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## Genome-wide association study of cardiotoxicity in NCCTG N9831 (Alliance) Adjuvant Trastuzumab Trial

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### Abstract

**Objectives**—The major clinical side-effect of the *ERBB2* targeted breast cancer therapy, trastuzumab, is a decline in left ventricular ejection fraction (LVEF). Improved markers are needed to better identify patients susceptible to cardiotoxicity.

**Methods**—The NCCTG N9831 trial compared adjuvant doxorubicin and cyclophosphamide followed by either weekly paclitaxel (Arm A); paclitaxel then trastuzumab (Arm B); or concurrent paclitaxel and trastuzumab (Arm C) in patients with HER2-positive breast cancer. A GWAS was performed on all patients with available DNA (N=1,446). We used linear regression to identify SNPs associated with decline in LVEF, adjusting for age, baseline LVEF, anti-hypertensive medications and the first two principle components.

**Results**—618,863 SNPs passed quality control (QC) and DNA from 1,191 patients passed genotyping QC and were identified as whites of non-Hispanic origin. SNPs at six loci were associated with a decline in LVEF ( $p=7.73\times 10^{-6}$  to  $8.93\times 10^{-8}$ ), *LDB2*, *BRINP1*, chr6 intergenic, *RAB22A*, *TRPC6* and *LINC01060*, in patients who received chemotherapy plus trastuzumab (Arms BC, N=800). None of these loci were significant in patients who received chemotherapy only (Arm A, N=391) and did not increase in significance in analysis of all patients combined. We did not observe association,  $p<0.05$  with SNPs previously associated with trastuzumab induced cardiotoxicity at *ERBB2*, I655V and P1170A. We replicated association,  $p<0.05$ , with SNPs previously associated with anthracycline cardiotoxicity at *CBR3* and *ABCB1*.

**Conclusions**—Our study identified six putative novel cardiotoxicity loci in patients treated with combination chemotherapy and trastuzumab that require further investigation and confirmed known associations of anthracycline-induced cardiotoxicity.

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## Keywords

trastuzumab; doxorubicin; breast cancer; GWAS; cardiotoxicity; cardio-oncology; ERBB2; CBR3; ABCB1

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## Introduction

The targeted ERBB2 therapy, trastuzumab, has had a tremendous impact on the management of patients with HER2+ advanced and early breast cancer. The major adverse event for this drug is cardiotoxicity, but symptoms are not uniform across patients and differ from the well-known dose-dependent effect of anthracyclines<sup>1</sup>.

There are no clinically proven markers to predict which patients will experience cardiotoxicity following trastuzumab treatment. Left ventricular ejection fraction (LVEF) <55%, age >60 years, and antihypertensive medication prior to treatment are known risk factors<sup>2</sup>. The observed variability of cardiac symptoms in humans and animal models suggest a genetic influence. ErbB2-deficient conditional mutant adult mice develop a phenotype consistent with dilated cardiomyopathy (DCM)<sup>3,4</sup>. Some forms of heart disease presenting with decreased LVEF in humans, such as familial dilated and hypertrophic cardiomyopathy, are known to have a strong heterogeneous genetic basis<sup>5</sup>. 197 published rare variants in more than 30 causative genes have been reported in the literature<sup>6</sup>. Common variants in these genes are over-represented in sporadic cases<sup>7–9</sup> suggesting the existence of modifying or risk variants that could be exacerbated by environmental stress factors such as trastuzumab and/or chemotherapy. To date, two common genetic polymorphisms within *ERBB2*, Ile655Val and Pro1170Ala, have been associated with an increased risk of trastuzumab-related cardiotoxicity, but sample sizes were small (N ranging 61–140 patients)<sup>10–14</sup>. Polymorphisms in several genes including *ABCB1*, *CBR3*<sup>15,16</sup>, *RAC2*, *NCF4*<sup>17,18</sup> and *SLC28A3*<sup>19,20</sup> have been associated with anthracycline-related cardiotoxicity.

Trastuzumab-related cardiotoxicity commonly manifests as an asymptomatic decline in LVEF that is mostly reversible when treatment is stopped. However, it remains unclear whether trastuzumab therapy could have long-lasting effects on cardiac function such as progressive cardiac remodeling and DCM. This adverse effect of trastuzumab has resulted in strict eligibility criteria in clinical trials and/or discontinuation prior to completion of treatment; 5% of almost 3,500 eligible patients in the NSABP-B31 and N9831 trials were not allowed to initiate trastuzumab therapy and a further 5% of initially eligible women discontinued treatment due to cardiac side effects<sup>21,22</sup>. Data from multiple randomized adjuvant trials and observational studies suggest that the rate of discontinuation of trastuzumab treatment ranges between 6–31%, mainly due to cardiotoxicity. In patients aged 65 years old, this figure increases to a notable and clinically relevant rate of 41%<sup>23</sup>. Identification of genetic variants that predict which patients are predisposed to cardiotoxicity could improve patient treatment and management.

Observations of trastuzumab related cardiotoxicity are further complicated by the use of anthracyclines prior to trastuzumab treatment, particularly as anthracyclines can result in

late-onset cardiac dysfunction<sup>24</sup>. All patients in the N9831 study received anthracyclines and paclitaxel prior to trastuzumab. The patients who had LVEF dysfunction at 6 years follow-up were the same patients who had LVEF dysfunction during trastuzumab-paclitaxel therapy<sup>25</sup>. In addition, LVEF dysfunction at 6 years was not statistically different among the chemotherapy only and chemotherapy plus trastuzumab treatment arms, so long-term LVEF dysfunction may not be related to trastuzumab but, possibly, to exposure to anthracyclines and/or increasing age. This is an important point because identification of genetic variants that predict which patients are predisposed to anthracycline cardiotoxicity could allow these patients to use a different chemotherapy regimen, allowing them to be eligible for and to complete trastuzumab therapy. The large size, design and detailed cardiac data collected in N9831 allowed us to examine the genetic influence of trastuzumab on cardiotoxicity in the context of both anthracycline chemotherapy and combination chemotherapy and trastuzumab.

## Methods

Detailed information about the methods and study population are available in supplemental material. Briefly, genome-wide genotyping was performed by Affymetrix Axiom array from which 618,793 SNPs passed genotyping quality control. Primary analysis consisted of patients from N9831, Arms BC (N=800 individuals) who received doxorubicin, paclitaxel and trastuzumab (Figure 1). We performed statistical analyses using R version 3.1.1, PLINK version 1.07 and Locus Zoom<sup>26</sup>. Linear regression was used for change in LVEF (lowest recorded LVEF - baseline LVEF), adjusting for age, baseline LVEF, anti-hypertensive medications and the first two principle components in the 800 patients in Arms BC who received combination chemotherapy and trastuzumab.

We report novel associations at  $p < 1 \times 10^{-5}$  in these patients. We also report on published associations of trastuzumab-related cardiotoxicity of the variants *ERBB2* Ile655Val and Pro1170Ala<sup>10,12–14</sup> from patients in Arms BC and on published associations of anthracycline-related cardiotoxicity at the *ABCB1*, *CBR3*, *RAC2*, *NCF4*, *SLC28A3*, *RARG* and *UGT1A6* loci<sup>27</sup> in patients from Arms A, B and C combined, who all received doxorubicin and paclitaxel.

## Results

### Suggestive loci associated with combination chemotherapy and trastuzumab induced decline in LVEF

Seeking modifying/susceptibility genes for cardiotoxicity in patients undergoing treatment for HER2-positive breast cancer, we used a genome-wide association approach. Our primary analysis consisted of DNA samples from 800 patients (Arms BC) who received doxorubicin, paclitaxel and trastuzumab and were determined to be of White/non-Hispanic origin with complete LVEF data from baseline (prior to doxorubicin) and up to two years post treatment.

This analysis yielded six regions at  $p < 1 \times 10^{-5}$ , with associated variants within or close to *LDB2*, *BRINP1*, an intergenic region on chromosome 6, *RAB22A*, *TRPC6* and *LINC01060* (Table 1), (QQ and Manhattan plots, Supplementary Figure 2). Locus zoom plots of each

region are shown in Supplementary Figure 3. The most significant variant, rs55756123,  $p=8.93\times 10^{-8}$  mapped within 500bp of the 3' UTR of *LDB2*. Imputation did not identify any additional variants at this level of significance or any additional coding variants at  $p<0.05$ . In the subset of patients with congestive heart failure (CHF) (N=10) versus those without (N=789), a Fisher's exact test of rs55756123 genotype counts was significant,  $p=0.007$ , suggesting enrichment of the associated allele in individuals with the worst cardiac outcome.

We observed a total of 18 SNPs,  $p<1\times 10^{-5}$  at the *BRINP1* locus; the most significant an intronic variant, rs62568637,  $p=6.01\times 10^{-7}$  and variants in the 3' flanking region, rs10117876 and rs7038023,  $p=5.86\times 10^{-7}$  in high LD,  $r^2=0.815$ . Of note, rs62568637 and flanking SNPs on either side showed positive GERP scores (ranging 1.46–4.51) suggestive of sequence conservation (Supplementary Table 4). Imputation at this region identified a more significant 3' flanking variant, rs7037449,  $p=1.87\times 10^{-7}$  in high LD with the other 3' flanking variants.

rs4305714,  $p=1.39\times 10^{-6}$  maps to an intergenic region on chromosome 6p22.3. Imputation across the region was supportive, yielding an additional 18 variants at  $p<1\times 10^{-5}$  and two additional variants in high LD, rs72836853 and rs10485148 at  $p=9.85\times 10^{-7}$ .

A common variant (MAF=0.40) within the 3' UTR of *RAB22A*, rs707557, was associated at  $p=5.62\times 10^{-6}$ . The positive GERP score for this variant (2.47) may suggest some level of sequence conservation at this locus. Imputation across this region yielded two additional SNPs at the same level of significance in very high LD, rs1045408 and rs6128327,  $p=3.48$  and  $3.38\times 10^{-6}$ , respectively, both of which map to sequences with very high GERP scores (ranging 4.32–5.40).

An intronic variant, rs77679196, within *TRPC6* was associated at  $p=7.72\times 10^{-6}$ . Imputation across the region yielded one additional SNP in the 5' flanking region, with a slightly stronger association, rs75865789,  $\beta=-8.62$  (95% CI -12.07 to -5.178),  $p=1.14\times 10^{-6}$ . In the subset of patients with CHF (N=10) versus those without (N=789), a Fisher's exact test of rs77679196 genotype counts was significant ( $p=0.019$ ) suggesting enrichment of the associated allele in individuals with the worst cardiac outcome.

Associated variants in the final significant region were entirely intergenic, the closest gene being *LINC01060*. Imputation supported the most significant SNP, rs7698718, with an additional variant at  $p<1\times 10^{-5}$  all in high LD, but no variants of greater association were identified.

We reasoned that variants associated with cardiotoxicity specifically from doxorubicin would be significant or show a trend in the chemotherapy Arm only and report these data separately in Table 1. None of the six regions from our discovery analyses of patients who received chemotherapy in combination with trastuzumab (N=800) were significant at  $p<0.05$  (1 tailed) in the patients who received chemotherapy only (N=391). *LDB2* and *RAB22A* showed an effect in the opposite direction in patients who received chemotherapy only, and combined analyses of all patients (N=1,191) became less significant,  $p=1.81\times 10^{-6}$  and  $p=0.0015$ , respectively. The most significant SNPs at *BRINP1* and the intergenic region close to *LINC01060* showed effects in the same direction and became slightly more

significant in combined analyses of all patients (N=1,191), *BRINP1*, rs62568637,  $p=4.73 \times 10^{-6}$ ; LINC01060,  $p=1.63 \times 10^{-6}$ . The intergenic SNP, rs4305714 became less significant in the combined analysis of all patients,  $p=1.20 \times 10^{-5}$ . rs7769196 in intron 1 of *TRPC6* did show a similar effect size in the same direction and a trend for association ( $p=0.06$ , 1-tailed) in patients who received chemotherapy only, increasing in significance in combined analysis of all patients,  $p=1.63 \times 10^{-6}$ .

### Replication of variants associated with ERBB2-induced cardiotoxicity

Our study failed to find association of decline in LVEF with either of the published associated *ERBB2* variants, rs1136201 (I655V) and rs1058808 (P1170A)<sup>10-14</sup>. To be consistent with published associations of the I655V variant, we also performed genotypic analyses of the AG+GG genotypes versus the AA genotype on a case control basis, where cases were defined as a decrease of at least 10% points from baseline with a resulting LVEF of less than 50%, a decrease of 15% with respect to baseline, or any decrease resulting in LVEF less than 45% at least once during the treatment or clinical manifestation of CHF<sup>14</sup>. We did not observe any association of the I655V or P1170A variants with either linear or logistic models (Table 2).

We also assessed association at *ERBB2* by imputation including 200kb flanking region and linear regression of decline in LVEF, plotted in Locus Zoom (Supplementary Figure 4) in those patients who received combination chemotherapy and trastuzumab (Arms BC). This analysis identified 95/943 SNPs at  $p < 0.05$ , although none of these remained significant following correction for multiple testing.

### Variants associated with anthracycline-induced cardiotoxicity

Several SNPs have been associated with anthracycline-induced cardiotoxicity<sup>16,19,20,27,28</sup>. Under our linear model of decline in LVEF, we observed a significant association of the *CBR3* V244M variant in combined analyses of all patients (N=1,191),  $p=0.004$ , Beta=0.84 when A (Met) allele is reference<sup>15,16</sup>. We also observed association with the published *ABCB1* variant rs2235047 ( $p=0.018$ ) and a trend for association of rs17863783 variant in *UGT1A6* ( $p=0.078$ ). We also looked for enrichment of the associated allele in the subset of individuals with CHF (N=17). There was no evidence of enrichment at either the *CBR3* or *ABCB1* loci. However, we did observe enrichment of reported associated alleles at the *NCF4* and *RARG* loci in patients with CHF. Allele A of the *NCF4* 5' flanking SNP, rs1883112, presented with a frequency of 0.44 in all patients who received chemotherapy (N=1,191) but only 0.24 (8/34 alleles) in patients with CHF, OR 0.35,  $p=0.011$ . The leucine allele of rs2229774 at the *RARG* locus was enriched in CHF patients relative to all patients who received chemotherapy (0.15 versus 0.07) and showed a trend for association,  $p=0.076$ . Locus zoom plots are shown in Supplementary Figure 5.

## Discussion

Cardiotoxicity is a major clinical side-effect of both anthracycline and trastuzumab therapy, with a higher incidence in patients receiving combination treatment compared to those receiving only anthracycline<sup>25,29</sup>. The extent and frequency of cardiotoxicity is not uniform

across patients, leading us to hypothesize a genetic influence. We performed a genome-wide association of decline in LVEF in patients treated with trastuzumab from the N9831 trial. This trial led to the use of trastuzumab as a standard of care in early onset HER2+ breast cancer. Our first approach used a quantitative analysis of the maximum observed decline in LVEF and DNA variants in patients from Arms BC (N=800) who received doxorubicin, paclitaxel and trastuzumab. We corrected for multiple testing with Benjamini-Hochberg and report those variants at  $q < 0.2$  for a total of 23 variants ( $p < 1 \times 10^{-5}$ ), from six novel loci: limb domain binding 2, (*LDB2*), an adapter molecule of transcriptional regulatory complexes<sup>30,31</sup>; BMP/retinoic acid-inducible neural-specific protein 1, (*BRINP1*), a suppressor of cell cycle progression<sup>32</sup>; transient receptor potential cation channel, subfamily C, member 6, (*TRPC6*), a central mediator of hypertrophic cardiomyopathy, cardiac remodeling<sup>33–36</sup>; a member of the RAS oncogene family, (*RAB22A*) involved in endocytic trafficking<sup>37–39</sup>; a LINC RNA, (LINC10160); and an intergenic region on chromosome 6.

Patients in Arms BC received both doxorubicin and trastuzumab. Therefore in an effort to discriminate between doxorubicin and trastuzumab-mediated cardiotoxicity, we reasoned that loci associated with decline in LVEF induced specifically by doxorubicin would be replicated in Arm A alone. However, none of the top variants were significant,  $p < 0.05$ , in the patients in Arm A (N=391) (Table 1). We did observe a trend for association of decline in LVEF for the intronic variant in *TRPC6* in patients who received chemotherapy only. This association was in the same direction and similar effect size as observed in patients receiving chemotherapy and trastuzumab,  $p = 0.06$  (1-tailed), suggesting that the *TRPC6* variant could be a result of chemotherapy. Evidence from the literature could support this hypothesis, because *TRPC6* expression which is increased in cardiac tissue from patients with dilated cardiomyopathy<sup>35</sup> is also up-regulated by doxorubicin<sup>40</sup>. The failure to replicate other variants in patients in Arm A could infer that the novel associations in Arms BC are induced by combination chemotherapy and trastuzumab, although we acknowledge that Arm A (N=391) is smaller than Arms BC (N=800) and may lack statistical power to replicate. We do not believe associations in Table 1 are a statistical artifact of different cumulative doses of doxorubicin as all patients in this analysis received the same dose ( $4 \times 60 \text{ mg/m}^2$ ) regimen completing chemotherapy with post-anthracycline LVEF remaining above 50% to continue with trastuzumab treatment.

To further discriminate between chemotherapy and trastuzumab-mediated cardiotoxicity, we also performed an exploratory analysis of each novel region. We hypothesized that true risk alleles of cardiotoxicity might be enriched in the subset of patients with CHF. At *LDB2*, *BRINP1* and *TRPC6* (low frequency variants,  $\text{MAF} < 0.02$ ), we observed five-fold enrichment in the subset of patients with CHF who received trastuzumab (N=10). Interestingly, in those patients with CHF who received chemotherapy only (N=7) we did not observe any of the putative risk alleles at these loci, suggesting these associations may be trastuzumab-mediated with the caveat that our subset of patients with CHF is underpowered. In contrast, for the most common associated variants in Table 1, chr6 intergenic region, *RAB22A* and *LINC01060*, we observed enrichment in those patients with CHF who received chemotherapy only (MAF 0.50, 0.57 and 0.36, respectively). We did not observe the same trend in the patients receiving trastuzumab who also developed CHF. If these variants are truly associated with doxorubicin and/or trastuzumab-mediated cardiotoxicity,



one could hypothesize the existence of large effect size, low frequency variants that increase susceptibility to cardiotoxicity with combination chemotherapy and trastuzumab, and the existence of common cardiac risk variants that increase susceptibility to anthracycline-related cardiotoxicity.

Our GWAS data also included variants from the current literature that were reported to be associated with cardiotoxicity from either, trastuzumab, anthracycline or combination therapy. We failed to observe genome-wide significant association with any variant *ERBB2*, the target of trastuzumab. We did not observe any association at  $p < 0.05$  for SNPs reported to be associated with trastuzumab-related cardiotoxicity in the current literature (i.e., rs1136201, I655V and rs1058808, P1170A). There are several possible reasons for this failure to replicate previous findings. We ruled out genotyping error based on concordant genotypes from multiple probes at rs1136201, genotypic data in Hardy-Weinberg equilibrium, observed allele frequencies in line with other reported studies and adequate cluster plots (Supplementary Figure 7).

We next considered our definition of cardiotoxicity as a reason for lack of replication with *ERBB2* I655V and P1170A. Our primary analysis used a quantitative model of maximum decline in LVEF. Published associations used a binary definition of cardiotoxicity or no cardiotoxicity, but with different definitions in each case, including decrease in LVEF of at least 20% points<sup>10</sup>; decrease in LVEF of at least 10% points to less than 50% or any decrease to  $< 45\%$ <sup>11</sup>; decrease  $> 10\%$  points to  $< 50\%$  or any decrease  $> 15\%$  or any decrease resulting in  $< 45\%$  or diagnosis of CHF<sup>14</sup>. Hence we also looked for association with the same binary definition as Gomez Pena<sup>14</sup>. Again, we did not observe any associations,  $p < 0.05$ . Acknowledging the caveat of the arbitrary definition of cardiotoxicity and the models we used, it is possible that our lack of replication was simply due to a smaller sample size in previous studies. Our data set from N9831 patients treated with trastuzumab (N=800) was over 2-fold greater compared to the four published studies combined (N=344, ranging 61–132 patients<sup>14</sup>), which showed large odds ratios ranging from 3.80 to 5.80. We note that there are no published negative studies of these variants with cardiotoxicity, which may suggest a publication bias towards positive associations in when using small sample sizes.

Our analysis of published SNPs associated with anthracycline cardiotoxicity in all patients who received doxorubicin (Arms A, B and C combined, N=1,191) was more successful in that we did observe association of the *CBR3* V244M variant in our quantitative analysis of maximum decline in LVEF ( $p = 0.004$ ) with supporting evidence from multiple SNPs at this locus. Carbonyl reductases (CBRs) catalyze reduction of anthracyclines to cardiotoxic alcohol metabolites and polymorphisms in *CBR1* and *CBR3* influence the synthesis of these metabolites. *CBR3* V244M was previously associated with a dose-dependent risk of anthracycline-related cardiomyopathy in childhood cancer survivors<sup>15</sup>, and with cardiotoxicity in a cohort of breast cancer patients<sup>16</sup>. In a literature review entitled, 'Recommendations for genetic testing to reduce the incidence of anthracycline-induced cardiotoxicity' it was deemed that *CBR3* required further validation. Our study confirms association of this variant in patients with breast cancer.

Similarly, we observed association of a variant in *ABCB1*, rs2235047, also previously associated with anthracycline-induced cardiotoxicity in children, where 266 were controls (defined as systolic function >30% during and after therapy, with follow-up of more than five years after completion of anthracycline therapy) and 78 cases (defined as systolic function <26% at any time during or after anthracycline therapy)<sup>19</sup>. This variant was not confirmed in a further study of children, consisting of 46 cases and 131 controls, analysed with the same methodology from the same group<sup>20</sup>, perhaps due to lack of power. However, in this study we observed an association of similar magnitude (Beta = -2.34, p=0.018) in our linear model of decline in LVEF in 1,191 patients receiving doxorubicin, adding further evidence for this association. Certainly, this gene is a reasonable candidate due to its involvement in drug excretion in the kidney, liver, and heart<sup>41,42</sup>. Specifically, changes in *ABCB1* expression or function prolong the presence of both doxorubicin in cardiac tissue and its cardiotoxic metabolite, doxorubicinol, within cardiac tissue<sup>43</sup>. Furthermore, the ERBB2 inhibitor, lapatinib, has been shown to inhibit the function of *ABCB1*<sup>44</sup>, suggesting a possible mechanism for increased cardiotoxicity as a result of doxorubicin in combination with HER2 targeted therapy.

We did not observe an association of published SNPs associated with doxorubicin-induced cardiotoxicity in *SLC28A3*, *NCF4*, *RAC2*, *RARG* and *UGT1A6* in our linear model of decline in LVEF in 1,191 patients receiving doxorubicin (Table 3). Given these were associations with CHF rather than decline in LVEF, we also tested for these variants in the limited subset of patients (N=17) with CHF. Aminkeng et al<sup>28</sup> reported a highly significant association of the 427Leu variant within retinoic acid receptor  $\gamma$ , *RARG* (rs2229774, Ser427Leu) with CHF in three studies of children (cases N=32, N=22 and N=19, respectively) treated with anthracyclines (either doxorubicin, daunorubicin, idarubicin, epirubicin or mitoxantrone). In our smaller subset of CHF patients from the N9831 trial treated with doxorubicin, we observed a weak association of the 427Leu variant, OR=2.39, p=0.038 (1-tailed). We also observed association of the *NCF4* rs1883112 A allele, OR 0.35, p=0.01 (2-tailed) (A allele frequency in CHF patients = 0.24 vs 0.44 in patients who were not confirmed with CHF). However, our finding is in the opposite direction to other reported associations. The AA genotype of this variant was previously associated with CHF in adult non-Hodgkin lymphoma patients treated with doxorubicin, OR 2.00, p=0.018<sup>18</sup> and the A allele was associated with CHF in cancer patients treated with anthracyclines, OR 5.11, p=0.016<sup>45</sup>.

A weakness of our study is the inability to validate our findings using other methods. Recognizing this weakness, we scanned the literature for reported cardiac modifying genes in animal models. This led us to examine four genes (*SORBS2*, *RXRA*, *DNAJB6* and *ANO5*) reported as cardiac modifiers of doxorubicin-induced cardiotoxicity in zebrafish and/or mouse models<sup>46,47</sup>. Suggesting that our data can be replicated we also identified variants in our study of N9831 in *SORBS2* and *RXRA* and p<0.01 in *DNAJB6*, p<0.001 (Supplementary Table 3). These findings suggest that these genes require further study as genes modified by chemotherapy treatments that could increase the risk for cardiomyopathy in patients.



In summary, our study confirmed previously reported associations of chemotherapy-induced cardiotoxicity with variants in the *ABCB1* and *CBR3* genes. We failed to replicate previous findings at *ERBB2* with any model of cardiotoxicity (linear or logistic); however, our sample size was significantly larger than other published studies suggesting that previous associations may have occurred due to small sample size. In an effort to identify novel loci relevant to patients receiving combination chemotherapy and trastuzumab, we identified six potential susceptibility loci. Further study is needed to confirm the role of these loci in trastuzumab-induced cardiomyopathy.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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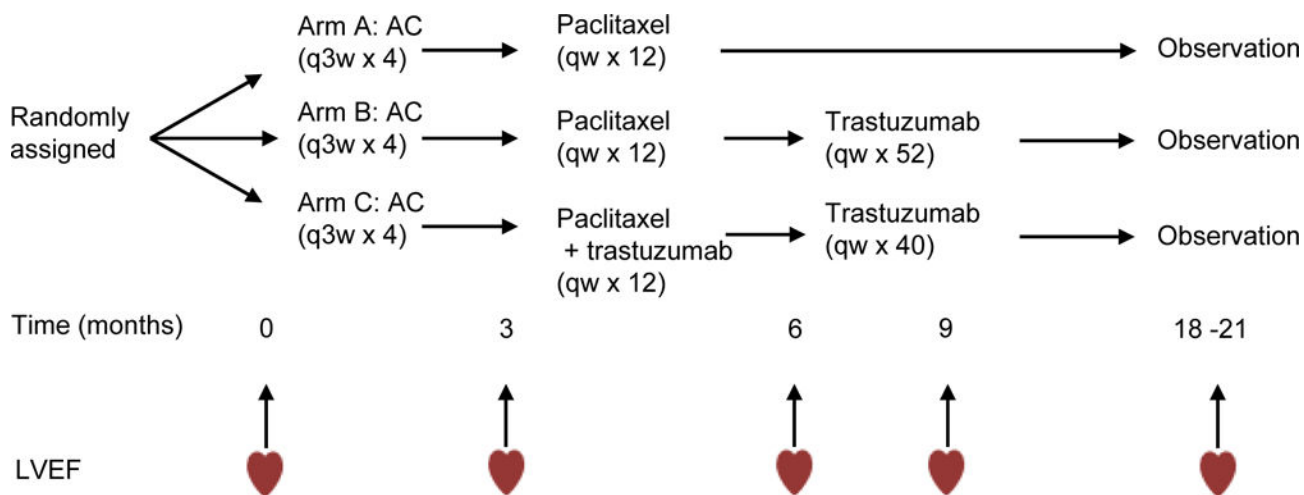
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**Figure 1. Treatment Arms and cardiac monitoring of N9831**

A, Doxorubicin 60 mg/m<sup>2</sup>; C, Cyclophosphamide 600mg/m<sup>2</sup>; paclitaxel 80 mg/m<sup>2</sup>; trastuzumab 4 mg/kg loading dose followed by 2 mg/kg weekly (qw); LVEF, left ventricular ejection fraction.

Table 1

Linear regression analysis of maximum decline in LVEF

SNP ID, Chr, chromosome and Hg19 position. MAF, minor allele frequency, L95, lower and U95 upper 95% confidence intervals and P, p-value, is shown for Arms BC and Arm A additive linear regression analysis. Genotype count for each SNP is shown in the subset of patients with CHF, congestive heart failure, separated for Arms BC and Arm A, with genotype counts of allele 1 homozygote/heterozygote/allele 2 homozygote and minor allele frequency in parentheses.

ID	Chr	Position Hg19	Locus	Arms BC- Max Drop in LVEF (N=800)			Arm A- Max Drop in LVEF (N=391)			CHF (n=17) genotype count and (MAF)	
				MAF	Beta (L95-U95)	P (2-tail)	MAF	Beta (L95-U95)	P (2-tail)	Arms BC	Arm A
rs55756123	4	16,502,767	LDB2	0.02	-6.11 (-8.32 to -3.89)	8.93E-08	0.01	1.61 (-3.82 to 7.04)	0.56	0/3/7 (0.15)	0/0/7 (0.00)
rs10117876	9	121,859,107	BRINP1	0.01	-7.79 (-10.82 to -4.76)	5.86E-07	0.01	2.12 (-3.35 to 7.59)	0.44	0/1/9 (0.05)	0/0/7 (0.00)
rs7038023	9	121,859,198	BRINP1	0.01	-7.79 (-10.82 to -4.76)	5.86E-07	0.01	1.83 (-3.26 to 6.92)	0.48	0/1/9 (0.05)	0/0/7 (0.00)
rs7041012	9	121,859,463	BRINP1	0.01	-7.18 (-10.06 to -4.29)	1.34E-06	0.01	1.16 (-3.92 to 6.24)	0.66	0/1/9 (0.05)	0/0/7 (0.00)
rs1160584	9	121,861,009	BRINP1	0.01	-7.91 (-11.12 to -4.70)	1.62E-06	0.01	2.85 (-3.64 to 9.35)	0.38	0/1/9 (0.05)	0/0/7 (0.00)
rs230145	9	121,879,897	BRINP1	0.01	-7.91 (-11.12 to -4.70)	1.62E-06	0.01	-1.76 (-7.70 to 4.18)	0.56	0/1/9 (0.05)	0/0/7 (0.00)
rs230144	9	121,882,710	BRINP1	0.01	-7.90 (-11.12 to -4.69)	1.70E-06	0.01	-1.76 (-7.70 to 4.19)	0.56	0/1/9 (0.05)	0/0/7 (0.00)
rs230142	9	121,883,736	BRINP1	0.01	-7.91 (-11.12 to -4.70)	1.62E-06	0.01	-1.76 (-7.70 to 4.18)	0.56	0/1/9 (0.05)	0/0/7 (0.00)
rs62573809	9	121,892,448	BRINP1	0.01	-6.88 (-9.84 to -3.93)	5.70E-06	0.01	-2.25 (-7.69 to 3.20)	0.42	0/1/9 (0.05)	0/0/7 (0.00)
rs16908078	9	121,893,380	BRINP1	0.01	-7.49 (-10.61 to -4.38)	2.95E-06	0.01	-1.76 (-7.70 to 4.18)	0.56	0/1/9 (0.05)	0/0/7 (0.00)
rs7851490	9	121,894,217	BRINP1	0.01	-7.48 (-10.60 to -4.36)	3.12E-06	0.01	-1.76 (-7.70 to 4.18)	0.56	0/1/9 (0.05)	0/0/7 (0.00)
rs7854066	9	121,899,889	BRINP1	0.01	-7.49 (-10.61 to -4.37)	3.04E-06	0.01	-1.76 (-7.70 to 4.18)	0.56	0/1/9 (0.05)	0/0/7 (0.00)
rs62573837	9	121,900,834	BRINP1	0.01	-7.50 (-10.62 to -4.38)	2.88E-06	0.01	-1.76 (-7.70 to 4.18)	0.56	0/1/9 (0.05)	0/0/7 (0.00)
rs76586195	9	121,902,640	BRINP1	0.01	-7.73 (-10.95 to -4.52)	2.90E-06	0.01	-1.76 (-7.70 to 4.18)	0.56	0/1/9 (0.05)	0/0/7 (0.00)
rs7027658	9	121,904,365	BRINP1	0.01	-7.49 (-10.61 to -4.38)	2.95E-06	0.01	-1.76 (-7.70 to 4.18)	0.56	0/1/9 (0.05)	0/0/7 (0.00)
rs75912020	9	121,909,304	BRINP1	0.01	-7.49 (-10.61 to -4.38)	2.95E-06	0.01	-1.77 (-7.71 to 4.18)	0.56	0/1/9 (0.05)	0/0/7 (0.00)
rs76890184	9	121,910,252	BRINP1	0.01	-7.49 (-10.61 to -4.38)	2.95E-06	0.01	-1.76 (-7.70 to 4.18)	0.56	0/1/9 (0.05)	0/0/7 (0.00)
rs58944852	9	121,912,974	BRINP1	0.01	-7.49 (-10.61 to -4.38)	2.87E-06	0.01	-1.76 (-7.70 to 4.18)	0.56	0/1/9 (0.05)	0/0/7 (0.00)
rs62568637	9	121,917,144	BRINP1	0.01	-8.50 (-11.81 to -5.19)	6.01E-07	0.01	-1.77 (-7.72 to 4.18)	0.56	0/1/9 (0.05)	0/0/7 (0.00)
rs4305714	6	22,957,737	intergenic	0.24	-1.87 (-2.63 to -1.12)	1.39E-06	0.24	-0.51 (-1.64 to 0.63)	0.38	0/5/5 (0.25)	1/5/1 (0.50)
rs707557	20	56,942,312	RAB22A	0.40	1.46 (0.83 to 2.08)	5.62E-06	0.38	-0.31 (-1.33 to 0.71)	0.55	1/3/6 (0.25)	1/6/0 (0.57)
rs77679196	11	101,453,508	TRPC6	0.01	-7.00 (-10.05 to -3.96)	7.72E-06	0.01	-5.29 (-11.95 to 1.38)	0.12	0/2/8 (0.10)	0/0/7 (0.00)
rs7698718	4	189,555,947	LINC01060	0.22	1.78 (1.01 to 2.56)	7.73E-06	0.22	0.11 (-1.02 to 1.23)	0.86	1/2/7 (0.20)	0/5/2 (0.36)



**Table 2**

**SNPs previously associated with ERBB2 cardiotoxicity (Arms BC)**

Association analyses were performed assuming an additive effect in both linear and logistic regression models. For logistic regression, we report genotype counts, odds ratio (OR) and P-values (P) using the more stringent definition of cases as per Gomez Pena et al, 2015: Decrease of at least 10% points from baseline with a resulting LVEF of less than 50% or decrease of 15% with respect to baseline, or any decrease resulting in LVEF less than 45% at least once during the treatment or clinical manifestation of CHF.

SNP ID	AA change	Linear Max drop in LVEF		Logistic LVEF literature			
		Beta, L95-U95	P	genotype case	OR, L95-U95	P	
rs1136201	I655V	-0.33 (-1.07 to 0.41)	0.39	GG=11	GG=39	0.941 (0.71 to 1.25)	0.67
				GA=59	GA=228		
				AA=103	AA=360		
rs1058808	P1170A	-0.45 (-1.14 to 0.24)	0.20	CC=21	CC=65	1.034 (0.80 to 1.34)	0.80
				CG=78	CG=293		
				GG=73	GG=268		

**Table 3**  
**SNPs previously associated with anthracycline cardiotoxicity (Arms ABC)**

Additive effect linear regression analyses in all Arms combined. Logistic regression was performed in those cases (N=17) with congestive heart failure (CHF) versus all other patients. P-values <0.05 are highlighted in bold.

Gene	rs ID	Function	Linear Max drop in LVEF			Logistic CHF (N=17)		
			Freq A1 (N=1191)	Beta, L95-U95	P	Freq A1 (N=17)	OR, L95-U95	P
ABCB1	rs2235047	intronic	0.02	-2.34 (-4.28 to -0.40)	<b>0.018</b>	0.00	NA	0.996
SLC28A3	rs7853758	L464L	0.16	-0.11 (-0.84 to 0.62)	0.766	0.12	0.71 (0.25 to 2.02)	0.523
CBR3	rs1056892	V244M	0.35	0.84 (0.26 to 1.41)	<b>0.004</b>	0.35	1.01 (0.49 to 2.08)	0.972
NCF4	rs1883112	5' flanking	0.44	0.19 (-0.36 to 0.72)	0.492	0.24	0.35 (0.15 to 0.78)	<b>0.011</b>
RAC2	rs13058338	intronic	0.26	0.05 (-0.55 to 0.66)	0.861	0.29	1.21 (0.58 to 2.57)	0.611
RARG	rs2229774	S427L	0.07	0.44 (0.61 to 1.48)	0.412	0.15	2.39 (0.91 to 6.23)	0.076
UGT1A6	rs17863783	V209V	0.02	-1.67 (-3.52 to 0.18)	0.078	0.00	NA	0.996