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Genetic Polymorphisms and Correlation with Treatment-Induced Cardiotoxicity and Prognosis in Patients with Breast Cancer

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ABSTRACT

Purpose: Cardiac toxicity is a serious potential complication of HER2-directed therapies and anthracyclines. *HER2* codon 655 and *SLC28A3* gene polymorphisms have been reported to be associated with cardiac toxicity from anti-HER2 and anthracycline therapy, respectively. Association of the polymorphism at *HER2* codon 655 with prognosis has also been reported.

Patients and Methods: Whole blood samples from patients treated on a randomized adjuvant breast cancer trial (BCIRG-006) that compared chemotherapy with or without trastuzumab plus either anthracycline or nonanthracycline chemotherapy were tested for genetic polymorphisms in *HER2* codon 655 and *SLC28A3*. Genotypes were correlated with cardiac function and disease-free survival (DFS) outcomes.

Results: Of 3,222 patients enrolled in BCIRG-006, 662 patient samples were successfully genotyped for the rs1136201 allele in

HER2 (codon 655): 424 (64%) were AA, 30 (4.5%) were GG, and 208 (31%) were AG genotype. In addition, 665 patient samples were successfully genotyped for the rs7853758 allele in the *SLC28A3* gene: 19 (3%) were AA, 475 (71%) were GG, and 171 (26%) were AG genotype. Follow-up time was 10 years. No correlation between DFS, cardiac event rate, or mean left ventricular ejection fraction (LVEF) and rs1136201 genotype was seen in the trastuzumab-treated or non-trastuzumab-treated patients. Moreover, mean LVEF and cardiac event rates were similar in all rs7853758 genotype groups treated with anthracycline-based therapy.

Conclusions: In the largest study to date to evaluate whether two polymorphisms are associated with DFS and/or cardiac toxicity in HER2-positive breast cancer treated with trastuzumab and/or anthracyclines, we observed no correlation.

Introduction

While outcomes for patients with early stage HER2-positive breast cancer have significantly improved with the use of HER2-targeted therapies combined with chemotherapy (1), cardiac toxicity remains an unpredictable yet serious complication of systemic therapy. In

addition, while other typical toxicities of chemotherapy such as nausea are now mostly preventable and treatable, the same cannot be said for cardiomyopathy. Rates of clinically evident congestive heart failure associated with trastuzumab range from 0.4% to 4% depending on whether an anthracycline is used (2–4). Sustained, subclinical loss of mean left ventricular ejection fraction (LVEF) has been reported in up to 18% and can persist for many years (2). The mechanism of trastuzumab-induced cardiac toxicity is not fully understood and is not dose-dependent. HER2 receptors in cardiomyocytes appear to have a significant role in growth and survival-related pathways. ErbB2, via activation of the phosphoinositide 3-kinase, protein kinase A and mitogen-activated protein kinase pathways, has a protective role in stress-related conditions, as well as maintenance of normal cardiac structure and function. Anti-HER2 treatments may disrupt these pathways, weakening the heart's cardioprotective and repair systems (3).

Various genetic polymorphisms in the *HER2* gene have been described with reports of single nucleotide polymorphisms (SNP) associated with prognosis as well as cardiac toxicity. One such polymorphism, originally reported in 1991 at codon 655 (rs1136201), results in placement of valine (GTC) instead of isoleucine (ATC) in the transmembrane domain of the *HER2* protein (4). It has been postulated that the less bulky valine isoform stabilizes the formation of dimers and enhances downstream signaling of the *HER2* protein (5). Based on this model, it is theorized that the Ile/Val (AG) isoform is more active than the Ile/Ile isoform and may lead to more aggressive tumors with greater sensitivity to trastuzumab therapy (5).

Cardiotoxicity is also a well-known, dose-dependent, yet unpredictable complication of anthracycline chemotherapy that appears to be caused from multiple stress-mediated mechanisms that generate

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Translational Relevance

In clinical practice, although there are some biomarker and imaging strategies to help identify subclinical cardiotoxicity in patients with breast cancer receiving trastuzumab and/or anthracycline, the reliable prediction of which patients with normal baseline cardiac function will go on to develop cardiac toxicity is challenging and unclear. In our study, we examined two genetic polymorphisms that have been reported in the literature to be associated with cardiac toxicity and/or disease-free survival (DFS). We found no correlation with either polymorphism with cardiac toxicity or DFS.

reactive oxygen species causing oxidative damage, along with inhibition of topoisomerase β causing breaks in DNA (6). When given in combination with anti-HER2 treatments that compromise repair mechanisms, this can be a synergistic “double-hit” on the heart making cardiac dysfunction more probable. Cardioprotective strategies have had limited efficacy in attenuating risk during treatment (7).

Association between a synonymous coding variant rs7853758 within the solute carrier family 28 member 3 (*SLC28A3*) gene and anthracycline-induced cardiotoxicity have been reported, primarily in pediatric patients. The minor allele (A) of rs7853758 affects *SLC28A3* mRNA levels and is thought to be cardioprotective compared to the G allele. *SLC28A3* (also known as human concentrative (Na⁺)-nucleoside cotransporter 3, CNT3) plays an important role in mediating the cellular entry of a broad array of nucleosides and synthetic anticancer nucleoside analog drugs (8). It may play a role in transport of anthracyclines into cells and hence its possible role in cardiac toxicity from these drugs (9).

Using samples from a large, randomized adjuvant clinical trial of trastuzumab based-therapy in which both anthracycline and non-anthracycline regimens were tested, we aimed to evaluate whether the rs1136201 polymorphism in *HER2* is associated with cardiac toxicity with or without use of trastuzumab and whether the rs7853758 coding variant in *SLC28A3* is correlated with the development of cardiomyopathy in anthracycline-treated patients. We also aimed to evaluate whether the *HER2* codon 655 SNP (rs1136201) is associated with disease-free survival (DFS) with or without therapy with trastuzumab.

Patients and Methods

Patients

As previously reported, from April 2001 through March 2004, 3,222 patients with HER2-positive, high-risk breast adenocarcinoma were enrolled on BCIRG-006 and randomly assigned to receive a standard adjuvant anthracycline–taxane chemotherapy regimen (doxorubicin/cyclophosphamide followed by docetaxel, AC-T), the same regimen plus trastuzumab (AC-TH), or a non-anthracycline, trastuzumab-based regimen with docetaxel and carboplatin (TCH) as its backbone (2). Of these patients, 1,286 signed an optional consent upon enrollment to have blood samples sent to central laboratory for exploratory analysis. These samples were stored at -80 degrees. A total of 666 patients had adequate samples for additional analysis for this study (Fig. 1). The study was conducted according to Institutional Review Board/Ethics Committee approved protocols. Outcome data from BCIRG-006 came from the final analysis with a median follow-up of 10.4 years (10).

This study was conducted in accordance with Declaration of Helsinki and International Ethical Guidelines for Biomedical Research Involving Human Subjects. Informed written consent was obtained from each subject.

Genotyping analysis

DNA was purified from whole blood samples using a QIAamp DNA Blood Mini Kit (Qiagen) and used for nested PCR amplification of regions containing the rs1136201 and rs7853758 sequences. PCR was carried out using Phusion Hot Start High-Fidelity DNA Polymerase (New England Biolabs) according to the manufacturer’s recommended protocols. The PCR products were purified using a Qiagen PCR Clean Kit (Qiagen) and then sequenced on an ABI3730XL (Applied Biosystems) using BigDye Terminator v3.1 chemistry. PCR products were also analyzed on a MassARRAY 114 analyzer (Sequenom) using Sequenom’s iPLEX Gold assay for *HER2* A>G polymorphism at amino acid codon 655 (rs1136201) and variant rs7853758 (L461L) within the *SLC28A3* gene.

Cardiac safety

An intensive cardiac-monitoring schedule was incorporated into the BCIRG-006 study in which the LVEF was measured for each patient seven times throughout the study for up to 10 years to detect congestive heart failure or sustained asymptomatic subclinical loss of mean LVEF, which was defined as a relative reduction from baseline of more than 10% at any of the measured time points. An independent cardiac review panel whose members were unaware of study-group assignments reviewed all cases of suspected cardiac events.

Statistical considerations

The primary aim was to determine genotype polymorphisms of rs1136201 and rs7853758 in a large trial of patients with non-metastatic *HER2*-amplified breast cancer treated with trastuzumab and/or anthracycline-based therapy and to assess correlation of either polymorphisms with the development of cardiac toxicity. Secondary aim was to correlate the *HER2* polymorphisms with DFS in trastuzumab-treated patients. DFS was calculated from first exposure to trastuzumab or anthracycline to the time of disease progression or death from any cause. DFS curves were estimated using the method of Kaplan–Meier. The effect of trastuzumab and the prognostic impact of genotype were assessed using the log-rank test. Comparison of baseline characteristics between the genotypes were tested using χ^2 test for discrete data or Analysis of Variance for continuous data. A model for repeated measures including genotype, time and interaction between time and genotype was used to assess the effect of genotype on LVEF overtime. An alpha of 0.05 was used as the cut off for significance for two-sided statistical testing.

Data were generated by the authors and included in the article.

Results

Patient population

Of 3,222 patients enrolled in the BCIRG-006 trial, 1,286 provided consent for additional blood sample collection and, after other correlative studies were performed on these samples, adequate sample was remaining for genotyping for this study from 666. Of the 666 patients analyzed, 216 patients were treated with AC-T, 226 with TCH, and 224 with AC-TH. Baseline characteristics of genotyped patients were fairly balanced across treatment arms (Table 1). The clinical and tumor characteristics of the patients genotyped in our study compared

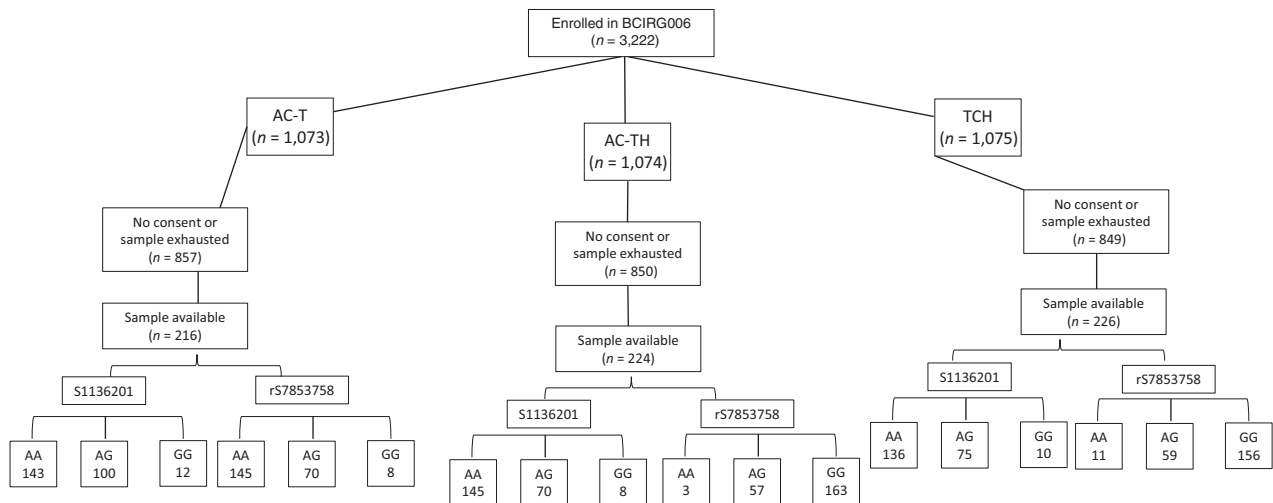


Figure 1.

CONSORT diagram. Schematic showing overall patient population in BCIRG006 trial, the number of patients in each arm and number who had samples available for analysis in the current study. AC-T: Adriamycin, Cytosan, Taxol; AC-TH: AC-T plus trastuzumab; TCH: Taxotere, carboplatin, trastuzumab.

with the patients who were not genotyped also appear similar (Supplementary Table S1). Compared with the overall results of the BCIRG-006 study, in the subset of patients genotyped in this analysis, a less robust improvement in DFS was observed for the trastuzumab arms than control arm [HR, 0.82; 95% confidence interval (CI), 0.57–1.18]. When stratified for prognostic features, the hazard ratio in favor of trastuzumab was consistent with that of the overall study (HR, 0.67; 95% CI, 0.45–1.01).

Genotype frequency

Samples from 662 patients were successfully genotyped for rs1136201. Of these, 424 (64%) were AA, 30 (4.5%) were GG, 208 (31%) were AG genotype. Samples from 665 patients were successfully

genotyped for rs7853758. Of these, 19 (3%) were AA, 475 (71%) were GG, and 171 (26%) were AG genotype. **Table 2** shows baseline patient and tumor characteristics for each genotype. The frequency of alleles was in conformity with Hardy Weinberg Equilibrium (Supplementary Table S2). No statistically significant difference in characteristics were found among the genotypes.

Genotype and DFS

DFS associated with the rs1136201 genotype in non-trastuzumab-treated patients and trastuzumab-treated patients are shown in **Figs. 2** and **3**, respectively. Although the Kaplan–Meier curve suggests a worse DFS with the GG genotype in the first 3 years in patients who did not receive trastuzumab, the 10-year DFS in patients was similar among

Table 1. Demographic and tumor characteristics for patients included in current analysis according to treatment received.

		AC-T(%, N = 216	AC-TH (%, N = 224	TCH(%, N = 226	Genotyped, Trastuzumab- containing arms (N = 450)	Genotyped, Anthracycline- containing arms (N = 440)	All trastuzumab- containing arms (n = 2,149)
Age	Median	49.7	48.5	47.9	48.2	49.1	
Menopausal status	Premenopausal	105 (49%)	102 (46%)	86 (38%)	188 (42%)	207 (47%)	
	Postmenopausal	111 (51%)	122 (54%)	140 (62%)	262 (58%)	233 (52.3%)	
Tumor grade	G1	3 (1.4%)	2 (0.9%)	2 (0.9%)	4 (0.9%)	5 (1.1%)	
	G2	55 (25%)	61 (27%)	63 (28%)	124 (28%)	116 (26.4%)	
	G3	154 (71%)	157 (70%)	158 (70%)	315 (70%)	311 (70.7%)	
Hormone receptor status	ER and PR negative	113 (52%)	127 (57%)	132 (58%)	259 (58%)	240 (54.5)	992 (46%)
	ER and/or PR positive	103 (48%)	97 (43%)	94 (42%)	191 (42%)	200 (45.5%)	1,157 (54%)
Tumor size	≤2 cm	92 (43%)	84 (40%)	88 (39%)	177 (39%)	176 (40%)	844 (39%)
	>2 cm	124 (57%)	135 (60%)	138 (61%)	273 (61%)	259 (58.9%)	1,303 (61%)
Number of positive lymph nodes	0	68 (31%)	71 (32%)	75 (33%)	146 (32%)	139 (31.6%)	613 (29%)
	1, 2, or 3	80 (37%)	88 (39%)	87 (38%)	175 (39%)	168 (38.2%)	825 (38%)
	4 or more	68 (31%)	65 (29%)	64 (38%)	129 (29%)	133 (30.2%)	711 (33%)
Cardiac signs or symptoms baseline ^a		55 (25%)	67 (30%)	58 (26%)	125 (28%)	122 (28%)	

Abbreviations: ER, estrogen receptor; PR, progesterone receptor.

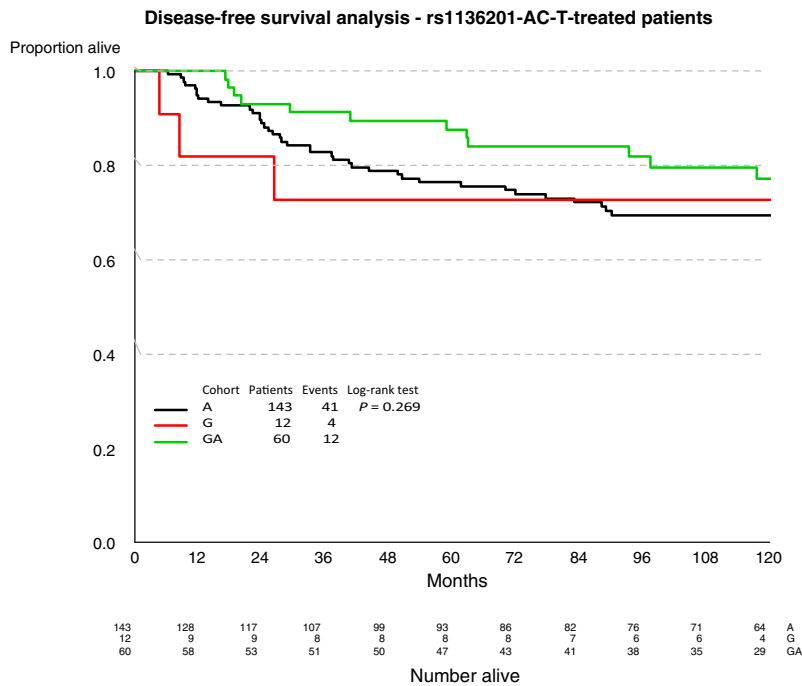
^aPreexisting conditions mostly consisting of hypertension, tachycardia, history of valve disorders, etc. and not heart failure.

Table 2. Demographic and tumor characteristics for patients according to genotype allele.

		Ile655Val			rs7853758		
		A	G	GA	A	G	GA
		N = 424	N = 30	N = 208	N = 19	N = 475	N = 171
Age	Median	49	45.5	49	51	49	48
Menopausal status	Premenopausal	194 (46%)	10 (33%)	88 (42%)	8 (42%)	215 (45%)	70 (41%)
	Postmenopausal	230 (54%)	20 (67%)	120 (58%)	11 (58%)	260 (55%)	101 (59%)
Hormone receptor status	ER and PR negative	246 (58%)	12 (40%)	111 (53%)	12 (63%)	264 (56%)	96 (56%)
	ER and/or PR positive	178 (42%)	18 (60%)	97 (47%)	7 (37%)	211 (44%)	75 (44%)
Tumor size	≤2 cm	258 (61%)	21 (70%)	117 (56%)	13 (68%)	279 (59%)	104 (61%)
	>2 cm	166 (39%)	9 (30%)	91 (44%)	6 (32%)	196 (41%)	67 (39%)
Number of positive lymph nodes	0	137 (32%)	7 (23%)	68 (33%)	6 (32%)	155 (33%)	53 (31%)
	1, 2, or 3	160 (38%)	8 (27%)	86 (41%)	10 (53%)	177 (37%)	68 (40%)
	4 or more	127 (30%)	15 (50%)	54 (26%)	3 (16%)	143 (30%)	50 (29%)
Treatment arm	AC-T	143 (34%)	12 (40%)	60 (29%)	5 (26%)	156 (33%)	55 (32%)
	AC-TH	145 (34%)	8 (27%)	70 (34%)	3 (16%)	163 (34%)	57 (33%)
	TCH	136 (27%)	10 (33%)	78 (38%)	11 (58%)	156 (33%)	59 (35%)
Cardiac signs or symptoms at baseline ^a		116 (27%)	6 (20%)	57 (27%)	4 (21%)	129 (27%)	47 (27%)

Abbreviations: ER, estrogen receptor; PR, progesterone receptor.

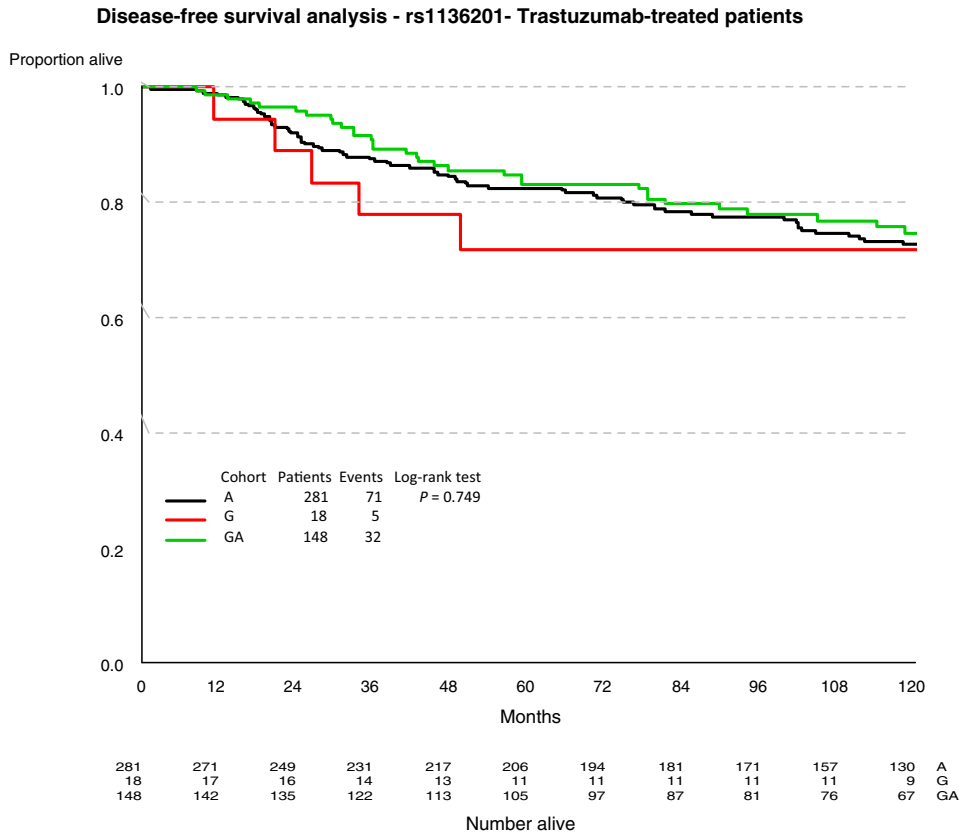
^aPreexisting conditions mostly consisting of hypertension, tachycardia, history of valve disorders, etc. and not heart failure.



Genotype	DFS at 10 years	95% Lower CI	95% Upper CI
A	0.69508	0.60654	0.76750
G	0.72727	0.37079	0.90283
GA	0.77132	0.62993	0.86425

Figure 2.

DFS outcome based on rs1136201 genotype in non-trastuzumab (AC-T)-treated patients.



Genotype	DFS at 10 years	95% Lower CI	95% Upper CI
A	0.72675	0.66640	0.77801
G	0.71795	0.44877	0.87194
GA	0.74601	0.65831	0.81436

Figure 3. DFS outcome based on rs1136201 genotype in trastuzumab (AC-TH, TCH)-treated patients.

the different genotypes (AA, 70%; GG, 73%; GA, 77%; $P = 0.27$; **Fig. 2**). Thus, the worse prognosis associated with the G-allele in HER2-positive breast cancer in the absence of trastuzumab was not confirmed (11). There was no difference in DFS among the genotypes in trastuzumab-treated patients (AA, 73%; GG, 72%; GA, 75%; $P = 0.75$; **Fig. 3**).

Genotype and heart function

There was no correlation seen between mean LVEF and HER2 genotype at rs1136201 in patients treated with trastuzumab as confirmed by the use of a model for repeated measures on LVEF over time ($P = 0.70$; **Fig. 4A**). Cardiac dysfunction more broadly defined as >10% decline in LVEF or clinical congestive heart failure developed in 16% of patients with AA, 17% of patients with GG, and 20% of patients with AG ($P = 0.65$). Similar to decline in LVEF, there was no correlation between the more broadly defined cardiac dysfunction and HER2 genotype. When evaluating the AC-TH cohort separately,

there was a small statistically significant difference in cardiac dysfunction with the addition of trastuzumab to anthracyclines, in the rs1136201, GA ($P = 0.047$) allele (**Fig. 4B**). No difference was seen in the TCH cohort among the 3 alleles ($P = 0.1216$; **Fig. 4C**).

There was also no correlation between mean LVEF and variant rs7853758 (*SLC28A3*) in patients treated with an anthracycline as confirmed by the use of a model for repeated measures on LVEF over time ($P = 0.55$; **Fig. 5A**). The percentage of patients who developed cardiac dysfunction was 13%, 17%, and 21% in AA, GG, and AG genotypes respectively ($P = 0.34$), without a correlation. No difference was seen when patients treated with AC-T or AC-TH were analysed separately (**Fig. 5B and C**).

Discussion

In the largest study to date of over 660 patients evaluating the relationship between cardiac toxicity and DFS with HER2

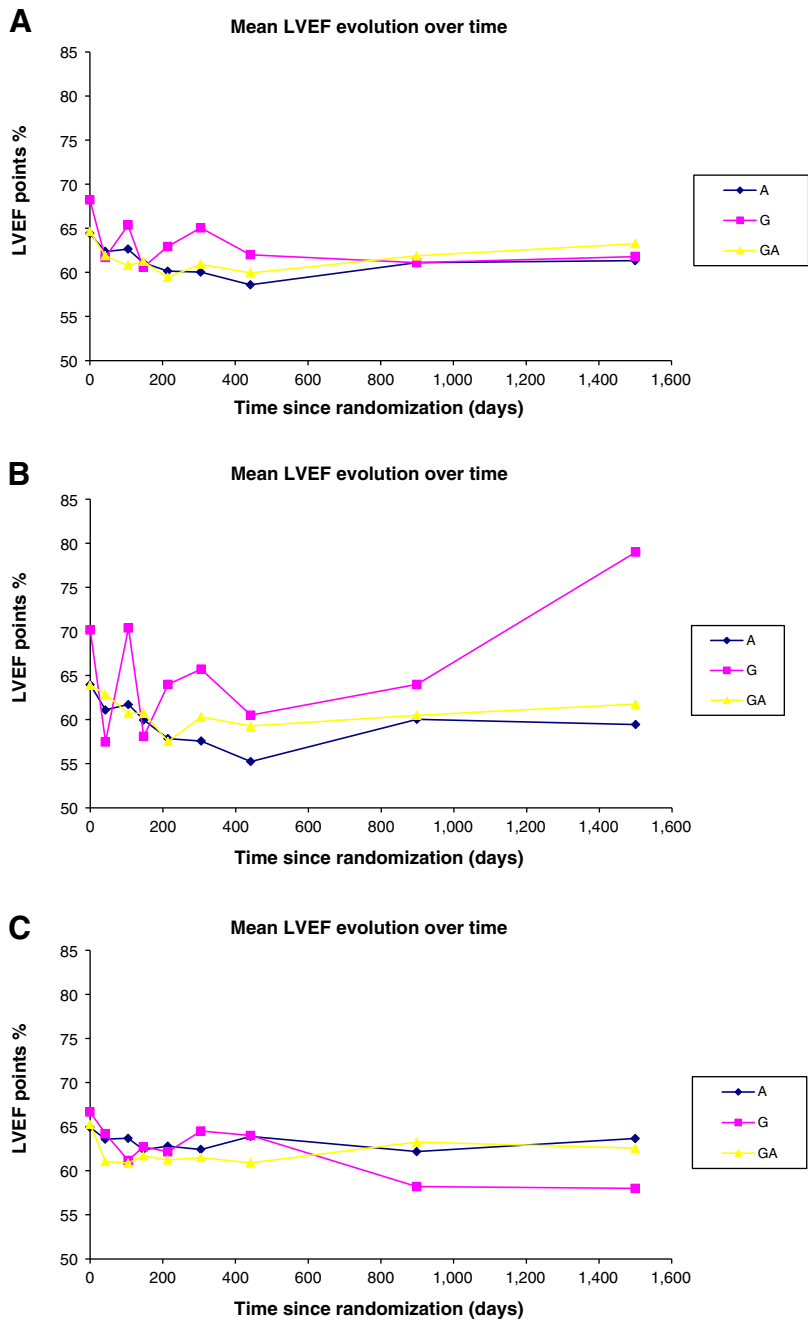


Figure 4. **A**, Mean LVEF according to rs1136201 genotype in patients treated with trastuzumab (AC-TH and TCH arms). **B**, Mean LVEF according to rs1136201 genotype in patients treated with AC-TH. **C**, Mean LVEF according to rs1136201 genotype in patients treated with TCH.

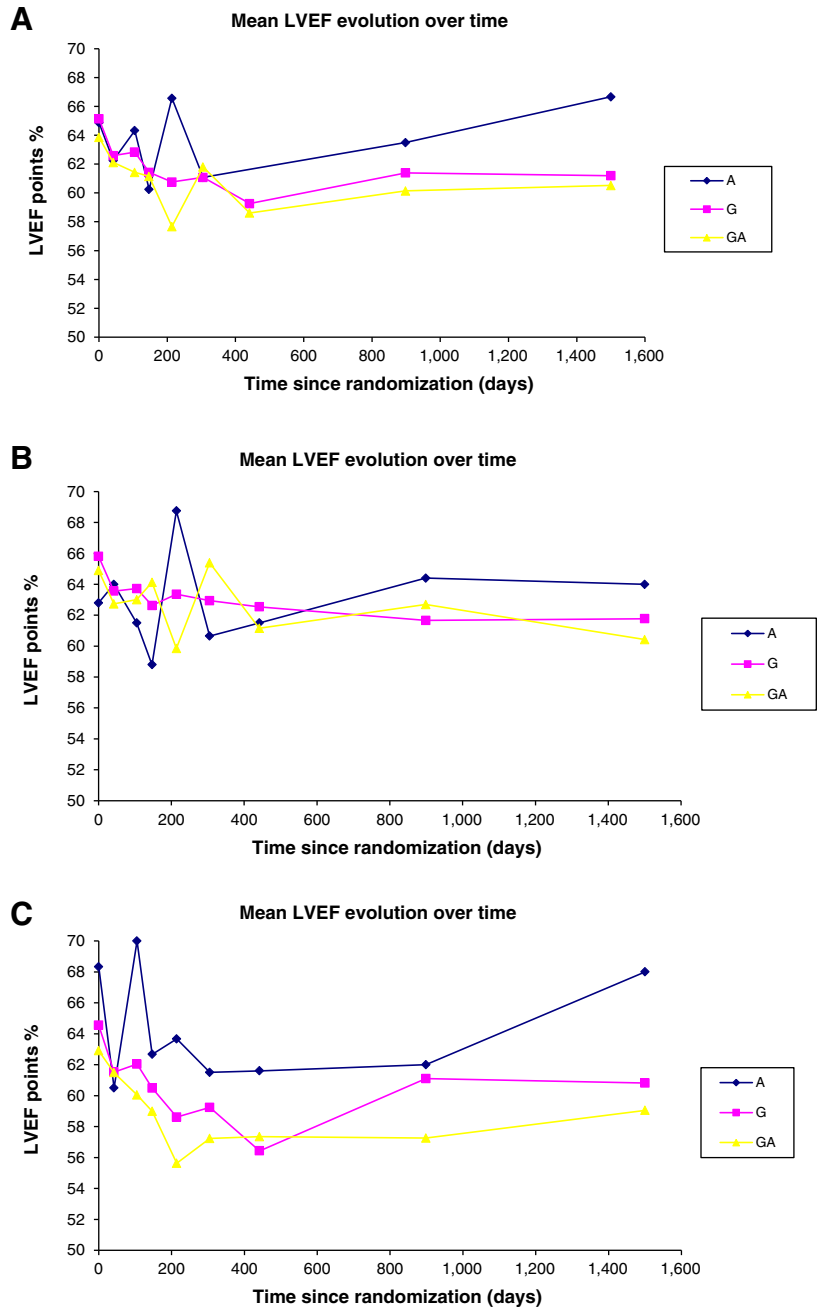
polymorphism in trastuzumab-treated patients, we did not find a correlation with *HER2* codon 655 (rs1136201) genotype and trastuzumab-induced cardiac toxicity or DFS. Neither did our study show a correlation between *SLC28A3* variant rs7853758 (L461L) and anthracycline-induced cardiotoxicity. Another study of over 1,000 patients published this year similarly did not show a correlation between *HER2* codon 655 and cardiac toxicity with our results (12). This study did not assess for association for DFS.

Previously reported studies showing a possible association between these polymorphisms and either cardiac toxicity or DFS had smaller sample sizes. A study of 140 patients published in 2015 was negative for cardiac toxicity correlation while an earlier study published in 2007 of

61 patients found all the cardiac toxicity to occur in patients with AG genotype (8, 7). Another study published in 2015 of 78 patients with *HER2*-positive early breast cancer treated with trastuzumab reported a correlation of the AG genotype with cardiac toxicity (13). Another study of 91 patients (14) and one involving 66 patients showed similar findings with the AG/GG genotype showing a correlation with increased cardiac toxicity (15). Similarly, there have been contradictory results in terms of survival correlation with this polymorphism. In one meta-analysis study, the *HER2* rs1136201 polymorphism was evaluated for association with DFS in 4,167 patients with operable breast cancer (11). In patients with *HER2*-positive disease who did not receive trastuzumab ($N = 816$), presence of the G allele was associated

Figure 5.

A, Mean LVEF according to rs7853758 genotype in patients treated with an anthracycline (AC-T and AC-TH arms). **B,** Mean LVEF according to rs7853758 genotype in patients treated with AC-T. **C,** Mean LVEF according to rs7853758 genotype in patients treated with AC-TH.



with a worse DFS compared with those who were homozygous for A, while in patients who received trastuzumab ($N = 212$), presence of the G allele was associated with a better DFS compared with those with the AA genotype. On the other hand, the previously positive study for correlation with cardiac toxicity was negative for a link with DFS (7).

Limitation of our study is that while it is the largest to date for trastuzumab-treated patients, it included only 21% of patients from the BCIRG-006 study for the analysis. That said, baseline tumor and patient characteristics in genotyped patients were fairly representative of the overall population and characteristics were also evenly distributed amongst the treatment arms. Moreover, in contrast to other published studies, this analysis has over 10-year follow-up for both DFS and cardiac outcomes associated with it. Polymorphisms are not

reported in the context of known cardiac risk and while we did assess for correlation of other clinical factors associated with cardiac disease, this will be the subject of another manuscript.

The underlying pathophysiology of cardiac toxicity from trastuzumab is not completely clear. A hypothesized mechanism for trastuzumab-induced cardiac dysfunction is the neuregulin (NRG) ERBB pathway. Neuregulin is a ligand for the HER3 and HER4 receptors that, upon binding, causes dimerization with HER2 and subsequent activation of the ERBB pathway (16). This pathway appears linked to myocyte survival and especially response to stress (17). Patients with stable chronic heart failure have been found to have high levels of circulating NRG (18). This hypothesis has not yet been proven. Preclinical data showed possible enhanced dimerization of the HER2

molecule based on rs1136201 Ile/Val polymorphism and was the basis for the hypothesis that it could affect cardiac toxicity and DFS (5). The mechanism of cardiac toxicity from anthracyclines is clearer and likely related to formation of free radicals during metabolism. The reactive oxygen species that are produced by doxorubicin metabolism in cardiomyocytes subsequently cause cell death through apoptotic pathways (19). Cardiotoxicity from trastuzumab, in the absence of anthracyclines, is largely reversible upon discontinuation and not dose-dependent while that of anthracyclines—including in combination with anti-HER2 treatments—is cumulative, dose-dependent and often irreversible if not identified and treated promptly with cardioprotective agents (20). Although SLC28A3 appears to play a role in transport of anthracyclines into cells and has been implicated in the development of anthracycline-induced cardiac toxicity, we failed to confirm this association (21). In three studies in children treated with anthracyclines, the rs7853758 variants were linked to increased risk of cardiac toxicity (9, 22, 23). Those studies reported conflicting results, however, with the minor A allele found to be protective for cardiac toxicity in one (9) but associated with increased toxicity in another (23).

The question of whether risk of cardiac toxicity by anthracyclines and anti-HER2 therapy can be predicted remains to be answered. We did not detect an association between two genotypes and the development of cardiac toxicity in a large population. However, this does not rule out whether different genomic polymorphisms in the same genes or different genes altogether play a role in cardiac toxicity. Rapid and efficient next-generation sequencing methods are needed that can identify a potential pattern amongst many genes that can correlate with cardiac toxicity. The search for meaningful markers continues.

Authors' Disclosures

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Authors' Contributions

P.F. Peddi: Conceptualization, writing—review and editing. **P.A. Fasching:** Conceptualization, writing—review and editing. **D. Liu:** Writing—review and editing. **E. Quinaux:** Methodology. **N.J. Robert:** Writing—review and editing. **V. Valero:** Writing—review and editing. **J. Crown:** Writing—review and editing. **C. Falkson:** Writing—review and editing. **A. Brufsky:** Writing—review and editing. **J.M. Cunningham:** Writing—review and editing. **R.M. Weinshilboum:** Writing—review and editing. **T. Pienkowski:** Writing—review and editing. **W. Eiermann:** Writing—review and editing. **M. Martín:** Writing—review and editing. **V. Bee:** Writing—review and editing. **X. Wang:** Writing—review and editing. **L. Wang:** Writing—review and editing. **E. Yang:** Writing—review and editing. **D.J. Slamon:** Writing—review and editing. **S.A. Hurvitz:** Conceptualization, writing—review and editing.

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