Single nucleotide polymorphisms in aldo-keto and carbonyl reductase genes are not associated with acute cardiotoxicity after daunorubicin chemotherapy.

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Running Title: SNPs in reductases are not markers of AIC

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Abstract

**Background:** Evidence suggests that inter-patient 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 demonstrated that 13 of the naturally occurring non-synonymous (ns)SNPs in the aldo-keto and carbonyl reductases (AKRs and CBRs) reduce anthracycline metabolic rate *in vitro*. Here, we test these SNPs individually and jointly for association with daunorubicin (DAUN) induced cardiotoxicity in acute myeloid leukemia (AML) patients.

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

**Conclusions:** Despite the demonstrated *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.

**Introduction**

Anthracyclines are effective anti-cancer drugs; however, their use is limited by side effects including life-threatening cardiotoxicity. The large inter-patient 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 AKRs and CBRs (1). A cardioprotective role for CBRs has been demonstrated 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 AIC. We have previously shown that 13 of the naturally occurring nsSNPs 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 AML patients undergoing DAUN-based chemotherapy.

Material and Methods

Peripheral blood samples were obtained from 185 AML patients after informed consent and with approval from the Clinical Research Ethics Board of the University of British Columbia. All patients were Caucasian, an average age of 46 years old (range 14 – 74 years old), 99 females and 86 males. Patients received DAUN in combination with cytarabine for initial remission induction and subsequent consolidation therapy (DAUN average cumulative dose: 323 mg/m²; range: 60-780 mg/m²).

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

All patients were genotyped for 13 nsSNPs in 4 AKR and 2 CBR genes (Table 1) using the Sequenome genotyping system (Sequenome Inc., San Diego, CA). As quality control (QC) only SNPs with frequency above 1%, in Hardy-Weinberg Equilibrium, and with genotyping rate above 95% were included in statistical analysis.
Initially, a base model for predicting LVEF % drop was built from the non-genetic variables (ie. gender, age, and cumulative dose) using stepwise addition based on Akaike’s Information Criterion. Only the non-genetic 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 performed 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 QC filters and were used in statistical analysis (bolded SNPs in Table 1). Initial tests for association of non-genetic variables with percent 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 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 demonstrated in vitro effect on anthracycline metabolism are associated with AIC in a population of AML patients. Since serial measurements of LVEF are commonly used to monitor asymptomatic cardiotoxicity during anthracycline chemotherapy (1), we used the percent 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 et al. but not by Visscher et al. (7, 8). Similar, to Visscher et al. we did not find an association between the SNP and AIC in a population of adult AML patients. 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 demonstrated in vitro effect on anthracycline metabolism, the nsSNPs in reductase genes were not associated with DAUN 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.

Grant Support

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References


Table 1. Non-synonymous SNPs in reductase genes with demonstrated in vitro effect on anthracycline metabolism.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Amino Acid change</th>
<th>Freq in CEU</th>
<th>Freq in AML population</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBR1</td>
<td>rs1143663</td>
<td>V88I</td>
<td>0.005</td>
<td>0.000</td>
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<tr>
<td></td>
<td>rs41557318</td>
<td>P131S</td>
<td>0.017</td>
<td>0.000</td>
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<td>CBR3</td>
<td>rs1056892</td>
<td>V244M</td>
<td>0.300</td>
<td>0.331</td>
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<tr>
<td></td>
<td>rs8133052</td>
<td>C4Y</td>
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<td>0.470</td>
</tr>
<tr>
<td></td>
<td>rs2835285</td>
<td>V93I</td>
<td>0.017</td>
<td>&lt;80% GR</td>
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<td></td>
<td>rs4987121</td>
<td>M235L</td>
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<td>AKR1C3</td>
<td>rs35575889</td>
<td>R170C</td>
<td>NA</td>
<td>Not in HWE</td>
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<tr>
<td></td>
<td>rs34186955</td>
<td>P180S</td>
<td>NA</td>
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</tr>
<tr>
<td></td>
<td>rs4987102</td>
<td>A106T</td>
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<tr>
<td>AKR1C4</td>
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<td>L311V</td>
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<tr>
<td>AKR7A2</td>
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<td>A142T</td>
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<tr>
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<td>0.000</td>
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<tr>
<td></td>
<td>rs2229540</td>
<td>N52S</td>
<td>0.044</td>
<td>0.058</td>
</tr>
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</table>

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP rs#</th>
<th>Amino acid change</th>
<th>Test on</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
<td>AKR1C4</td>
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<td>L311V</td>
<td>SNP, SNP+SNP*dose</td>
<td>0.9556, 0.7646</td>
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<tr>
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<td>rs1043657</td>
<td>A142T</td>
<td>SNP, SNP+SNP*dose</td>
<td>0.4379, 0.7398</td>
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<tr>
<td>CBR3</td>
<td>rs1056892</td>
<td>V244M</td>
<td>SNP, SNP+SNP*dose</td>
<td>0.6988, 0.2927</td>
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<td></td>
<td>rs8133052</td>
<td>C4Y</td>
<td>SNP, SNP+SNP*dose</td>
<td>0.7788, 0.8492</td>
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<tr>
<td>AKR1A1</td>
<td>rs2229540</td>
<td>N52S</td>
<td>SNP, SNP+SNP*dose</td>
<td>0.1667, 0.3405</td>
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</tbody>
</table>
Tables Legends

Table 1. Non-synonymous SNPs in reductase genes with demonstrated *in vitro* effect on anthracycline metabolism. Bolded SNPs were tested for association with DAUN induced cardiotoxicity. *a*CEU – one of HapMap populations representing Utah residents with Northern and Western European ancestry. *b*GR=genotyping rate. NA=not available.

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

“SNP+SNP*dose” = a test for effect of SNP including linear interaction terms with dose.
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