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Association Of A *HER2 Ile655Val* (Rs1136201) Polymorphism in Breast Cancer in A Mexican Population

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***Abstract*-EGFR (epidermal growth factor receptor) has tyrosine kinase activity and plays an important role in processing carcinogens. Data on the *HER2 Ile655Val* polymorphism have revealed associations with cancer, suggesting that this polymorphism contributes to tumor promotion in breast cancer (BC). We examined the role of the *HER2 Ile655Val* genotype by comparing the genotypes of 225 healthy Mexican women with 400 Mexican women with BC. The genotype frequencies observed in the controls and patients with BC were 14.6 and 21%, respectively, for the *A/G* (*Iso/Val*) genotype. The obtained odds ratio (OR) was 1.5, with a 95% confidence interval (CI) from 1.01-2.4, P = 0.04. The association was also evident upon analysis of the distributions from the *A/G-G/G* genotype in patients with nulliparity and non-chemotherapy response who were positive for HER2 tumor expression (OR 2.5, 95%CI = 1.06-7.78, P = 0.032). This study suggests that the A/G genotype *HER2 Ile655Val* polymorphism is associated with BC susceptibility in the Mexican population.**

***Index Terms*-HER2, *Ile655Val* polymorphism. breast cancer, non-chemotherapy response, Mexican population.**

I. Introduction

Breast cancer (BC) is characterized by the accumulation of genetic and epigenetic events that promote the uncontrolled growth of cells that invade and destroy breast ducts or lobules and can spread through the lymphatic or blood stream to form metastases [1], [2]. Each year, 1.67 million new cases and 522,000 deaths occur from breast cancer worldwide [2]. BC is a major health problem in Mexico; in 2013, the incidence of breast cancer was estimated at 26/100,000 women over 20 years. The incidence of BC in Mexico has great heterogeneity; there are regional differences, and it is often observed with more frequency in the northern and central states of the country [3], particularly the state of Jalisco, which reported an incidence of 50 in every 100,000 women in 2013. These data are higher than the national average, with only 10% of all of the cases being detected early in stage I [4], [5]. BC has been classified as a multifactorial disease with family history, lifestyle, reproductive history and genetic mutations influencing the development of this pathology [6]. In recent years, many studies have explored BC pathogenesis, particularly with respect to epidermal growth factor receptor-2 (HER2), a member of the human epidermal growth factor receptor family [7] that has tyrosine kinase activity, activates intracellular signaling pathways in response to extracellular signals, and has been related to the development and progression of multiple tumors [8]. The *HER2* (*ERBB2*) gene is located on chromosome 17q21 and is translated into the HER2 proto-oncogene as a transmembrane glycoprotein [9]. It has also been shown that *HER2* oncogene over-expression occurs in approximately 25% of cases of BCs; this fact is associated with more aggressive diseases and thus poor prognoses [10]. Different polymorphisms have been described in functionally relevant regulatory regions that could have an effect on protein expression. One of the most studied variants is a single nucleotide polymorphism at codon 655 (*HER2* Ile655Val; rs1136201). This polymorphism is described as a strong candidate for susceptibility to BC; it has also been demonstrated that cells expressing valine show a higher growth rate compared to cells expressing isoleucine [11]-[13]. However, associations with the *Ile655Val* polymorphism have shown contradicting results in different studies [13]-[16]. Cresti et al, 2016 reported on 361 patients with breast cancer in the UK and found the following genotypic frequencies: (1) *AA (Ile / Ile)* 61.8%, (2) *AG (Ile / Val)* 34.3% and (3) *GG (Val / Val)* 3.9%; these results were similar to a previous study (63.75%, 32.5% and 3.75% for the *AA*, *AG* and *GG* genotypes, respectively) described by Watrowski et al., 2015 that concluded that genotypic variation at *HER2* codon 655 does not alter the risk of BC in Austrian Caucasian women [13]. Another study observed that the Ile655Val polymorphism affects the

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function of the *HER2* gene alone in patients with HER2-positive BC. Patients carrying the Val variant have more aggressive phenotypes but respond to treatment with trastuzumab [12]. Only two studies have been completed in the Mexican population that describe the prevalence of the *Val/Val* genotype in the control groups with values of 1.9% [17] and 21% [18]; the first study included gastric cancer patients, and the second included HER2 positives BC patients. Despite the reported variability in the genotype frequencies of the *Ile655Val* polymorphism, the goal of this study was to evaluate the association of the *Ile655Val HER2* polymorphism in Mexican women with BC.

II. MATERIAL AND METHODS

Sample collection and patient information

DNA samples (400 from women with BC and 225 from healthy women of the Mexican general population) were obtained from genomic libraries from projects previously approved by ethical committee #1305, Western Biomedical Center Research, Western National Medical Center, Mexican Institute of Social Security, Guadalajara, Jalisco, Mexico. All of the samples were obtained after a written informed consent form was signed by the participants. Clinical and demographic data were obtained using written questionnaires. All of the patients were also interviewed to determine their occupational exposure as well as the use of pharmacological therapies. This study was conducted respecting national and international ethical standards. Efforts were made to ensure that siblings of individuals who had already been sampled were excluded. The BC patient database and the DNA samples have been examined for other polymorphisms [4], [5], [19]-[21].

Genotyping

The genomic DNA was obtained previously using a standard protocol [22]. A PCR method was used to detect the *HER2 Ile655Val* polymorphism in the genomic DNA samples from the study groups using the following primers: (1) forward 5'-AGAGCGCCAGCCCTCTGACGTCCAT-3' and (2) reverse 5'-TCCGTTTCCTGCAGCAGTCTCCGCA-3' (23). The PCR amplifications were performed in a total volume of 15 µL containing 0.2 mM dNTPs (Invitrogen, Carlsbad, CA, USA), 7.5 pmol primers, 2.5 mM MgCl2, 2.5 U of Taq polymerase (Invitrogen, Carlsbad, CA, USA), and 50 ng of genomic DNA. The PCR conditions were as follows: (1) 94 °C for 4 min; (2) 35 cycles of 94 °C for 5 min; (3) 35 cycles of 94 °C for 1 min, 59 °C for 1 min and 72 °C for 1 min; and (4) a final extension at 72 °C for 7 min. The 148-bp long amplicon was digested with the BsmAI restriction enzyme (New England BioLabs) at 37 °C for 12 h. Allele discrimination was performant using 8% polyacrylamide gel (19:1) electrophoresis followed by silver staining. Reduction of the amplicons to 122- and 26-bp fragments indicated the presence of the wild-type genotype (*AA, Ile/Val*), whereas three 90-, 32- and 22-bp fragments were present for the polymorphic genotype (*GG, Val/Val*) (Figure 1).

The genotypes were confirmed by sequencing six samples (two from each genotype) (Figure 2).

Statistical analysis

The allele frequency was obtained by direct counting. The chi-square test was used for the Hardy-Weinberg equilibrium. The odds ratio and 95% confidence intervals were calculated using SPSS 20.0 (SPSS, Chicago, IL, USA).

III. RESULTS

Clinical and demographic characteristics of the study groups

The comparative epidemiological data from the patients with BC and control individuals are shown in Table I. The average ages were 54.037 and 43.92 years in the patient and control groups, respectively. Menarche presented at a mean age of 12.66 years in the patients and at 12.08 years in the controls. Postmenopause (OR = 15.9; 95% CI = 10.1-25.1; P < 0.0001) and oral contraceptive use (OR = 2.0; 95% CI = 1.3-3.1; P=0.002) appeared to promote BC. Conversely, alcohol consumption seems to be a protective factor (OR = 0.47; 95% CI = 0.27-0.83, P = 0.009).

Table II provides the general clinical characteristics of the BC patient group. We observed that 10.5 % of the patients exhibited nulliparity, 32 % presented abortion, 43% presented obesity I-III, 66 % displayed stage III-IV tumors, 89 % had ductal histology, and 47 % were Luminal A positive.

Genotype frequencies of the study groups

The genotype and allele frequencies of the *Ile655Val HER2* gene polymorphism were different in the control and patient groups (Table III). The heterozygous genotype (*A/G*) was observed in 21 % (85/400) of patients and in 14.6 % (33/225) of controls (OR = 1.5; 95 % CI = 1.01-2.4, P = 0.04). The allele *G* was different between the BC patients and the controls (OR = 1.5; 95 % CI = 1.01-2.2, P = 0.042), and the over-dominant genetic model (*AG* vs. *AA+GG*) was also different (OR = 1.5; 95 % CI = 1.01-2.43, P = 0.043). The genotype distribution of the control group was in Hardy-Weinberg equilibrium. All of the samples were analyzed, and genotypes of all of the participant genotypes (for 225 controls and 400 BC patients) were obtained.

The clinical characteristics of the BC patients with the *A/G-G/G HER2* polymorphism genotype are listed in the Table II. Only the *A/G-G/G* genotypes associated with nulliparity (adjusted OR = 3.46; 95 % CI = 1.77-6.74; p<0.0001) were found to be risk factors. We also observed that the *A/G-G/G* variant genotype served as a risk factor in the non-chemotherapy response (received chemotherapy in combination with trastuzumab treatment) of BC patients who were positive for HER2 tumor expression (OR = 2.8; 95 % CI = 1.06-7.78; P = 0.032) (data not shown).

IV. DISCUSSION

BC is considered a major public health problem in the world. In Mexico, BC represents one of the leading causes of death in working-age women [4], [5], [19], [20], [24]-[26]. These facts are consistent with the observations made in the current study, in which the average age of patients with BC was 54.037 (± 11.75) years. In our study, when the groups of patients were stratified by menopause stage, as showing either a premenopausal or menopause status, the use of oral contraceptives and tobacco consumption emerged as risk factors. It has been suggested that the effects of menopause on BC risk might be due to the function of endogenous ovarian hormones that could be more relevant for estrogen receptor-positive disease than for estrogen receptor-negative disease and appear to be more relevant for lobular rather than for ductal tumors [27]. In contrast, alcohol consumption has emerged as a protective factor; these data agree with Frydenberg et al., 2015, who described that moderate alcohol consumption could be a key point for BC prevention. Alcohol consumption was also positively associated with daily endogenous estrogen levels and mammographic density in premenopausal women [26].

The transmembrane HER2 gene encodes a glycoprotein with tyrosine kinase activity that participates in signal transduction that mediates tumor cell proliferation and/or motility and has been the focus of many cancer investigations [28]. HER2 has been associated with tumor aggressiveness, lymph node metastasis, cardiotoxicity to trastuzumab, and poor patient clinical outcomes in BC [12], [13], [29].

The *HER2 Ile655Val* polymorphism in the transmembrane domain-coding region has shown association with breast cancer [13]. [30], [31]. Different studies have demonstrated that valine-expressing cells display a higher growth rate compared to isoleucine-expressing cells, which might accelerate neoplastic processes [29]. Therefore, studies to identify whether the Val/Val genotype is associated with BC susceptibility have been performed in various populations [15], [30]-[34].

In the present study, we observed a similar frequency for the *G/G* (*Val/Val*) genotype *HER2 Ile655Val* polymorphism in our control group (0.4 %) compared with a previously reported study in Mexico. The previous study described a 1.9 % (n = 103) frequency for the *G/G* genotype among individuals in the general Mexican population compared with gastric cancer and no found association [17]. The heterozygous (*A/G*) genotypic frequencies were 14.6 and 21 % in the control group and in patients with BC, respectively, suggesting a marginal association of this allele as a BC risk factor. These data were also evaluated with the allele frequency using the over-dominant model. However, the association between the *HER2 Ile655Val* polymorphism remains controversial and depends on the population studied [31]-[35]. For example, Chen et al., 2014 found an association between the *HER2 Ile655Val* polymorphism and BC in a meta-analysis study that included 14,926 cases and 15,768 controls from 29 studies that suggested that the *HER2 Ile655Val* polymorphism is marginally associated with breast cancer susceptibility in populations worldwide with additive and dominant models, but not a recessive model [31].

Lemieux et al., 2013 observed an association between heterozygous (*A/G*) in BC with heavy alcohol use during the course of trastuzumab treatment and concluded that the *HER2 Ile655Val* genotype may constitute risk factors for cardiac toxicity [33]. However, the association of *HER2 Ile655Val* polymorphism with BC in this study has been controversial [14], [35]. In a meta-analysis of 22 studies that included 9,209 cases and 10,132 controls, Ma et al., 2011 [14] reported an association between the *HER2* codon 655 polymorphism and breast cancer susceptibility (for *Val/Ile* vs. *Ile/Ile*: OR = 1.069, 95% CI = 0.976-1.172; for *Val/Val* vs. Ile/Ile: OR = 1.191, 95% CI = 0.922-1.538; for the dominant model: OR = 1.093, 95% CI = 0.991-1.206; for the recessive model: OR = 1.141, 95% CI = 0.902-1.444). However, a modest association between the *HER2 Ile655Val* polymorphism and an Asian population was found (*Val/Ile* vs. *Ile/Ile*: OR = 1.207, CI = 1.006-1.450). In another meta-analysis study using 33 case-control studies and reporting data with an additive genetic model (20,461 cases/23,832 controls), Dahabreh and Murray, 2011 [35] found evidence of an association between rs1136201 and breast cancer (OR=1.05, 95% CI, 0.99-1.11) that suggested that heterogeneity is present between the study types and concluded that laboratory artifacts, a lack of genotyping quality control or blinding and publication bias appear to have influenced the results of published manuscripts and needs to be addressed in the design of future studies. However, the association between the *HER2 Ile655Val* polymorphism remains controversial and depends on the population studied.

In this study, we also observed a risk association between the *G/A-G/G* genotypes with nulliparity in BC. In fact, the risk association appears to have a heterozygous genotype. It is known that nulliparity is associated with an increased risk for certain reproductive malignancies, including breast, ovarian and uterine cancers. Existing evidence suggests that the risk is related to the increased number of ovulatory cycles in nulliparous women, meaning that it might be preventable by using oral contraceptives [30], [36], [37]. Other theories suggest that there are synergistic effects between nulliparity and obesity in elderly women that contribute to breast cancer risk [38].

Similar results have been described by Butt et al., 2009 [36]; they found an association between nulliparity with HER2-positive tumors of BC (OR 3.24: 1.02-10.25) and concluded that both nulliparity and late first childbirth are associated with relatively more aggressive breast cancer subgroups. Different genomic changes in the breast epithelium of women have also been evaluated. The results

from this inquiry found that the genome in parous women with no breast cancer is different from both nulliparous and parous women with breast cancer.

Recent studies by Asztalos et al., 2015 [37] described the expression of five genes, *TGFB3, ESR1, PGR, TIMP2*, and *ERBB2*, in nulliparous groups and suggest that pregnancy-associated changes persist in human breast tumors for as long as 5–10 years post-partum. The presence of the *Val* in the *Ile655Val* polymorphism probably causes selective advantages in growing neoplastic cells in BC patients with nulliparity, with these cells contributing to the development of cancer.

We also observed that the *AG-GG* variant genotype could be a risk factor in the non-response to chemotherapy (trastuzumab) in BC patients who were positive for HER2 tumor expression. Similar results were observed by Arce et al., 2010 in a study of 113 Mexican BC patients; 55 % of these patients overexpressed Her2 and received trastuzumab. They observed an association between the *HER2 Ile655Val* polymorphism with non-pathological responses that suggested the possibility of a new mechanism for trastuzumab resistance due to structural changes in the polymorphic protein [18].

Our results showed that the frequency of the AG heterozygous genotype is significantly different in controls versus patients with BC. The risk association with nulliparity and non-chemotherapy (trastuzumab) response were also evident in patients carrying the *AG-GG* genotype, which might contribute significantly to BC susceptibility in the analyzed sample from a Mexican population. Nevertheless, further studies are required to confirm or reject these conclusions.

A. *Figures and Tables*

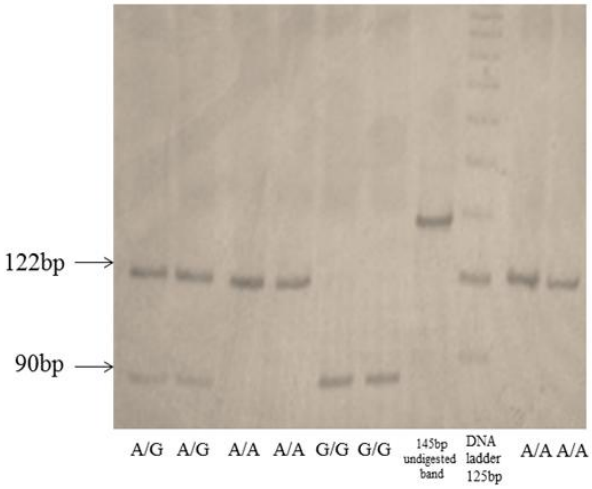

Figure 1. Polyacrylamide gel electrophoresis (8% (19:1) detection of the *A/A* (122 bp), *A/G* (90 and 122 bp), and *G/G* (90 bp) *HER2 Ile655Val* polymorphism genotypes.

Table I. Demographic data for the studied groups.

	BC (n=400)		**Controls** (n=225)		**OR (CI 95%)***	***p-value***
Age (years)						
Means ± SD	54.037±11.75		43.92±14.53			<0.0001
< 50 years, (n)%	(138)	34,5	(142)	63		
≥ 50 years, (n)%	(262)	65.5	(83)	37	3.2 (2.3-4.5)	<0.0001
Menarche						
Means ± SD	12.66±1.62		12.08±0.79			<0.0001
7-10, (n)%	(27)	7	(0)	0		
11-13, (n)%	(254)	64	(213)	95	0.13(0.07-0.26)	<0.0001
14-18, (n)%	(119)	29	(12)	5		
Menopause						
Premenopausal, (n)%	(126)	32	(198)	88		
Postmenopausal, (n)%	(274)	68	(27)	12	15.9 (10.1-25.1)	<0.0001
Oral contraceptive use						
Yes, (n)%	(177)	44	(73)	32	2.0 (1.3-3.1)	0.002
No, (n)%	(223)	56	(152)	68		
Tobacco consumption						
Yes, (n)%	(106)	27	(48)	21		
No, (n)%	(294)	73	(177)	79		
Alcohol consumption						
Yes, (n)%	(59)	15	(53)	24	0.47 (0.27-0.83)	0.009
No, (n)%	(341)	75	(172)	76		

*OR (odds ratio) from the adjusted regression analysis, SD (Standard deviation), CI (confidence interval).

Table II. Clinical data for the BC patients

	(n)	%		(n)	%
Nulliparity			**Histology**		
Yes	(42)	10	Ductal	(356)	89
No	(358)	90	Lobular	(37)	9
Abortion			Mixed	(7)	2
Yes	(127)	32	**Tumor markers**		
No	(273)	68	Luminal A	(189)	47
Breastfeeding			Luminal B	(65)	16
< 6 months	(71)	18	HER 2	(56)	14
≥ 6 months	(229)	57	Triple negative	(90)	23
No	(100)	25	**Tumor localization**		
Body Mass Index *			Unilateral	(381)	95
18.5-24.9 (normal)	(82)	20	Bilateral	(19)	5
≥25-29.9 (overweight)	(146)	37	**Lymph node status**		
≥30-40.0 (obesity I-III)	(171)	43	Yes	(282)	71
Stage			No	(118)	29
I-II	(137)	34	**Chemotherapy**		
III-IV	(263)	66	Response	(91)	23
			Non response**	(281)	70
			Partial response	(28)	7

* According to OMS classifications. (Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Ginebra (Suiza): World Health Organization, 2004), ** Non-response and non-response by recurrence.

Table III. The genotype distribution of the rs1136201 HER2 polymorphism in BC patients and healthy controls.

	Groups						
	BC (400)		**Controls** (225)*		**BC *vs.* Controls**		
Genotypes**	**(n)**	**%**	**(n)**	**%**	**OR**	**CI95%**	**p value**
A/A	(312)	78	(191)	85	1		
A/G	(85)	21	(33)	14.6	1.5	(1.01-2.4)	0.04
G/G	(3)	1	(1)	0.4			1.0
Models:	Over-dominant						
AA + GG	(315)	79	(192)	85			
AG	(85)	21	(33)	15	1.5	(1.01-2.43)	0.04
	Recessive						
AA	(312)	78	(191)	85			
GG + AG	(88)	22	(34)	15	1.5	(1.02-2.44)	0.03
Alleles							
A	(709)	0.886	(415)	0.922	0.65	(0.43-0.98)	0.04
G	(91)	0.113	(35)	0.077	1.5	(1.01-2.2)	0.04

*Hardy-Weinberg equilibrium in controls (chi-square test = 0.1126; p = 0.737). **Marker informativity of 0.50 assessed within a range from 0-1; markers with a score higher than 0.85 were considered highly informative, whereas markers with a value of 0.44 were considered moderately informative [7-8, 20-23].

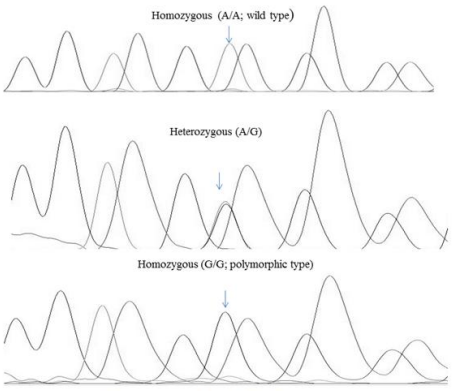

Figure 2. Sequencing of the *HER2Ile655Val* polymorphism genotypes.

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