

Pharmacogenetics Driving Personalized Medicine: Analysis of Genetic Polymorphisms in FCGR2A (Rs1801274) And ERBB2 (Rs1136201) Related to Breast Cancer Medications in Iraqis Populations

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Abstract

Background:Breast-cancer is the most common cancer in women characterized by a high-variable-clinical-outcome among individuals-treated with equivalent-regimens and novel-targeted-therapies.We investigated associations between FCGR2A(rs1801274)andERBB2(rs1136201)polymorphism and clinical-outcomes in Arab and Kurdish population of Iraqi-breast-cancer-patients withHER2-negative-breast-cancer,and in women withHER2-positive-breast-cancer who received-trastuzumab.**Methods:**FCGR2A(rs1801274)andERBB2(rs1136201)polymorphism determined byARMS-PCRassay in-160Arab andKurdish population of Iraqi-breast-cancer-patients withHER2-negative-breast-cancer,and in women withHER2-positive breast-cancer who received-trastuzumab.**Results:**FCGR2Ars1801274 andERBB2(rs1136201)genotype frequencies didnot differ significantly between study-patients and controls.But in follow-up of 5-years,in1801274-Patients withAA-genotype may have increased response to trastuzumab and longer progression-free survival and inrs1136201-Patients withAA-genotype and breast-cancer may have an increased-response to treatment with trastuzumab.**Conclusions:**All these findings increased-knowledge on prevalence of specific variants related with breast-cancer-treatment-responsiveness.

Keyword: Breast Cancer; Genetic Polymorphism; Pharmacogenetics; Trastuzumab; Targeted therapies

1. Introduction

Breast cancer is the most common cancer in women worldwide. Breast cancer has become a major threat to female health in Iraq, where it is the leading cause of death after cardiovascular diseases among women, with a cancer-related mortality rate of 23%.1,4,8,9 It has been the highest-ranked malignancy among the Iraqi population in general since 1986 [1]. An early detection combined with an appropriate treatment has proved to be effective in reducing risk of death and relapse. Pharmacogenetics can be used to select the best treatment for each patient. Pharmacogenetics is the study of how people respond differently to drug therapy based upon their genetic makeup or genes [2] Single Nucleotide Polymorphisms (SNPs) holds the key in defining the risk of an individual's susceptibility to various illnesses and response to drugs. There is an ongoing process of identifying the common, biologically relevant SNPs, in particular those that are associated with the risk of disease [3] The humanized HER-2/neu immunoglobulin G (IgG) 1 monoclonal antibody (mAb) trastuzumab is an

effective treatment of HER-2/neu-positive breast cancer. However, large differences in clinical outcome remain among patients treated with trastuzumab [4, 5] Identifying molecular markers that can select patients who are to benefit from trastuzumab treatment is crucial for avoiding chemotherapy toxicity and reducing treatment costs. In this study, we examine the relationship between the two polymorphisms rs1801274 and rs1136201 and the response to the drug trastuzumab in the Arab and Kurdish population of Iraqi breast cancer patients. s1801274 is a SNP in the Fc fragment of IgG, low affinity IIa, receptor (CD32) FCGR2A gene. rs1801274(C) encodes the arginine (R) allele, with the (T) allele encoding the variant histidine (H). The (H) isoform is considered high-binding to IgG2 and IgG3, while the (R) isoform is considered low-binding. This SNP is known in the literature by many names, including A519C and H131R. FcγR isoforms expressed on immune system cells have been linked to the pathogenic consequences triggered by autoantibodies or immune complexes in autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), as well as to the efficacy of

some immunotherapeutic treatments such as rituximab [6, 7] rs1136201 (Ile655Val) is a SNP within ERBB2/HER2. The HER2 gene is subjected to somatic mutations and to germinal polymorphism [8]. The most investigated germinal polymorphism at clinical level concerns codon 655 (GTC/valine to ATC/isoleucine, transmembrane domain of the HER2 protein) [9]. Xie et al. reported that women with the Ile/Val or Val/Val genotype had a high risk of breast cancer [10]. In this study, we evaluated the genotypes of polymorphisms FCGR2A H131R and ERBB2 I655V with the TARMS-PCR method in 120 people with breast cancer treated with trastuzumab. The results of the two-polymorphism genotype were compared and evaluated with response to treatment during treatment. As controls, we evaluated 40 consecutive patients with metastatic breast cancer, not selected for HER-2/neu amplification, who not received trastuzumab over the same period. The Response Evaluation Criteria in Solid Tumors were used for disease response assessment.

2. Method and Materials

We investigated the associations between the FCGR2A (rs1801274) and ERBB2 (rs1136201) polymorphism and clinical outcomes in the Arab and Kurdish population of Iraqi breast cancer patients with HER2- negative breast cancer, and in women with HER2- positive breast cancer who received trastuzumab. Genotyping of FCGR2A (rs1801274) and ERBB2 (rs1136201) variants was performed on genomic DNA by multiplex Tetra-Primer Amplification Refractory Mutation System (T-ARMS) PCR and the results were confirmed by sequence-based typing (SBT). The population studied in this study included: 120 people (61 arab and 59 kurdish) with breast cancer treated with trastuzumab as a target group and 40 patients with metastatic breast cancer who did not use trastuzumab as a control group. Each person received 5 ml of peripheral blood in tubes containing the anti-coagulant EDTA. Genomic DNA were extracted from peripheral blood mononuclear cells (PBMCs) using a conventional proteinase K protocol, as previously described [11]. The PCR reaction was performed in a final volume of 20 µl containing ≈25 ng genomic DNA, 1.5 mM Mg²⁺, 200 µmol/L dNTPs, 0.8 µL Elongase® Enzyme Mix (Life Technologies), and 0.5 µmol/L of each primer. PCR products were analyzed on 2 % agarose gel containing Eurosafe Nucleic Acid Stain (EuroClone, Milan, Italy) and the remaining amount was purified and diluted at 1:20; then, to confirm the specificity of FCGR2A and ERBB2 amplification, a short amplicon was analyzed by direct sequencing (using the outer primers) (Table 1). Patients' status was monitored over a 5-year period Statistical analyzes were performed by SPSS software version 19 and the results were considered significant with P.value≤0.05.

Table 1: TARMS-PCR primers sequence

Gene polymorphism	Primer name	Primer sequence
FCGR2A rs1801274	outer forward	5'-ACAACAGCCTGACTACCTATTACCTT-3'
	outer reverse	5'-CATATTTGTGTCITTCAGAATGGC-3'
	Normal	5'-GGAACATCCCAGAAATTCACA-3'
	mutant	5'-GAACATCCCAGAAATTCACG-3'
ERBB2 rs1136201	outer forward	5'-ACCCTCCGACTCCCTTC-3'
	outer reverse	5'-TGTACTCCGGATCTCTGCTG-3'
	Normal	5'-CAGCCCTCTGACGTCCATAA-3'
	mutant	5'-GCCAACACCAGAGGC-3'

3. Results

Clinical and pathologic features of patients treated with trastuzumab and are presented in Table 2. The FCGR2A rs1801274 genotype frequencies did not differ significantly between study patients and controls (Table3). 46% (55 of 120) of the patients were homozygous for the AA (H/H), 49% (58 of 120) were heterozygous AG (H/R), and 6% (7 of 120) were homozygous GG (R/R) (Tables 2). Patients with the AA genotype may have increased response to trastuzumab and longer progression-free survival in people with Breast cancer as compared to patients with genotype AG or GG. Other genetic or clinical factors may also influence the response to trastuzumab. The ERBB2 rs1136201 genotype frequencies did not differ significantly between study patients and controls (Table2). 75% (90 of 120) of the patients were homozygous for the AA (Ilu/Ilu), 23% (28 of 120) were heterozygous AG (Ilu/Val), and 2% (2 of 120) were homozygous GG (Val/Val). Patients with the AA genotype and breast cancer may have an increased response to treatment with trastuzumab as compared to patients with the AG or GG genotypes. Other genetic and clinical factors may also affect a patient's response to trastuzumab. Also the Ile655Val polymorphism was found to be associated with PR status (p=0.031); whereas no significant associations were found between the Ile655Val genotype and other clinicopathological characteristics.

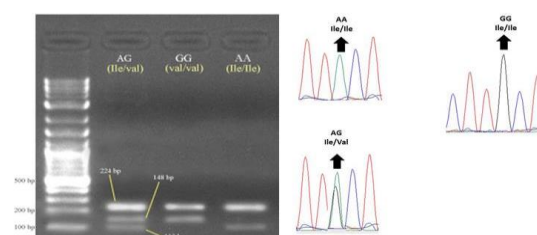


Fig1. The state of polymorphism rs1136201 was genotyped by TARMS-PCR. Band 244 was identified as the control band, band 113 as the AA genotype, band 148 as the GG genotype, and three bands 244, 113 and 148 as the heterozygous genotype of the AG. The results were confirmed by the PCR product sequence method of external primers.

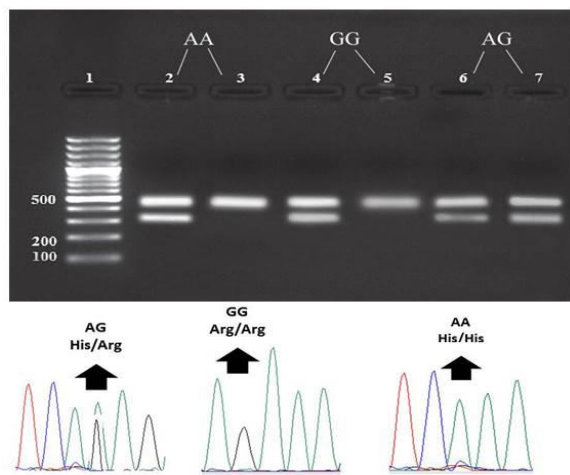


Fig2. The status of polymorphism 1 was genotyped by ARMS-PCR method. For each sample, two reactions were performed on the RCR, the 492 band was considered as the control band, and the 316 band was considered as the AA and GG bands in each reaction. The PCR product of each sample was sequenced to confirm the results.

Table2: Clinical and pathologic features of patients treated with trastuzumab and control

Characteristic	patients		control	
	No	%	No	%
Number of patients	120	100	40	100
age average	58		61	
Histological grade				
1	6	5	3	7
2	37	31	12	29
3	72	60	23	58
Unknown	5	4	2	6
ER status				
Pos	73	61	27	69
Neg	47	39	13	31
PR status				
Pos	66	55	19	49
Neg	54	45	21	51
Her2 neu status				
Pos	120	100	-	-
Neg	-	-	40	100
Objective response				
Complete	12	10	2	5
Partial	37	31	12	30
Stable disease	61	51	16	40
Progressive disease	10	8	10	25

Table3: Genotype frequency between patient who received trastuzumab and patient who did not received trastuzumab

Polymorphism	Genotype frequency in patient who received trastuzumab			Genotype frequency in patient who did not received trastuzumab			Sig.
	Total	Arab	Kurdish	Total	Arab	Kurdish	
FCGR2A	H/H 55 (46%)	39%	41%	H/H 48 (40%)	38%	41%	0.412
Rs1801274	H/R 58 (49%)	46%	43%	H/R 61 (51%)	48%	43%	
	R/R 7 (5%)	15%	16%	R/R 11 (9%)	14%	16%	
ERBB2	Ilu/Ilu 90 (75%)	81%	74%	Ilu/Ilu 29 (73%)	79%	78%	0.341
Rs1136201	Ilu/Val 28 (23%)	18%	26%	Ilu/Val 10 (24%)	19%	21%	
	Val/Val 2 (2%)	1%	0%	Val/Val 1 (3%)	2%	1%	

4. Discussion

Although most of the studies have been focusing on tumor characteristics, it is clear that host's genetic makeup can influence treatment tolerability and outcome [12]. In this study, we examined the relationship between polymorphisms FCGR2A H131R and ERBB2 I655V and the response to trastuzumab. The results of this study could help doctors prescribe this drug. In the case of the ERBB2 rs1136201 polymorphism, our results showed that patients with the AA genotype and breast cancer may have an increased response to treatment with trastuzumab as compared to patients with the AG or GG genotypes. Also the Ile655Val polymorphism was found to be associated with PR status (p=0.031); whereas no significant associations were found between the Ile655Val

genotype and other clinicopathological characteristics. The nucleotide changes in codon 655 gene ERBB2 (HER2), in which the amino acid isoleucine is converted to valine, increases the activity of the protein tyrosine kinase gene HER2. Trastuzumab (herceptin), a humanized, recombinant monoclonal antibody that binds to the extracellular domain of HER2, has been shown to selectively exert anti-tumor effects in cancer models and patients with HER2-amplified breast cancer, and not in tumors with normal HER2 expression [13]. Although trastuzumab is generally well tolerated, its use is often associated with a clinically relevant cardiotoxicity thus a particular caution in the qualification for treatment is necessary and the genotype information could improve the physician's decision making process. A recent investigation has shown that protein modification induced by rs1136201 ERBB2 polymorphisms may render cardiomyocytes dependent upon HER2 signaling and more sensitive to trastuzumab-mediated toxicity [14]. X.han et al among 212 HER2-positive patients who received chemotherapy in combination with trastuzumab treatment, patients with the Val/Ile or the Val/Val genotype had a significantly better disease-free survival and distant disease-free survival than patients with the Ile/Ile genotype[15] Toomey et al By studying two different populations, they proved that minor allele of ERBB2-I655V has a negative impact on response to adjuvant trastuzumab therapy [16] Damien Cote et al showed Allele G is associated with decreased response to carboplatin, docetaxel and trastuzumab in women with Breast Neoplasms [17]. In the case of the FCGR2A rs1801274 polymorphism, our results showed that Patients with the AA genotype may have increased response to trastuzumab and longer progression-free survival in people with Breast cancer as compared to patients with genotype AG or GG. Fcgr isoforms expressed on immune system cells have been linked to the pathogenic consequences triggered by autoantibodies or immune complexes in autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), as well as to the efficacy of some immunotherapeutic treatments [18] In a study of HIV-infected men, those with a FCGR2a CC genotype progressed to a CD4+ cell count of <200/mm3 at a faster rate than individuals with either of the other genotypes (relative hazard = 1.6; p = 0.0001).[20] However, the authors noted that the progression to AIDS was less impacted by this SNP, largely because TT homozygotes had an increased risk of pneumonia as an AIDS-defining illness [19] Tamura et al. In their study on the population of breast cancer patients in East Asia showed that The AA genotype is associated with increased survival and suppression of tumor progression during treatment with trastuzumab [20]. Ying et al in their study showed Genotype GG is associated with increased overall survival when treated with cetuximab in people with Colorectal Neoplasms as compared to genotypes AA + AG [21].Also In a study of 39 patients with metastatic colorectal cancer treated with cetuximab, the rs1801274(A) allele was associated with longer progression-free survival (PFS; p = 0.055), by perhaps 1-2 months. Studies

involving infliximab [22] Another study by Morales et al. On the European population with rheumatoid arthritis found that Allele A is not associated with response to tocilizumab in people with Arthritis, Rheumatoid as compared to allele G.

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