

# Pharmacokinetics and pharmacogenomics of daunorubicin in children: a report from the Children's Oncology Group

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## Abstract

**Purpose** We explored the impact of obesity, body composition, and genetic polymorphisms on the pharmacokinetics (PK) of daunorubicin in children with cancer.

**Patients and methods** Patients  $\leq 21$  years receiving daunorubicin as an infusion of any duration  $< 24$  h for any type of cancer were eligible. Plasma drug concentrations were measured by high-performance liquid chromatography. Body composition was measured by dual-energy X-ray absorptiometry. Obesity was defined as a BMI  $> 95$  % for age or as body fat  $> 30$  %. NONMEM was used to perform PK model fitting. The Affymetrix DMET chip was used for genotyping. The impact of genetic polymorphisms was

investigated using SNP/haplotype association analysis with estimated individual PK parameters.

**Results** A total of 107 subjects were enrolled, 98 patients had PK sampling, and 50 patients underwent DNA analysis. Population estimates for daunorubicin clearance and volume of distribution were  $116 \text{ L/m}^2/\text{h} \pm 14 \%$  and  $68.1 \text{ L/m}^2 \pm 24 \%$ , respectively. Apparent daunorubicin clearance and volume of distribution were  $26.8 \text{ L/m}^2/\text{h} \pm 5.6 \%$  and  $232 \text{ L/m}^2 \pm 10 \%$ , respectively. No effect of body composition or obesity was observed on PK. Forty-four genes with variant haplotypes were tested for association with PK. FMO3-H1/H3 genotype was associated with lower daunorubicin clearance than FMO3-H1/H1,  $p = 0.00829$ . GSTP1\*B/\*B genotype was also associated with lower daunorubicin clearance compared to GSTP1\*A/\*A,  $p = 0.0347$ . However, neither of these associations was significant after adjusting for multiple testing by either Bonferroni or false discovery rate correction.

**Conclusions** We did not detect an effect of body composition or obesity on daunorubicin PK. We found suggestive associations between FMO3 and GSTP1 haplotypes with daunorubicin PK that could potentially affect efficacy and toxicity.

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## Introduction

Daunorubicin is an anthracycline antineoplastic drug widely used in childhood cancer. Despite its frequent use, however, daunorubicin's pharmacokinetics (PK) and pharmacodynamics (PD) have not been studied widely

in children and very little is known about the relationship between PK parameters and pharmacogenetic markers or covariates like obesity, body composition, age, gender, or ethnicity.

Daunorubicin is administered intravenously over a wide range of doses and schedules. As with other anthracyclines, daunorubicin is extensively metabolized through a complex process involving reduction to the C-13 alcohol metabolite daunorubicinol as well as conversion of both daunorubicin and daunorubicinol to various aglycones, with daunorubicinol the primary metabolite reported in human plasma (reviewed in Robert [1]). Daunorubicinol is more than a log less cytotoxic in vitro than the parent drug daunorubicin [2]. However, alcohol metabolites of anthracyclines may contribute significantly to cardiac toxicity, an important late effect of treatment [3–10].

Recent reports have begun to elucidate the genetic contribution to variability in anthracycline PK/PD, predominantly focusing on doxorubicin (reviewed in Lau 2010 [11]). Among the genes involved in anthracycline uptake and efflux in cells are *MDR1* [12]; the glutathione conjugate transporter *RLIP76* [13]; and the organic cation transporter *SLC22A16* [14]. Genes implicated in anthracycline metabolism include the carbonyl reductases *CBR1* and *CBR3* [15], and the pregnane X receptor *PXR* [16]. Anthracyclines and their metabolites may also act as substrates for the glutathione S-transferases (GSTM1, GSTP1, and GSTT1) [17, 18]. To our knowledge, studies of the impact of these genes on daunorubicin PK in children have not been performed. Furthermore, the influence of body size on anthracycline PK and treatment outcomes is unclear [19–22]. We therefore performed a study to describe daunorubicin PK in children and to explore the impact of obesity, body composition, and genetic polymorphisms on daunorubicin PK.

## Patients and methods

### Subjects and clinical methods

IRB approval and written informed consent were obtained according to federal and institutional guidelines. Patients  $\leq 21$  years of age, receiving daunorubicin as an infusion of any duration  $< 24$  h on either a 1-day or 2-day schedule, were eligible. There were no restrictions on diagnosis or concomitant chemotherapy, and there were no organ function requirements as the decision to administer daunorubicin was not part of the study. Pregnant and breast feeding patients and those with a large prosthesis that might interfere with body composition testing were excluded.

Height and weight were measured and used to calculate body surface area (BSA) and body mass index (BMI).

Body composition was measured by dual-energy X-ray absorptiometry (DEXA) in subjects enrolling at institutions with this capability. A CBC, serum creatinine, ALT, AST, bilirubin, total protein, albumin, alkaline phosphatase, and gamma glutamyl transferase were obtained at baseline. Patients were on numerous concomitant medications, and it was not possible to analyze their effect.

### Pharmacokinetic samples and assay

Blood samples were obtained in sodium heparin tubes stored on wet ice until centrifugation at 4 °C within 15 min of collection to separate the plasma, which was then frozen at  $-20$  to  $-80$  °C until analysis. For single dose regimens, samples were obtained prior to the drug infusion, at the midpoint of the infusion for infusions  $\geq 30$  min in duration, and at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, and 72 h after the end of the infusion. For 2 day dosing, samples were obtained on day 1 prior to the infusion, at 0, 0.5, 1, 2, 4, and 6 h after the end of the day 1 infusion, immediately prior to the day 2 infusion, and at 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 48, and 72 h after the end of the day 2 infusion. Samples were initially collected only from a site different from the daunorubicin infusion site. Subsequently, a catheter-clearing procedure was validated to permit samples to be obtained from a central venous catheter that had been used to administer drug. Plasma concentrations of daunorubicin and its metabolite daunorubicinol were measured using a validated high-performance liquid chromatography assay with fluorescence detection [23, 24]. The recovery of daunorubicin and daunorubicinol was  $> 90$  %. The limit of quantitation was 17 nM for daunorubicin and 10 nM for daunorubicinol. The intraday coefficient of variation was  $< 3$  %, and the interday variation was  $< 7$  % for both daunorubicin and daunorubicinol.

### Pharmacokinetic model

We modeled the PK of daunorubicin and daunorubicinol using NONMEM VI (ICON Development Solutions, Elliott City, MD) on a personal computer using a DIGITAL Visual FORTRAN compiler (version 6.1). Final model selection was based on visual inspection of the fit of the model to the data. A stepwise modeling approach similar to that described Hempel et al. [25] was used.

For daunorubicin, a two-compartment model was built using the ADVAN5 subroutine. Exponential error models were used to describe the interindividual variance in each PK parameter, with a proportional residual error model. To analyze the pharmacokinetics of the metabolite daunorubicinol, the pharmacokinetic parameters for daunorubicin were fixed to the estimated parameters and used as inputs to estimate the parameters of daunorubicinol. A

one-compartment model was used for daunorubicinol with the NONMEM subroutine ADVAN7. Exponential error models were used to describe the interindividual variance in the apparent volume of distribution and apparent clearance for daunorubicinol. The residual error model included both proportional and additive terms. The final model was used to generate estimates of the individual PK parameters for each patient. These estimates were then used in post hoc statistical analyses to explore the impact of obesity, body composition, and genetic polymorphisms on the PK parameters. PK parameters were linearly scaled based on BSA.

#### Analysis of the effect of body composition

Two approaches were used to evaluate potential body size or composition effects. First, we incorporated BMI, BMI percentile, z-BMI, and body fat content as covariates in the NONMEM base models, screening each PK parameter for significant covariate effects graphically using S-PLUS and LOESS curve-fitting. Promising covariates were then incorporated into the model, and we used the likelihood ratio test to evaluate the improvement relative to the base model. A change in the objective function of at least 3.84 for each degree of freedom (corresponding to a  $p < 0.05$ ) was required to consider the improvement statistically significant.

Second, we conducted a post hoc analysis comparing the individual PK parameters of obese versus non-obese subjects. The analysis was repeated using two different definitions of obesity: (1) BMI greater than the 95th percentile and (2) for the subset of patients ( $n = 48$ ) who had DEXA measurement of body composition, body fat  $>30\%$ . To make these comparisons, we used an unpaired  $t$  test. All  $p$  values are two-sided, and we considered a  $p < 0.05$  to be statistically significant. To screen for the effect of other factors on PK, we used S-PLUS to examine plots of each parameter against the available clinical variables (age, gender, race/ethnicity, and baseline laboratory values).

#### Other analyses of potential variables effecting PK

We investigated the potential effect of other clinical variables (age, gender, race/ethnicity, and baseline laboratory data) on each PK parameter graphically using S-PLUS and LOESS curve-fitting.

Covariates that showed potential relationships with PK parameters were then evaluated by direct assessment within NONMEM. With the incorporation of each covariate, a likelihood ratio test was used to evaluate the improvement relative to the base model. A change in the objective function of at least 3.84 for each degree of freedom (corresponding to a  $p < 0.05$ ) was required to consider the improvement statistically significant.

#### Analysis of the effect of genetic polymorphisms

Approximately 5 mL of whole blood was collected in K<sub>2</sub>-EDTA-coated vacutainers. DNA was isolated using the QIAamp DNA Blood Maxi Kit (Qiagen Inc, Valencia, CA) according to manufacturer's protocol. DNA was analyzed via Nanodrop 8000 (Thermo Scientific, Wilmington, DE) spectrophotometric readings (260/280) for quantity and quality and diluted to 60 ng/μL. Samples were evaluated by PicoGreen after dilution, and those that did not contain enough DNA were amplified using the Repli-g method (Qiagen Inc). Sufficient high-quality DNA was available for 50 individuals to be genotyped on Affymetrix DMET Plus arrays. Of the 1,834 successfully genotyped SNPs, 595 had a minor allele frequency  $>0.05$  and were in Hardy–Weinberg equilibrium ( $p > 0.001$ ). These 595 SNPs were tested for association with daunorubicin and daunorubicinol clearance (L/m<sup>2</sup>/h) using a linear additive model in the software PLINK [26]. The clearances were log<sub>2</sub>-transformed prior to association testing, which created approximately normal distributions. The DMET Plus software also provides haplotypes (i.e., \*1/\*2 alleles) for 57 of the genes on the array. Forty-four genes had at least one individual with a variant haplotype, and these haplotypes were tested for association with daunorubicin and daunorubicinol clearance. Only haplotypes classified as unique (“UNIQ” by the DMET Plus software) were used in the association tests. Linear regression was performed using R statistical software.

#### Results

One hundred and seven subjects enrolled; seven withdrew prior to the completion of PK sampling. PK data were analyzable for 98 patients. Subject characteristics are shown in Table 1. The median age was 12 years (range 0.5–20.4), median weight was 51.4 kg (range 7.5–126), and median BMI was 20.2 (range 13.2–39.9). Five patients were underweight (BMI  $<10$ th percentile for age), 65 were normal weight (BMI 10th–85th percentile), twelve were overweight (BMI 85th–95th percentile), and 16 were obese (BMI  $>95$ th percentile). Forty-eight patients had body composition measured by DEXA scan. Median body fat was 26% (range 12–46), and 15 patients had body fat  $>30\%$ .

The PK model consisted of two compartments for daunorubicin and one for daunorubicinol. The population PK parameters are shown in Table 2. Daunorubicin clearance and volume of distribution were 116 L/m<sup>2</sup>/h  $\pm$  14% and 68.1 L/m<sup>2</sup>  $\pm$  24%. The apparent daunorubicinol clearance and volume of distribution were 26.8 L/m<sup>2</sup>/h  $\pm$  5.6% and 232 L/m<sup>2</sup>  $\pm$  10%. Interindividual variability was large, particularly for the volume of distribution parameters for both daunorubicin and daunorubicinol.

**Table 1** Patient characteristics ( $n = 98$ )

Age (years)	
Median	12
Range	0.5–20.4
Gender	
Male	67
Female	31
Race	
White	70
Black	12
Other/unknown	16
Ethnicity	
Non-Hispanic	75
Hispanic	15
Other/unknown	8
Diagnoses	
ALL	60
AML	32
Lymphoma	5
Other	1
Weight (kg)	
Median	51.4
Range	7.5–126
BMI ( $\text{kg}/\text{m}^2$ )	
Median	20.2
Range	13.2–39.9
Body fat (%; $n = 48$ )	
Median	26.4
Range	11.9–45.7
Infusion time (min)	
$\leq 15$	33
15–30	31
60	1
3 h	1
6 h	32

The diagnostic plots for the model are shown in Fig. 1. Figure 1a and b shows the population (PRED) and individual estimated (IPRED) model concentrations plotted against the measured plasma concentrations (DV) for daunorubicin. Figure 1c and d shows the population (PRED) and individual estimated (IPRED) model concentrations plotted against the measured plasma concentrations (DV) for daunorubicinol.

No significant effects of body composition covariates on PK parameters were identified. Table 3 shows the PK parameters in the patients defined as obese based on BMI criteria (BMI greater or equal to 95 %) compared with non-obese patients. Table 4 shows the PK parameters in patients defined as obese on the basis of body fat measurements (body fat >30 %) compared with non-obese patients. No

**Table 2** Population pharmacokinetic model parameters for patients receiving short and long daunorubicin infusions

Parameter	Population mean	Interindividual variability
CL—Daunorubicin ( $\text{L}/\text{h}/\text{m}^2$ )	116 ( $\pm 14$ %)	63.6 % $\pm$ 24.1 %
$V_1$ —Daunorubicin ( $\text{L}/\text{m}^2$ )	68.1 ( $\pm 24$ %)	151 % $\pm$ 23 %
$V_2$ —Daunorubicin ( $\text{L}/\text{m}^2$ )	128 ( $\pm 25$ %)	80.1 % $\pm$ 26 %
Q—Daunorubicin ( $\text{L}/\text{hr}/\text{m}^2$ )	312 ( $\pm 15$ %)	99.0 % $\pm$ 31 %
$\text{CL}_m/\text{F}$ —Daunorubicinol ( $\text{L}/\text{h}/\text{m}^2$ )	26.8 ( $\pm 5.6$ %)	63.6 % $\pm$ 22.6 %
$V_m/\text{F}$ —Daunorubicinol ( $\text{L}/\text{m}^2$ )	232 ( $\pm 10$ %)	108.6 % $\pm$ 27.9 %

statistically significant differences were observed in either comparison.

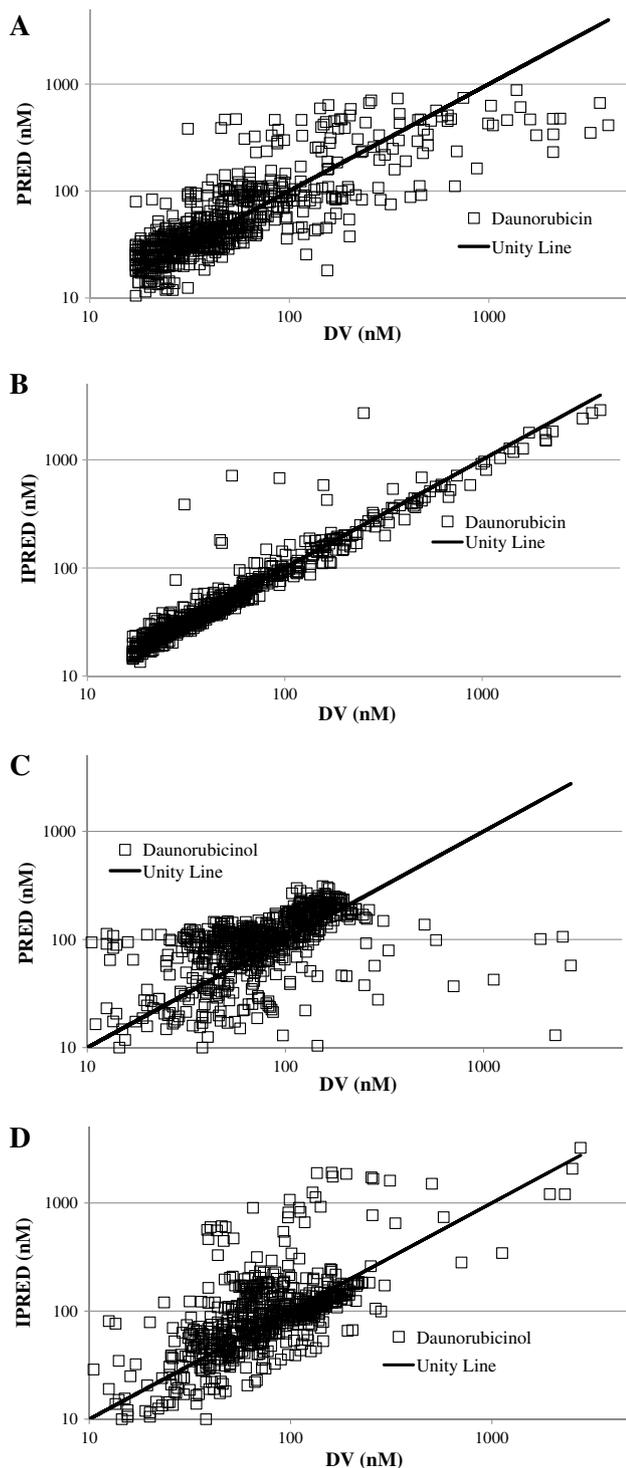
No significant relationships between age, gender, race/ethnicity, or baseline laboratory values and PK were observed. For laboratory values, this was an expected result since the overwhelming majority of patients enrolled in the study had no significant abnormalities in organ function or baseline laboratory values. Patients were on numerous concomitant medications, and it was not possible to analyze their effect on PK.

#### PK–PG results

Of the 1,834 genotyped SNPs from 50 patients, 595 had a minor allele frequency (MAF) >0.05 and were in Hardy–Weinberg equilibrium ( $p > 0.001$ ). These 595 SNPs were tested for association with daunorubicin and daunorubicinol clearance using a linear additive model. Eighteen SNPs associated with daunorubicin clearance (Supplemental Table S1,  $p < 0.05$ ) and 37 associated with daunorubicinol clearance (Supplemental Table S2,  $p < 0.05$ ). None of the associations remained significant after adjusting for multiple comparisons. Of 44 genes with variant haplotypes tested for association with the two phenotypes, the best results ( $p < 0.05$ ) were for FMO3 and GSTP1. In univariate analyses, individuals with FMO3-H1/H3 ( $n = 7$ ) have significantly lower daunorubicin clearance than individuals with FMO3-H1/H1 ( $n = 8$ ),  $p = 0.00829$ . Individuals with GSTP1\*B/\*B ( $n = 7$ ) have significantly lower daunorubicin clearance than individuals with GSTP1\*A/\*A ( $n = 19$ ),  $p = 0.0347$ . Figure 2 illustrates the effect on clearance. However, neither of these associations was significant after adjusting for multiple testing by either Bonferroni or false discovery rate correction.

#### Discussion

In this study, we described daunorubicin PK in children with cancer and examined the influence of obesity, body



**Fig. 1** **a** Comparison of population predicted (PRED) and measured daunorubicin concentrations (DV). **b** Comparison of individual predicted (IPRED) and measured daunorubicin concentrations (DV). **c** Comparison of population predicted (PRED) and measured daunorubicinol concentrations (DV). **d** Comparison of individual predicted (IPRED) and measured daunorubicinol concentrations (DV)

composition, and genetic polymorphisms on daunorubicin PK. There were no significant differences in daunorubicin clearance and daunorubicinol clearance between obese and non-obese patients. Associations between FMO3-H1/H3 and GSTP1\*B/\*B genotypes and lower daunorubicin clearance were suggested, though neither of these associations was significant after adjusting for multiple testing.

Despite daunorubicin's use in the treatment of a wide range of malignancies, there are limited data available in the literature on its PK. Our population model has several similarities to what has been previously reported for daunorubicin PK in other studies. Hempel et al. [25] developed a population model for daunorubicin and daunorubicinol in infants with leukemia. Their model was similar structurally (two compartments for daunorubicin and one for daunorubicinol), and they estimated daunorubicin clearance to be  $43.9 \text{ L/m}^2/\text{h} \pm 65 \%$ . The apparent clearance of daunorubicinol was  $19.1 \text{ L/m}^2/\text{h} \pm 9 \%$ , similar to what we report. In a study in adults with sixteen patients receiving daunorubicin  $50 \text{ mg/m}^2$  as a 10-min infusion, Callies et al. estimated somewhat higher clearances of  $168 \text{ L/h} \pm 36 \%$  for daunorubicin and  $54.3 \text{ L/h} \pm 27 \%$  for daunorubicinol [27]. Assuming an average BSA of  $1.73 \text{ m}^2$ , the normalized estimates from the Callies study are  $97.1$  and  $31.3 \text{ L/m}^2/\text{h}$ . In our study, large interindividual variability was observed in the volume of distribution parameter estimates. This probably reflects the wide variation of drug doses and schedules administered. Estimates of volume of distribution, particularly for daunorubicin, are influenced by the early concentration time points and may be influenced by the infusion time and the sampling schedule.

Glutathione S-transferases (GSTs) are a polygenic group of enzymes involved in the phase 2 detoxification of a wide range of xenobiotics and chemotherapeutic agents by catalyzing the conjugation of reactive electrophiles to glutathione [28]. Anthracyclines can be substrates for the glutathione S-transferases (GSTM1, GSTP1, and GSTT1) [17, 18], and GSTs are also involved in the detoxification of reactive oxygen species, which can be formed from anthracyclines [29, 30]. *GSTP1* is polymorphic with two single-nucleotide substitutions in the coding sequence (1578A>G and 2293C>T), giving rise to Ile105Val and Ala114Val amino acid substitutions [30]. Both amino acid residues lie within the substrate-binding site of *GSTP1* [31]. Three functional haplotypes have been identified: *GSTP1*\*A (105Ile;114Ala), *GSTP1*\*B (105Val;114Ala), and *GSTP1*\*C (105Val;114Val) [32]. Therefore, *GSTP1*\*B is a haplotype containing the variant at 105, a polymorphism close to the substrate-binding site that changes the enzymatic activity for particular substrates. In our study,

**Table 3** Effect of BMI on daunorubicin and daunorubicinol pharmacokinetic parameters

Parameter (units)	BMI <95 % (median ± SD) N = 82	BMI ≥95 % (median ± SD) N = 16	p value
CL—Daunorubicin (L/h/m <sup>2</sup> )	125 ± 73.6	121 ± 54.3	0.80
V <sub>1</sub> —Daunorubicin (L/m <sup>2</sup> )	54.8 ± 214	76.5 ± 258	0.92
V <sub>2</sub> —Daunorubicin (L/m <sup>2</sup> )	317 ± 266	370 ± 154	0.65
Q—Daunorubicin (L/h/m <sup>2</sup> )	137 ± 214	198 ± 102	0.66
CL <sub>m</sub> /F—Daunorubicinol (L/h/m <sup>2</sup> )	31.9 ± 20.7	27.9 ± 10.5	0.083
V <sub>m</sub> /F—Daunorubicinol (L/m <sup>2</sup> )	329 ± 186	258 ± 252	0.058

**Table 4** Effect of percentage body fat on daunorubicin and daunorubicinol pharmacokinetic parameters

Parameter (units)	FAT % <30 (median ± SD) N = 33	FAT % >30 (median ± SD) N = 15	p value
CL—Daunorubicin (L/h/m <sup>2</sup> )	123 ± 83.6	112 ± 54.3	0.39
V <sub>1</sub> —Daunorubicin (L/m <sup>2</sup> )	62.7 ± 180	37.5 ± 142	0.13
V <sub>2</sub> —Daunorubicin (L/m <sup>2</sup> )	309 ± 227	261 ± 178	0.14
Q—Daunorubicin (L/h/m <sup>2</sup> )	149 ± 130	106 ± 73.1	0.053
CL <sub>m</sub> /F—Daunorubicinol (L/h/m <sup>2</sup> )	36.0 ± 26.1	30.6 ± 8.8	0.23
V <sub>m</sub> /F—Daunorubicinol (L/m <sup>2</sup> )	387 ± 211	424 ± 174	0.18

those carrying the variant had lower adjusted daunorubicin clearance than individuals with *GSTP1*\*A/\*A or wild type.

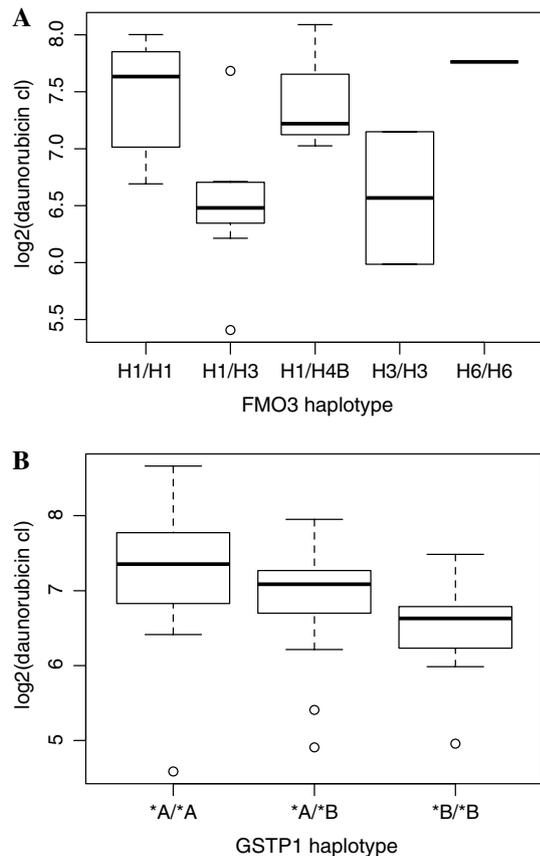
There is a paucity of published data on the role of *GSTP1* and daunorubicin; however, data from studies with doxorubicin indicate that GSTs have an important role in anthracycline pharmacology. In support of our hypothesis, previous work has shown that breast cancer patients carrying the homozygous *GSTP1* 105Val variant had a lower risk of chemoresistance when treated with doxorubicin [33]. Our results are also compatible with other studies in multiple myeloma, breast and colon cancer, and Hodgkin's lymphoma that showed better outcomes (e.g., progression-free survival or overall survival) for patients with *GSTP1* 105Val following chemotherapy with drugs known to be *GSTP1* substrates [34–37]. Similarly, a study of 68 children with intermediate- and high-risk ALL found that patients who were homozygous for the valine variant were at lower risk for CNS relapse [38]. The authors suggested that impaired detoxification ability in patients with the homozygous valine mutation led to increased active drug exposure and reduced the risk of CNS relapse.

Our work supports the hypothesis that the significance of *GSTP1* polymorphisms may be explained at least in part by pharmacokinetic effects. Lower clearance of parent drug in individuals homozygous for the valine allele, as seen in our study, could result in increased exposure to active drug in these patients, which in turn could improve outcomes. In addition, previous work has shown that polymorphisms in GSTs have important pharmacodynamic effects. In studies with cultured human lymphocytes, the genetic damage induced in vitro by doxorubicin was strongly

influenced by *GSTP1* genotype. Lymphocytes from individuals homozygous for the valine variant had a significant increase in chromosomal damage compared with individuals with at least one wild-type allele [39].

Compared to the GSTs, significantly less is known about the flavin-containing monooxygenase (FMO) enzymes. *FMO3* is a hepatic microsomal enzyme that oxidizes a host of drugs, xenobiotics, and other chemicals. Numerous variants in the gene encoding *FMO3* have been identified. Some of the variants alter enzymatic activity and, consequently, substrate metabolism. [40] Most notably, *FMO3* plays an important role in the N-oxidation of the antipsychotic drug olanzapine. [41].

Although *GSTP1* and *FMO3* genotype may have an impact on anthracycline pharmacology, it is clearly not the only important genetic factor. Doxorubicin clearance has shown to be lower and AUC higher in patients with genetic variations such as at least one *ABCB1* c.1236C>T allele [42], or homozygosity for c.146A>G and c.312T>C polymorphisms in the influx transporter *SLC22A16* [43]. Accordingly, doxorubicin clearance is higher and AUC lower in patients with a CC-GG-CC genotype at the *ABCB1* 1236-2677-3435 loci [42]. In our study, *ABCB1* genotype did not affect daunorubicin clearance; however, the C allele of another SNP in *ABCB1* (rs2235033), which is linked to the C allele of *ABCB1* c.1236C>T, was associated with increased daunorubicinol clearance in the univariate analysis (Table S2). The clinical impact of this change is unknown, but it is possible that patients with faster clearance and lower daunorubicinol exposure could have less risk of cardiotoxicity.



**Fig. 2** **a** Boxplot of *FMO3* genotype versus daunorubicin clearance. Individuals with *FMO3*-H1/H3 ( $n = 7$ ) have significantly lower daunorubicin clearance than individuals with *FMO3*-H1/H1 ( $n = 8$ ),  $p = 0.00829$ . **b** Boxplot of *GSTP1* genotype versus daunorubicin clearance. Individuals with *GSTP1*\*B/\*B ( $n = 7$ ) have significantly lower daunorubicin clearance than individuals with *GSTP1*\*A/\*A ( $n = 19$ ),  $p = 0.0347$

In summary, our current work is the largest pharmacokinetic study of daunorubicin and its metabolite daunorubicinol in children. The study explored the effect of obesity, body composition, and drug metabolizing genotypes on daunorubicin and daunorubicinol PK. Our study design did not permit analysis of the toxicity or efficacy of daunorubicin, so it is still possible that obesity, body composition, and other drug metabolizing genotypes may have important clinical effects. Future studies should be conducted to further evaluate these factors and to evaluate the influence of *GSTP1* and *FMO3* haplotype on daunorubicin PK. Understanding the impact of these clinical and genetic factors on pharmacokinetics and pharmacodynamics may lead to safer and more effective therapy with anthracyclines in children.

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