hormonal status | Oral/injection[2] | (150) | 46.0 | | | |
| | HRT[2] | (54) | 16.0 | | | |
| BMI (kg/m$^2$)*** | Normal (18.5-24.9 kg/m$^2$)[2] | (93) | 28.0 | | | |
| | Overweight (25-29.9 kg/m$^2$)[2] | (109) | 33.0 | | | |
| | Obesity I (30-34.9 kg/m$^2$)[2] | (92) | 28.0 | | | |
| | Obesity II (35-39.9 kg/m$^2$)[2] | (20) | 7.0 | | | |
| | Obesity III (40-45.9 kg/m$^2$)[2] | (14) | 4.0 | | | |
| PPA | Mastitis chronic[2] | (4) | 1 | | | |
| | Breast fibrosis[2] | (36) | 11 | | | |
| | Uterine myomas[2] | (46) | 14 | | | |
| | DM2[2] | (45) | 14 | | | |
| | SAH2 | (55) | 17 | | | |
| | DM2/SAH[2] | (19) | 6 | | | |
| Metastatic lymph Nodes | Positive[2] | (156) | 48 | | | |
| Type | Ductal | (306) | 93 | | | |
| | Lobular | (21) | 6.7 | | | |
| | Mixed | (1) | 0.3 | | | |
| Histological type3 | Luminal A[2] | (99) | 30 | | | |
| | Luminal B[2] | (106) | 33 | | | |
| | HER2/neu[2] | (66) | 20 | | | |
| | Triple negative[2,4] | (57) | 17 | | | |
| Stage | I[2] | (24) | 7 | | | |
| | II[2] | (109) | 33 | | | |
| | III[2] | (107) | 33 | | | |
| | IV[2] | (88) | 27 | | | |

Standard deviation (SD), hormonal replacement therapy (HRT), type 2 diabetes mellitus (DM2), Systemic Arterial Hypertension (SAH), Body mass index (BMI), Pathology personal antecedent (PPA). *5 cigarretes-2 pack per day, **3-8 drinks per week, ***BMI, according to OMS classification (appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Genève (Switzerland): World Health Organization, 2004). [1]Mann-Whitney U test, [2]positive on base n=328, [3]Luminal A: estrogen receptor (ER, +), progesterone receptor (PR, +), 50% of BC are invasive, good response to endocrine therapy and good prognosis. Luminal B: ER (+), PR (+), human epidermal growth factor receptor (HER2/neu, +), 20% of BC are invasive, tamoxifen and aromatase inhibitors response less than Luminal A and prognosis is less than Luminal A. HER2/neu: HER2/neu (+), 15% of BC are invasive, trastuzumab (Herceptin) and anthracycline response, and unfavorable prognosis. TNBC: ER (-), PR (-), HER2/neu (-), ~15% of BC are invasive, no response to endocrine therapy or trastuzumab, and worse prognosis[47]. Triple negative BC: age less than 43 years old 58% (33/57), overweight 33% (19/57), obesity 42% (24/57), ductal type 98% (56/57), metastasis lymph node 47% (27/57), advances stages III-IV 61% (35/57) and metastasis 33% (19/57)[4].

hormonal replacement therapy (HRT; 16%) consumption, body mass index (BMI) normal (28%), overweight (33%), obesity I (28%), obesity II (7%), obesity III (4%), presence of DM2 (14%) and systemic arterial hypertension (SAH; 17%), metastatic lymph nodes (48%), luminal B (33%), and stage IV tumor (27%). In this sense, we observed a prevalent pattern in BC patient of this study, the majority were overweight and obese (72%; 235/328), with metastatic lymph nodes (48%; 156/328), luminal A/B subtypes (63%; 205/328), and advanced stages (III-IV; 60%; 195/328).

Additional data observed were in triple negative breast cancer (TNBC) patients: age 58% (33/57) were less of 43 years old, overweight 33% (19/57), obesity 42% (24/57), ductal type histological 98% (56/57), metastatic lymph node 47% (27/57), advances stages III-IV 61% (35/57) and metastasis 33% (19/57) (data not shown).

The rs1008562 polymorphism in the *CXCR1* gene did not show significant differences between BC and control groups (Table II). However, the rs2234671 was significantly different between BC patients and controls. The genotypes *CC* (OR 2.32, 95% CI 1.02-5.27, $p$ =0.037) and *GC/GG* (heterozygous model; OR 1.54, 95% CI 1.07-2.22, $p$ =0.018), *GC/CC* (dominant model; OR 1.64, 95% CI 1.16-2.32, $p$ =0.004) and *C* allele (OR 1.59, 95% CI 1.18-2.12, $p$ =0.001) were observed as risk factors for developing BC. The genotype distribution of the rs3138060 polymorphism in *CXCR1* was significantly different between the study groups. The genotype *CG/GG* (dominant model, OR 1.60, 95% CI 1.05-2.44, $p$

**Table II.** Genotype and allelic distributions of rs1008562, rs2234671, and rs3138060 polymorphisms of the *CXCR1* gene in BC patients and controls.

| Polymorphism | | BC* | | Controls* | | | | |
|---|---|---|---|---|---|---|---|---|
| **rs1008562** | **Genotype** | **(n = 257)** | **%** | **(n = 200)** | **%** | **OR** | **95% (CI)** | ***p*-value** |
| | *GG* | (54) | 21 | (55) | 28 | 1.0 | | |
| | *GC* | (132) | 51 | (97) | 48 | 1.12 | (0.77-1.62) | 0.608 |
| | *CC* | (71) | 28 | (48) | 24 | 1.20 | (0.79-1.84) | 0.441 |
| Dominant | *GC/CC* | (203) | 79 | (145) | 72 | 1.42 | (0.92-2.19) | 0.132 |
| | Allele (2n=514) | | | (2n=400) | | | | |
| | G | (240) | 0.4670 | (207) | 0.5175 | 0.81 | (0.62-1.06) | 0.146 |
| | C | (274) | 0.5330 | (193) | 0.4825 | 1.22 | (0.94-1.59) | 0.146 |
| **rs2234671** | **Genotype** | **(n = 328)** | **%** | **(n = 243)** | **%** | | | |
| | *GG* | (185) | 56 | (165) | 68 | 1.0 | | |
| | *GC* | (119) | 36 | (70) | 29 | 1.41 | (0.99-2.02) | 0.056 |
| | CC | (24) | 8 | (8) | 3 | 2.32 | (1.02-5.27) | **0.037** |
| Heterozygous | GC *vs*. GG | (119) | 36 | (69) | 29 | 1.54 | (1.07-2.22) | **0.018** |
| Dominant | GC/CC | (143) | 44 | (78) | 32 | 1.64 | (1.16-2.32) | **0.004** |
| | Allele (2n=556) | | | (2n=486) | | | | |
| | G | (489) | 0.8794 | (400) | 0.8230 | 0.6296 | (0.47-0.84) | **0.001** |
| | C | (167) | 0.1206 | (86) | 0.177 | 1.59 | (1.18-2.12) | **0.001** |
| **rs3138060** | **Genotype** | **(n = 257)** | **%** | **(n = 200)** | **%** | | | |
| | *CC* | (172) | 67 | (153) | 76.5 | 1.0 | | |
| | *CG* | (75) | 29 | (44) | 22 | 1.47 | (0.95-2.25) | 0.097 |
| | GG | (10) | 4 | (3) | 1.5 | 2.6 | (0.72-9.79) | 0.161 |
| Dominant | *CG/GG* | (85) | 96 | (47) | 98.5 | 1.60 | (1.05-2.44) | **0.032** |
| | Allele (2n=514) | | | (2n=400) | | | | |
| | *C* | (419) | 0.8151 | (350) | 0.8750 | 0.63 | (0.43-0.91) | **0.018** |
| | *G* | (95) | 0.1848 | (50) | 0.1250 | 1.58 | (1.09-2.29) | **0.018** |

Odds ratio (OR), confidence intervals (CI), significant $p$-value < 0.05. *Hardy-Weinberg equilibrium in controls of rs1008562 (chi-square test=0.166, $p$=0.6836) and BC patients (chi-square test=0.2591, $p$=0.6107), controls of rs2234671 (chi-square test=0.034, $p$=0.8523 and BC patients (chi-square test=0.6370, $p$=0.4247), and controls of the rs3138060 (chi-square test = 0.0065, $p$=0.9355) and BC patients (chi-square test=0.2554, $p$=0.6132) of CXCR1 gene polymorphisms.

**Table III.** Association of the rs1008562, rs2234671, and rs3138060 polymorphisms of the *CXCR1* gene with alcohol consumption in BC patients and controls.

| Polymorphism | | Genotype* | Variables | OR | 95% (CI) | *p*-value |
|---|---|---|---|---|---|---|
| rs1008562 | Downstream | *GC/CC* | Alcohol consumption | 2.2 | (1.16-4.34) | 0.016 |
| | | | Tobacco consumption | 2.3 | (1.14-4.22) | 0.015 |
| rs2234671 | +860 Exon 2 | *GC/CC* | Alcohol consumption | 2.2 | (1.03-4.6) | 0.039 |
| | | | Tobacco consumption | 1.9 | (1.11-3.3) | 0.033 |
| rs3138060 | -1566 Intron 1 | *CG/GG* | Alcohol consumption | 3.2 | (1.78-6.0) | 0.001 |
| | | | Tobacco consumption | 2.2 | (1.34-4.5) | 0.003 |

*Dominant model, **5 cigarretes-2 pack per day, ***3-8 drinks per week.

=0.032) and *G* allele (OR 1.58, 95% CI 1.09-2.29, $p$=0.018) were observed as risk factor for developing BC (Table II). The rs1008562, rs2234671, and rs3138060 polymorphisms in the *CXCR1* gene were in Hardy-Weinberg equilibrium in the studied groups (Table II).

Significant differences were observed when comparing the of rs1008562, rs2234671, and rs3138060 polymorphisms of the *CXCR1* stratified by tobacco and alcohol consumption between BC patients and controls ($p$<0.05) (Table III).

No significant differences were observed when comparing rs1008562, rs2234671, and rs3138060 polymorphisms of the *CXCR1* stratified by hormonal (oral/injection) consumption, BMI, type histological, and metastatic lymph node ($p$>0.05), between BC patients. However, the *GC/CC* genotype of rs2234671 polymorphism showed significant statistical differences with HTR consumption (OR 2.1, 95% CI 1.17-3.86, $p$=0.016) in BC patients (data not showed).

The genotype distribution of rs1008562, rs2234671, and rs3138060 polymorphisms of the *CXCR1* gene classified by molecular subtype of BC patients is presented in Table IV. No significant differences were observed when comparing the polymorphisms stratified by molecular subtypes of BC, except, in the *GC* genotype of rs3138060 polymorphism with molecular subtypes HER2/neu than TNBC (OR 2.5, 95% CI 1.02-6.54, $p$=0.043).

Table V represents the genotype distribution of rs1008562, rs2234671, and rs3138060 polymorphisms of the *CXCR1* gene classified by stages of BC patients. No significant differences were observed when comparing the polymorphisms stratified by stages of BC, except, in the *GC* genotype of rs18008562 polymorphism with stage IV than stage I (OR 3.2, 95% CI 1.04-9.56, $p$=0.028).

The genotype distribution of rs1008562, rs2234671, and rs3138060 polymorphisms of the *CXCR1* gene classified by molecular subtype and

**Table IV.** Genotype distributions of rs1008562, rs2234671, and rs3138060 polymorphisms of the CXCR1 gene classified by histological type in BC patients.

| | Luminal A | | Luminal B | | HER2/neu | | TNBC | |
|---|---|---|---|---|---|---|---|---|
| **rs1008562** | **(n)** | **%** | **(n)** | **%** | **(n)** | **%** | **(n)** | **%** |
| *GG* | (17) | 18 | (13) | 19 | (12) | 29 | (12) | 23 |
| *GC* | (51) | 53 | (34) | 51 | (23) | 55 | (24) | 46 |
| *CC* | (28) | 29 | (20) | 30 | (7) | 16 | (16) | 31 |
| **rs2234671** | | | | | | | | |
| *GG* | (54) | 55 | (65) | 61 | (38) | 58 | (28) | 49 |
| *GC* | (37) | 37 | (33) | 31 | (24) | 36 | (25) | 44 |
| *CC* | (8) | 8 | (8) | 8 | (4) | 6 | (4) | 7 |
| **rs3138060** | | | | | | | | |
| *CC* | (63) | 66 | (43) | 64 | (24) | 57 | (42) | 81 |
| *CG** | (29) | 30 | (20) | 30 | **(16)** | **38** | **(10)** | **19** |
| *GG* | (4) | 4 | (4) | 6 | (2) | 5 | (0) | 0 |

**CG* genotype of rs3138060 polymorphism was significant different when comparing HER2/neu than TNBC (OR 2.5, 95% CI 1.02-6.54, $p$=0.043).

**Table V.** Genotype distributions of rs1008562, rs2234671, and rs3138060 polymorphisms of the *CXCR1* gene classified by stage in BC patients.

| | Stage I | | Stage II | | Stage III | | Stage IV | | Stage I+II | | Stage III+IV | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **rs1008562** | **(n)** | **%** | **(n)** | **%** | **(n)** | **%** | **(n)** | **%** | **(n)** | **%** | **(n)** | **%** |
| GG | (1) | 5 | (24) | 30 | (20) | 23 | (26) | 37 | (25) | 26 | (46) | 30 |
| GC* | (13) | 69 | (42) | 53 | (49) | 55 | (28) | 40 | (55) | 56 | (77) | 49 |
| CC | (5) | 26 | (13) | 17 | (20) | 22 | (16) | 23 | (18) | 18 | (36) | 21 |
| **rs2234671** | | | | | | | | | | | | |
| GG | (11) | 46 | (66) | 61 | (60) | 56 | (48) | 55 | (77) | 58 | (108) | 55.5 |
| GC | (11) | 46 | (39) | 36 | (37) | 35 | (32) | 36 | (50) | 37.5 | (69) | 35.5 |
| CC | (2) | 8 | (4) | 8 | (10) | 9 | (8) | 9 | (6) | 4.5 | (18) | 9 |
| **rs3138060** | | | | | | | | | | | | |
| CC | (1) | 5 | (3) | 4 | (3) | 3 | (3) | 4 | (4) | 4 | (6) | 4 |
| CG | (6) | 32 | (23) | 29 | (21) | 24 | (25) | 36 | (29) | 30 | (46) | 29 |
| GG | (12) | 63 | (53) | 67 | (65) | 73 | (42) | 60 | (65) | 66 | (107) | 67 |

**CG* genotype of rs18008562 polymorphism was significant different when comparing stage IV than stage I (OR 3.2, 95% CI 1.04-9.56, $p$=0.028).

stages of BC patients is presented in Figure 1. Below describes, the polymorphisms of rs1008562, rs2234671, and rs3138060 polymorphisms of the *CXCR1* gene showed a risk association for advanced stage (III-IV) BC stratified by the molecular subtype: rs1008562 polymorphism with molecular subtype luminal B than TNBC (OR 8.0, 95% CI 0.94-67.97, $p$=0.037), and HER2/neu than TNBC (OR 10.2, 95% CI 1.19-87.27, $p$=0.015) in carrying the *CC* genotype. The rs2234671 poly-

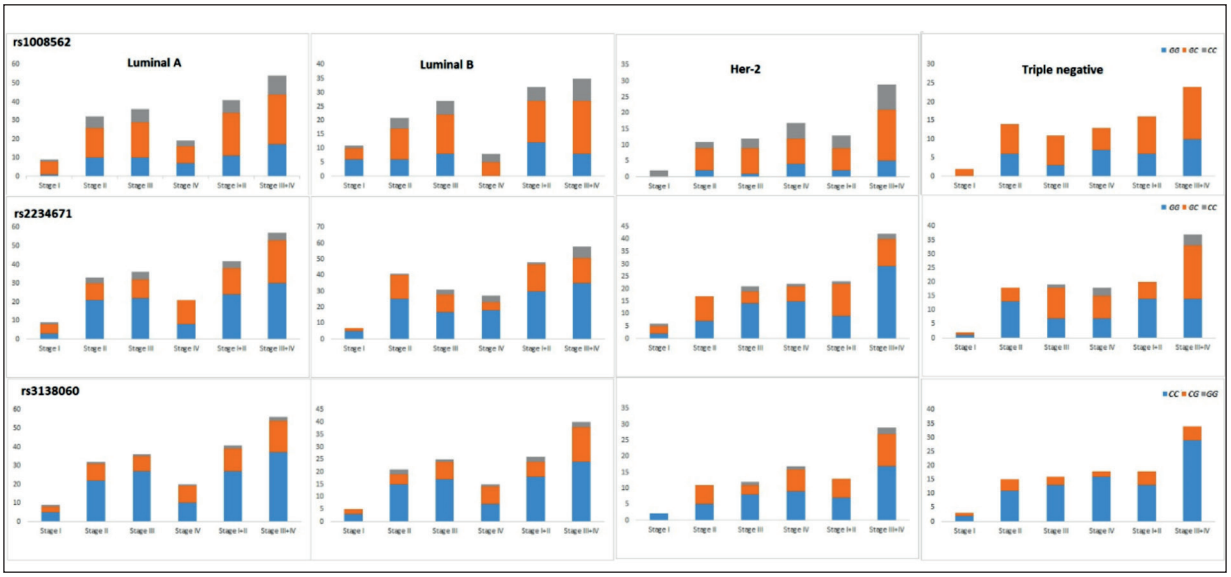


**Figure 1.** Genotype distributions of rs1008562, rs2234671, and rs3138060 polymorphisms of the *CXCR1* gene classified by histological type and stage in BC patients.**rs1008562 polymorphism:** A risk association for BC was observed in patients carrying the *CC* genotype with advanced stage (III-IV) with molecular subtype luminal B compared than TNBC (OR 8.0, 95% CI 0.94-67.97, $p$=0.037), and HER2/neu compared than TNBC (OR 10.2, 95% CI 1.19-87.27, $p$=0.015) carrying the *CC* genotype. **rs2234671 polymorphism:** observed as a risk factor in patients carrying the *GC* genotype with advanced cancer (III-IV) with molecular subtype luminal A compared than triple luminal B (OR 2.5, 95% CI 1.11-5.66, $p$=0.040), HER2/neu TNBC (OR 2.7, 95% CI 1.14-6.80, $p$=0.038), TNBC compared than luminal B (OR 2.7, 95% CI 1.16-6.57, $p$=0.033), and TNBC compared than HER2/neu (OR 3.0, 95% CI 1.19-7.86, $p$=0.032). **rs3138060 polymorphism**: associated as a risk factor in patients carrying *CG+GG* genotype with luminal B compared than TNBC (OR 3.8, 95% CI 1.23-12.09, $p$=0.031), and HER2/neu compared than TNBC (OR 4.0, 95% CI 1.22-13.63, $p$=0.036) carrying *CG+GG* Genotype.

morphism in BC with subtype luminal A than luminal B (OR 2.5, 95% CI 1.11-5.66, $p$=0.040), luminal A than HER2/neu (OR 2.7, 95% CI 1.14-6.80, $p$=0.038), TNBC than luminal B (OR 2.7, 95% CI 1.16-6.57, $p$=0.033), and TNBC than HER2/neu (OR 3.0, 95% CI 1.19-7.86, $p$=0.032) in carrying the *GC* genotype. The rs3138060 polymorphism in BC luminal B than TNBC (OR 3.8, 95% CI 1.23-12.09, $p$=0.031), and HER2/neu than TNBC (OR 4.0, 95% CI 1.22-13.63, $p$=0.036) in carrying *CG*/*GG* Genotype.

The haplotype frequency and association of *CXCR1* polymorphisms are presented in Table VI. The most frequent haplotype was *GGC* 38% in BC and 40% in control group, however, no statistical differences were observed between the study groups. Nonetheless, evident differences were observed with the haplotypes *CGC* (OR 0.63, 95% CI 0.45-0.87, $p$=0.008), *CCC* (OR 1.75, 95% CI 1.03-2.97, $p$=0.046), and *GGG* (OR 3.73, 95% CI 1.61-8.65, $p$=0.0018).

We observed that D' and $r^2$ values of rs1008562 *vs.* rs2234671 were 0.3426 and 0.1966; rs1008562 *vs.* rs3138060 of 0.3017 and 0.0671; and rs2234671 *vs.* rs3138060 of 0.4123 and 0.2254, respectively.

Haplogenotype association between BC patients and controls have shown that the *GCGG-CC* haplogenotype was associated with protective susceptibility (OR 0.42, 95% CI 0.25-0.69, $p$=0.0008). It has been suggested that the *GCGG-CG* haplogenotype (OR 3.41, 95% CI 0.97-11.94), $p$=0.0510) has shown to be with in the limit of associated BC risk. The association of clinical variables with haplogenotypes in the group of patients with BC did not show statistically significant differences (data not shown).

## Discussion

In Mexico, BC is one of the leading causes of death in adult women[1,2]. BC was observed to occur at an average age of 50 years[2,3], which is consistent with data from this study since the mean age was 52.48 years. In this study, we observed the differences in tobacco and alcohol consumption between BC patients and controls. The relationship between these two factors with cancer development is well established[3,21,22]. We also observed a prevalent pattern in BC patient, the presence of overweight-obesity, DM2-SAH, metastasis lymph node, molecular subtype A/B and advanced stages (III-IV) as predominant clinical characteristic BC patients of this study. In this context, BC is a multifactorial disease with a complex etiology and, these factors have been previously associated with BC[23-26]. In fact, Gómez Flores et al[25] and Ramos et al[26] associated obesity with BC risk. The authors, has been attributed this association to the participation of leptin, insulin, adipocytes, and other molecules that mediate the inflammatory process, peripheral circulating estrogens, and metabolic syndrome; these may in turn activate molecular processes that are mitogenic in breast epithelial cells and thereby stimulate neoplasia[25,26]. A familial history of DM2-SAH was found to be a risk factor for BC. It has been observed that DM2 is a serious health problem that affects more than 7% of adults in developed countries, that up to 16% of patients with BC have DM2, and that the two major risk factors for DM2, old age and obesity are also associated with BC[27,28].

**Table VI.** Polymorphism rs1008562, rs2234671, and rs3138060 haplotype frequencies in the study groups.

| | Haplotypes* | | | BC (n = 368) | | Controls (n = 313) | | OR | |
|---|---|---|---|---|---|---|---|---|---|
| | rs1008562 | rs2234671 | Frequency rs3138060 | (n) | % | (n) | % | (95% CI) | *p*-value |
| 1 | *G* | *G* | *C* | (139) | 38 | (126) | 40 | 1 | |
| 2 | *C* | *G* | *C* | (95) | 26 | (111) | 35 | 0.63 (0.45-0.87) | **0.008** |
| 3 | *C* | *C* | *C* | (45) | 12 | (23) | 7 | 1.75 (1.03-2.97) | **0.046** |
| 4 | *G* | *C* | *C* | (23) | 6 | (14) | 5 | 1.42 (0.71-2.81) | 0.395 |
| 5 | *G* | *G* | *G* | (29) | 8 | (7) | 2 | 3.73 (1.61-8.65) | **0.0018** |
| 6 | *C* | *C* | *G* | (16) | 4 | (14) | 5 | 0.97 (0.46-2.02) | 1.0 |
| 7 | *C* | *G* | *G* | (13) | 4 | (13) | 4 | 0.84 (0.38-1.85) | 0.8253 |
| 8 | *G* | *C* | *G* | (8) | 2 | (5) | 2 | 1.36 (0.44-4.22) | 0.7895 |

*rs1008562 *vs.* rs2234671 D'= 0.3426, $r^2$=0.1966; rs1008562 *vs*. rs3138060 D'= 0.3017, $r^2$=0.0671, and rs2234671 *vs*. rs3138060 D'=0.4123, $r^2$=0.2254. Breast cancer (BC), Odds ratio (OR), Confidence Interval (CI), n=alleles.

In this regard, BC patients with stage III-IV tumors and presence of lymph node metastases has been associated as they were risk factors for poor prognostic in BC patients. In fact, the BC tumor stage is important for determining treatment and a predictor of survival. There is evidence[1,2,29] showing that patients with micrometastases in axillary lymph nodes have an increased risk of distant metastases compared to patients without axillary lymph node metastases.

Concerning TNBC, Marra et al[30], described that approximately 15-20% of all breast carcinomas are triple negative type and are associated with earlier age of onset, aggressive clinical course, and dismal prognosis compared to hormone receptor and HER2/neu positive breast carcinomas. These data are in agreement with the results observed in the present study.

In this study, we observed the differences in tobacco and alcohol consumption between BC patients and controls. The relationship between these two factors with cancer development has been confirmed[3,21,22]. The over expression of *CXCR1* has been observed frequently in the cytoplasm and nucleus of BC cells and it has been suggested that CXCR1 may play an important role in the innate immune system that stimulates the growth and progression of the tumor[5,31]. The *CXCR1* gene has multiplex binding sites for different transcription factors that function as a ligand to activate the transcription and participate in the inflammation processes[5].

Some studies[10-12] of the rs1008562 and rs2234671 polymorphisms have been reported to have an association with increased colorectal cancer risk. The rs3138060 polymorphism has showed an association with visceral leishmaniasis[32]. However, there are no studies on the association of rs1008562, rs2234671 and rs3138060 polymorphisms with the risk of BC.

Moreover, little is known about the association of the rs1008562, rs2234671 and rs3138060 polymorphisms of the *CXCR1* gene in Mexican BC patients. In our study, the rs1008562 polymorphism has not observed an association with susceptibility to BC. However, the frequency of *CC*, *GC* (heterozygous model), *GC/CC* (dominant model) genotypes and *C* allele of rs2234671 polymorphism have shown statistically significant differences between BC patients and controls ($p<0.05$) and were associated with the risk of developing BC.

Moreover, the frequency of *GG* genotype and *G* allele of rs3138060 polymorphism showed statistically significant differences between BC patients and controls ($p<0.05$) and were associated with the risk of developing BC.

This is the first report where the association of the rs1008562, rs2234671 and rs3138060 polymorphisms of the *CXCR1* in BC was analyzed. The expression of *CXCR1* in BC has been analyzed in different studies[4,11,33-36]. Although there are researches over regulatory mechanisms in the development of BC, still are more information to be completely. The *C* and *G* alleles of rs1008562, rs2234671, and rs3138060 polymorphisms, likely have changes in splicing regulation and post-translation of *CXCR1*, that might affect the CXCR1 activity enzyme, and as a result alter the angiogenic properties of endothelial cells that permit tumor growth[35,36]. Moreover, CXCR1, one of two high-affinity receptors for IL-8 has been found to be associated with mechanisms of invasion, metastasis and drug resistance in several types of solid tumor that included BC[37]. In our study, the association in the dominant model of the rs1008562 (*GC/CC*), rs2234671 (*GC/CC*), and rs3138060 (*CG/GG*) polymorphisms as risk factors in BC stratified by tobacco and alcohol consumption was also demonstrated.

One study[12] determined the association GEIs (characterization of gene-environment interactions) in rectal cancer demonstrated the association of *CXCR1* gene with smoking and alcohol, and concluded that the angiogenic genes and smoking, alcohol consumption, and animal protein impact on rectal cancer risk. The IL-8 function mitogenic in the tumor cells are mediated mainly by CXCR1 receptor[38]. Additional data from the *C* and *G* alleles of the rs1008562, rs2234671, and rs3138060 polymorphisms of the *CXCR1* gene, respectively, could deregulate the promoter activity and the function of CXCR1 receptor. Therefore, this receptor participates in the development of cancer.

Based on the above results, we might suggest that the consumption of tobacco and alcohol, a greater production of free radicals (by nicotine of tobacco), could be generated in people carrying the *C* and/or *G* alleles of these polymorphisms in the *CXCR1* gene, and thus, acting to stimulate angiogenesis molecules in breast tissue has had increased susceptibility to cancer[12,27].

In this investigation it also was observed the GC/CC genotype as a risk factor in BC with HTR consumption, but no existing studies in literature that corroborate this associations. However, hormone therapy is widely used to relieve symptoms

of menopause, it has been associated with coronary heart disease, stroke and increased invasive BC risk[39]. It is known that, extrinsic factors and other cells that form part of the tumor microenvironment are responsible for regulating and promoting the tumor activity. The interleukin-8 (IL-8) participate, as deregulated expression of multiple inflammatory cytokines, in malignant breast disease. IL-8 can promote tumor invasion, metastases, and treatment resistance, through the CXCR1/2 receptors[40]. Moreover, Jiang et al[37] suggests that the increased secretion of IL-8, stimulated by estrogen, might promote the metastatic activities of the ER-negative BC cells *via* G protein-coupled estrogen receptor 1 (GPER1) and CXCR1 activation, and IL-8 may represent a novel therapeutic target for estrogen driven breast carcinogenesis and tumor progression[41]. Association of *GC* genotype of rs3138060 and rs18008562 polymorphisms of *CXCR1* gene as risk factor between HER2/neu than TNBC and stage IV than stage I respectively, were observed. Moreover, the rs1008562, rs2234671, and rs3138060 polymorphisms of the *CXCR1* gene were associated as risk factor in stage advanced III-IV of BC in: rs1008562 polymorphism Luminal B than TNBC, Her2/neu than TNBC carrying *CC* genotype. rs2234671 polymorphism luminal A than luminal B, luminal A than HER2/neu, TNBC than luminal B and TNBC than HER2/neu, carrying *GC* genotype and rs3138060 luminal B than TNBC and HER2/neu than TNBC, carrying *GC/GG* genotype. However, the confidence intervals were wide due to the simple BC stratification or small sample size. There are no studies on the association of rs1008562, rs2234671, and rs3138060 polymorphisms in *CXCR1* gene and the molecular type and stage in BC. However, the expression of CXCR1/2 have been observed in TNBC patients[42-44], and these data could support the results observed in this study. Ilgın et al[42], hypothesized a difference in expression of CD133 among different subtypes BC. CD133 expression was at the periphery and CXCR1 expression at the central area of the tumor, which is prominent in the TNBC.

Yang et al[43] found that the knockout of the *CXCR4* and *CXCR7* genes affects the binding capacity and functions of CXCL12, inhibits the malignant progression of triple negative BC cells significantly, and may become a potential target for the treatment of TNBC.

Wu et al[44] detected that high expressions of the gene *CXCL1/2* and its receptor CXCR4 and CXCR7 in HER2/neu and TNBC might be strongly associated with their poor prognosis. Inhibition of their expressions in HER2/neu positive BC and TNBC may provide a strategy for treating BC in clinic.

TNBC is characterized by high metastasis and rapid progress compared with the other BC subtypes. The chemokine CXCL1/2 and its receptors CXCR4 and CXCR7 play an important role in tumorigenesis and metastasis; CXCR4 and CXCR7 affect the progression of TNBC through different signaling pathways. The clinical data also have showed that high expression levels of CXCL1/2 and its receptors CXCR4 and CXCR7 were associated with easy metastasis and poor prognosis of TNBC. Wu et al[43] have proposed that CXCR4 and CXCR7 promote cell migration, invasion, angiogenesis, and tumorigenesis through different modes of signal transduction based on their respective characteristics. Finally, the tumor microenvironment is an important factor in cancer treatment response, it is considered as a target for combination therapy of BC. The signaling pathways (CD47 up-regulation; NF-κB; STAT-1; STAT-3; TNF) and immune evasion, tumor growth and progression, prediction of clinical outcome and prediction of response, or resistance to chemotherapy are important factors involved in development of BC. These altered pathways related to the immune system open clinical opportunities for prognosis or treatment of patients[45,46].

In addition, the haplotype and haplogenotype association of rs1008562, rs2234671, and rs3138060 polymorphisms of the *CXCR1* gene, were determined between BC patients and control groups. The haplotypes showed linkage disequilibrium with each other. We observed that the *CCC* (OR 1.75; 95% CI 1.03-2.97, $p$=0.046) and *GGG* (OR 3.73; 95% CI 1.61-8.65, $p$=0.0018 haplotypes and haplogenotype *GCGGCG* (OR 341; 95% CI 0.97-11.94, $p$=0.0510) were associated with the susceptibility to BC; however, it should be noted that the confidence intervals were high due to the small sample size.

To our knowledge, this is the first study to report the association by subtypes, stages of rs1008562, rs2234671, and rs3138060 polymorphisms of the *CXCR1* gene with BC. However, we could elucidate that the progression of cancer is associated with adverse clinical outcomes and it may modify the expression of different molecular factors including angiogenesis process regulated by interactions of multiples genes, which could alter the regulation of cellular processes[12].

## Conclusions

Shortly, our results showed a pattern prevalent in BC patients, to our knowledge it is the first study where it is has been shown that, the rs2234671 and rs3138060 polymorphisms were associated with BC risk when comparing controls and BC patients for the genotypes *CC*, *GC* (heterozygous model) *GC/CC* (dominant model) and *G allele*, and *CG/GG* (dominant model) and allele *G*, respectively. In addition, there were evident differences within patients and controls with the dominant model of the rs1008562 (*GC/CC*), rs2234671(*GC/CC*), and rs3138060 (*CG/GG*) polymorphisms with tobacco, alcohol, HTR consumption, molecular subtype and stage in BC. The haplotypes *CCC* and *GGG* were observed to be a risk factor for BC. Previous evidence indicates that these factors significantly contribute to BC susceptibility in the analyzed sample from a Mexican population. However, further studies are required to confirm these observations.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

### Acknowledgements

The authors would like to thank the nurses for assisting in the blood sampling process. This research was financially supported by FIS/IMSS/PROT/PRIO/19/111 and CIBO, IMSS grants.

### Authors' Contribution

GAMP contributed in the design, analysis, experimentation, data collection and financing; CJBZ, JYSL, PPAM, DSJI contributed to data collection; FLE, ZGGM, GMBC, MFH contributed to the design and analysis of the manuscript. All the authors have read and approved the final manuscript.

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