Null Results in Brief

Cancer Epidemiology, Biomarkers & Prevention

Single-Nucleotide Polymorphisms in *Aldo-Keto* and *Carbonyl Reductase* Genes Are Not Associated with Acute Cardiotoxicity after Daunorubicin Chemotherapy

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Abstract

Background: Evidence suggests that interpatient variability in anthracycline metabolic rate may contribute to the cardiotoxicity associated with anthracycline-based chemotherapy. Therefore, polymorphisms in the anthracycline metabolizing enzymes have been proposed as potential biomarkers of anthracycline-induced cardiotoxicity (AIC).

Methods: We have previously shown that 13 of the naturally occurring nonsynonymous single-nucleotide polymorphisms (nsSNP) in the *aldo–keto reductases (AKR)* and *carbonyl reductases (CBR)* reduce anthracycline metabolic rate *in vitro*. Here, we test these SNPs individually and jointly for association with daunorubicin-induced cardiotoxicity in patients with acute myeloid leukemia (AML).

Results: Five of the 13 nsSNPs exhibiting an *in vitro* effect on anthracycline metabolism were detected among the 185 patients with AML. No association was found between the SNPs and daunorubicin-induced cardiotoxicity in either individual or joint effect analyses.

Conclusions: Despite the shown *in vitro* effect of nsSNPs in reductase genes on anthracycline metabolic rate, on their own these SNPs do not explain enough variability in cardiotoxicity to be useful markers of this adverse event.

Impact: The results of this study provide important information for biomarker studies on side effects of anthracycline chemotherapy. *Cancer Epidemiol Biomarkers Prev; 21(11); 2118–20. ©2012 AACR.*

Introduction

Anthracyclines are effective anticancer drugs; however, their use is limited by side effects including life-threatening cardiotoxicity. The large interpatient variability in sensitivity to anthracyclines correlates with the wide range of pharmacokinetic values reported for these drugs, which is largely a reflection of differences in the phase I conversion of anthracyclines to their metabolites catalyzed by the aldo–keto reductases (AKR) and carbonyl reductases (CBR; ref. 1). A cardioprotective role for CBRs has been shown in mouse models (2, 3), and it has been proposed that polymorphisms in these genes are likely to affect anthracycline metabolic rates, and thus contribute to anthracycline-induced cardiotoxicity (AIC). We have previously shown that 13 of the naturally occurring nonsynonymous single-nucleotide polymorphisms (nsSNP) in the reductase genes have a significant effect on the metabolism of anthracyclines *in vitro* (4). Here, we evaluate these nsSNPs for their association with AIC in a population of patients with acute myeloid leukemia (AML) undergoing daunorubicin-based chemotherapy.

Material and Methods

Peripheral blood samples were obtained from 185 patients with AML after informed consent and with approval from the Clinical Research Ethics Board of the University of British Columbia (Vancouver, BC). All patients were Caucasian, an average age of 46 years old (range 14–74 years old), 99 females and 86 males. Patients received daunorubicin in combination with cytarabine for initial remission induction and subsequent consolidation therapy (daunorubicin average cumulative dose: 323 mg/m^2; range: 60–780 mg/m^2).

Cardiac function was monitored by left ventricular ejection fraction (LVEF) measurements pre- and postadministration of the treatment. Percentage drop in LVEF was used as a quantitative cardiac outcome after daunorubicin treatment.

All patients were genotyped for 13 nsSNPs in 4 *AKR* and 2 *CBR* genes (Table 1) using the Sequenome genotyping system (Sequenome Inc.). As quality control, only SNPs

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doi: 10.1158/1055-9965.EPI-12-1037

Table 1. Nonsynonymous SNPs in reductase genes with shown *in vitro* effect on anthracycline metabolism

Gene	SNP	Amino acid change	Frequency in CEU[a]	Frequency in AML population
CBR1	rs1143663	V88I	0.005	0.000
	rs41557318	P131S	0.017	0.000
CBR3	**rs1056892**	**V244M**	**0.300**	**0.331**
	rs8133052	**C4Y**	**0.536**	**0.470**
	rs2835285	V93I	0.017	<80% GR[b]
	rs4987121	M235L	0.000	0.000
AKR1C3	rs35575889	R170C	NA[c]	Not in HWE
	rs34186955	P180S	NA[c]	Failed genotyping
	rs4987102	A106T	0.000	0.000
AKR1C4	**rs17134592**	**L311V**	**0.158**	**0.149**
AKR7A2	**rs1043657**	**A142T**	**0.097**	**0.087**
AKR1A1	rs6690497	E55D	0.000	0.000
	rs2229540	**N52S**	**0.044**	**0.058**

NOTE: Bolded SNPs were tested for association with daunorubicin-induced cardiotoxicity.
[a]CEU, one of HapMap populations representing Utah residents with Northern and Western European ancestry.
[b]GR, genotyping rate.
[c]NA, not available.

with frequency more than 1%, in Hardy–Weinberg equilibrium, and with genotyping rate more than 95% were included in statistical analysis.

Initially, a base model for predicting LVEF% drop was built from the nongenetic variables (i.e., gender, age, and cumulative dose) using stepwise addition based on Akaike information criterion. Only the nongenetic variables predictive of the outcome were included in the genetic model.

The joint effect of all SNPs on LVEF% drop was assessed using a global test of association, and the individual effects of each SNP were assessed with standard likelihood ratio tests (5). All analyses were adjusted for gender and cumulative dose after applying a logarithmic transformation to stabilize the outcome variance.

All statistical analyses were conducted within the R software environment for statistical computing, using the GlobalTest package (6).

Results

Of the 13 nsSNPs included in the study, 5 passed the quality control filters and were used in statistical analysis (bolded SNPs in Table 1). Initial tests for association of nongenetic variables with percentage drop in LVEF identified only cumulative dose and gender to be predictive of LVEF drop. Therefore, all tests for the effect of the SNPs on the outcome were adjusted for cumulative dose and gender. The likelihood ratio tests for association of each SNP individually found no association for any of the 5 SNPs with LVEF drop (Table 2). Furthermore, no

Table 2. Results of likelihood ratio tests for the effect of each SNP on the drop in LVEF

Gene	SNP rs#	Amino acid change	Test on	*P* value
AKR1C4	rs17134592	L311V	SNP	0.9556
			SNP+SNP*dose	0.7646
AKR7A2	rs1043657	A142T	SNP	0.4379
			SNP+SNP*dose	0.7398
CBR3	rs1056892	V244M	SNP	0.6988
			SNP+SNP*dose	0.2927
	rs8133052	C4Y	SNP	0.7788
			SNP+SNP*dose	0.8492
AKR1A1	rs2229540	N52S	SNP	0.1667
			SNP+SNP*dose	0.3405

NOTE: "SNP+SNP*dose", a test for effect of SNP including linear interaction terms with dose.

association was found when all 5 SNPs were tested for joint effect ($P = 0.889$). Given that cumulative dose could potentially be a modifying variable, we also tested the effect of the SNPs individually and jointly including linear interaction terms with dose. These tests similarly did not detect any significant associations (Table 2; $P = 0.581$ for the global test of joint effect of 5 SNPs and 5 interactions of SNPs and dose).

Discussion

The aim of this study was to determine if any of the nsSNPs in reductase genes with a shown *in vitro* effect on anthracycline metabolism are associated with AIC in a population of patients with AML. Because serial measurements of LVEF are commonly used to monitor asymptomatic cardiotoxicity during anthracycline chemotherapy (1), we used the percentage drop in LVEF as a measure of acute cardiotoxicity. We found no association between the nsSNPs in reductase genes and AIC in either individual or joint effects models.

To the best of our knowledge of the SNPs investigated here, only rs1056892 (V244M) in *CBR3* has been previously tested for association with AIC, and was found to be associated in survivors of childhood cancers by Blanco and colleagues but not by Visscher and colleagues (7, 8). Similar to Visscher and colleagues, we did not find an association between the SNP and AIC in a population of adult patients with AML. However, our study differs from previous studies in that it is looking at acute and not chronic cardiotoxicity in adult population.

In summary, despite their shown *in vitro* effect on anthracycline metabolism, the nsSNPs in reductase genes were not associated with daunorubicin-induced cardiotoxicity. However, given that AIC is a complex phenotype, it is possible that these variants may improve predictive power of polygenic models in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Grant Support

The study was funded by Canadian Institutes for Health Research (Grant #21R45100).

Received September 7, 2012; accepted September 12, 2012; published OnlineFirst September 20, 2012.

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