

# Association of HER2 codon 655 polymorphism with ovarian cancer

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**Abstract** The role of the human epidermal growth factor receptor 2 (HER2) codon 655 (Ile655Val) polymorphism in ovarian cancer is not fully understood. Two studies indicated a possible association between the Val allele and elevated risk or reduced prognosis of ovarian cancer. We investigated the HER2 codon 655 (rs1136201) polymorphism in 242 Austrian women—142 ovarian cancer patients and 100 healthy controls—by polymerase chain reaction and pyrosequencing. Associations between Ile655Val polymorphism and clinicopathological variables (e.g., age, FIGO stage, grading, serous vs. non-serous histology) were evaluated. The genotype distributions in ovarian cancer patients and controls were: AA; 66.2 %, AG; 25.35 %, GG; 8.45 %, and AA; 63 %, AG; 34 %, GG; 3.7 %, respectively (OR 1.15, CI 95 % 0.67–1.96). We observed a non-significant trend toward elevated cancer risk in Val/Val genotype (OR 2.98, CI 95 % 0.82–10.87,  $p=0.10$ ). Of note, 11 out of 12 Val/Val homozygotes were postmenopausal. The link between the Val/Val homozygosity and age over 50 years at diagnosis (OR 0.15, CI 95 % 0.02–1.2) was barely significant ( $p=0.056$ ). Summarizing, our data indicated a non-significant trend toward increased ovarian cancer risk in the Val/Val homozygosity, especially in women aged

above 50 years. Further large-cohort studies focusing on the role of the HER2 codon 655 Val allele are needed.

**Keywords** HER2 · Codon 655 · HER2 Ile655Val · Ovarian cancer · Polymorphism

## Introduction

Ovarian cancer is the most lethal genital malignancy in women [1, 2]. 238,719 new cases and 151,900 deaths were estimated in 2012 [1]. About 1.5 % of women will be faced with the diagnosis of ovarian cancer in their lifespan [2, 3]. In 65–70 % of cases, ovarian cancer is diagnosed at an advanced stage, when the 5-year survival rate is 30–50 % [2, 3].

Human epidermal growth factor receptor 2 (HER2, ERBB2) is a member of the epidermal growth factor receptor (EGFR) family belonging to the receptor tyrosine kinases (RTKs) superfamily [4, 5]. HER2 is expressed in normal tissues and is involved in signal transduction and cell proliferation [4, 5]. The overexpression of HER2 is an unfavorable prognostic factor occurring in 15–30 % of breast cancers [4, 6], but it offers a therapeutic target for HER2 antibodies, which—in turn—improves the overall survival [6]. HER2 is also overexpressed, e.g., in 10–50 % of gastric cancer cases [7–10] and in 2–80 % of colorectal cancers [11–13]. In those cancers, the HER2 overexpression may be associated with worsened prognosis [7], but the data are less coherent as in breast cancer [7–13]. Also in non-mammary carcinomas HER2 can be therapeutically targeted [14, 15].

In respect to ovarian cancer, the overexpression of HER2 has been described in ovarian cancer cells in vitro and in vivo. The overexpression of HER2 occurs in 7–30 % of ovarian cancers [5, 16–20] and seems to promote cancer development through partially different signaling pathways as in breast cancer [17]. Statistically significant effects of HER2 overexpression on

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ovarian cancer prognosis, found in retrospective studies [16, 17], could not be confirmed in prospective studies and—therefore—remain still controversial [20]. Similarly, therapeutic targeting of HER2 in ovarian cancer is still the subject of clinical trials and not yet established in clinical routine [21].

The HER2 molecule consists of an extracellular ligand-binding domain, a transmembrane domain, and an intracellular domain [4, 5]. Papewalis et al. identified a single nucleotide polymorphism (SNP) in the transmembrane domain-coding region (codon 655), resulting in an A to G transition. In consequence, the amino-acid isoleucine (Ile), which is encoded with ATC, is replaced with valine (Val), encoded with GTC [22]. The presence of 655Ile in the transmembrane domain of HER2 has been attributed to impaired dimerization of active HER2 proteins with other HER-family members, which could result in reduced signal transduction as compared to the 655Val variant [23]. Fleishman et al. described an opposing effect of the HER2 activating oncogenic point mutation and the Ile655Val SNP due to a shift in the equilibrium between the active and inactive HER2 states [23]. So far, four studies investigated the association between the HER2 Ile655Val polymorphism and ovarian cancer [24–27]. Two of these studies indicated that the Val/Val homozygosity could be linked to increased cancer risk [27] or impaired prognosis [24] in ovarian cancer. The aim of the present study was to determine the association of the Ile655Val (rs1136201) polymorphism within the HER2 gene with ovarian cancer risk and clinicopathological variables in Caucasian women.

## Materials and methods

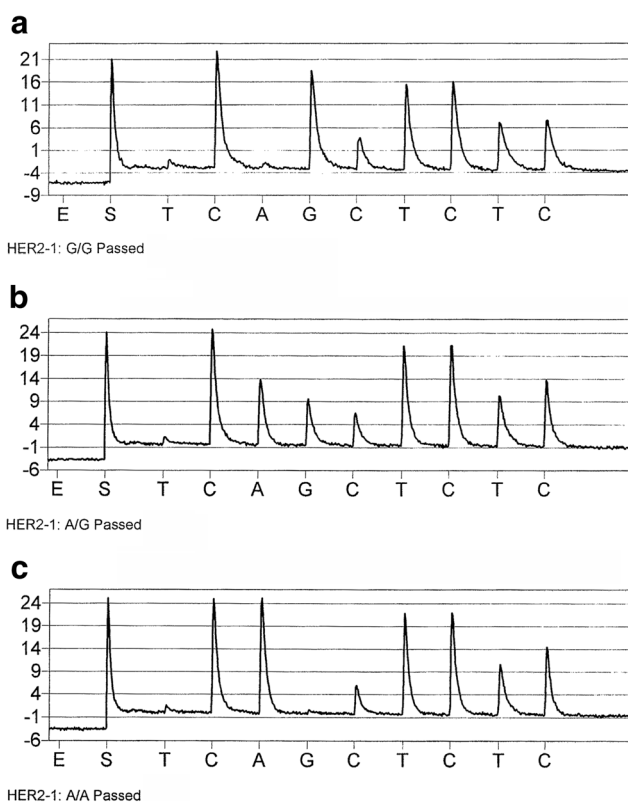
### Patients and samples

EDTA-blood samples were obtained from 142 patients with first-diagnosed ovarian cancer 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. Clinicopathological classification and staging were carried out according to the WHO (2003) [28] and FIGO [29] classifications. All patients and all controls were of Caucasian origin. None of the patients received preoperative systemic treatments. Informed consent was obtained from all individual participants included in the study. 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'-CCCAAAGTAGCCCTCAATC-3') with HER2-AS (5'-AGACCACGACCAGCAGAATG-3') were used to amplify a 96-bp fragment of the HER2 DNA. Information of DNA sequences was obtained from UCSC Genome Bioinformatics (<http://genome.ucsc.edu>). 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, England, UK), which contains 2.5 units of puReTaq DNA polymerase, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 µM dATP, dCTP, dGTP and dTTP, and stabilizers, including BSA. The reaction was performed on a Perkin-Elmer GeneAmp PCR system 9600 9600 (Applied Biosystems, Foster City, California, USA) with 35 cycles at 94 °C for 30 s, at 51 °C for 30 s and 72 °C for 30 s. The reaction was preceded by a primary denaturation step at 94 °C for 1 min and incubated at 72 °C for 7 min at least. The HER2 Ile655Val polymorphism was investigated using a Pyrosequencer PSQ 96 and the PSQ 96 SNP Reagent Kit (Uppsala, Sweden). 25 µl PCR product was used for pyrosequencing according to the instruction of the manufacturer. 5 pmol of the sequencing primer (HER2-SEQ: 5'-CCCTCTGACGTCAT-3') were applied to detect the corresponding polymorphism, Fig 1.



**Fig. 1** Representative pyrograms of the pyrosequencing results. **a** GG phenotype. **b** AG phenotype. **c** AA phenotype

## Statistical methods

Differences in allelic frequencies and clinicopathological parameters between patients and controls were assessed by the chi-squared test (with Yates correction where appropriate) or Fisher's 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  $\leq 0.05$  was considered statistically significant. Hardy-Weinberg equilibrium was tested by chi-squared tests comparing observed and expected genotype frequencies by use of an online calculator [30]. For other statistical analyses, we used the software package Statistica 12 Test Version (StatSoft Europe GmbH, Hamburg, Germany) and the online calculator VassarStats: Website for Statistical Computation [31].

## Results

The mean age of patients was 54.2 (median 54, SD 13.5; range 22–83) years. Ninety (63.4 %) patients were older than 50 years, while 50 (35.2 %) patients were younger than 50 years. The most tumors ( $n=90$ , 63.4 %) presented serous histology. Every second of the 44 non-serous carcinomas was of endometrioid type ( $n=22$ , 16.2 %). In the majority of cases, the tumor was diagnosed in FIGO stage III ( $n=75$ , 52.8 %).

The most tumors were poorly differentiated ( $n=59$ , 41.5 %), while 35 (24.6 %) of the tumors were moderately differentiated, and 34 (23.9 %) presented a well differentiation grade.

As shown in Table 1, the genotype distributions in cases and controls were: AA; 66.2 %, AG; 25.35 %, GG; 8.45 % vs. AA; 63 %, AG; 34 %, GG; 3.7 %, respectively. The number of Val/Val homozygotes in cancer cases doubled the values expected by the Hardy-Weinberg equilibrium ( $p=0.004$ ). Accordingly, the Val/Val (GG) genotype was non-significantly ( $p=0.1$ ) linked to

the presence of cancer (OR 2.98, CI 95 % 0.82–10.87). The distributions of genotypes for the 655 A/G within the controls were in Hardy-Weinberg equilibrium ( $p=0.53$ ). The allelic frequencies (G allele; 21 % in cases and 20 % in the control group) did not differ between patients and controls. Accordingly, no significant cancer risk alteration could be observed in respect to the presence of the Val allele (AA vs. AG+GG;  $p=0.6$ ; OR 1.15, CI 95 % 0.67–1.96).

Patient and tumor characteristics broken down by the distribution of the 655 AA, AG, and GG genotypes are presented in Table 2. We observed a barely significant ( $p=0.056$ ) association between Val/Val homozygosity and older age at diagnosis ( $\leq 50$  vs.  $>50$  years) (OR 0.15, CI 95 % 0.02–1.2). No other associations between the HER-2 Ile655 Val allelic variants and FIGO stage (I vs. II-IV,  $p=0.34$ ), serous vs. non-serous histology ( $p=0.73$ ), or differentiation grade (G1 vs. G2-G3,  $p=0.48$ ) were observed.

## Discussion

The HER2 Ile655Val polymorphism and the role of the 655Val allele have been most widely studied in respect to breast cancer, but several studies yielded conflicting results [32–35]. In African women, the Val/Val genotype was associated with a significantly increased breast cancer risk [35]. In Caucasian populations, a weak association between at least one 655Val allele and a modest association between the Val/Val genotype and risk of breast cancer or benign breast fibroadenoma have been also confirmed [32, 36, 37]. Recently, a non-significant trend toward the presence of the 655Val allele and more aggressive cancer types has been supposed [38]. Beyond breast cancer, the prevalence of at least one Val allele was associated with advanced cervical cancer.

**Table 1** Genotype and allele frequencies of the Her-2/neu codon 655 polymorphism in breast cancer patients and controls

	Ovarian cancer $n=140$ (100 %)	Controls $n=100$ (100 %)	$p$ ( $\chi^2$ ) <sup>a</sup>	OR (CI 95 %) <sup>b</sup>
Genotypes				
655 AA	94 (66.2 %)	63 (63 %)	0.61 (0.26) <sup>c</sup>	1.15 (0.67–1.96)
655 AG	36 (25.35 %)	34 (34 %)		
655 GG	12 (8.45 %)	3 (3 %)	0.1 <sup>d,e</sup>	2.98 (0.82–10.87)
Alleles				
A	224	160	0.76 (0.09)	0.93 (0.60–1.46)
G	60	40		
G allele frequency	0.21	0.2		

<sup>a</sup> Pearson's chi-squared test

<sup>b</sup> Odds ratio (OR) and 95 % confidence interval (CI 95 %)

<sup>c</sup> calculated for AA vs. AG+GG

<sup>d</sup> calculated for GG vs. AA+AG

<sup>e</sup> two-tailed Fisher exact test

**Table 2** Patient and tumor characteristics and distribution of 655 A/G genotypes

Parameter	No. of samples or patients <sup>a</sup>	Genotype distribution <sup>b</sup>			AA vs. AG+GG		GG vs. AG+AA	
		655 AA	655 AG	655 GG	<i>p</i> ( $\chi^2$ )	OR (CI 95 %)	<i>p</i> ( $\chi^2$ )	OR (CI 95 %)
No. of patients	142	94 (66.2 %)	36 (25.35 %)	12 (8.45 %)				
Histological type								
Serous	90 (63.4 %)	60 (66.7 %)	22 (24.4 %)	8 (8.9 %)	0.73 (0.12) <sup>c</sup>	1.14 (0.54–2.43)	1.0**	0.98 (0.28–3.43)
Non-serous	44 (31 %)	28 (63.6 %)	12 (27.3 %)	4 (9.1 %)				
Endometrioid	22 (16.2 %)							
Mucinous	9 (6.6 %)							
Clear cell	8 (5.9 %)							
Others	5 (3.7 %)							
Not available	8 (5.6 %)	6 (75 %)	2 (25 %)	0 (0 %)				
Differentiation grade								
Well (G1)	34 (23.9 %)	25 (75.5 %)	6 (17.65 %)	3 (8.8 %)	0.48 (0.5) <sup>d,*</sup>	0.67 (0.28–1.59)	1.0**	0.96 (0.24–3.86)
Moderate (G2)	35 (24.6 %)	25 (71.4 %)	8 (22.9 %)	2 (5.7 %)				
Poor (G3)	59 (41.5 %)	36 (61 %)	17 (28.8 %)	6 (10.2 %)				
Not available	14 (9.9 %)	8 (57.1 %)	5 (35.7 %)	1 (7.1 %)				
Tumor stage								
FIGO I	42 (29.6 %)	30 (71.4 %)	9 (21.4 %)	3 (7.1 %)	0.34 (0.88) <sup>c</sup>	0.69 (0.31–1.51)	1.0**	1.25 (0.32–4.98)
FIGO II	16 (11.3 %)	10 (62.5 %)	4 (25 %)	2 (12.5 %)				
FIGO III	75 (52.8 %)	46 (61.3 %)	23 (30.7 %)	6 (8 %)				
FIGO IV	4 (2.8 %)	4 (100 %)	0 (0 %)	0 (0 %)				
Not available	5 (3.5 %)	4 (80 %)	0 (0 %)	1 (20 %)				
Age								
≤50 years	50 (35.2 %)	34 (68 %)	15 (30 %)	1 (2 %)	0.61 (0.26)	1.21 (0.58–2.51)	0.056**	0.15 (0.02–1.2)
>50 years	90 (63.4 %)	58 (64.4 %)	21 (23.3 %)	11 (12.2 %)				
Not available	2 (1.4 %)	2 (100 %)	0 (0 %)	0 (0 %)				

Statistical analysis by chi-squared test

<sup>a</sup>Distribution (%) within the whole sample (within column), <sup>b</sup>Genotype distribution (%) within subgroup (within line), <sup>c</sup>Serous vs. non-serous, <sup>d</sup>G1 vs. G2+G3, <sup>e</sup>FIGO I vs. FIGO II-IV

\*After Yates correction

\*\*Fisher's exact probability test

This association was most pronounced in Val/Val (GG) homozygotes [39]. Similarly, homozygous Val/Val genotype of HER2 codon 655 SNP has been linked to increased susceptibility for gall bladder cancer in females, but not in males [40].

So far, four studies with cumulative 298 patients and 564 controls investigated the association between the HER2 Ile655Val polymorphism and ovarian cancer. Their results are summarized in Table 3. Shanmughapriya et al. [27]

**Table 3** HER2 codon 655 (GTC/valine to ATC/isoleucine) polymorphisms and ovarian cancer

First author (publication year)	Number of patients/controls	Country	Effect
Pinto (2005) [24]	111/146	Portugal	No data regarding cancer risk. Reduced overall and progression-free survival in Val/Val (GG) homozygotes
Puputti (2006) [25]	8/0	Finland	No valid results (low number of patients)
Mojtahedi (2013) [26]	107/130	Iran	No association
Shanmughapriya (2013) [27]	72/288	India	Association between ovarian cancer risk and Val allele, especially in Val/Val (GG) homozygotes
Watrowski (present study)	142/100	Austria	Non-significant trend ( <i>p</i> =0.1) toward association between Val/Val (GG) homozygosity and ovarian cancer risk

reported a positive association between ovarian cancer risk and the Val allele, and especially the Val/Val (GG) homozygosity in Indian women. Pinto et al. [24] described reduced overall and progression-free survival in Val/Val (GG) homozygous ovarian cancer patients from Portugal (73.3 months for AA, 70.7 months for AG, and 35.3 months for GG). The estimated 5-year survival rate was 68.8 % for women carrying AA or AG genotypes compared with 25.0 % for patients carrying the GG genotype.

Our results indicate that the Val/Val genotype may be associated with increased ovarian cancer risk in Caucasian women, especially in patients aged above 50 years. Similarly, in a Turkish collective in which no overall association between HER2 genotype and breast cancer was seen, a positive association between the Ile/Val+Val/Val (vs. Ile/Ile genotypes) and breast cancer was reported for women older than 60 years [41]. On the other hand, a moderate association with elevated breast cancer risk was reported for Val/Val+Ile/Val vs. Ile/Ile genotypes in young women (<45 years) with familial history of breast cancer [42].

Similar to data obtained from ovarian [24, 27], cervical [39], or gall bladder cancers [40], the HER2 Ile/Val SNP was not associated with the histological type or grade. However, this lack of associations should be interpreted with caution in respect to the sample numbers used in our case control study.

Our patient cohort is the largest sample of ovarian cancer patients in which the HER2 Ile655Val SNP was studied so far. Nevertheless, the sample numbers used in our case control study were still limited. Our results, together with some earlier observations [24, 27], indicate that large-cohort studies focusing on the role of the Val allele of the HER2 codon 655 in ovarian cancer are needed.

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#### Compliance with ethical standards

**Conflicts of interest** None.

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