HER2 Codon 655 (Ile/Val) Polymorphism and Breast Cancer in Austrian Women

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Abstract. *Background: The overexpression of the human epidermal growth factor receptor 2 (HER2) in breast cancer (BC) is associated with impaired prognosis. Data concerning the HER2 codon 655 polymorphism (Ile/Val) and BC risk are conflicting. Materials and Methods: We studied the HER2 codon 655 (rs1136201) polymorphism in 80 Austrian patients with BC and 100 healthy volunteers by pyrosequencing and polymerase chain reaction. Associations between codon 655 allelic variants and clinicopathological variables (e.g. age, stage of disease, tumor type, grading, and receptor status) were studied with 2×2 tables. Results: The genotypic distributions in patients with BC (AA: 63.75%, AG: 32.5%, GG: 3.75%) and controls (AA: 63%, AG: 34%, GG: 3.7%) were virtually identical (odds ratio=1.03, 95% confidence interval=0.56-1.90). A non-significant link between carrying at least one G allele and more aggressive tumor type (estrogen receptor-negative p=0.08, G3 tumor p=0.19) was observed. Conclusion: Genotypic variation within the codon 655 of HER2 does not alter the BC risk in Caucasian Austrian women. The association between the G allele and more aggressive tumor types requires further investigation.*

Breast cancer (BC) is the most common cancer diagnosis in women worldwide, with an estimated 1.7 million cases and 521,900 deaths in 2012 (1). BC accounts for 25-29% of new diagnosed malignancies and 15% of all cancer-related deaths in women (1, 2). The lifetime risk of developing BC in developed countries is 8% (1). Human epidermal growth factor receptor 2 (*HER2, ERBB2*) is a part of the ERBB-like oncogene family and belongs to the tyrosine kinase superfamily. It is expressed in normal tissues and is involved in signal transduction and cell proliferation (3, 4). Overexpression of *HER2* occurs in 15-30% of BC (3-5). Without anti-*HER2* therapy, *HER2*-overexpressing BC had a shortened time to locoregional recurrence (6), earlier metastasis (7) and an overall worsened prognosis (3, 4). In contrast, therapeutic targeting of *HER2* with, *e.g.* trastuzumab, can improve the overall survival (4). The *HER2* gene is located at chromosome 17q21 (8). Papewalis *et al.* described a single nucleotide polymorphism (SNP) resulting in the A to G transition in the transmembrane domain-coding region (codon 655) of *HER2*. In consequence, isoleucine (Ile) (encoded by ATC) is replaced with valine (Val) (encoded by GTC) within the transmembrane domain-coding region (9). The presence of 655Ile in the transmembrane domain of *HER2* may impair the dimerization of active *HER2* proteins with other *HER* family members and result in reduced signal transduction compared to the 655Val variant (10). Fleishman *et al.* described the opposing effects of the *HER2* activating oncogenic point mutation and the Ile655Val SNP which were linked to reduced risk of BC and explained this fact in terms of "shifts in the equilibrium between the active and inactive states of *HER2*" (10). The role of the *HER2* Ile655Val SNP has been studied in relation to BC risk, with inconclusive results (8, 11-14). According to both most recent meta-analyses (13, 14), a weak association between 655Val (G) allele and increased BC risk in Caucasian populations and between the Val/Val (G/G) genotype and elevated BC risk in African women can be assumed. Additionally, it has been confirmed that the Ile655Val polymorphism influences the response to trastuzumab therapy (15) and trastuzumab-induced cardiotoxicity (16, 17).

The primary goal of the present study was to investigate the association of the Ile655Val (rs1136201) polymorphism of the *HER2* gene with the risk of sporadic BC in Austrian women. The

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Table I. *Genotypic and allelic frequencies of the human epidermal growth factor receptor 2 (HER2) codon 655 polymorphism in patients with breast cancer and controls.*

	Breast cancer n=80 (100%)	Controls n=100 (100%)	*p* (Chi2)[a]	OR (95% CI)[b]
Genotype				
655 A/A	51 (63.75%)	63 (63%)	0.92 (0.01)	1.03 (0.56-1.90)
655 A/G	26 (32.5%)	34 (34%)		
655 G/G	3 (3.75%)	3 (3%)		
Alleles				
A	128	160	1 (0)	1 (0.59-1.68)
G	32	40		
G allelic frequency	0.2	0.2		

[a]Calculated for A/A *vs.* A/G + G/G, [b]odds ratio (OR) and 95% confidence interval (CI) calculated for A/A *vs.* A/G + G/G.

secondary goal was to investigate possible associations between allelic variants of *HER2* codon 655 and clinicopathological variables.

Materials and Methods

Patients and samples. EDTA blood samples were obtained from 80 patients with sporadic BC and 100 healthy age-matched volunteers at the Department of Obstetrics and Gynecology and the Department of Blood Group Serology and Transfusion Medicine, University of Vienna, respectively. Clinicopathological data were obtained by chart review. Staging was carried out according to the TNM classification (18). The protein levels of estrogen receptor (ER) and progesterone receptor (PgR) in cancer tissues were determined quantitatively as described previously (19). All patients and all controls were of Caucasian origin. None of the patients had received preoperative systemic treatments. Informed consent was obtained from all patients, and all procedures were approved by the Institutional Review Board of the Medical University of Vienna, Vienna, Austria (EK 366-2003).

DNA preparation and genotyping of HER2 Ile655Val polymorphism. DNA was isolated from blood using commercially available kits (DNA Extraction System II; ViennaLab, Vienna, Austria). Primer pair HER2-SE (5'-CCCAAACTAGCCCTCAATC-3') with HER2-AS (5'-AGACCACGACCAGCAGAATG-3') were used to amplify a 96 bp fragment of the *HER2* DNA. Information of DNA sequences is from UCSC Genome Bioinformatics (http://genome.ucsc.edu). Polymerase chain reaction (PCR) was carried out in a total volume of 25 μl including 25 ng template, 5 pmol of each sense and antisense primers and puReTaq Ready-To-Go PCR Beads (Amersham Biosciences, UK), which contains 2.5 units of puReTaq DNA polymerase, 10 mM Tris-HCl (pH 9.0 at room temperature), 50 mM KCl, 1.5 mM MgCl2, 200 μM dATP, dCTP, dGTP and dTTP, and stabilizers, including bovine serum albumin. The reaction was performed on a Perkin-Elmer GeneAmp PCR system 9600 (Applied Biosystems, Foster City, CA, USA) with 35 cycles at 94˚C for 30 seconds, at 51˚C for 30 sec and 72˚C for 30 seconds. The reaction was preceded by a primary denaturation step at 94˚C for 1 min, with final incubation at 72˚C for 7 min. Polymorphisms were detected using the Pyrosequencer PSQ 96 and PSQ 96 SNP Reagent Kit (Uppsala, Sweden). PCR product (25 μl) was used for pyrosequencing according to the instruction of the manufacturer; 5 pmol of the sequencing primer (HER2-SEQ: 5'-CCCTCTGACGTCCAT-3') were applied to detect the corresponding polymorphisms.

Statistical methods. Differences in allelic frequencies and clinicopathological parameters between patients and controls were assessed by the chi-square test (with Yates correction where appropriate) or Fisher exact test. Results are presented as *p*- and chi values and as odds ratios (OR) with 95% confidence interval (95% CI). A two-sided *p*-value of 0.05 or less was considered statistically significant. Hardy–Weinberg equilibrium was tested by chi-square tests comparing observed and expected genotypic frequencies (20). For all other statistical analyses, we used the online calculator VassarStats: Website for Statistical Computation (21).

Results

The mean age of patients was 57.6 (SD=13.1; range=28-84) years. Fifty-six (70%) patients were older than 50 years, and 24 (30%) patients were 50 years or younger. The most common histological type was invasive ductal carcinoma (n=58, 72.5%), followed by invasive lobular type (n=20, 27.5%). An almost equal number of tumors were smaller than 2 cm (pT1) (n=37; 46.3%) or between 2 and 5 cm (pT2) (n=34; 42.5%). A total of 65% of patients (n=52) had nodal-negative (pN0) disease. Most tumors had a moderate differentiation grade (n=55, 68.75%) and were positive for ER and PgR (58.8% and 50%, respectively). The distributions of genotypes for the 655 A/G within cancer cases and controls were in Hardy–Weinberg equilibrium (p=0.89 and 0.53, respectively).

As shown in Table I, both genotypic distributions (p=0.92) and allelic frequencies (p=1.0) were identical between patients and controls. Patient and tumor characteristics in relation to the distribution of 655 A/G genotypes are presented in Table II. A non-significant trend towards more aggressive tumor characteristics (ER negative p=0.08, G1/G2 *vs.* G3 p=0.19) in the presence of at least one G allele was noted. No significant

Table II. *Distribution of human epidermal growth factor receptor 2 (HER2) codon 655 A/G genotypes according to patient and tumor characteristics.*

Parameter	No. of samples or patients[a]	Genotype[b]			p (Chi^2)[c]	OR (95% CI)[c]
		655 A/A	655 A/G	655 G/G		
No. of patients	80	51 (63.75%)	26 (32.5%)	3 (3.75%)		
Histological type						
Invasive ductal carcinoma	58 (72.5%)	38 (65.5%)	18 (31%)	2 (3.5%)	0.57 (0.33)[d]*	0.64 (0.23-1.81)
Invasive lobular carcinoma	20 (25%)	11 (55%)	8 (40%)	1 (5%)		
Other	2 (2.5%)	2 (100%)	0 (0%)	0 (0%)		
Differentiation grade						
Well (G1)	7 (8.75%)	5 (71.4%)	2 (28.6%)	0 (0%)	0.7e**	0.62 (0.11-3.43)
Moderate (G2)	55 (68.75%)	36 (65.5%)	17 (30.9%)	2 (3.6%)	0.19 (1.73)[f]*	0.38 (0.12-1.25)
Poor (G3)	14 (17.5%)	6 (42.9%)	7 (50%)	1 (7.1%)		
Not available (Gx)	4 (5%)	4 (100%)	0 (0%)	0 (0%)		
Tumor stage						
pT1 (<2 cm)	37 (46.3%)	24 (64.9%)	13 (35.1%)	0 (0%)	0.6 (0.28)[g]	0.78 (0.31-1.97)
pT2 (2-5 cm)	34 (42.5%)	19 (55. 9%)	12 (35.3%)	3 (8.8%)		
pT3 (≥5 cm)	3 (3.75%)	3 (100%)	0 (0%)	0 (0%)		
pT4	2 (2.5%)	1 (50.0%)	1 (50.0%)	0 (0%)		
Not available (pTx)	4 (5%)	4 (100%)	0 (0%)	0 (0%)		
Nodal status						
pN0	52 (65%)	32 (61.5%)	18 (34.6%)	2 (3. 9%)	0.86 (0.03)[h]*	1.04 (0.38-2.82)
pN1	22 (27.5%)	14 (63.6%)	7 (31.8%)	1 (4.6%)		
pN2	2 (2.5%)	1 (50%)	1 (50%)	0 (0%)		
Not available	4 (5%)	4 (100%)	0 (0%)	0 (0%)		
Estrogen receptor						
Positive	47 (58.75%)	27 (57.5%)	18 (38.3%)	2 (4.3%)	0.08**	3.7 (0.94-14.5)
Negative	18 (22.5%)	15 (83.3%)	3 (16.7%)	0 (0%)		
Not available	15 (18.75%)	9 (60%)	5 (33.3%)	1 (6.7%)		
Progesterone receptor						
Positive	40 (50%)	24 (60%)	15 (37.5%)	1 (2.5%)	0.47 (0.52)*	1.7 (0.58-5.04)
Negative	25 (31.3%)	18 (72%)	6 (24%)	1 (4%)		
Not available	15 (18.8%)	9 (60%)	5 (33.3%)	2 (6.7%)		
Age						
<50 years	24 (30%)	15 (62.5%)	8 (33.3%)	1 (4.2%)	0.92 (0.01)*	0.93 (0.34-2.49)
≥50 years	56 (70%)	36 (64.3%)	18 (32.1%)	2 (3.6%)		

[a]Distribution (%) within the whole sample (within column), [b]distribution (%) within subgroup (within line), [c]calculated for AA *vs*. A/G + G/G, [d]invasive ductal *vs*. invasive lobular, [e]G1 *vs*. G2 + G3, [f]G1 +G2 *vs*. G3, [g]pT1 *vs*. pT1+, hpN0 *vs*. pN+, *after Yates correction, **Fisher's exact probability test.

associations were observed between the presence of at least one G allele and patient age (<50 years, p=0.92), tumor stage (pT1 *vs*. >pT1, p=0.6), nodal status (pN0 *vs*. pN+, p=0.86), histological type (ductal *vs*. lobular, p=0.57), or positivity for PgR (p=0.47).

Discussion

Despite an increasing number of studies, the evidence regarding the possible role of Ile655Val SNP for *HER2* remains inconclusive. Meta-analyses published in the last five years reported conflictingly that an association between *HER2* Ile655Val and BC risk exists (8, 13, 14), and does not (11, 12). According to some researchers the codon 655 polymorphism may contribute to breast cancer risk particularly in Caucasian women (8, 13, 14). While the Val (G) allele has been attributed to increased susceptibility to BC in Caucasian women, the Val/Val homozygosity has been identified as a risk factor for breast cancer in African individuals (8, 13).

Frank *et al*. demonstrated that the inconclusiveness regarding the role of the Ile655Val SNP could additionally result from methodological issues (use of TaqMan allele load analysis instead of direct sequencing) because the presence of Val in position 654 could result in an erroneous identification of Val655Val homozygotes (22). In our study, we used the pyrosequencing method.

We did not observe any association of the *HER2* Ile655Val polymorphism and the susceptibility to BC in Austrian women of Caucasian origin. Neither the stage nor most clinicopathological parameters correlated with any of the allelic variants. The sample numbers used in our case–control study were limited. However, the allelic distributions were virtually

identical in both groups. Therefore, significant changes in the results with an increased number of patients seem not to be realistic. Otherwise, a non-significant trend towards ER negativity (p=0.08) and poor differentiation grade (p=0.19) in presence of the G allele was noted: In our collective, eight out of 14 (57%) patients with G3 carcinomas carried at least one G allele. The possible link between carrying at least one G allele and estrogen receptor-negativity is of interest. On the one hand, the Ile655Val SNP has been attributed to functional changes in HER2 protein (10), and on the other hand, BC progression may be more stringently controlled by *HER2* upon loss of hormone dependency (5). Summarizing, our results suggest that the *HER2* codon 655 SNP is unlikely to be associated with BC risk in Caucasian women. A possible association between *HER2* Ile655Val SNP, *HER2* amplification and more aggressive cancer sub-types should be elucidated in further, large cohort and multi-ethnic studies.

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Conflicts of Interest

The Authors declare no conflicts of interest.

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