Evidence for association of SNPs in *ABCB1* and *CBR3*, but not *RAC2*, *NCF4*, *SLC28A3* or *TOP2B*, with chronic cardiotoxicity in a cohort of breast cancer patients treated with anthracyclines

Aims: Validation of associations for SNPs in *RAC2*, *NCF4* and *SLC28A3*, identification of a novel association with a *TOP2B* SNP and screening 23 SNPs putatively relevant to anthracycline-induced cardiotoxicity. **Patients & methods:** A total of 166 breast cancer patients treated with doxorubicin underwent echocardiogram, including 19 cases with systolic dysfunction (ejection fraction <55%) and 147 controls. Four high priority SNPs were tested in the primary analysis, with appropriate statistical correction, and 23 additional SNPs were screened in an uncorrected secondary analysis. **Results:** Previously reported associations for *RAC2*, *NCF4* and *SLC28A3* could not be validated and a novel association with *TOP2B* was not discovered in this cohort (all $p > 0.05$), likely due to inadequate power. Two SNPs were identified in the uncorrected secondary analysis including a protective SNP in *ABCB1* (3435C>T, $p = 0.049$) and a risk allele in *CBR3* (V244M, $p = 0.012$). **Conclusion:** The associations reported in prior publications and those discovered in this secondary analysis require further replication in independent cohorts.

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In the era of targeted therapies, anthracycline chemotherapy remains one of the most important weapons in the arsenal against multiple different cancers. A well-known limitation to the use of anthracyclines is the toxic effect anthracyclines can have on the heart, both acutely and over years after chemotherapy has been completed [1,2]. Cardiomyopathy induced by anthracyclines primarily manifests as systolic dysfunction causing a drop in left ventricular ejection fraction (EF) and increased myocardial strain [3]. The incidence of anthracycline-induced cardiomyopathy in adults varies in the literature, but is reported to be between 1 and 7% [1,2,4,5].

A patient's genetic makeup is a major determinant of cancer therapy failure and adverse drug reactions [6]. Differences among patients' ability to tolerate anthracy-

cline chemotherapy may be attributable to genetic variation, in particular the presence of common germline SNPs. SNPs in genes relevant to anthracycline metabolism and elimination may contribute to differences in drug exposure, which may account for variability in the development of cardiomyopathy. Alternatively, variation in genes related to the pharmacological mechanism of cardiotoxicity could dictate a patient's sensitivity to the development of cardiomyopathy [7].

Although several studies have evaluated the influence of patient genetics on anthracycline-induced cardiomyopathy, no SNPs have been validated to be clinically useful predictors of cardiotoxicity risk. Utilizing a cohort of patients assembled to investigate physiological biomarkers of chronic anthracycline-induced cardiotoxicity, we attempted to replicate the

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influence of candidate SNPs on the development of cardiomyopathy after receiving an anthracycline in the curative setting. We prioritized SNPs in genes that were either previously reported to be associated with cardiotoxicity (*RAC2*, *NCF4*, *SLC28A3*) [8–12] or that had compelling mechanistic rationale but had not been previously investigated to our knowledge (*TOP2B*) [13]. Additional variants with putative relevance to anthracycline-induced cardiotoxicity, anthracycline exposure, or previous reports of significant association with cardiotoxicity were screened for an association with systolic dysfunction in an uncorrected secondary analysis. Successful validation of SNPs that predict a patient's susceptibility to anthracycline-induced cardiomyopathy may enable clinicians to provide genotype-directed treatment to minimize toxic side-effects.

Patients & methods

Cohort

We conducted a cross-sectional observational study of anthracycline-induced cardiomyopathy in a cohort of women recruited from the University of Michigan Comprehensive Cancer Center. Detailed information regarding the parent clinical study cohort, procedures and findings can be found in the primary publication [14]. Briefly, patients seen in the Breast Oncology Program who had completed neoadjuvant or adjuvant chemotherapy with a doxorubicin-based regimen for stage I–III breast cancer and received their last dose of doxorubicin at least 12 months prior were eligible for enrollment. No patients in this study had congestive heart failure (CHF) prior to doxorubicin treatment as this is a strict contraindication. Patients with a previous or second cancer that required chemotherapy or radiation to the chest, had a breast cancer recurrence or metastatic disease, or were receiving trastuzumab at the time of enrollment were ineligible. Patients with known anthracycline-induced cardiac dysfunction were eligible for this study, but this was not used to identify potential subjects, as one of the objectives of the parent study was to document the prevalence of chronic cardiomyopathy in an unselected cohort of doxorubicin treated patients. This study was approved by the Institutional Review Board of the University of Michigan Medical School (MI, USA).

After informed consent was obtained, individuals underwent a physical exam, blood draw, urine collection and cardiovascular health questionnaire. Blood and urine samples were analyzed for highly sensitive physiological biomarkers of heart strain including brain natriuretic peptide (BNP), troponin and NT-proBNP [15,16]. Individuals with known doxorubicin-induced cardiomyopathy or an abnormal biomarker, physical exam, or symptom inventory suggestive of

cardiac dysfunction were referred for an echocardiogram and eligible for this secondary pharmacogenetic analysis. An extensive medical record review was conducted for evaluation of relevant patient, disease and treatment characteristics such as age, comorbid conditions, breast cancer stage and treatment received. Continuous quality checks of the data were performed. All clinical data were collected prior to genetic analysis.

Genotyping

Whole blood was collected and stored at -80°C until DNA extraction using the QIAamp® DNA blood kit (Qiagen Inc., CA, USA) according to manufacturer's instructions. DNA quality and quantity was determined using a Nanodrop Spectrophotometer™ (Thermo Scientific, Inc., Wilmington, DE, USA) and 300 ng submitted to the University of Michigan Medical School's DNA Sequencing Core for genotype determination using the Sequenom MassARRAY iPLEX® platform (Agena Bioscience, San Diego, CA, USA). SNP assays were designed using the Sequenom software (both online Assay Design Suite tools and desktop Assay Design 4.0) and assays were performed using a Sequenom MassARRAY Compact instrument, according to manufacturer's standard protocols. Results were processed to generate SNP calls automatically, using Sequenom TyperAnalyzer software, and then manually reviewed by the operator to validate the allele calls. Automatic SNP calls that were of concern, based on questionable spectra, were removed. A total of 16 unique SNPs were determined using Sequenom MassARRAY and an additional eight SNPs of interest (*ABCB1*: rs1128503, *RAC2*: rs13058338, *NCF4*: rs1883112, *TOP2B*: rs10865801, *CYP3A4*22*: rs35599367, *ABCB4*: rs1149222, *CBR3*: rs1056892, *CYBA*: rs4673) were determined using the Applied Biosystems' (CA, USA) Taqman® Allelic Discrimination Assay as previously described [17].

Statistical analyses

Genotyping quality control was conducted for the 24 SNPs selected for analysis including call rate (>95%), Hardy–Weinberg equilibrium ($p > 0.002$ [0.05/24]), and resequencing of 10% of samples genotyped by Taqman™ Allelic Discrimination Assay (Roche Molecular Systems Inc., Pleasanton, CA, USA). Individuals were dichotomized into cases and controls for pharmacogenetic association testing with the primary endpoint, systolic dysfunction defined as an EF <55%, patients with EF ≥55% were classified as controls. The threshold EF of 55% was chosen based on the American Society of Echocardiographer's Guidelines [18]. Demographics were compared between cases and controls using Fisher's exact tests for categori-

cal variables and Wilcoxon rank sum tests for age, BMI and cumulative anthracycline dose.

From the 24 SNPs included in the analysis, four were selected *a priori* as co-primary hypotheses based on previous successful replication (*RAC2* rs13058338, *NCF4* rs1883112, *SLC28A3* rs7853758) [8–12] and biological rationale (*TOP2B* rs10865801) [13]. For each co-primary hypothesis a predicted genetic model and direction of effect was selected based on prior literature. An analysis that has a predefined direction of effect is analyzed using a one-sided p-value. The typical threshold for a one-sided p-value is $p = 0.10$, which is equivalent to the familiar $p = 0.05$ for a two-tailed test (which can be significant in either direction). In this analysis we performed four one-sided association tests using an appropriately corrected significance threshold of $\alpha = 0.025$ ($0.10/4$). A secondary, uncorrected screen was performed including all SNPs to test for an association with EF <55% assuming an additive genetic model without defining a direction of effect. No statistical correction was applied to this hypothesis-generating exploratory analysis. SNP association analyses were

conducted using univariate logistic regression, followed by multivariable regression for any positive associations adjusting for relevant clinical covariates (age at enrollment, hypertension, trastuzumab treatment, cumulative anthracycline dose and left sided radiation) [19]. All analyses were performed using SAS® v9.3 (SAS, NC, USA) and R 3.0.2 (genetics package).

Results

Among the 293 subjects enrolled into the parent clinical study [14], 166 underwent echocardiogram due to known anthracycline-induced CHF or an abnormal biomarker suggestive of CHF. Of these 166 echocardiograms, 19 cases of systolic dysfunction (EF <55%) were identified for comparison with the control group of 147 patients who had a normal echocardiogram (Figure 1). Demographic data for the entire cohort, the controls and the cases (stratified by EF <55%) are reported in Table 1. The patient population was predominantly non-Hispanic white (95%) with a median age of 50 years at the time of anthracycline treatment and 56 years at the time of study enrollment. More

Figure 1. Flowchart of patients from enrollment into the parent study to classification of cases and controls for this pharmacogenetic analysis. Among the 293 patients enrolled on the clinical study, 166 underwent an echocardiogram, 19 of which were documented to have EF <55% (cases). Patients with normal echocardiograms (EF ≥55%; $n = 147$) were included as controls.

CHF: Congestive heart failure; EF: Ejection fraction determined by transthoracic echocardiogram.

Table 1. Demographic data for all patients included in pharmacogenetic analysis, and stratified into cases and controls.

Characteristic		All women (n = 166)	Controls (n = 147)	Cases (n = 19)	p-value
Race	White	157 (95%)	140 (95%)	17 (89%)	0.13
	Black	3 (2%)	1 (1%)	2 (11%)	
	Other	6 (4%)	6 (4%)	0	
Age (years)	At anthracycline treatment	50 (24–80)	50 (24–80)	50 (35–64)	0.90
	At enrollment	56 (31–85)	56 (31–85)	56 (37–69)	0.46
Size	BMI (kg/m ²)	30 (15–70)	30 (15–70)	30 (23–59)	0.95
Smoking	Positive history	10 (6%)	9 (6%)	1 (5%)	1.0
Anthracycline	Cumulative dose (mg/m ²)	240 (120–366)	240 (120–366)	240 (240–350)	0.63
Supplementary treatment	Trastuzumab	29 (17%)	24 (16%)	5 (26%)	0.33
	Radiation	137 (83%)	120 (82%)	17 (89%)	0.74
	Left-sided radiation	72 (43%)	62 (42%)	10 (53%)	0.40
Hypertension	Present	38 (23%)	36 (24%)	2 (11%)	0.25
Other comorbidities	Coronary disease	2 (1%)	2 (1%)	0	1.0
	Diabetes	8 (5%)	8 (5%)	0	0.60
	Any lung disease	6 (4%)	6 (4%)	0	1.0
	Sleep apnea	8 (5%)	7 (5%)	1 (5%)	1.0
	Atrial fibrillation	3 (2%)	3 (2%)	0	1.0
	Valvular heart disease	7 (4%)	5 (3%)	2 (11%)	0.18
	Any other comorbidity	34 (20%)	30 (20%)	4 (21%)	1.0

Data reported as count (%) or median (range).

than 80% of individuals received radiation, with approximately half of that directed at the left side of the chest, and less than 20% of patients received trastuzumab. No patients were given trastuzumab concurrently with doxorubicin and no patients were being treated with trastuzumab at the time of study enrollment. No significant differences in demographic, treatment, or clinical factors were identified between the cases and controls ($p > 0.05$).

One candidate SNP determined by Sequenom failed quality assurance (*CYP3A4*1B*, rs12705060) and was excluded leaving 23 SNPs for analysis. None of the four co-primary hypotheses were significantly associated with EF <55% with the selected genetic model and direction of effect ($p > 0.025$, Table 2). The full results for each SNP including the genotype frequency in the cases and controls and the association results for different genetic models (additive, recessive and dominant) are shown in Supplementary Table 1. The choice of genetic model did not meaningfully change the results, nor did adjustment for clinical covariates or restricting analysis to white patients only ($n = 157$) ($p > 0.025$, data not shown).

All 23 candidate SNPs were then tested for an association with EF <55% in a secondary pharmacogenetic

screening analysis assuming an additive genetic effect using an uncorrected significance threshold of $p < 0.05$. In unadjusted analyses assuming an additive genetic effect two SNPs had a nominally significant association with low EF (Table 3 & Figure 2). The variant allele of *ABCB1* 3435C>T (rs1045642) had an additive protective effect (odds ratio [OR] = 0.48; 95% CI: 0.23–1.00; $p = 0.049$). The second association was for the V244M variant in *CBR3* (rs1056892) which increased risk of EF <55% (OR = 2.50; 95% CI: 1.22–5.11; $p = 0.012$), and an even stronger recessive genetic effect (OR = 6.19; 95% CI: 1.94–19.76; $p = 0.002$) (Supplementary Table 1). Each additive association remained nominally significant after correction for clinical covariates ($p = 0.048$, $p = 0.012$, respectively) and when both genetic variables were included in the model simultaneously with the clinical covariates ($p = 0.029$, $p = 0.008$, respectively).

Discussion

Cardiotoxicity is a well-known side effect of doxorubicin that has led to a decline in use of this drug and substitution for nonanthracycline-based alternatives over the last few decades [20]. The exact mechanisms responsible for anthracycline-induced cardiomyopathy are not com-

pletely understood but multiple hypotheses including the creation of reactive oxygen species, the toxic effects of alcohol metabolites on cardiomyocytes, and the contribution of topoisomerase 2-beta (*TOP2B*) [21] have been proposed. Common variants in the genes involved in these processes could, in theory, affect the development of anthracycline-induced cardiotoxicity. Therefore, the identification of genetic variants that predict anthracycline-induced cardiotoxicity would enable clinicians to continue anthracycline use in patients most likely to benefit and least likely to experience toxicity. Unfortunately, this study was unable to validate the influence of three high priority candidate SNPs (*SLC28A3*, *RAC2*, *NCF4*) and did not detect the predicted association of a novel SNP in *TOP2B* with the occurrence of doxorubicin-induced cardiotoxicity in this cohort.

Two SNPs that may be associated with doxorubicin-induced cardiotoxicity were identified in an uncorrected exploratory secondary analysis. A common synonymous SNP (rs1045642, 3435C>T, Ile1145Ile) in *ABCB1*, which encodes the p-glycoprotein (P-gp) efflux transporter, had a cardioprotective effect in this cohort. Three *ABCB1* SNPs that are in relatively strong linkage disequilibrium ($r^2 > 0.8$) were included in this analysis. These SNPs have been reported to increase systemic doxorubicin clearance [22], potentially explaining the decreased risk of systolic dysfunction in patients. Alternatively, cardiomyocytes are known to express P-gp [23] and cardiac tissue samples of *ABCB1* variant carriers have greater P-gp expression [24], suggesting that enhanced doxorubicin efflux within cardiomyocytes may provide the cardioprotective effect. However, a previous pharmacogenetic analysis that included rs1045642 did not detect a cardioprotective effect similar to that detected in our cohort [8].

The second SNP identified in this analysis is a non-synonymous variant in *CBR3* (rs1056892, 730G>A, V244M). Previous pharmacogenetic analyses have reported a protective effect of this SNP, particularly at lower cumulative doxorubicin doses [25,26], or detected no association for this variant with anthracycline-induced cardiotoxicity [12,27]. Conversely, in our study this variant was more common in patients with systolic dysfunction than in control patients. The *CBR3* gene encodes for carbonyl reductase, which metabolizes doxorubicin to a toxic alcohol metabolite, doxorubicinol. This nonsynonymous SNP has lower doxorubicin metabolic activity *in vitro* [28], but patients carrying the variant genotype have been reported to have higher doxorubicinol exposure [29]. These intriguing but discordant findings, both clinically and preclinically, reveal a need for further investigation into the activity of this variant SNP and its importance in cardiotoxicity development.

Table 2. Association of primary hypothesis SNPs with systolic dysfunction (ejection fraction <55%).

Gene	SNP Information		Predicted association		Pharmacogenetic association results		
	rsID	Nucleotide change	Effect of variant on systolic dysfunction risk	Genetic model	OR (95% CI)	Univariate p-value	Adjusted p-value*
<i>RAC2</i>	rs13058338	T>A	Increase	Additive	0.75 (0.31–1.82)	0.52	0.38
<i>NCF4</i>	rs1883112	G>A	Increase	Recessive	1.44 (0.51–4.07)	0.49	0.60
<i>SLC28A3</i>	rs7853758	G>A	Decrease	Additive	0.55 (0.16–1.91)	0.35	0.43
<i>TOP2B</i>	rs10865801	C>T	Increase	Additive	1.32 (0.67–2.61)	0.43	0.47

*p-value adjusted for age at enrollment, hypertension, trastuzumab treatment, cumulative anthracycline dose, and left sided radiation.
OR: Odds ratio.

Table 3. Association of all SNPs with ejection fraction <55% assuming additive genetic effects.

Gene variant	rsID	Nucleotide change	MAF	HWE p-value	Association p-value
<i>RAC2</i>	rs13058338	T>A	0.22	0.60	0.52
<i>NCF4</i>	rs1883112	G>A	0.47	0.08	0.93
<i>SLC28A3</i>	rs7853758	G>A	0.13	0.67	0.35
<i>TOP2B</i>	rs10865801	C>A	0.48	0.95	0.43
<i>CYP3A4*22</i>	rs35599367	G>A	0.04	0.13	0.67
<i>CYP3A5*3</i>	rs776746	C>T	0.07	0.16	0.13
<i>NR1I2</i>	rs1523127	C>A	0.40	0.37	0.44
	rs3732357	G>A	0.31	0.005	0.26
	rs1523130	T>C	0.42	0.26	0.76
<i>ABCB1*2</i>	rs1128503	C>T	0.42	0.34	0.14
	rs2032582	G>T/A [†]	0.44	0.49	0.35
	rs1045642	C>T	0.48	0.62	0.049 [‡]
<i>SLC22A16</i>	rs714368	T>C	0.23	0.14	0.60
	rs6907567	A>G	0.22	0.15	0.51
	rs723685	A>G	0.10	0.16	0.29
	rs12210538	A>G	0.24	0.54	0.48
<i>CBR3 V244M</i>	rs1056892	G>A	0.32	0.92	0.012 [‡]
<i>ABCC1</i>	rs4148350	G>T	0.06	0.46	0.92
<i>CBR1</i>	rs9024	G>A	0.10	0.16	0.30
<i>UGT1A6</i>	rs17863783	G>T	0.02	0.81	NA
<i>ABCB4</i>	rs1149222	G>T	0.23	0.79	0.88
	rs4148808	T>C	0.15	0.61	0.58
<i>CYBA</i>	rs4673	A>G	0.31	0.46	0.45

[†]Tri-allelic SNP, A and T variants collapsed into single variant group.
[‡]Surpassed exploratory significance threshold $p < 0.05$.
HWE: Hardy–Weinberg equilibrium; MAF: Minor allele frequency; NA: Could not be estimated due to separation of data (those with systolic dysfunction all had same set of alleles).

None of the four SNPs selected as co-primary hypotheses were associated with cardiotoxicity in this cohort. A synonymous variant in *SLC28A3* (rs7853758, L461L) has been previously reported to be cardioprotective in multiple patient cohorts, with odds ratios of 0.35–0.42 [9,10]. In our cohort, the effect of this SNP was in a consistent direction with a similar odds ratio (0.55), however, with a wide confidence interval (0.16–1.91). If this is a true effect, this silent polymorphism is either tagging a functionally consequential SNP, or this could be another potential example of a synonymous variant that affects protein activity [30]. Risk variants in *RAC2* (rs13058338) and *NCF4* (rs1883112) were first reported in a large case-control analysis by Wojnowski *et al.* [8] and replicated in independent cohorts [11,12]. Similar to the nominal concordance of the *SLC28A3* results, the *NCF4* SNP that had been previously reported to increase risk was found

more commonly in patients with cardiotoxicity in our cohort, with a slightly lower magnitude of association (1.44 vs 1.88–2.70). Again, this point estimate had a wide confidence interval (0.51–4.07), indicative of the imprecision of the estimate, which is likely due to the small number of cardiotoxicity events ($n = 19$) in this cohort. Alternatively, the nonsignificant results for *RAC2* and *TOP2B* were in the opposite direction of that previously published or hypothesized. Thus we were unable to replicate the associations of these SNPs with chronic anthracycline-induced cardiotoxicity in our cohort of breast cancer patients.

The fourth SNP selected as a primary hypothesis is an intronic SNP in *TOP2B* (rs2363520) that we hypothesized would be found more frequently in systolic dysfunction cases than controls. *TOP2B* expression is correlated with cellular sensitivity to doxorubicin [31] and the TOP2B protein is necessary for development of

Figure 2. Association of SNPs in *ABCB1* and *CBR3* with systolic dysfunction. Two SNPs were significantly associated with systolic dysfunction (EF <55%) in the secondary pharmacogenetic screen of 23 candidate SNPs. The *ABCB1* 3435C>T variant (rs1045642, Figure 2A) was more common in controls than systolic dysfunction cases, providing an additive protective effect (odds ratio [OR] = 0.48; 95% CI: 0.23–1.00; $p = 0.049$). The *CBR3* V244M (rs1056892, Figure 2B) SNP was found more often in cases than controls, with significantly increased risk in either an additive (OR = 2.50; 95% CI: 1.22–5.11; $p = 0.012$) or recessive (OR = 6.19; 95% CI: 1.94–19.76; $p = 0.002$) genetic model. Note: Numbers do not add up to 166 due to missing genotypes in some patients (*ABCB1*: 4; *CBR3*: 3). EF: Ejection fraction.

anthracycline-induced cardiotoxicity in mice [13]. Using bioinformatics tools we identified a SNP (rs2363520) that is highly associated with *TOP2B* expression ($p = 9 \times 10^{-6}$) in lymphoblastoid cell lines [32]. The SNP we interrogated (rs10865801) in this analysis is in near perfect linkage disequilibrium with rs2363520 ($r^2 = 0.98$) and is significantly associated with *TOP2B* gene expression in human heart left ventricle tissue specimens ($p = 0.02$) [33]. Other groups have previously investigated SNPs in *TOP2B* and not found associations with anthracycline-induced cardiotoxic-

ity [8], however, we are the first to use a bioinformatics approach to directly interrogate a SNP known to affect *TOP2B* expression. Interestingly, a recent genome-wide association study (GWAS) of anthracycline-induced cardiotoxicity in childhood cancer identified a non-synonymous variant in *RARG* (rs2229774) that affects *TOP2B* expression, providing further evidence that *TOP2B* is a key regulator of this toxicity [34]. Based on the preclinical and GWAS evidence the genetic predictors of *TOP2B* expression and function should be further investigated in pharmacogenetic analyses.

Critical limitations of this analysis, and differences with prior analyses, may explain the lack of replication of previously reported candidate SNPs. Among the 166 patients who received doxorubicin and underwent echocardiogram, few ($n = 19$) cases of doxorubicin-induced systolic dysfunction were identified, severely restricting study power. Due to the low study power for pharmacogenetic analysis it is only possible to conclude that the previously reported pharmacogenetic associations could not be validated in this cohort, not that the associations do not exist. The low event rate is likely due to the rarity of this toxicity at the doses of anthracyclines used in the adjuvant treatment of breast cancer and also may partly be a consequence of the relatively young, healthy patient cohort enrolled on our study. Another important limitation to consider is that our analyses were restricted to patients who are still alive after treatment of their cancer years ago. Patients who died from cancer recurrence, other causes, or most importantly anthracycline-induced cardiotoxicity would not have been included in this analysis so the event rate may have been higher if participation had not been conditional on survival. Furthermore, this analysis differs from the previous pharmacogenetic discovery and replication studies in critical ways. Prior associations for *SLC28A3* and cardiotoxicity were reported in cohorts of anthracycline-treated children [9,10], whereas our cohort included only adults. The mechanisms and/or predictors of anthracycline-induced cardiotoxicity are likely to be distinct within these two treatment settings. The patients included in the analyses of *NCF4* and *RAC2* [8,11] received doxorubicin concurrently with cyclophosphamide, vincristine and prednisone (CHOP) with or without rituximab (R-CHOP) on standard lymphoma protocols. The drugs and regimens used in lymphoma treatment are different from those used in the breast cancer patients in our cohort. Twenty percent of the patients in our cohort were treated with sequential use of cardiotoxic trastuzumab and 80% of patients received chest radiation, either of which may contribute to the inability to replicate these associations. It is possible that genetic predictors of anthracycline-induced cardiotoxicity are distinct in patients of different ages, tumor types and/or treatment regimens and pharmacogenetic discovery and validation will need to be conducted within large cohorts of similar patients receiving standardized treatment for a single tumor type, such as prospective clinical trial cohorts.

Conclusion

The association of four candidate SNPs with putative effects on anthracycline-induced cardiotoxicity

could not be validated in an independent cohort of doxorubicin-treated breast cancer patients. A secondary pharmacogenetic screen of 23 SNPs detected associations for SNPs in *ABCB1* and *CBR3* with systolic dysfunction, though these uncorrected associations require additional replication in larger cohorts of patients with doxorubicin-induced cardiotoxicity. Validation of these SNPs as predictors of cardiotoxicity would enable treatment individualization, via selection of alternative regimens, enhanced cardiotoxicity monitoring, modification of maximum cumulative doses, or possibly initiation of cardioprotective agents [35] to reduce the occurrence of anthracycline-induced cardiotoxicity in women with breast cancer.

Future perspective

Individualized prediction of anthracycline-induced cardiotoxicity will slowly make its way into clinical care. Over the next decade, analyses of large clinical trial cohorts will discover useful risk predictors that can be successfully validated in independent patient cohorts. These predictors will not be limited to genetics, but include clinical and biochemical features. These risk predictors will eventually be useful for estimating an individual patient's maximum anthracycline dose or selecting alternative therapeutic approaches for high-risk patients.

Supplementary data

To view the supplementary data that accompany this paper, please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/PGS.15.162

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Disclaimer

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive Summary

- Cardiotoxicity is a rare but serious adverse effect of anthracycline treatment.
- Genetic predictors of doxorubicin-induced cardiotoxicity have been reported, but have not been sufficiently validated for clinical translation. The most promising candidates based on prior literature are SNPs in *RAC2* (rs13058338), *NCF4* (rs1883112) and *SLC28A3* (rs7853758).
- The exact mechanism of anthracycline-induced cardiotoxicity is unknown, but recent evidence strongly implicates Topoisomerase 2–Beta in the pathopharmacologic process. Using publicly available bioinformatics tools we discovered a common SNP (rs10865801) that is associated with *TOP2B* expression in cell lines and human heart tissue.
- The SNPs in *RAC2*, *NCF4*, *SLC28A3* and *TOP2B* were not associated with risk of anthracycline-induced cardiotoxicity in this cohort.
- There are several potential reasons for this lack of replication. The most likely reason is low study power caused by a small number of events in this cohort. Other potential explanations include differences in the patients, tumors, treatment, or phenotypes, particularly the use of adult breast cancer patients in this analysis and children or lymphoma patients in prior studies.
- Pharmacogenetic screening of 23 SNPs identified a potential protective association for *ABCB1* 3435C>T (rs1045642, odds ratio = 0.48; $p = 0.049$) and a risk association for *CBR3* V244M (rs1056892, odds ratio = 2.50; $p = 0.012$) that require additional replication in independent patient cohorts.
- The *ABCB1* association can be explained by increased systemic or myocardial cell efflux of doxorubicin for carriers of the variant genotype. The *CBR3* effect, on the other hand, is in the opposite direction of previous associations for this SNP with similar phenotypes. This discordance is further complicated by discrepant findings in preclinical and clinical analyses of the influence of *CBR3* V244M on doxorubicin metabolism and doxorubicinol exposure.
- More comprehensive understanding of the mechanistic basis of anthracycline-induced cardiotoxicity will enhance our ability to intelligently select candidate SNPs for pharmacogenetic association testing.
- This phenotype is particularly difficult to study due to the low incidence rate and the delay between treatment and the onset of toxicity. Large cohorts of patients will be required to collect enough events for sufficiently powered discovery and validation pharmacogenetic studies, particularly for less common genetic variants.

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