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Association of Polymorphisms in *FCGR2A* and *FCGR3A* With Degree of Trastuzumab Benefit in the Adjuvant Treatment of ERBB2/HER2–Positive Breast Cancer:

Analysis of the NSABP B-31 Trial

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Abstract

IMPORTANCE—Preclinical models and studies in the metastatic and neoadjuvant settings suggest that single nucleotide polymorphisms in *FCGR3A* and *FCGR2A* may be associated with differential response to trastuzumab in the treatment of ERBB2/HER2–positive breast cancer, by modulating antibody-dependent cell-mediated cytotoxic effects.

OBJECTIVE—To evaluate the effect of *FCGR2A* and *FCGR3A* polymorphisms on trastuzumab efficacy in the adjuvant treatment of ERBB2/HER2–positive breast cancer.

DESIGN, SETTING, AND PARTICIPANTS—This is a retrospective analysis of patients enrolled in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 trial, a phase 3 cooperative group study conducted between 2000 and 2005. The NSABP B-31 trial randomized 2119 women with surgically resected node-positive, ERBB2/HER2–positive breast cancer to treatment with doxorubicin and cyclophosphamide followed by paclitaxel or the same regimen with the addition of 1 year of weekly trastuzumab. Patients were accrued at cooperative group sites across the United States and Canada. This analysis was performed between 2013 and 2016.

INTERVENTIONS—Doxorubicin and cyclophosphamide followed by paclitaxel or the same regimen with the addition of 1 year of weekly trastuzumab.

MAIN OUTCOMES AND MEASURES—Disease-free survival.

RESULTS—The genotyped cohort (N = 1251) resembled the entire B-31 cohort based on clinical variables and the degree of benefit from trastuzumab. Median follow-up time was 8.2 years in the genotyped samples. The disease-free survival probability at 3, 5, and 8 years was 74% (95%CI, 71%–79%), 66% (95%CI, 62%–71%), and 58% (95%CI, 54%–63%) in patients who received ACT and 86% (95%CI, 83%–89%), 82% (95%CI, 79%–85%), and 78% (95%CI, 74%–81%) in patients who received ACTH. Addition of trastuzumab significantly improved patient outcome (hazard ratio [HR], 0.46; 95%CI, 0.37–0.57; $P < .001$). The expected trend for interaction between polymorphisms and trastuzumab was observed for both genes, but only *FCGR3A*-158 polymorphism reached statistical significance for interaction ($P < .001$). As hypothesized, patients with genotypes *FCB3A*-158V/V or *FCB3A*-158V/F received greater benefit from trastuzumab (HR, 0.31; 95%CI, 0.22–0.43; $P < .001$) than patients who were homozygous for the low-affinity allele (HR, 0.71; 95%CI, 0.51–1.01; $P = .05$).

CONCLUSIONS AND RELEVANCE—The *FCGR3A*-158 polymorphism is predictive of trastuzumab efficacy in this cohort of patients with early ERBB2/HER2–positive breast cancer. Patients who are homozygous for phenylalanine at this position represent a considerable proportion of the population and, in contrast to previously reported analyses from similarly designed trials, our results indicate that trastuzumab may be less efficacious in these patients.

TRIAL REGISTRATION—clinicaltrials.gov Identifier for NSABP B-31: NCT00004067

Clinical trial National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31, analyzed jointly with the North Central Cancer Treatment Group (NCCTG) N9831, established the benefit of adding trastuzumab, a monoclonal antibody targeting the ERBB2/HER2 protein, to standard chemotherapy in patients with early stage ERBB2/HER2–positive breast cancer. However, only about one-third to one-half of patients benefit from this therapy in advanced or adjuvant settings, respectively.^{1,2} Although treatment is generally well tolerated, its high

cost, associated cardiotoxic effects, and the availability of promising alternatives warrant an attempt to identify patients who will not benefit.

Although many mechanisms have been attributed to the success of trastuzumab, the original patent described 2 primary potential mechanisms. First, by binding to ERBB2/HER2 on the cancer cell membrane, it prevents ERBB2/HER2 dimerization, blocks downstream signaling, and subsequently blocks proliferation. Second, it triggers the host immune system to attack and kill trastuzumab-bound tumor cells. This immune response, known as antibody-dependent cell-mediated cytotoxic effect (ADCC), is initiated when the FC γ receptor on natural killer cells (NK) binds to the Fc portion of trastuzumab. As a result, NK cells release factors including IFN- γ , perforins, and granzymes, which cause tumor cell death via apoptosis. A similar process, known as antibody-dependent cellular phagocytosis (ADCP), is initiated when the FC γ receptor on a macrophage binds to trastuzumab and results in tumor cell death by phagocytosis.

Early studies in ERBB2/HER2–positive breast cancer cell lines demonstrated potent ADCC caused by trastuzumab in vitro.³ Experiments in mice have demonstrated reduced trastuzumab efficacy if the Fc fragment is removed from the antibody or if the mouse is deficient in FC γ receptors.^{4,5} In addition, an increase in the infiltration of lymphoid cells into tumor samples was observed after trastuzumab treatment compared with paired pretreatment samples.⁶ Collectively, these findings support a strong role of ADCC or ADCP in the mechanism of trastuzumab efficacy.

Additional preclinical studies have demonstrated an association of single-nucleotide polymorphisms in the genes encoding FC γ receptors with the strength of the immune response. Many studies have focused on position 158 of *FCGR3A*, which encodes a valine or phenylalanine, and position 131 of *FCGR2A*, which encodes a histidine or arginine (reviewed in Mellor et al).⁷ In vitro ADCC assays demonstrated greater trastuzumab-mediated ADCC with the *FCGR3A*-158 valine (V)/V genotype and a trend for association with the *FCGR2A*-131 histidine (H)/H genotype.⁸ Analyses of paired pretreatment and posttreatment peripheral blood mononuclear cells (PBMCs) demonstrated more differential changes in gene expression from patients with the V/V or H/H genotypes than did patients with phenylalanine (F)/F or arginine (R)/R genotypes, indicating a difference in the molecular response to trastuzumab according to genotype.⁹ These observations are consistent with the observation that the FC γ RIIA-131H and FC γ RIIA-158V have a higher affinity to immunoglobulin (IgG) than the proteins encoded by alternate alleles.¹⁰ Cells bearing the high-affinity alleles mediate ADCC more effectively.⁷

Clinical studies using therapeutic monoclonal antibodies such as rituximab for the treatment of lymphoma and cetuximab for the treatment of colon cancer have shown association of the *FCGR3A* and *FCGR2A* genotypes with patient outcomes.^{11–15} However, clinical studies examining the association of these single-nucleotide polymorphisms with trastuzumab benefit for patients with breast cancer are not clear. Tamura and colleagues¹⁶ demonstrated improved response rates to trastuzumab in patients with a *FCGR2A*-131 H/H genotype in the neoadjuvant setting and improved objective response rate in the metastatic setting. Musolino and colleagues⁸ observed improved objective response rate and progression-free

survival for patients with the *FCGR3A*-158 V/V genotype.⁸ However, while conducting our study, 2 large studies of patients enrolled in the NCCTG-N9831¹⁷ and BCIRG-006¹⁸ clinical trials found no association of these loci with trastuzumab efficacy in the adjuvant treatment of ERBB2/HER2–positive breast cancer.

To explore this discrepancy, we retrospectively examined the *FCGR3A*-158 and *FCGR2A*-131 genotypes in all available pretreatment blood specimens from NSABPB-31. Our prespecified primary objective was to determine whether patients with breast cancer with *FCGR3A*-158 V/V or *FCGR3A* 158 V/F received greater benefit from trastuzumab than patients with the *FCGR3A* F/F genotype.

Methods

Patient Cohort

Detailed patient characteristics, eligibility criteria, adverse events, and clinical trial results have previously been reported.^{2,19,20} The NSABPB-31 trial randomly assigned women with surgically resected node-positive ERBB2/HER2–positive breast cancer to treatment with doxorubicin and cyclophosphamide followed by paclitaxel (ACT) or the same regimen with the addition of 1 year of weekly trastuzumab (ACTH). Patients were accrued between February 2000 and April 2005 at cooperative group sites across the United States and Canada. Treatment assignments were balanced according to nodal status, planned hormonal therapy, type of surgery, intended radiotherapy, and institution, with the use of a biased-coin minimization algorithm. Additional inclusion requirements for the current study include availability of clinical follow-up, appropriate informed consent, and availability of pretreatment blood specimens. Informed consent forms were approved by a local human investigations committee in accordance with an assurance filed with and approved by the US Department of Health and Human Services to permit use of banked tissue and blood samples. These trials were approved by local human investigations committees or institutional review boards in accordance with assurances filed with and approved by the Department of Health and Human Services. Written informed consent was obtained from participants in each trial.

Genotyping

Whole blood was collected before treatment and stored at –80° C. Participant DNA was prepared from 100 µL of whole blood using the Mag-Bind Blood DNA HDQ kit (Omega Biotek) and quantified with picogreen (ThermoFisher). Genotyping of rs1801274 (*FCGR2A*-131 R/H) and rs396991 (*FCGR3A*-158 V/F) was performed using iPLEX Pro chemistry and mass spectrometry (Agena) according to the manufacturer's instructions. Nested polymerase chain reaction was used for *FCGR3A*-158 to achieve specificity against its homologue *FCGR3B*. Genotypes were determined with Typer software using default settings after autoclustering (Agena). Two-thirds of the rs396991 assays were performed in duplicate. Bacterial artificial chromosome clones RP11-5K23 (*FCGR3A*), RP11-100D4 (*FCGR3B*), and an equimolar mixture of the 2 were used as controls to ensure the rs396991 primers specifically amplified the 3A homologue. Detailed reaction conditions and primer

sequences are provided in eMethods in the Supplement. All assays were performed blinded to clinical outcome using deidentified specimens.

Statistical Methods

The use of patient blood specimens and a detailed statistical analysis plan were approved by the Cancer Therapy Evaluation Program (CTEP) of the National Cancer Institute (NCI) before starting the current investigation. The primary end point for survival analyses was disease-free survival. Disease-free survival events included local, regional, and distant recurrence; contralateral breast cancer; a second primary cancer; or death from any cause. Follow-up included events recorded before June 30, 2012. In 2005, during a planned joint analysis with NCCTG-N9831, the early-stopping boundary was reached and patients on the ACT arm were offered trastuzumab. Patients who crossed over to the trastuzumab arm were censored at the time of crossover. The treatment benefit and prognostic effect for each genotype was first investigated using the Kaplan-Meier method and treatment arms were compared using log-rank tests. As the primary aim, *FCGR3A* genotype by treatment interaction was tested using proportional hazard regression models. For purposes of the interaction tests, the high-affinity genotypes were combined (*FCGR3A*-158 V/V or V/F vs. F/F). Similar analyses were performed for *FCGR2A* as a secondary objective. Survival analyses provided here were adjusted for estrogen receptor (ER) (positive or negative), progesterone receptor (PR) (positive or negative), age (years), tumor size (cm), and nodal status (1–3, 4–9, or 10 positive lymph nodes). Univariable models are reported in the Supplement or are clearly indicated as such. Missing values in the covariates resulted in the exclusion of 8 cases for multivariable analyses of *FCGR3A* and 7 cases for *FCGR2A*. The Fisher exact test was used to test deviation from Hardy Weinberg Equilibrium and to test the association of genotype with clinical characteristics. Linkage disequilibrium was investigated in the subset of patients with results for both assays. All statistical tests were 2-sided and performed in R.

Results

Patient Characteristics

Among 2006 eligible patients with follow-up and informed consent, pretreatment blood specimens were available for 1253 and DNA was successfully prepared for 1251. Details regarding the reasons for sample loss are shown in eFigure 1 in the Supplement. Clinical and pathological characteristics were well balanced in the treatment arms of the study population and closely resembled the entire B-31 cohort (eTable 1 and eTable 2 in the Supplement). Median follow-up time was 8.2 years in the genotyped samples. The disease-free survival probability at 3, 5, and 8 years was 74% (95% CI, 71%–79%), 66% (95% CI, 62%–71%), and 58% (95% CI, 54%–63%) in patients who received ACT and 86% (95% CI, 83%–89%), 82% (95% CI, 79%–85%), and 78% (95% CI, 74%–81%) in patients who received ACTH. Addition of trastuzumab significantly improved patient outcome (hazard ratio [HR], 0.46; 95% CI, 0.37–0.57; $P < .001$).

Genotyping Results

Of 883 assays performed in duplicate, 3 discordances were observed (0.3%) for *FCGR3A*-158 (rs396991). These 3 observations were excluded from further analysis. Approximately 2% of *FCGR2A*-131 and 7% of *FCGR3A*-158 assays failed default genotyping quality controls in the Typer software, yielding 21 and 87 missing values, respectively. Some of the *FCGR3A* reactions excluded by the software may actually represent heterozygous patients with 3 germline copies of the gene.²¹ However, these wells are indistinguishable from wells with contamination or poor reaction conditions and their expected frequency of 2.5% limits our power to detect meaningful clinical significance. For these reasons, any sample that failed quality control was eliminated. Thirteen samples failed both assays, suggesting poor-quality DNA. The patients genotyped showed 25% H/H, 49% H/R, and 26% R/R alleles for *FCGR2A*-131 and 46% F/F, 42% F/V, and 12% V/V for *FCGR3A*-158. Both loci conform to the Hardy Weinberg Equilibrium in our data. In 1156 specimens with both assays successful, linkage disequilibrium analysis indicated the *FCGR3A* and *FCGR2A* genes are strongly linked ($D' = 0.30$; $P < .001$) as expected from their close genomic proximity and previous studies.¹⁸

FCGR Polymorphisms and Clinical Characteristics

FCGR3A-158 single-nucleotide polymorphisms were not associated with nodal status, ER, PR, tumor size, or race (eTable 3 in the Supplement). However, patients with the homozygous V/V genotype tended to be older ($P < .001$). The mean age was 52 years for V/V patients and 49 to 50 years for the other genotypes. *FCGR2A*-131 was not associated with nodal status, ER, PR, tumor size, or age. However, it was weakly associated with race (eTable 4 in the Supplement).

FCGR3A Polymorphism and Trastuzumab Efficacy

Patients with genotypes that included the higher-affinity alleles *FCGR3A*-158 V/V or V/F received greater benefit from trastuzumab (HR, 0.31; 95% CI, 0.22–0.43; $P < .001$) than patients who were homozygous for the low-affinity allele (HR, 0.71; 95% CI, 0.51–1.01; $P = .05$) (Figure 1A and B). Genotype by treatment-interaction test indicates an association between *FCGR3A*-158 and benefit from trastuzumab ($P < .001$). In an exploratory analysis, the *FCGR3A*-158, high-affinity V/V homozygous patients received the most benefit (HR, 0.12; 95% CI, 0.05–0.28; $P < .001$), heterozygous F/V patients received intermediate benefit (HR, 0.34; 95% CI, 0.23–0.48; $P < .001$), and homozygous low-affinity F/F patients received the least benefit (HR, 0.71; 95% CI, 0.51–1.01; $P = .05$) (Figure 2). Similar results were observed using invariable models.

FCGR2A Polymorphism and Trastuzumab Efficacy

The *FCGR2A* polymorphism demonstrated the trends expected from preclinical models (Figure 2). *FCGR2A*-131, high-affinity H/H homozygous patients received the most benefit (HR, 0.31; 95% CI, 0.19–0.49; $P < .001$), heterozygous H/R patients received intermediate benefit (HR, 0.50; 95% CI, 0.37–0.68; $P < .001$), and homozygous low-affinity R/R patients received the least benefit (HR, 0.57; 95% CI, 0.35–0.92; $P = .02$) (Figure 2). However,

treatment interaction tests indicate no difference in trastuzumab efficacy according to *FCGR2A*-131 ($P = .24$ for H/H or H/R vs. R/R).

***FCGR* Polymorphism and Prognosis**

In an exploratory analysis in the ACT arm, patients with *FCGR3A*-158 V/For V/V had worse prognosis than patients with F/F (HR, 1.57; 95% CI, 1.15–2.14; $P = .005$) (eFigure 2 in the Supplement). On the other hand, in the ACTH arm, patients with *FCGR3A*-158 V/F or V/V had better prognosis than patients with F/F (HR, 0.68; 95% CI, 0.48–0.96; $P = .03$). Univariable models demonstrated similar results. *FCGR2A*-131 genotypes showed no evidence of prognosis in either arm.

Combined Genotypes

Additional exploratory analyses revealed approximately 15% of the population are homozygous for both of the low-affinity alleles (*FCGR3A*-158 F/F and *FCGR2A*-131 R/R; $n = 179$) (Figure 3). Trastuzumab appears less effective in these patients (HR, 0.81; 95% CI, 0.41–1.58; $P = .53$). However, treatment interaction tests of this population were not significant ($P = .06$ for F/F and R/R vs others).

Discussion

Analysis of *FCGR3A*-158 polymorphism in NSABP B-31 demonstrated differential trastuzumab benefit predicted by preclinical models, studies of other antibodies, and smaller studies of trastuzumab in the metastatic and neoadjuvant settings.⁷ Treatment interaction tests indicate polymorphisms at this position are associated with variability in the degree of benefit from adjuvant trastuzumab. Patients with the 158 F/F genotype have a better prognosis when treated with ACT and received less relative benefit (HR, 0.71) from the addition of trastuzumab, where as patients with 158 F/V or V/V have a worse prognosis with ACT and receive significantly more relative benefit from trastuzumab (HR, 0.31). These results indicate that ADCC may play a substantial component in the efficacy of trastuzumab for the treatment of breast cancer in the adjuvant setting; ADCC also activates tumor-antigen-specific cellular immunity via intercellular crosstalk among NK and dendritic cells, which may also enhance the efficacy of anti-ERBB2/HER2 therapy.^{22,23}

We also observed that the *FCGR2A*-131 polymorphism showed the expected trend for trastuzumab benefit but treatment interaction tests indicated no evidence of differential trastuzumab treatment effect according to *FCGR2A* genotypes. Perhaps this may be owing to the lack of expression of FC γ RIIa on NK cells because NK cells are thought to be the main effectors of ADCC; although cytotoxic effects mediated through other effector cells via FC γ RIIa have been reported.²⁴ Our observation that the significant differential benefit from trastuzumab was limited to the *FCGR3A*-158 polymorphism and was not seen in the *FCGR2A*-131 single-nucleotide polymorphisms may also be owing to the fact that FC γ RIIIa-158V was found to bind to IgG1 immune complexes at low concentrations but did not bind to FC γ RIIIa-158F. Conversely, no binding differences were detected between FC γ RIIIa-131H and 131R at low IgG1 concentrations.²⁵

Our results differ from recent reports from the NCCTG-N9831¹⁷ and BCIRG-006 trials,¹⁸ which found no association of *FCGR3A*-V158 and benefit from trastuzumab. These trials also examined the addition of trastuzumab to ACT in the adjuvant treatment of ERBB2/HER2-positive breast cancer and differed from NSABP B-31 primarily in the timing of the treatments. Both studies had sample sizes remarkably similar to our own and demonstrated substantial benefits from the addition of trastuzumab to chemotherapy. The methodologies used were rigorous: BCIRG-006 measured each single-nucleotide polymorphisms in quadruplicate (duplicate measurements on 2 platforms) and NCCTG-N9831 had 100% concordance in one-third of the population measured in duplicate. However, both of the previous *FCGR* studies suffered from sampling bias. In the BCIRG-006 study, the 1286 patients who were genotyped did not show significant benefit from trastuzumab (HR, 0.84; $P = .19$) and the authors acknowledged that they cannot exclude a contribution of *FCGR* polymorphisms to the outcome of trastuzumab-treated patients due to the sampling bias. *FCGR3A*-158 also deviated from the Hardy Weinberg Equilibrium in the BCIRG-006 study. Blood for genotyping was collected from NCCTG-N9831 patients after the trial closed to accrual, leading to differences from the study population and the intent-to-treat population. The NCCTG-N9831-genotyped cohort included a greater proportion of trastuzumab-treated patients, less nodal disease, smaller tumors, a greater number of hormone receptor-positive tumors, and patients who were slightly older than the entire NCCTG-N9831 cohort. As a result, the genotyped patients had a substantially better disease-free survival than the entire NCCTG-N9831 cohort. For both studies, these biases resulted in a loss of events and as a result reduced power to detect genotype-treatment interaction.

In our exploratory analysis, the observation that the *FCGR3A* low-affinity allele was associated with a better prognosis than the high-affinity allele in the ACT arm was unexpected. Chemotherapy has been shown to induce an immune response and possibly the differential binding affinity of FC γ RIIIa-158V and FC γ RIIIa-158F proteins to intrinsic antibodies may result in differential benefit from chemotherapy. Polymorphisms in other immune molecules such as TLR4²⁶ and P2RX7²⁷ have been reported as candidate biomarkers predicting progression-free survival associated with chemotherapy-elicited cell death in breast cancer. The observed direction for prognosis is in the opposite direction that might be expected based on the binding affinities of the 2 alleles and might represent a chance finding. However, the considerably diverse expression of this receptor on a number of different immune cells (macrophages, γ T cells, and many others) taken together with a wide variety of functions that often vary depending on the local microenvironment suggests that clinical outcomes can be very difficult to predict.

We have shown that patients with breast cancer with 1 or 2 of the *FCGR3A*-158V alleles receive greater benefit from trastuzumab than patients who are homozygous for the *FCGR3A*-158F allele. Although multivariable analysis showed that patients with the F/F genotype had a slightly higher residual risk, this difference was not remarkable. Thus, genotyping does not seem to be sufficient for selection of patients for additional treatment.

Currently, many therapies, including new anti-ERBB2/HER2 monoclonal antibodies or pan-tyrosine kinase inhibitors are being used in addition to trastuzumab to treat patients with breast cancer in the metastatic and neoadjuvant settings. These agents also engage the

immune system and boost trastuzumab-induced ADCC.^{28–30} Recent investigation of additional immune checkpoint inhibitors to anti-ERBB2/HER2 therapies in patients with ERBB2/HER2–positive metastatic breast cancer is based on the hypothesis that a substantial component of trastuzumab benefit is mediated through ADCC and cross-priming of antigen-specific cytotoxic T cells,^{22,23,31} which could enable the inhibition of immune checkpoints to improve the long-term efficacy of anti-ERBB2/HER2 therapy. However, carefully designed clinical trials would be needed to determine if additional agents enhance trastuzumab activity in specific genotypes.

Studies on NK cell mediated therapies suggest that the Fc region on monoclonal antibodies such as trastuzumab could be re-engineered to augment the affinity for a variety of inhibitory and activating FCGR allelotypes.^{10,32,33} Single amino acid changes to the antibody at the binding site can strongly influence the binding strength. This notion also requires additional clinical studies.

Conclusions

This study supports the hypothesis that ADCC activity plays an important role in the efficacy of trastuzumab. However, a great number of different molecules are involved in determining ADCC activity, therefore it may be useful to collect peripheral blood monocytes from patients in future clinical trials so that functional ADCC activity can be assessed and associated with treatment efficacy of trastuzumab and other therapeutic monoclonal antibodies.³⁴

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

Question

Do polymorphisms in FC γ receptors affect trastuzumab efficacy?

Findings

In this secondary analysis of 1251 patients with early-stage ERBB2/HER2–positive breast cancer enrolled in a randomized clinical trial, patients homozygous for *FCGR3A*-158 F benefited less from the addition of trastuzumab to chemotherapy than did patients with F/V or V/V genotypes.

Meaning

Significant association between polymorphisms of *FCGR3A* and trastuzumab benefit suggests that antibody-dependent cell-mediated cytotoxic effect plays a role in determining the efficacy of trastuzumab in the adjuvant treatment of ERBB2/HER2–positive breast cancer.

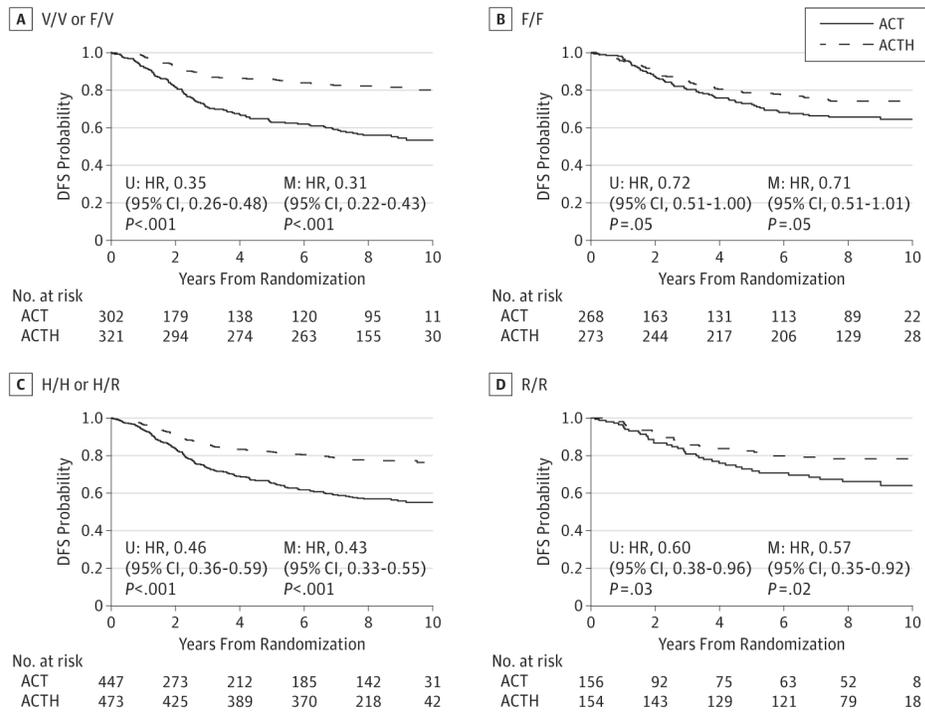


Figure 1. Treatment Effect According to FCGR Polymorphism
 ACT indicates doxorubicin and cyclophosphamide followed by paclitaxel; ACTH, doxorubicin and cyclophosphamide followed by paclitaxel with the addition of 1 year of weekly trastuzumab; DFS, disease-free survival; HR, hazard ratio; M, multivariable; U, univariable.

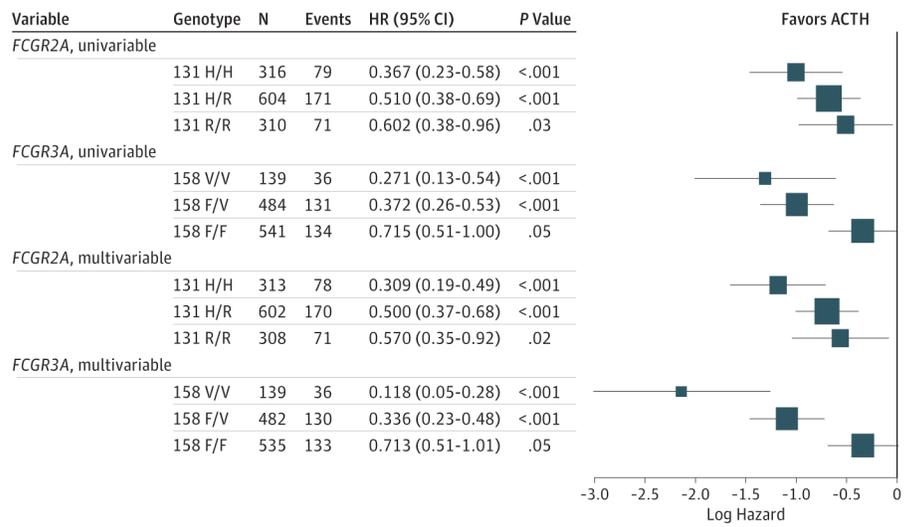


Figure 2. Cox Models Examining Treatment Affect According to Genotype
 The box size is proportional to the precision. ACTH, doxorubicin and cyclophosphamide followed by paclitaxel with the addition of 1 year of weekly trastuzumab; HR, hazard ratio.

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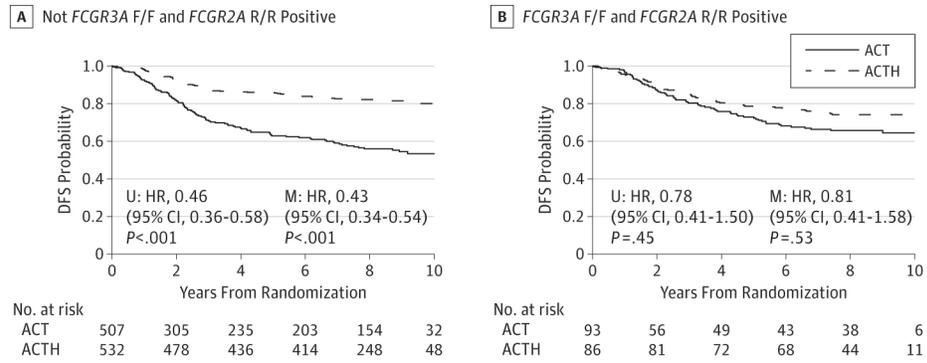


Figure 3. Combined Genotypes

ACT indicates doxorubicin and cyclophosphamide followed by paclitaxel; ACTH, doxorubicin and cyclophosphamide followed by paclitaxel with the addition of 1 year of weekly trastuzumab; DFS, disease-free survival; HR, hazard ratio; M, multivariable; U, univariable.