

Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

Polymorphisms in *FCGR3A* Predict Trastuzumab Efficacy in the Adjuvant Treatment of HER2 Positive Breast Cancer

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eMethods

SNP Analysis of *FCGR2A* and *FCGR3A* Polymorphisms Using the Sequenom Platform

We have developed an assay for the relevant single nucleotide polymorphism (SNP) in the *FCGR3A* gene (rs396991). This G/T SNP results in an amino acid change at position 158. The G allele codes for a valine and the T for a phenylalanine. Because there is extensive homology between *FCGR3A* and *FCGR3B*, it was necessary to do a nested PCR amplification. In the first round of amplification the forward and reverse primers were GCTGCAGGGCCAGAACCCAG and CACTCCGTGGCCACCGTCAC, respectively. Specificity for the *FCGR3A* gene is due to the reverse primer, which has 3 bases unique to the *FCGR3A* gene. These PCR products were used in a second round of amplification using the following PCR primers: ACGTTGGATGTTACAGTCTCTGAAGACAC, ACGTTGGATGTCCAAAAGCCACACTCAAAG and extension primer GACACATTTTTACTCCCAA.

All Sequenom PCR primers contain the same 10 bases on the 5-prime ends to enhance the specificity of the PCR reaction. The polymorphism in the *FCGR2A* gene is a C/T polymorphism at amino acid position 131 and is also known as SNP rs1801274. The (C) allele encodes arginine (R) and the (T) allele encodes histidine (H). The (H) isoform has a higher-binding affinity to IgGs, whereas the (R) isoform is considered to be low-binding. Genomic DNAs were amplified with ACGTTGGATGCTGTGACTGTGGTTTGCTTG and ACGTTGGATGCTTCCAGAATGGAAAATCCC and extended with AGAAGGTGGGATCCAAA. All reactions were performed according to the manufacturer's instructions regarding cycling conditions and concentrations.

eTable 1. Clinical Characteristics According to Treatment Arm

Variable	Category	ACT	ACTH	P
Total		616 (49.2%)	635 (50.8%)	
Age				
	<60	519 (84.3%)	534 (84.1%)	0.99
	≥60	97 (15.7%)	101 (15.9%)	
Tumor size				
	≤2 cm	238 (38.6%)	246 (38.7%)	0.13
	2.1 – 5 cm	332 (53.9%)	325 (51.2%)	
	>5 cm	42 (6.8%)	63 (9.9%)	
	Unknown	4 (0.6%)	1 (0.2%)	
Positive lymph nodes				
	1 - 3	362 (58.8%)	371 (58.4%)	0.76
	4 - 9	179 (29.1%)	178 (28.0%)	
	≥10	75 (12.2%)	86 (13.5%)	
Estrogen Receptor				
	Negative	301 (48.9%)	310 (48.8%)	0.99
	Positive	313 (50.8%)	324 (51.0%)	
	Unknown	2 (0.3%)	1 (0.2%)	
Race				
	White	496 (80.5%)	528 (83.1%)	0.2
	Black	57 (9.3%)	47 (7.4%)	
	Hispanic	35 (5.7%)	24 (3.8%)	
	Other	28 (4.5%)	36 (5.7%)	
	Unknown	0 (0%)	0 (0%)	

ACT = doxorubicin and cyclophosphamide followed by paclitaxel
 ACTH = ACT plus one year of weekly trastuzumab

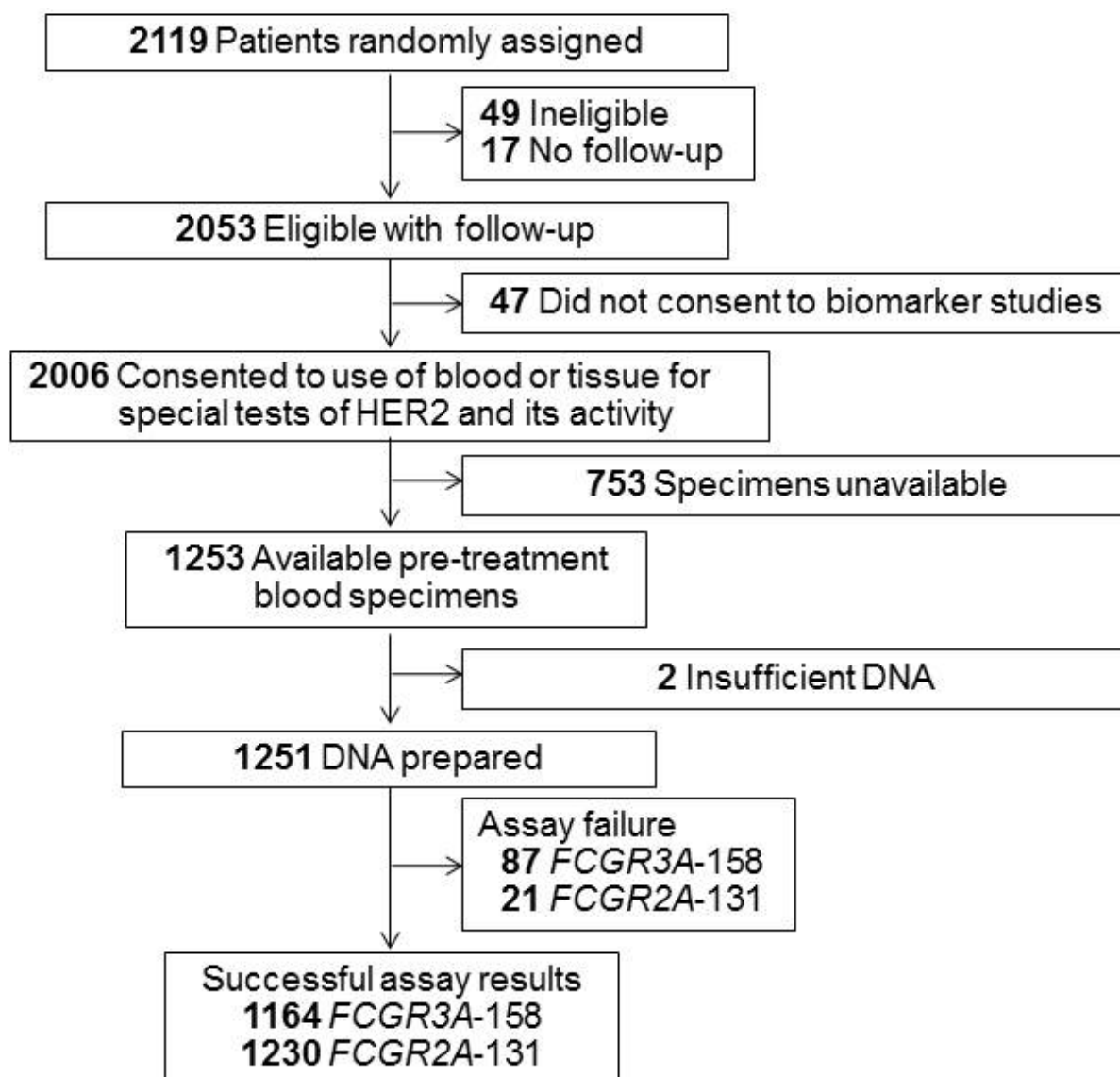
eTable 2. Association of Clinical Characteristics with FCGR3A-158

	Total	158 V/V	158 F/V	158 F/F	P
Age					0.0009
< 60	975 (83.8%)	110 (79.1%)	428 (88.4%)	437 (80.8%)	
≥ 60	189 (16.2%)	29 (20.9%)	56 (11.6%)	104 (19.2%)	
Tumor size					0.52
≤2 cm	457 (39.3%)	50 (36.0%)	193 (39.9%)	214 (39.6%)	
2.1 – 5 cm	603 (51.8%)	72 (51.8%)	249 (51.4%)	282 (52.1%)	
>5 cm	99 (8.5%)	17 (12.2%)	41 (8.5%)	41 (7.6%)	
Missing	5 (0.4%)	0 (0.0%)	1 (0.2%)	4 (0.7%)	
Positive lymph nodes					0.79
1-3	686 (58.9%)	78 (56.1%)	283 (58.5%)	325 (60.1%)	
4-9	329 (28.3%)	39 (28.1%)	139 (28.7%)	151 (27.9%)	
≥10	149 (12.8%)	22 (15.8%)	62 (12.8%)	65 (12.0%)	
Estrogen receptor					0.51
Negative	573 (49.2%)	73 (52.5%)	229 (47.3%)	271 (50.1%)	
Positive	588 (50.5%)	66 (47.5%)	253 (52.3%)	269 (49.7%)	
Missing	3 (0.3%)	0 (0.0%)	2 (0.4%)	1 (0.2%)	
Progesterone Receptor					0.23
Negative	731 (62.8%)	95 (68.3%)	308 (63.6%)	328 (60.6%)	
Positive	429 (36.9%)	44 (31.7%)	174 (36.0%)	211 (39.0%)	
Missing	4 (0.3%)	0 (0.0%)	2 (0.4%)	2 (0.4%)	
Race					0.64
Black	100 (8.6%)	11 (7.9%)	39 (8.1%)	50 (9.2%)	
Hispanic	54 (4.6%)	4 (2.9%)	20 (4.1%)	30 (5.5%)	
Other	59 (5.1%)	10 (7.2%)	25 (5.2%)	24 (4.4%)	
White	951 (81.7%)	114 (82.0%)	400 (82.6%)	437 (80.8%)	

eTable 3. Association of Clinical Characteristics with FCGR2A-131

	Total	131 H/H	131 H/R	131 R/R	<i>P</i>
Age					0.91
< 60	1,036 (84.2%)	268 (84.8%)	509 (84.3%)	259 (83.5%)	
≥ 60	194 (15.8%)	48 (15.2%)	95 (15.7%)	51 (16.5%)	
Tumor size					0.35
≤2 cm	475 (38.6%)	123 (38.9%)	229 (37.9%)	123 (39.7%)	
2.1 – 5 cm	649 (52.8%)	159 (50.3%)	323 (53.5%)	167 (53.9%)	
>5 cm	102 (8.3%)	32 (10.1%)	52 (8.6%)	18 (5.8%)	
Missing	4 (0.3%)	2 (0.6%)	0 (0.0%)	2 (0.6%)	
Nodal Status					0.12
1-3	724 (58.9%)	175 (55.4%)	361 (59.8%)	188 (60.6%)	
4-9	347 (28.2%)	106 (33.5%)	156 (25.8%)	85 (27.4%)	
≥10	159 (12.9%)	35 (11.1%)	87 (14.4%)	37 (11.9%)	
Estrogen receptor					0.87
Negative	602 (48.9%)	152 (48.1%)	294 (48.7%)	156 (50.3%)	
Positive	626 (50.9%)	162 (51.3%)	310 (51.3%)	154 (49.7%)	
Missing	2 (0.2%)	2 (0.6%)	0 (0.0%)	0 (0.0%)	
Progesterone receptor					0.22
Negative	770 (62.6%)	210 (66.5%)	369 (61.1%)	191 (61.6%)	
Positive	456 (37.1%)	104 (32.9%)	233 (38.6%)	119 (38.4%)	
Missing	4 (0.3%)	2 (0.6%)	2 (0.3%)	0 (0.0%)	
Race					0.024
Black	104 (8.5%)	22 (7.0%)	44 (7.3%)	38 (12.3%)	
Hispanic	58 (4.7%)	16 (5.1%)	26 (4.3%)	16 (5.2%)	
Other	64 (5.2%)	25 (7.9%)	29 (4.8%)	10 (3.2%)	
White	1004 (81.6%)	253 (80.1%)	505 (83.6%)	246 (79.4%)	

eFigure 1 REMARK Study Flow Diagram



eFigure 2. Proportional Hazard Models Examining the Prognostic Effect of Genotype According to Treatment Arm

For this analysis, the hi-affinity genotypes were combined (*FCGR2A*-HH or HR vs RR; *FCGR3A*-VV or VF vs FF) and multivariable estimates were adjusted for estrogen receptor (ER) (positive or negative) and nodal status (1-3, 4-9, or ≤ 10 positive lymph nodes). Due to missing ER status two patients were excluded from multivariable analysis of *FCGR2A* and three were excluded for *FCGR3A*. The box size is proportional to the precision.

