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Research Article

Cancer Immunology Research

Association Studies of Fcγ Receptor Polymorphisms with Outcome in HER2$^+$ Breast Cancer Patients Treated with Trastuzumab in NCCTG (Alliance) Trial N9831

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Abstract

Patients with HER2$^+$ breast cancer treated with trastuzumab and chemotherapy have superior survival compared with patients treated with chemotherapy alone. Polymorphisms within *FCGR2A* and *FCGR3A* are associated with binding affinity of natural killer cells to the IgG1 portion of trastuzumab, and a polymorphism in *FCGR2B* (I232T) is associated with impaired regulatory activity. The association of these polymorphisms with clinical response among trastuzumab-treated patients is equivocal, with both positive and negative associations. We performed genotyping analysis on the *FCGR3A* V158F, *FCGR2A* R131H, and *FCGR2B* I232T polymorphisms in 1,325 patients from the N9831 clinical trial. Patients in arm A ($N = 419$) received chemotherapy only. Patients in arms B ($N = 469$) and C ($N = 437$) were treated with chemotherapy and trastuzumab (sequentially in arm B and concurrently in arm C). Using log-rank test and Cox proportional hazard models, we compared disease-free survival (DFS) among genotypic groups within pooled arms B/C. We found no differences in DFS between trastuzumab-treated patients who had the *FCGR3A* 158 V/V and/or *FCGR2A* 131 H/H high-affinity genotypes and patients without those genotypes. Furthermore, there was no significant interaction between *FCGR3A* and *FCGR2A* and treatment. However, there was a difference in DFS for *FCGR2B* I232T, with I/I patients deriving benefit from trastuzumab ($P < 0.001$), compared with the T carriers who did not ($P = 0.81$). The interaction between *FCGR2B* genotype and treatment was statistically significant ($P = 0.03$). Our analysis did not reveal an association between FcγR high-affinity genotypes and outcomes. However, it seems that the *FCGR2B* inhibitory gene may be predictive of adjuvant trastuzumab benefit. *Cancer Immunol Res; 2(10); 962–9. ©2014 AACR.*

Introduction

In the adjuvant setting, patients with HER2 overexpression or amplification (HER2$^+$) breast cancer treated with trastuzumab along with chemotherapy have superior disease-free survival (DFS) and overall survival compared with patients treated with chemotherapy alone (1–4). However, trastuzumab effectiveness is limited, as approximately 20% of adjuvant patients develop tumor relapse within 10 years of receiving treatment (1). In the metastatic setting, only approximately 25% to 30% of patients derive significant tumor shrinkage after single-agent trastuzumab treatment, which is improved to 50% to 60% upon the addition of chemotherapy (5).

Understanding the mechanism of action of trastuzumab and chemotherapy is essential to better identify those patients most likely to respond to treatment and potentially to better engineer the drug itself. One of the accepted mechanisms of action is Fcγ receptor (FcγR) antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by various immune effectors, such as macrophages and natural killer cells (5–9). ADCC occurs when the Fc portion of the tumor-bound antibody is recognized by the FcγRs (10). In mice deficient in activating FcγR genes, the antitumor effects of trastuzumab are significantly blunted (11). Consistent with these observations, engineered anti-HER2 antibodies with disabled Fc domains fail to induce tumor responses *in vivo*, despite retained HER2 binding and growth inhibition *in vitro*. Conversely, antitumor antibodies are 10-fold more effective in mice deficient in inhibitory FcγRs, and antitumor antibody potency is greatly

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increased by engineering Fc domains to antibody that binds activating Fc receptors with greater avidity than inhibitory Fc receptors (11).

Some common single-nucleotide polymorphisms (SNP) in the coding regions of the FcγR genes have been associated with differential antibody-binding affinities and functional outcomes. A coding polymorphism, V158F, located in the extracellular domain of the FcγRIIIa and in an area that directly interacts with the lower hinge region of IgG1, is known to affect binding (12, 13). The histidine allelic variant at amino acid position 131 (H131) in the FcγRIIa extracellular domain confers higher binding affinity than the arginine (R131) variant (14). An *in vitro* study of these polymorphisms using peripheral blood mononuclear cells (PBMC) subsequently demonstrated that PBMCs homozygous for H131 or V158 showed significantly higher trastuzumab-mediated cytotoxicity than PBMCs with other genotypes (15). A third functional polymorphic allele encodes a loss-of-function variant of the inhibitory receptor, FcγRIIb (I232T). The 232T variant is unable to associate with lipid rafts and is therefore strongly impaired in its negative regulatory activity (16, 17).

Associations of FcγR gene polymorphisms with clinical response among trastuzumab-treated patients have been equivocal, some finding positive associations (15, 18), whereas others finding no associations (19). A study of 54 patients with HER2$^+$ metastatic breast cancer by Musolino and colleagues (15) reported a better objective response rate (ORR) in patients with the *FCGR3A* 158 V/V genotype as compared with patients with V/F and F/F genotypes. This study also showed a significantly higher response to trastuzumab therapy in patients with *FCGR3A* 158 V/V genotype and/or *FCGR2A* 131 H/H genotype and when combining both favorable 158 V/V and 131 H/H genotypes, progression-free survival (PFS) was significantly longer than for patients without either genotype. In some agreement with the Musolino study, Tamura and colleagues (18) reported a non–statistically significant trend in a smaller cohort of 35 HER2$^+$ patients with metastatic breast cancer in which patients with *FCGR3A* 158 V/V had a higher ORR than V/F and F/F patients. They also observed a significantly higher ORR and PFS period in patients with *FCGR2A* 131 H/H genotypes compared with H/R and R/R genotypes. In contrast with these studies, two other studies found no association between FcγRs with responses in the metastatic setting (19, 20). The contradictory results of these studies suggest that larger sample sizes are required to ascertain the role of FcγRs, which is now becoming a possibility with the maturation of several ongoing and recently completed adjuvant trials.

To date, the largest study of FcγR polymorphisms and clinical responses to trastuzumab involves genotyping of patients in the adjuvant trastuzumab-chemotherapy study BCIRG-006, which had DNA available from 1,286 patients (19). In that study, Hurvitz and colleagues reported that there was no association between *FCGR3A* and/or *FCGR2A* genotypes and trastuzumab efficacy or DFS in patients treated with trastuzumab. Despite the improved sample size, the cohort of trastuzumab patients genotyped in that study seemed to have had little trastuzumab benefit relative to the control arm, limiting power to detect associations with survival, meriting further investigations. Thus, in our own study, we aimed to determine whether the clinical benefit of trastuzumab is associated with polymorphisms in the FcγR genes in the well-powered N9831, adjuvant trastuzumab cohort, with long-term follow-up duration (1). As in the Hurvitz study, we genotyped the two polymorphisms associated with receptor affinity (*FCGR3A* and *FCGR2A*), but, in addition, we also genotyped the inhibitory polymorphism (*FCGR2B I232T*) in 1,325 patients with HER2$^+$ breast cancer (5).

Materials and Methods

Patient population

Patients in the N9831 trial ($n = 2{,}932$) were required to have histologically confirmed adenocarcinoma of the breast with 3+ immunohistochemical staining for HER2 or amplification of the HER2 gene by fluorescence *in situ* hybridization ($\geq$2.0 ratio) and with either lymph node–positive or high-risk lymph node–negative disease to be eligible for the study (1). The N9831 trial had three treatment arms. In arm A, patients were treated with doxorubicin and cyclophosphamide every 3 weeks for four cycles, followed by weekly paclitaxel for 12 weeks; in arm B, patients received sequential trastuzumab treatment with doxorubicin and cyclophosphamide every 3 weeks for four cycles, followed by weekly paclitaxel for 12 weeks, followed by 52 weeks of weekly trastuzumab; and in arm C, patients received concurrent trastuzumab treatment, doxorubicin and cyclophosphamide every 3 weeks for four cycles, followed by weekly paclitaxel plus concurrent trastuzumab for 12 weeks, followed by 40 more weeks of weekly trastuzumab.

Enrollment to N9831 closed in May 2005. DNA sample collection was not included in the original study design and was added as addendum 16 in November 2005. DNA samples from 1,325 patients (45% of the full N9831 cohort) were subsequently collected and genotyped for polymorphisms in the FcγR genes according to a protocol that was written and approved by the Mayo Clinic Institutional Review Board with written informed consent. DNA samples were not available from 1,607 patients in the N9831 trial before the addendum to extract germline DNA.

Genotyping assays

Germline DNA samples were genotyped at Stanford University (Stanford, CA; Pegram laboratory) for three SNPs in the FcγR genes as follows: *FCGR3A* V158F (rs396991), *FCGR2A* R131H (rs1801274), and *FCGR2B* I232T (rs1050501). For each SNP, genotyping was performed with TaqMan real-time PCR assays (Applied Biosystems). *FCGR3A* V158F and *FCGR2A* R131H were TaqMan assays on demand (C-25815666-10 and C-9077561-20, respectively). The *FCGR2B* I232T polymorphism was determined using a custom TaqMan SNP Genotyping Assay (Applied Biosystems) with the following primers: sense 5′-CCTAGCTCCCAGCTCTTCAC-3′ and antisense 5′-CCACTACAGCAGCAACAATGG-3′. TaqMan reactions were set up in 25-μL volumes as per the manufacturer's instructions (Applied Biosystems), with 10 ng DNA. Thermal cycling conditions were as follows: initial denaturation step at 95°C for

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10 minutes, followed by 50 cycles of denaturation at 92°C for 15 seconds, and annealing/extension step at 60°C for 1 minute. All samples were run in duplicate. Each reaction plate included a triplicate nontemplate control and internal positive controls. Allelic discrimination was performed with an ABI Prism 7900HT Sequence Detector system using SDS 1.2.3 Software (Applied Biosystems). Human DNA specimens (NA00131, NA00607, and NA00893) purchased from the Coriell Institute for Medical Research (Camden, NJ) were used as positive controls for each SNP.

Statistical analysis

The primary goal was to determine whether DFS differed among genotypic groups for three polymorphisms in the FcγR genes in patients treated with trastuzumab; arms B and C were pooled for this analysis. DFS was defined as the time from registration to the first disease-related event, which includes a local, regional, or distant breast cancer recurrence; contralateral breast cancer; a new primary cancer (except squamous or basal cell carcinoma of the skin, carcinoma *in situ* of the cervix, or lobular carcinoma *in situ* of the breast); or death from any cause (1). Kaplan–Meier curves were generated for groups of patients based on treatment arm and/or genotype information and compared with a log-rank test. Cox proportional hazard models were used to generate a point estimate and corresponding 95% confidence intervals (CI) to estimate hazard ratios (HR). Specific comparisons were made between genotypic groups within treatment arms and between treatment arms within genotypic groups. A test for an interaction between treatment arm and genotypic group was performed by including the interaction variable and main effects, in a Cox model. Comparisons of variables among the genotypic groups were made with a χ^2 test for categorical variables and a two-sample t test (or ANOVA for more than two groups) for continuous variables. Exact tests of the Hardy–Weinberg equilibrium (HWE; ref. 21) and linkage disequilibrium values of D' and r^2 were determined in Haploview (21). Analyses were performed with SAS v9.3, and P values less than 0.05 were considered statistically significant.

Results

Genotyping quality control

DNA was available and genotyped in 1,325 patients from 2,932 (45%) patients in the N9831 trial. The genotype success rate was 100% for 1,325 samples typed in *FCGR3A* and *FCGR2A*, and 99.8% for *FCGR2B*. Genotype distributions were within HWE for *FCGR3A* V158F and *FCGR2A* R131H ($P = 0.77$ and 0.64, respectively). We observed a slight excess of homozygous genotypes at the *FCGR3B* I232T SNP, HWE $P = 0.02$. Approximately one third of the DNA samples were genotyped in duplicate with 100% concordance.

Patient population

Only minor differences were observed for clinical and pathologic features of genotyped patients treated with trastuzumab (arms B and C) and patients treated with chemotherapy only (arm A; Table 1). Patients genotyped for FcγR gene polymorphisms in arms B/C showed superior DFS compared with patients in arm A. Eight years after randomization, the survival rate in patients in combined arms B and C was 84.6% (95% CI, 82.2%–87.1%), whereas survival in arm A was 76.0% (95% CI, 72.0%–87.1%; Fig. 1A). The patient subset from which DNA samples were available for analysis (resulting from a protocol addendum to re-consent patients for an additional blood draw for germline DNA extraction in 2005) demonstrated superior DFS relative to the 1,607 patients from whom DNA samples were not available for analysis (Fig. 1B). A breakdown of tumor and patient characteristics in the genotyped cohort versus the nongenotyped cohort is presented in Supplementary Table S1. The nongenotyped cohort had a greater proportion of patients treated in arm A, was slightly younger, had greater nodal disease, had larger tumors, and had a smaller proportion of hormone receptor–positive tumors compared with the genotyped cohort.

Of the individuals genotyped in this study, race was reported as follows: white, 88.4%; black or African American, 5.5%, Asian, 2%, and unknown/not given, 4.1%. We examined differences in patient demographics and tumor characteristics between each genotype group for each polymorphism (Supplementary Tables S2–S4). No meaningful differences were observed between genotypic groups for polymorphisms in *FCGR3A* and *FCGR2A*. For *FCGR2B* I232T, however, we observed a slight excess of 232T homozygous genotypes in nonwhites than in whites, $P = 0.004$ (Supplementary Table S4), although, as we have noted, the number of 232T homozygotes is small ($n = 30$).

FcγR gene allele frequencies and linkage disequilibrium

Allele frequencies and linkage disequilibrium measures for *FCGR3A* V158F and *FCGR2A* R131H were comparable with those previously reported by Hurvitz and colleagues (19). The minor allele frequencies (MAF) across 1,325 individuals for *FCGR3A* V158F, *FCGR2A* R131H, and *FCGR2B* I232T were $V = 0.354$, $R = 0.497$, and $T = 0.124$. *FCGR3A* V158F and *FCGR2A* R131H map 34.8 kb apart, and given the previous reports of positive association of both the 158V and 131H variants, we tested the degree of linkage disequilibrium between these loci. We found that the linkage disequilibrium was low, $D' = 0.37$ and $r^2 = 0.07$, suggesting that these variants are not correlated despite their close physical proximity.

We further examined allele frequencies of each polymorphism by race. *FCGR3A* V158F MAFs were 158V = 0.355, 0.377, and 0.389 in whites ($N = 1{,}170$), African Americans ($N = 73$), and Asians ($N = 27$), respectively. *FCGR2A* MAFs were 131R = 0.495, 0.479, and 0.481 in whites, African Americans, and Asians, respectively. *FCGR2B* I232T showed a significant difference in allele frequency by race with MAFs (232T) of 0.118, 0.171, and 0.185 in whites, African Americans, and Asians, respectively ($P = 0.019$, white vs. nonwhite). The genotypic distribution of *FCGR2B* I232T was within HWE for whites and African Americans ($P = 0.24$ in both). In the Asian group, genotype distributions were not in HWE ($P = 0.04$), with an excess of T homozygotes. This finding is in line with published data in which the frequency

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Table 1. Patient and clinical characteristics

	Arm A (*N* = 419)	Arm B/C (*N* = 906)	*P*
Age			0.75
N	419	906	
Mean (SD)	50.0 (10.0)	50.2 (10.3)	
Median	50	50	
Q1, Q3	43.0, 57.0	43.0, 57.0	
Range	(27.0–80.0)	(22.0–82.0)	
Age group			0.82
<40	62 (15%)	147 (16%)	
40–49	140 (33%)	278 (31%)	
50–59	145 (35%)	316 (35%)	
≥60	72 (17%)	165 (18%)	
Menopausal status			0.68
Premenopausal or <50	223 (53%)	471 (52%)	
Postmenopausal or ≥50	196 (47%)	435 (48%)	
Breast surgery			0.5
Breast sparing	177 (42%)	365 (40%)	
Mastectomy	242 (58%)	541 (60%)	
Positive nodes			0.99
Axdis 1–3 positive	164 (39%)	348 (38%)	
Axdis 4–9 positive	107 (26%)	246 (27%)	
Axdis 10+ positive	47 (11%)	99 (11%)	
Positive sent node	35 (8%)	80 (9%)	
Negative sent node	40 (10%)	82 (9%)	
Negative axdis	26 (6%)	51 (6%)	
Receptor status			0.4
ER-positive and/or PR-positive	226 (54%)	511 (56%)	
Other	193 (46%)	395 (44%)	
Tumor size, cm			0.61
≤2.0	170 (41%)	394 (43%)	
2.0–5.0 cm	218 (52%)	447 (49%)	
>5.0 cm	31 (7%)	65 (7%)	
Histology			0.23
Ductal	390 (93%)	860 (95%)	
Other	28 (7%)	46 (5%)	
Missing	1	0	
Tumor grade			0.61
High	301 (73%)	644 (72%)	
Low/intermediate	111 (27%)	254 (28%)	
Missing	7	8	
Tumor stage			0.61
1	170 (41%)	394 (43%)	
2	218 (52%)	447 (49%)	
3	31 (7%)	65 (7%)	
N stage			0.34
0	66 (16%)	133 (15%)	
1	334 (80%)	722 (80%)	
2	18 (4%)	51 (6%)	
3	1 (0%)	0 (0%)	
Agent			0.52
None	193 (46%)	396 (44%)	
Other	77 (18%)	158 (17%)	
Tamoxifen	149 (36%)	352 (39%)	

Abbreviations: Axdis, axillary lymph node dissection; ER, estrogen receptor; positive sent, positive sentinel node dissection; negative sent, negative sentinel node dissection; negative axdis, negative axillary node; PR, progesterone receptor.

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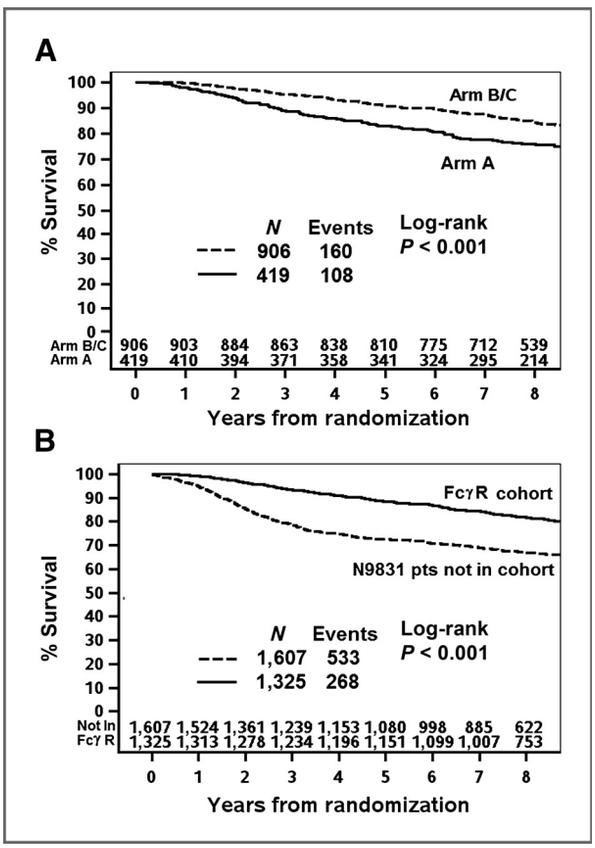

Figure 1. The therapeutic benefit of trastuzumab-containing regimens was observed in N9831 patients who provided DNA specimens for genetic analysis. A, Kaplan–Meier analysis of DFS by the treatment group with arms B and C combined. The inset numbers shown are the numbers of individuals considered at risk at the respective time points in each cohort. B, Kaplan–Meier analysis of DFS in patients who provided DNA for FcγR analysis (FcγR cohort) compared with patients who did not provide DNA and for whom genotyping data were not available.

of the *FCGR2B* 232T variant is increased in African and Asian populations, broadly corresponding to areas where malaria is endemic (22). It is possible that the observed 232T excess in our African-American and Asian populations and deviation from HWE in Asians results from positive selection of the 232T variant. Measures of linkage disequilibrium for each marker combination showed some variation by race, but linkage disequilibrium was low to moderate in all cases.

Association of genotype with DFS in patients treated with trastuzumab

There were no significant differences in DFS among the genotypic groups for *FCGR3A*, *FCGR2A*, and *FCGR2B* for patients treated with trastuzumab (Fig. 2A–C). Furthermore, there was no significant difference in DFS between patients who were homozygous for *FCGR3A* V/V and/or homozygous for *FCGR2A* H/H and patients who were not (HR, 1.01; 95% CI, 0.72–1.41; $P = 0.96$). Our results differ from published reports that did find an association with DFS and patients who were *FCGR2A* H/H and/or *FCGR3A* V/V (15, 18). An analysis of arms B and C separately also did not find significant associations of genotypic groups *FCGR3A*, *FCGR2A*, and *FCGR2B* (Supplementary Fig. S1A–S1C).

Impact of genotype on trastuzumab effect

Stratified Cox models were used to compare the DFS between trastuzumab-treated patients (arms B/C) and patients not treated with trastuzumab (arm A) within each genotypic group for *FCGR3A*, *FCGR2A*, and *FCGR2B* (Fig. 3). For *FCGR3A* and *FCGR2A*, the trastuzumab effect seems to be similar among the different genotypic groups. Nonsignificant *P* values for the tests for interaction of treatment arms (A vs. B/C) and *FCGR3A* and *FCGR2A* genotypes confirmed

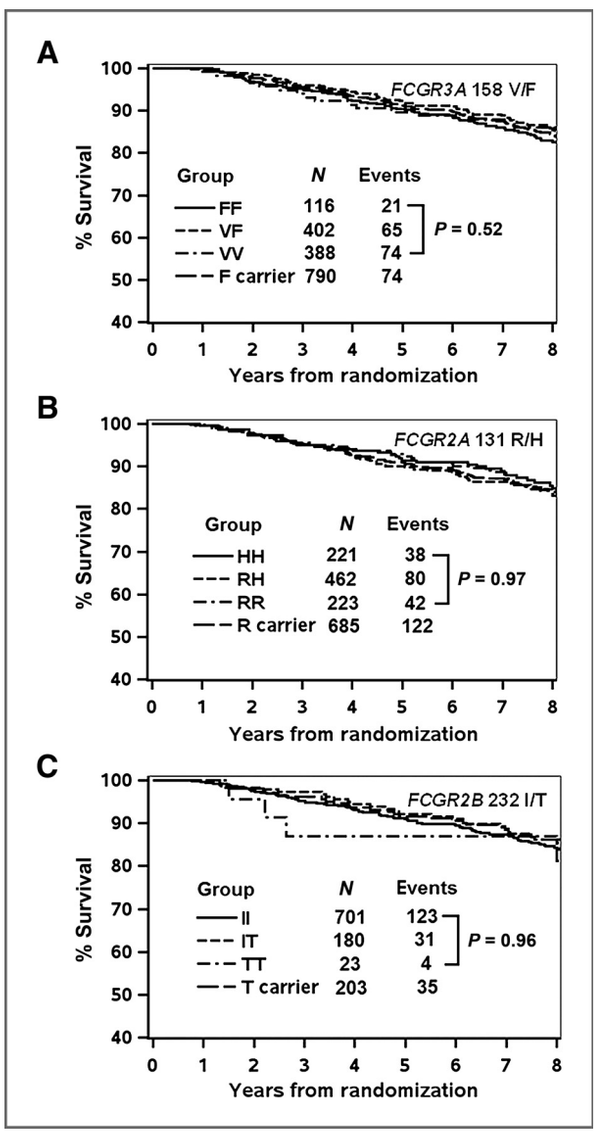

Figure 2. DFS was not improved for any genotypic group in the trastuzumab-containing regimens. Kaplan–Meier analyses of DFS by the genotype group for each of the three FcγR genes examined: *FCGR3A* V158F (A), *FCGR2A* R131H (B), and *FCGR2B* I232T (C).

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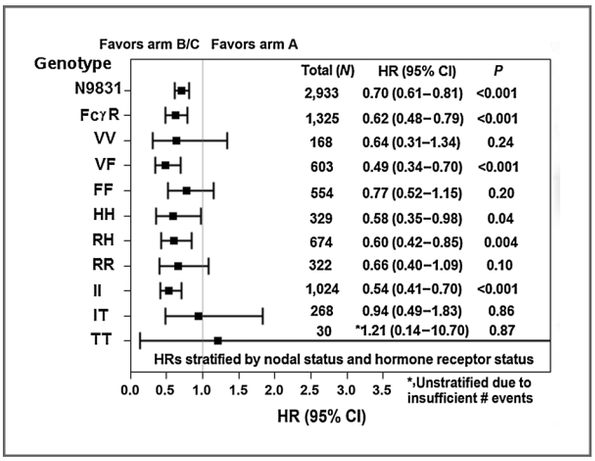

Figure 3. HRs did not differ by genotype in arm A versus arms B/C. HRs of arm B/C individuals compared with arm A patients as calculated by Cox regression analysis in the full N9831 cohort, N9831 subset genotyped for the FcγR cohort and each genotype.

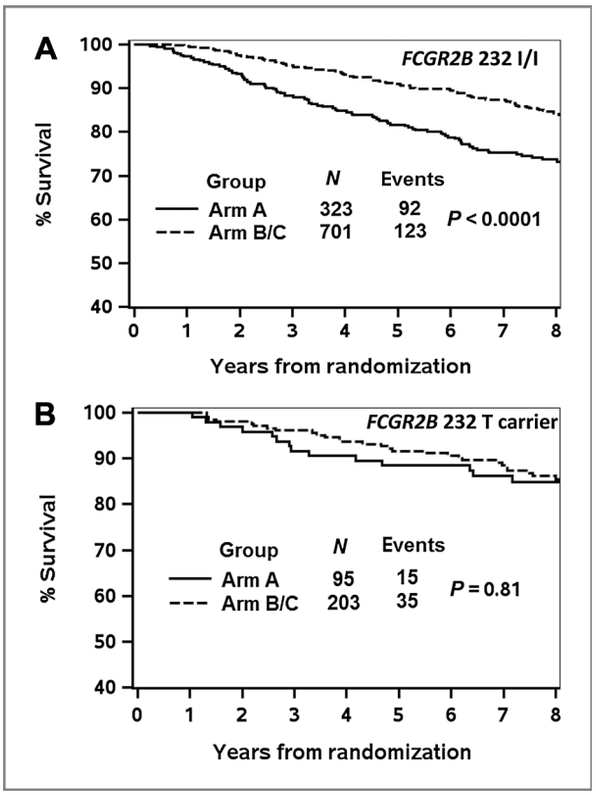

Figure 4. *FCGR2B* 232 T carriers may not benefit from the inclusion of trastuzumab into their adjuvant chemotherapy regimen. Kaplan–Meier analyses of DFS by genotype group for both arms A and B/C (A) *FCGR2B* 232 I/I homozygote genotype and (B) *FCGR2B* 232 T carrier.

this observation. However, it seems as though there might be an interaction between treatment and *FCGR2B* genotype from Fig. 4.

Given the relatively few patients with the T/T genotype for *FCGR2B*, an analysis was performed comparing patients who were I/I homozygous with those who were T carriers (I/T and T/T). The results of the test for interaction between treatment arms (A vs. B/C) and *FCGR2B* genotype (I/I vs. T carrier) were statistically significant ($P = 0.03$). Figure 4A shows that *FCGR2B* I/I patients treated with trastuzumab had better DFS than those not treated with trastuzumab ($P < 0.0001$). However, DFS does not seem to have differed between T carriers who were and were not treated with trastuzumab ($P = 0.81$; Fig. 4B), and T carriers who received chemotherapy alone showed similar survival to I/I homozygotes who received chemotherapy plus trastuzumab. An exploratory Kaplan–Meier analysis of patients treated with chemotherapy only showed improved survival in T carriers compared with I/I homozygotes ($P = 0.01$).

Discussion

Common genetic variants in the FcγR genes *FCGR3A* (V158F) and *FCGR2A* (R131H) are associated with affinity for the monoclonal antibody trastuzumab *in vitro*, (12–14), whereas the I232T variant is associated with loss of function in the inhibitory receptor encoded by *FCGR2B* (16). A positive association of these genetic variants with clinical outcome in patients treated with trastuzumab would support the use of genotyping to preselect patients most likely to respond to trastuzumab treatment, and further engineering of monoclonal antibodies with increased affinity for the FcγRs. Suggestions of positive associations with clinical outcome to date have arisen from multiple small studies of patients treated in the metastatic ($n = 35$–54) and neoadjuvant ($n = 15$) settings (15, 18).

Our study design tested for the association of three FcγR polymorphisms with DFS in the N9831 clinical trial of patients with early-stage HER2$^+$ breast cancer treated with chemotherapy alone (arm A) or chemotherapy in combination with trastuzumab (arms B/C). We did not find association of the *FCGR2A* and *FCGR3A* genes for any genotype or genotypic combination with improved DFS. However, *FCGR2B* I/I patients showed superior DFS in the trastuzumab arms as compared with I/I patients in the chemotherapy-only arm. In contrast, patients who carried the T allele did not show any benefit from trastuzumab, and their survival was nearly the same as that of I232 (I/I) patients who received trastuzumab. This is important because it suggests that the I232 variant may be predictive of trastuzumab benefit, or that the T allele is an independent DFS predictor in patients with breast cancer treated with chemotherapy alone.

We can only speculate on the biologic reasons as to why trastuzumab treated and nontreated T carriers had survival similar to trastuzumab-treated I/I homozygotes. *FCGR2B* plays an inhibitory role in the immune response, and the minor allele (232T) results in loss of function of the inhibitory response. Loss of the suppressive function results in increased resistance to infection (e.g., malaria) as well as increased susceptibility to autoimmunity (e.g., systemic lupus erythematosus; ref. 22). Thus, one possiblity is that the T allele could be associated with enhanced antitumoral immunity, possibly including a natural endogenous trastuzumab-like response.

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This natural response has been observed in some cases in HER2$^+$ breast cancer (23). Hence, we would predict T carriers to have good survival and not respond to trastuzumab because they already demonstrate natural endogenous trastuzumab-like immune response. In I/I patients, we would predict that the addition of trastuzumab could compensate for the lack of natural response, thus improving DFS.

The results of our study of 1,325 patients from N9831 are partially in agreement with those from Hurvitz and colleagues, which genotyped the *FCGR3A* (V158F) and *FCGR2A* (R131H) polymorphisms in approximately 1,200 patients from the BCIRG-006 adjuvant trastuzumab-based study. Taking this into consideration, our study is highly relevant for three reasons. First, the subset of BCIRG-006 patients who were genotyped showed little benefit from trastuzumab (HR, 0.842; $P = 0.1925$). Second, the genotypic data for *FCGR3A* polymorphism deviated significantly from that expected by HWE ($P < 0.001$). The lack of genotyping in a control (healthy population) cohort made it difficult to determine whether this deviation resulted from systematic genotyping error or putative genetic association with breast cancer. In our similarly sized cohort, both significant benefit from trastuzumab (arm A vs. arms B/C; $P < 0.001$) in the subset of individuals genotyped for the FcγR polymorphisms and nondeviation from HWE for either the *FCGR3A* or *FCGR2A* polymorphism were observed. Similarly, our MAFs and measures of linkage disequilibrium between the *FCGR3A* and *FCGR2A* polymorphisms are also in agreement with those of Hurvitz and colleagues, suggesting that the polymorphisms genotyped in these studies may have little or no effect on clinical outcome in patients treated with trastuzumab. Third, our study differed from that of Hurvitz and colleagues, as we also evaluated for impact of the inhibitory FcγR polymorphism *FCGR2B* I232T.

There are a number of limitations to our study that might explain a lack of association of FcγR high-affinity variants with outcome. First, germline DNA collection was not included in the original study design, but as a protocol addendum 5 months after the close of enrollment, hence DNA was available for only 1,325 of 2,932 (45%) patients. The subset of genotyped individuals showed significant benefit from trastuzumab to the same degree as the full N9831 cohort, but we also observed that patients in the genotyped subset showed superior DFS compared with patients who did not provide or consent for DNA analysis ($P < 0.001$). It is possible that the nongenotyped cohort had a more aggressive form of disease because this cohort had a greater proportion of patients treated in arm A, was slightly younger, had greater nodal disease, had larger tumors, and had a smaller proportion of hormone receptor–positive tumors compared with the genotyped cohort. This chance bias toward improved clinical outcome in the genotyped cohort could have potentially masked modest genotypic effects on outcome. Second, despite the large sample size, the total number of events in the genotyped cohort was 268 (160 in the trastuzumab arms and 108 in the chemotherapy-only arm), which, when further broken down by genotype, would limit the power to detect small or modest associations. This is also of relevance to the most significant finding of the study with the *FCGR2B* I232T variant. Within the trastuzumab-containing arms, only 35 events were observed among patients carrying the 232T variant. Third, our choice of polymorphisms was based on previous published associations with metastatic breast cancer, neoadjuvant breast cancer, and *in vitro* functional assays. There remains the possibility that these polymorphisms are associated with survival difference only in certain treatment settings such as the metastatic and perhaps neoadjuvant setting as reported by Musolino and colleagues (15). However, this argument is tempered by the results reported by Hurvitz and colleagues, which also examined patients with metastatic cancer and found no association of PFS with the polymorphisms (19). Alternatively, as Carlotti and colleagues (24) have postulated in the rituximab setting, the effects of the FcγR variants may only be relevant when antibodies are used as monotherapy. Finally, we did not perform comprehensive genotyping across the FcγR genomic locus, and there are potentially other genetic variants in this region that could influence response to trastuzumab or moderate the effect of the variants selected in this study, such as the three copy number variable regions spanning the *FCGR3A*, *FCGR2A*, *FCGR2C*, and *FCGR2B* genes (25).

In conclusion, our data did not identify any genetic association of *FCGR2A* and *FCGR3A* high-affinity alleles with superior DFS in patients treated with trastuzumab. Although this finding does not preclude a role for these FcγRs, it does suggest that these polymorphisms alone have little, if any, effect on the clinical outcome of trastuzumab in the adjuvant setting when delivered in combination or immediately following chemotherapy. However, our observation of *FCGR2*B as predictive of benefit to trastuzumab or, alternatively, as an independent predictor of DFS in patients treated with chemotherapy alone, has not been tested in other comparable studies and warrants further investigation.

Disclosure of Potential Conflicts of Interest

M. Pegram has received speakers' bureau honoraria and served as a consultant advisory board member for Genentech, Inc. E.A. Perez reports receiving a commercial research grant from Genentech (research funding directly to Mayo Clinic). No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R.M. Olson, M. Pegram, K.L. Knutson, E.A. Perez
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): N. Norton, M. Pegram, K. Tenner, K.V. Ballman, R. Clynes, K.L. Knutson, E.A. Perez
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