RESEARCH ARTICLE

HER2 Ile655Val polymorphism is negatively associated with breast cancer susceptibility

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Background: The *HER2* (human epidermal growth factor receptor-2) Ile655Val (rs1136201) genetic polymorphism can alter the receptor structure and its auto-activation, which can modify the signal transduction and, consequently, the cell cycle regulation. For this reason, this polymorphism has been extensively investigated as a candidate marker for breast cancer (BC). In this context, the aim of this study was to evaluate the possible influence of *HER2* Ile655Val in BC susceptibility and prognostic factors in a Brazilian population.

Methods: Polymorphism genotype was assessed through RFLP-PCR in 107 BC patients with clinicopathological data available and in 150 women with no evidence of neoplasia and with no familial history of BC as control group. Association between this polymorphism and BC susceptibility and clinical parameters was evaluated through odds ratio (OR) and chi-squared or Fisher's exact test, respectively.

Results: A significant negative association between valine allele and BC susceptibility in dominant model was found (OR 0.5; 95% CI 0.27-0.93, $P = .036$). No significant association was found in relation to BC clinicopathological features (tumor size, lymph nodes commitment, histological grade, *HER2* overexpression, hormonal receptors, p53, and Ki-67).

Conclusion: Although this polymorphism did not demonstrate potential as a prognostic marker, it may be a suitable susceptibility marker for BC.

KEYWORDS

breast cancer, clinicopathological features, genetic polymorphism, *HER2*, prognosis, single nucleotide polymorphism, susceptibility

1 | INTRODUCTION

HER2 is a transmembrane tyrosine kinase receptor belonging to a family of epidermal growth factor receptors (EGFR). The *HER2* gene, also known as *ERBB2/HER2*, is amplified in 20%-30% of breast cancers (BC) and is considered a marker of poor prognosis. Its overexpression is associated with an aggressive phenotype of tumor cells, low overall survival, and resistance to antihormonal therapy and cytotoxic therapies.¹

Homo- or heterodimerization of *HER* family receptors activates intracellular tyrosine kinase domain which promotes the autophosphorylation of cytoplasmic tail tyrosine residues, leading to activation of pathways that trigger cell survival and proliferation.² However, according to crystallographic analysis, *HER2* is already in a binding conformation even in the absence a ligand, explaining why no ligand for this receptor has been identified so far.³

Several polymorphisms have been described in the *HER2* gene, and among them, the Ile655Val (rs1136201) has been extensively

investigated as a susceptibility factor for BC. It was described by Papewalis and Nikitin⁴ and consists of a single nucleotide polymorphism (SNP) characterized by an adenine (ATC, isoleucine) to guanine (GTC, valine) change in the codon 655, in the transmembrane receptor domain. It has been described that Val allele is associated with enhanced HER2 signaling when compared to Ile⁵ and that Val-expressing cells show enhanced proliferation and decreased apoptosis *in vitro*.⁶

However, after several studies, the biological significance of this allelic variant in BC pathogenesis remains controversial. In this context, this study aimed to investigate the Ile655Val (rs1136201) polymorphism of *HER2* gene in BC patients and neoplasia-free women from a Brazilian cohort, to verify a possible impact on susceptibility and clinical presentation, in search for a new marker to this neoplasia in this unique population.

2 | MATERIAL AND METHODS

2.1 | Samples selection

This study was approved by the Ethics Committee for Research Involving Human Beings of the Londrina State University (CEP/UEL 189/2013 – CAAE 73557317.0.0000.5231). Peripheral blood samples were obtained from 107 BC patients who had undergone surgery at the Londrina Cancer Hospital, Parana, Brazil. All samples were obtained from patients free of adjuvant or neoadjuvant chemotherapy. The control group was composed of 150 neoplasia-free women from a Health Care Unit of Londrina, bearing recent (within the past two years from collection time) negative mammography, age nearby to the patient's group, and with no familial history of breast neoplasia. Both patients and controls were attended by Brazilian Public Health System, and they signed a free informed consent form prior to sample collection.

2.2 | Clinicopathological parameters

BC clinicopathological parameters were obtained from patients' health-care records maintained in Londrina Cancer Hospital, including: tumor size, lymph node commitment, histological grade, HER2 overexpression, hormonal receptors (estrogen (ER) and progesterone (PR)) status, p53, proliferation index (Ki-67 status), and clinicopathological staging (according to TNM classification of breast tumors⁷), which were determined according to the Union for International Cancer Control criteria. Immunohistochemical analyses of HER2, hormonal receptors, p53 and Ki-67 were performed by pathologists who followed the score criteria described in the American Society of Clinical Oncology/College of American Pathologists guideline recommendations.^{8,9}

2.3 | Genomic DNA extraction

DNA was obtained from peripheral blood of patients and controls using Biopur Mini Spin Kit (Biometrix Diagnostica, Curitiba, Parana, Brazil) according to the manufacturer's instructions. DNA was quantified by NanoDrop 2000c Spectrophotometer (Thermo Scientific, Wilmington, Delaware, EUA) at a wavelength of 260 nm, and

purity was assessed through the absorbance ratio in 260 nm and 280 nm. Extracted DNA was stored at -20°C and used as template in polymerase chain reactions (PCRs).

2.4 | Ile655Val genotyping

HER2 Ile655Val genotypes were determined by restriction fragment length polymorphism PCR-based assay (RFLP-PCR). The primers were manufactured according to Xie et al¹⁰ as follows: forward: 5'-AGAG CGCCAGCCCTCTGACGTCCAT-3'; reverse: 5'-TCCGTTTCCTGCAG CAGTCTCCGCA-3'. PCRs were performed using 100 ng of genomic DNA and the buffer kit plus 1.25 units of Taq polymerase (Invitrogen, Carlsbad, CA, USA) at 25 µL of final volume, in a A200 Gradient Thermal Cycler (LongGene, Hangzhou, China).

PCR conditions were as follows: denaturation at 94°C for 5 minutes, 35 cycles of 30 seconds at 94°C, 30 seconds at 61°C, and 30 seconds at 72°C, with final elongation of 10 minutes at 72°C. Amplicons of 148 base pairs (bp) were analyzed by electrophoresis in 2% agarose gel and visualized using UV fluorescence after staining with Blue Green Loading Dye (LGC Biotecnologia, São Paulo, Brazil). All reactions were conducted with a negative control to ensure no contamination with exogenous DNA during reactions preparation.

PCR products were then subjected to enzymatic restriction by incubating with 3U of *BsmAI* endonuclease (New England Biolabs, Beverly, Massachusetts, USA) for 4 hours at 55°C, according to the manufacturer's instructions. Restriction fragments were analyzed by electrophoresis on polyacrylamide gel (10%) and detected using a silver staining method. Three genotypes were verified: Ile/Ile (148 bp), Ile/Val (148 bp/116 bp), and Val/Val (116 bp).

The three genotypes observed through RFLP-PCR were confirmed by Sanger-based sequencing. PCR products were generated using 5 U/µL of Taq Platinum High Fidelity DNA polymerase (Invitrogen) and purified using PureLink™ PCR Purification Kit (Invitrogen), following manufacturer's instructions. The sequencing reaction was performed using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems®, Foster City, CA, USA), 50 ng of DNA template, and 5 µM of primer (forward or reverse) in a final volume of 10 µL. PCR conditions were as follows: 10 seconds at 95°C, 30 cycles of 20 seconds at 95°C, 20 seconds at 50°C, and 1 minutes at 60°C. The amplicons were sequenced in a 24-capillary 3500xl Genetic Analyzer (Applied Biosystems).

2.5 | Statistical analysis

For the case-control association study, odds ratios (OR) at 95% confidence interval (CI) were calculated along with Fisher's exact test, setting the prevalent genotype (Ile/Ile) as reference (OR = 1.0), using GraphPad Prism version 6.0 for Windows (GraphPad Software, La Jolla, California, USA). Fisher's exact tests or chi-squared (χ^2) tests were performed to assess possible association between *HER2* polymorphism and clinicopathological parameters using SPSS Statistics version 22.0 (SPSS Inc., Chicago, Illinois, USA). All tests were two-tailed, and *P* values lesser than .05 were considered statistically significant.

3 | RESULTS

3.1 | Sample characterization

The average age for breast cancer patients was 56.4 years. The highest incidence of BC was observed between 40 and 49 years. The average age for the control group was 50.5 years.

Most patients had tumor in stage II or III (82.75%). About 59.8% of the patients had tumor size between 1.5 and 3.0 cm, and the average tumor size was 3.10 cm. Additionally, 64.6% of patients were classified as HER2 negative, 43.26% were classified as luminal, and 8.65% were classified as triple negative (TN) subtypes.

3.2 | HER2 Ile655Val polymorphism and BC susceptibility

To confirm the genotypes obtained by RFLP-PCR, DNA sequencing of 3 amplified PCR fragments was performed, as shown in Figure 1.

Genotype distribution and association analyses are shown in Table 1. A negative association in relation to BC susceptibility was observed, in which the allelic variant valine (GTC) demonstrated protection for cancer development in the dominant model of analysis (Ile/Val + Val/Val vs Ile/Ile: OR = 0.5; 95% CI = 0.27-0.93; $P = .036$) (Table 1). Val allele frequency was also significantly different between control and BC groups (15.7% and 8.9%, respectively; OR = 0.52; 95% CI = 0.3-0.92; $P = .024$) (Table 1).

The genotype distribution for Ile655Val polymorphism from our control group was compared to a population of 609 elderly individuals from São Paulo, Brazil, deposited in the publicly available ABraOM database (<http://www.abraom.ib.usp.br/>),¹¹ and no significant difference was found (ABraOM genotype frequencies: Ile/Ile = 73.4%, Ile/Val = 24.3%, Val/Val = 2.3%; $\chi^2 = 0.28$; $P = .87$).

Allelic frequency was also compared between our control group and different populations worldwide using data from the 1000 Genomes projects, retrieved through the web-based application LDlink (<https://analysistools.nci.nih.gov/LDlink/>).¹² Allele frequency from our population was similar to that from all American admixed populations and to that of all Asian populations, except for Vietnamese population, in which Val

FIGURE 1 DNA sequencing of the HER2 Ile655Val genetic polymorphism. (A) Amplified sequence containing the adenine nucleotide. (B) Amplified sequence containing the guanine nucleotide. Recognition region of BsmAI restriction enzyme (GTCTCN) is underlined. The arrow indicates the cutting position. (C) Electropherogram of the three genotypes (IUPAC ambiguity code R = adenine or guanine). (D) Electrophoretic profile of Ile655Val genetic polymorphism. Lane 1, 100 bp Ladder; Lane 2, genotype AA (Ile/Ile); Lane 3 genotype AG (Ile/Val); Lane 4 genotype GG (Val/Val)

TABLE 1 Case-control association study for Ile655Val polymorphism in BC patients and neoplasia-free women

Genotypes	Controls n = 150	Patients n = 107	OR ^a	95% CI ^b	P value
Ile/Ile	107 (71.3%)	89 (83.2%)	1.00	Reference	-
Ile/Val	39 (26.0%)	17 (15.9)	0.52	0.28-0.99	.047*
Val/Val	4 (2.7%)	1 (0.9%)	0.30	0.03-2.74	.382
Dominant	43 (28.7%)	18 (16.8%)	0.50	0.27-0.93	.037*
Recessive	4 (2.7%)	1 (0.9%)	0.34	0.04-3.13	.405
Allele Ile count	253 (84.3%)	195 (91.1%)	1.00	Reference	-
Allele Val count	47 (15.7%)	19 (8.9%)	0.52	0.30-0.92	.024*

^aOR: odds ratio.

^bCI95%: confidence interval at 95%.

* $P < .05$ by Fisher's exact test.

frequency was 9.09% ($P = .04$). Val allele was significantly more frequent in our population than in all African populations (Val frequency = 0.08%, $P < .0001$ in pooled analysis), in which this allele was extremely rare or absent (for the majority of populations in African continent). Val allele frequency was significantly smaller in our population than in all European populations (Val frequency = 24.55%; $P = .001$ in pooled analysis), except for Italian population from Toscana (Val frequency = 12.5%, $P = .3$).

3.3 | HER2 Ile655Val polymorphism and BC clinical presentation

There was no significant association between Ile655Val in the dominant model of analysis and any clinicopathological parameter tested (Table 2). Recessive model analyses were hampered by the low frequency of Val/Val genotype.

TABLE 2 Ile655Val polymorphism distribution according to BC clinicopathological parameters

Parameters	Total [n (%)]	Ile/Ile [n (%)]	Ile/Val + Val/Val [n (%)]	P value
Age at diagnosis				
<40	7 (66.0)	7 (8.0)	0 (0.0)	.738 [†]
40-49	33 (31.1)	28 (31.8)	5 (27.8)	
50-59	23 (21.7)	19 (21.6)	4 (22.2)	
60-69	24 (22.6)	19 (21.6)	5 (27.8)	
>69	19 (17.9)	15 (17.0)	4 (22.2)	
Tumor staging				
Low (I/II)	33 (57.9)	27 (57.4)	6 (60.0)	.999 [‡]
High (III/IV)	24 (42.1)	20 (42.6)	4 (40.0)	
Tumor size				
0-1.5 cm	9 (8.8)	9 (10.5)	0 (0.0)	.129 [†]
1.5-3.0 cm	61 (59.8)	48 (55.8)	13 (81.3)	
>3.0 cm	32 (31.4)	29 (33.7)	3 (18.8)	
Lymph node commitment				
Absent	49 (49.5)	43 (51.8)	6 (37.5)	.295 [†]
Present	50 (50.5)	40 (48.2)	10 (62.5)	
Histological grade				
I	13 (13.1)	11 (13.3)	2 (12.5)	.928 [†]
II	33 (33.3)	27 (32.5)	6 (37.5)	
III	53 (53.6)	45 (54.2)	8 (50.0)	
HER2				
Negative	64 (64.6)	54 (65.1)	10 (62.5)	.844 [†]
Positive	35 (35.4)	29 (34.9)	6 (37.5)	
Estrogen receptor				
Negative	29 (28.2)	27 (31.4)	2 (11.8)	.142 [‡]
Positive	74 (71.8)	59 (68.6)	15 (88.2)	
Progesterone receptor				
Negative	48 (46.6)	39 (45.3)	9 (52.9)	.566 [†]
Positive	55 (53.4)	47 (54.7)	8 (47.1)	
p53				
Wild	60 (62.5)	52 (65.8)	8 (47.1)	.147 [†]
Mutated	36 (37.5)	27 (34.2)	9 (52.9)	
Ki-67				
Low	21 (25.6)	17 (26.2)	4 (23.5)	.449 [†]
Moderate	33 (40.2)	24 (36.9)	9 (52.9)	
High	28 (34.2)	24 (36.9)	4 (23.5)	

[†]P values obtained by χ^2 test.

[‡]P values obtained by Fisher's exact tests.

Possible associations between *HER2* Ile655Val and clinicopathological parameters were also tested considering only *HER2*-positive BCs, but no statistically significant association was found.

4 | DISCUSSION

The discovery of new markers is essential to improve prevention and prognosis of patients with BC. Molecular markers such as estrogen and progesterone receptors and *HER2* oncogene are well established in the clinic as markers that indicate prognosis and suitable treatments selection.¹³

Several case-control studies investigating Ile655Val polymorphism in BC have been performed; however, contradictory results have been found. Within this context, the present study evaluated a *HER2* allelic variant in Brazilian patients, seeking out for a susceptibility and/or prognosis marker.

Kara and Karakus¹⁴ analyzed Ile655Val in BC patients and free neoplasia controls in a Turkish population and found no significant association in relation to susceptibility or protection. Dahabreh and Murray¹⁵ conducted a meta-analysis based on 33 case-control studies (20,461 cases and 23,832 controls) and found no significant association in relation to BC development. On the other hand, the results demonstrated by Nelson and Gould¹⁶ in US Midwest population suggested that Val/Val genotype of Ile655Val is negatively associated with BC susceptibility. These results corroborate with the findings of the present study, in which the variant Val was negatively associated with BC development in the dominant model (Table 1).

On the other hand, Xie and Shu¹⁰ found that Val/Val and Ile/Val genotypes confer an increased susceptibility to this neoplasia (OR = 1.4; 95% CI: 1.0-2.0) in Chinese BC patients. This association was even higher for patients under the age of 45 years. In the Bangladesh population, the variant allele was also shown as a risk factor for BC (OR = 1.5; 95% CI: 1.0-2.2) and was significantly more frequent in patients under 45 years.¹⁷ Corroborating these results, Montgomery and Gertig¹⁸ also demonstrated in an Australian case-control study involving patients under age of 40 years that Val/Val genotype was positively associated with BC susceptibility.

In contrast, a study conducted by Baxter and Campbell,¹⁹ which examined British BC patients with an average age of 38 years, found no significant association, leading to the conclusion that this polymorphism has a large variation between different ethnic groups. In our study, we did not find differences in Val allele distribution among age groups. Furthermore, in our samples, patients below 40 years did not exhibit this allele, hampering the ability to study the possible association between this polymorphism and early onset BC (Table 2).

Based on other studies, this one included, we can observe that there is great variation in genetic markers among different populations. Some common polymorphism frequencies in Asian populations seem to be completely different from those that occur in European populations. Thus, the susceptibility to cancer depends on ethnicity and environmental risk factors, whose population is exposed. This probably explains why the present results, based on a Brazilian

cohort, corroborate Nelson and Gould's¹⁶ study, conducted in an American population, but not with other studies involving different populations, such as European or Asiatic. Indeed, the present study shows that Val allele frequency is significantly smaller in American and Asian populations than in majority European populations.

Regarding clinicopathological features, no significant association was found between Ile655Val polymorphism in dominant model and age at diagnosis, tumor size, lymph nodes commitment, histological grade, *HER2*, estrogen and progesterone receptors, p53 or Ki-67 status either in general BC group or in *HER2*-positive samples, indicating that the variation does not influence disease course. This result is in accordance with the work from Han et al²⁰, who showed no association between this polymorphism and clinicopathological features in a cohort of Chinese BC patients. However, in the same work, the authors showed that Val allele was an important prognostic factor in *HER2*-positive BCs, indicating a more aggressive tumor phenotype with worst outcome when treated with conventional adjuvant chemotherapy, but more favorable response to the targeted therapy with trastuzumab.²⁰ This highlights the clinical importance of studying this polymorphism in BC patients worldwide.

In conclusion, the present study suggests that *HER2* Ile655Val polymorphism may be a candidate marker for BC susceptibility. Further studies involving larger samples are necessary to validate its potential as a BC marker, emphasizing that its impact on disease susceptibility may diverge between different populations around the world. Although no association with prognostic factors were found in the present work, literature highlights the need to delineate prospective studies in different populations to evaluate the real potential prognostic role for this polymorphism, especially in *HER2*-positive BCs.

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REFERENCES

1. Banin Hirata BK, Oda JMM, Losi Guembarovski R, Ariza CB, Oliveira CECd, Watanabe MAE. Molecular markers for breast cancer: prediction on tumor behavior. *Dis Markers*. 2014;2014:1-12.
2. Citri A, Skaria KB, Yarden Y. The deaf and the dumb: the biology of ErbB-2 and ErbB-3. *Exp Cell Res*. 2003;284:54-65.
3. Lonardo F, Di Marco E, King CR, et al. The normal erbB-2 product is an atypical receptor-like tyrosine kinase with constitutive activity in the absence of ligand. *New Biologist*. 1990;2:992-1003.
4. Papewalis J, Nikitin A, Rajewsky MF. G to A polymorphism at amino acid codon 655 of the human erbB-2/HER2 gene. *Nucleic Acids Res*. 1991;19:5452.
5. Fleishman SJ, Schlessinger J, Ben-Tal N. A putative molecular-activation switch in the transmembrane domain of erbB2. *Proc Natl Acad Sci USA*. 2002;99:15937-15940.
6. Beauclair S, Formento P, Fischel JL, et al. Role of the HER2 [Ile655Val] genetic polymorphism in tumorigenesis and in the risk of trastuzumab-related cardiotoxicity. *Ann Oncol*. 2007;18:1335-1341.
7. Sobin LH, Gospodarowicz MK, Wittekind C. TNM classification of malignant tumours 2009.
8. Wolff AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J Clin Oncol*. 2013;31:3997-4013.
9. Hammond ME, Hayes DF, Wolff AC, Mangu PB, Temin S. American society of clinical oncology/college of American pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Oncol Pract/Am Soc Clin Oncol*. 2010;6:195-197.
10. Xie D, Shu XO, Deng Z, et al. Population-based, case-control study of HER2 genetic polymorphism and breast cancer risk. *J Natl Cancer Inst*. 2000;92:412-417.
11. Naslavsky MS, Yamamoto GL, de Almeida TF, et al. Exomic variants of an elderly cohort of Brazilians in the ABraOM database. *Hum Mutat*. 2017;38:751-763.
12. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics*. 2015;31:3555-3557.
13. Weigelt B, Reis-Filho JS. Molecular profiling currently offers no more than tumour morphology and basic immunohistochemistry. *Breast Cancer Research: BCR*. 2010;12(Suppl 4):S5.
14. Kara N, Karakus N, Ulusoy AN, Ozaslan C, Gungor B, Bagci H. P53 codon 72 and HER2 codon 655 polymorphisms in Turkish breast cancer patients. *DNA Cell Biol*. 2010;29:387-392.
15. Dahabreh IJ, Murray S. Lack of replication for the association between HER2 I655V polymorphism and breast cancer risk: a systematic review and meta-analysis. *Cancer Epidemiol*. 2011;35:503-509.
16. Nelson SE, Gould MN, Hampton JM, Trentham-Dietz A. A case-control study of the HER2 Ile655Val polymorphism in relation to risk of invasive breast cancer. *Breast Cancer Research: BCR*. 2005;7:R357-R364.
17. Parvin S, Islam MS, Al-Mamun MMA, et al. Association of BRCA1, BRCA2, RAD51, and HER2 gene polymorphisms with the breast cancer risk in the Bangladeshi population. *Breast Cancer*. 2016;24:229-237.
18. Montgomery KG, Gertig DM, Baxter SW, et al. The HER2 I655V polymorphism and risk of breast cancer in women < age 40 years. *Cancer Epidemiol Biomark Prev*. 2003;12:1109-1111.
19. Baxter SW, Campbell IG. Re: Population-based, case-control study of HER2 genetic polymorphism and breast cancer risk. *J Natl Cancer Inst*. 2001;93:557-559.
20. Han X, Diao L, Xu Y, et al. Association between the HER2 Ile655Val polymorphism and response to trastuzumab in women with operable primary breast cancer. *Ann Oncol*. 2014;25:1158-1164.

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