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# Novel risk factors for glucarpidase use in pediatric acute lymphoblastic leukemia: Hispanic ethnicity, age, and the *ABCC4* gene

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## Abstract

**Background—**Carboxypeptidase $G_2$ ($CPDG_2$; glucarpidase) is a rescue drug for patients at risk for kidney injury from high-dose methotrexate (MTX). As there are no strategies for predicting patients who will require $CDPG_2$, we evaluated the role of demographic, clinical, and genetic factors for $CPDG_2$ use.

**Procedure—**Cases who received $CPDG_2$ and controls who did not were identified by chart review of acute lymphoblastic leukemia (ALL) patients who received MTX doses between 1000–5000mg/m2 between 2010–2017. We used multivariable Bayesian logistic regression to evaluate the association of $CPDG_2$ use with demographic and clinical variables and, on a subset of patients, with genetic ancestry and 49 single nucleotide variants previously associated with MTX toxicity.

**Results—**We identified 423 patients who received 1592 doses of MTX. Of the 18 patients who received $CPDG_2$, 17(94%) were Hispanic. No patients who received 1000 or 2000 mg/m2 of MTX received $CPDG_2$. Hispanic ethnicity (odds ratio: 4.68; 95% compatibility interval:1.63–15.06) and older age (1.87[1.17–3.17]) were associated with receiving $CPDG_2$. Of the 177 patients in the genomic cohort, 11 received $CPDG_2$. Each additional G allele of rs7317112 in *ABCC4* increased the odds of requiring $CPDG_2$ (3.10[1.12–6.75]). Six other loci (*NTRK1*/rs10908521, *TSG1/*rs9345389, *STT3B/*rs1353327, *SCLO1B1*/rs4149056, *GATA3/*rs3824662, *ARID5B/*rs10821936) demonstrated probabilities of association between 88–97%.

**Conclusion—**We demonstrated that demographic characteristics, including Hispanic ethnicity and age, are associated with $CPDG_2$ use. Additionally, we provide evidence that inherited genetic variation is associated with risk of requiring $CPDG_2$. If validated in independent populations, this information could be leveraged to develop targeted toxicity prevention strategies for children with ALL.

## Previous abstracts:

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CONFLICT OF INTEREST

The authors have nothing to disclose.

DATA AVAILABILITY

Data are available from the Baylor College of Medicine institutional data access (contact via epicenter@bcm.edu) for researchers who meet criteria.

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Zobeck M, Bernhardt M, Kamdar K, Rabin K, Lupo P, Scheurer M. Novel risk factors for glucarpidase use in pediatric acute lymphoblastic leukemia (ALL): Hispanic ethnicity, age, and the ABCC4 gene. In: International Society of Pediatric Oncology; 2020, October 14–17; Virtual platform. 2020.

### Keywords

Glucarpidase; Pediatric Acute Lymphoblastic Leukemia; Hispanic ethnicity; Genetics; Ancestry; Methotrexate

## INTRODUCTION

Methotrexate (MTX) is an important drug for the successful treatment of pediatric acute lymphoblastic leukemia (ALL), lymphomas, and osteosarcoma. Acute kidney injury is a known adverse effect from MTX as renal excretion accounts for 70–90% of drug clearance.[1] Carboxypeptidase $G_2$ ($CPDG_2$; glucarpidase) is administered to patients at risk for severe or life-threatening kidney injury from MTX. $CPDG_2$ is a recombinant bacterial enzyme that cleaves circulating MTX into inactive metabolites that are eliminated through the liver, resulting in a rapid decrease of MTX blood concentration and avoidance of continued nephrotoxic effect.[2]

Patients who receive $CPDG_2$ may be considered the patients at highest risk of suffering severe acute kidney injury. Although institutional thresholds for administering $CPDG_2$ vary, two common indications include higher than expected MTX levels, indicating a high risk for developing kidney injury, and rising serum creatinine, indicating an acute and evolving kidney injury. Therefore, studying risk factors for requiring $CPDG_2$ can reveal important insights about which patients are at highest risk for nephrotoxic complications from MTX administration.

While much work has been done to evaluate risk factors for other types of MTX toxicity,[3–8] little is known about use patterns of $CPDG_2$. Christensen et al. reported that 1.8% of patients (22/1131) who received high-dose MTX in the range of 3300 – 12000 mg/m$^2$ at St. Jude Children's Hospital required $CPDG_2$.[9] Svahn et al. reported that 3.6% (47/1286) of pediatric ALL patients in Nordic countries who received 5000 mg/m$^2$ of MTX required $CDPG_2$.[10] A recent study from Texas Children's Hospital (TCH) demonstrated that a higher proportion of patients required $CPDG_2$ compared to the proportions reported by Christensen or Svahn. While this difference in proportion may be due to different in institutional parameters for administration, it was noted that 80% of patients who received $CPDG_2$ in the TCH study

were of self-reported Hispanic ethnicity, a proportion well over the total proportion of Hispanic patients seen at the Center (35–50% depending on the diagnosis), and a population likely underrepresented in the previous published estimates.[11] Although there appeared to be no difference in 24-hour MTX serum concentrations between Hispanic and non-Hispanic patients at the Center, they observed a cluster of Hispanic patients with higher 24-hour methotrexate concentrations compared to the other Hispanic and non-Hispanic patients.

To explain these findings, Schafer et al. hypothesized that MTX-related pharmacogenomic variants may be more frequent in Hispanic populations and thereby explain the increased rate of $CPDG_2$ administration in this cluster.[11] There is a growing body of evidence to support the role of genetic ancestry and pharmacogenomic factors in ALL treatment outcomes[12–14] and, more specifically, in MTX toxicity.[15–19] None of these studies, however, have evaluated the effects of genomic factors on risk for $CPDG_2$ use.

As little is known about the predictors of $CPDG_2$ use, using a case-control approach, we sought to interrogate the demographic and clinical risk factors associated with this treatment feature among ALL patients treated with ≥1000 mg/m$^2$ of MTX. Moreover, on a subset of patients, we evaluated the effect of genetic ancestry and a set of candidate single nucleotide variants (SNVs) on the risk of requiring $CPDG_2$.

## METHODS

### The Clinical Cohort

All patients with ALL at TCH who received between 1000–5000 mg/m$^2$ of MTX from the period of September 2010–December 2017 were identified through medical record review. The National Institutes of Health standard self-report form was used to collect patient self-reported race and ethnicity. Clinical information including age, sex, leukemia type, $CPDG_2$ and MTX administration records, and height and weight prior to each MTX cycle were extracted from the electronic medical record, cleaned, and manually reviewed for accuracy. Local, institutional guidelines were followed for $CPDG_2$ administration, except where guided by any clinical research protocol. For patients not enrolled on a clinical trial, $CPDG_2$ may have been considered in the following circumstances: at 24 hours post infusion initiation if MTX level was ≥ 150 μM or for creatinine > 25% of the baseline level, at 36 or 42 hours if the MTX level was ≥ 10 μM, or at 48 hours or more for MTX levels ≥ 6 μM.

### The Genomic Cohort

Genotype data were available for a subset of 154 patients in the clinical cohort. Informed consent and assent, when appropriate, were obtained for sample collection according to institutional review board-approved protocols. Briefly, peripheral blood samples were collected during routine clinical blood draws once participants reached complete remission. DNA was extracted using the PerkinElmer (PerkinElmer, Inc., Waltham, MA) Prepito instrument, and each SNV was genotyped using the Illumina (Illumina, Inc., San Diego, CA) Infinium Global Screening Array according to the respective manufacturer protocols for each instrument. Genetic ancestry was estimated with STRUCTURE version 2.3.4 software assuming an admixture model with four underlying subpopulations using HapMap Phase 3

reference populations of European, African, East Asian, or Native American ancestry.[20] Native American genetic ancestry was used as a proxy variable for self-reported Hispanic ethnicity, consistent with established methodology.[21]

## SNV selection

Candidate SNVs related to MTX pharmacokinetics and pharmacodynamics were selected from the Pharmacogenomics Knowledge Base (PharmGKB)[22] and from a review of the literature by the investigators. We queried the database in September 2018 and found 109 individual variants associated with MTX toxicity and 42 specifically associated with toxicity in ALL patients Through manual literature review, we identified an additional 11 SNVs from two genome-wide association studies (GWAS)[23,24] describing variants with increased risk of hyperuricemia in Hispanic patients, primarily due to defects in renal urate transporters, that were also included for analysis (see full SNV list in Table S5).

## Statistical Analysis

Descriptive statistics were calculated for each demographic and clinical variable for the group of subjects that received $CPDG_2$ and the group that did not. For both the clinical and genomic cohort, Bayesian logistic regression was used to estimate odds ratios for each of the variables and $CPDG_2$ requirement. The model for the clinical cohort was constructed first. First, univariable models for each predictor variable were fit and analyzed (Table S1). Through an iterative process of model comparison using the expected logarithmic pointwise probability density[25] (Table S2) along with considerations of biological plausibility, the final clinical model included ethnicity and age. Age was transformed into a standardized variable (individual age minus mean age divided by standard deviation) for computational purposes. The outcomes for patients who received 1000–2000 $mg/m^2$ MTX were collected and reported but not included in modeling due to the paucity of literature describing their risk for requiring $CPDG_2$ and due to institutional clinical experience demonstrating a lack of $CPDG_2$ requirement in these dose ranges. All infant ALL patients received 4000 $mg/m^2$, and both B- and T-ALL patients received 5000 $mg/m^2$. logistic regression, which showed no change in the conclusions, but more precise estimates of the Bayesian coefficients (Table S3).

During the process of model specification, prior probability distributions for the parameters were defined using data from the literature and expert knowledge, and the prior predictive distribution was evaluated. In brief, a prior distribution for the intercept term was specified such that probabilities for requiring $CPDG_2$ between 0–10% were most likely and higher values were possible but increasingly less likely. Prior distributions for the variable coefficients were weakly informative and normally distributed, wherein the model allows for, but remains skeptical of, strong effect sizes for the variables.[26–28] The joint prior predictive distribution was evaluated via standard methods (see Appendix A in the supplement for further explanation).[28,29]

The model was run for 4000 iterations, a sufficient number to ensure probability convergence.[30] Posterior probability distributions were simulated using the Hamiltonian Monte Carlo algorithm, and convergence diagnostics were monitored.[28,31] Odds ratios (ORs) and compatibility intervals (CIs) for the parameters were reported, and probability of

positive association (PPA) of the parameter of interest was calculated. This latter statistic is calculated as the number of simulated ORs that were greater than one for each variable divided by the total number of simulations. This statistic is analogous to a one-sided p-value; however, instead of estimating how extreme are the data if the null hypothesis is true, the PPA produces a direct estimate of the probability that the hypothesis "a variable is positively associated with risk for requiring $CPDG_2$" is true given the model, prior distributions, and data.[32,33]

The genomic models were fit following a similar process. First, a model was fit with the ancestry variables for African, European and Native American genetic ancestry data. Then SNV-specific effects were estimated using a series of 49 models, one for each variant, coded as an additive genetic model and adjusted for ancestry. Similar prior and posterior distribution analyses were used as in the clinical cohort.

All statistical analyses were performed in R version 3.5.1.[34] Bayesian modeling was performed using the "brms" packages,[35] and all visualizations were constructed using the "ggplot2", "tidybayes", and "bayesplot" packages.[36–38]

## RESULTS

### Clinical Cohort

A total of 423 patients who received 1592 doses of MTX were identified. Forty-eight patients received MTX doses of 1000 mg/m$^2$ or 2000 mg/m$^2$; none of these patients required $CPDG_2$. Of the 375 patients who received 4000–5000 mg/m$^2$, 18 (4.8%) required $CPDG_2$. Of these patients, 17 (94%) were Hispanic (Table 1). The median age of patients who received $CPDG_2$ was 12.7 years (interquartile range [IQR]: 11.4–15.2), compared to 9.4 years (4.7 −13.6) for those who did not require $CPDG_2$. Sixteen of the 293 (5.5%) patients with B-ALL required $CPDG_2$, as did one of the 67 (1.5%) patients with T-ALL and one of the 15 (6.7%) patients with infant ALL.

Multivariable Bayesian logistic regression analysis demonstrated that patients of self-reported Hispanic ethnicity were 4.68 times more likely (95% compatibility interval [CI]:1.63–15.06) to require $CPDG_2$ compared to self-reported non-Hispanic patients. Older patients were also more likely to require $CPDG_2$, with the odds increasing by 1.87 per one standard deviation (5.15 years) increase in age (95% CI:1.17–3.17). Leukemia type, BMI, race, and sex did not demonstrate a clear association with $CPDG_2$ requirement (See Table S1 for univariable estimates; see Table S2 for model comparisons using ELPD-LOO cross-validation).

Using the full model, predicted probabilities of each patient requiring $CPDG_2$ by age and ethnicity demonstrated an increasing probability for older Hispanic patients. For example, a 9-year-old patient of Hispanic ethnicity has a probability of requiring $CPDG_2$ of 5.6% (95% CI: 2.9%−9.1%) compared to 1.4% (95% CI: 0.4%−3.1%) for a similar non-Hispanic patient (Table 3).

### Genomic Cohort

Of the patients in the clinical cohort, 154 patients had genotype information available, and 11 of these patients received $CPDG_2$. Ethnic assignment based on Native American (NA) ancestry correlated with Hispanic ethnicity in 91 of the 95 (95.8%) self-reported Hispanic patients and 6 of the 59 (10.1%) self-identified non-Hispanic patients. A model of the ancestry data demonstrated that a 10% increase in Native American ancestry resulted in an increased odd of requiring $CPDG_2$ (OR = 1.11; 95%CI: 0.98 – 1.27) with a PPA of 94%. The Asian, African, and European ancestry variables did not demonstrate a notable directional association with $CPDG_2$ use.

Of the 53 candidate SNVs identified, 49 were included in the final analysis. One variant was excluded because it was an insertion-deletion, and three others were excluded due to insufficient coverage of genotyping (Table S5 for full list of SNVs; Table S6 for sample genotype frequencies). The models of the genomic loci, which were adjusted to account for the association of ethnicity, demonstrated one SNV with a notable association. Each additional G allele of rs7317112 in *ABCC4,* which codes for an MTX efflux transporter in renal cells, conferred an increase in the odds of $CPDG_2$ use by 3.10 (95%CI:1.12–6.75) with a PPA of 99.5%. Six other loci (NTRK1/rs10908521, *TSG1/*rs9345389, *STT3B/*rs1353327, SCLO1B1/rs4149056, *GATA3/*rs3824662, *ARID5B/*rs10821936) also demonstrated probabilities of association between 88–97% (Table 4; Figure 1 for the model coefficients for each SNV; Table S7 for risk alleles).

A combined model of ethnicity, age, and the top performing SNV, rs7317112, demonstrated associations for each variable and $CPDG_2$ requirement, although the coefficient for age was less precisely estimated than in the larger clinical cohort (Table 5). This combined model the best predictive performance when compared using ELPD-LOO cross-validation to models containing only genetic predictors and only clinical predictors, although the difference was small (Table S8). These results can be interpreted that Hispanic ethnicity, age, and rs7317112 independently contain predictive information for $CPDG_2$ requirement, although precisely estimating the amount of predictive information is limited in by the sample size.

## DISCUSSION

This study originated from our observations that Hispanic patients were the majority of recipients of $CPDG_2$ at our institution. The results of the multivariable model support the association of Hispanic ethnicity with risk for requiring $CPDG_2$ after accounting for leukemia type, BMI, age. The model estimated that the odds of a Hispanic patient requiring $CPDG_2$ are, on average, 4.7 times higher than for a similar non-Hispanic patient. Using the full posterior probability distribution, the model predicted the probability of requiring $CPDG_2$, for a patient of average age and BMI, is 5.6% for a Hispanic patient compared to 1.4% for a non-Hispanic patient.

Hispanic ethnicity has previously been implicated both in risk for MTX toxicity as well as for poor ALL treatment outcomes. Our center recently reported that risk of neurotoxicity after high-dose MTX was increased in self-reported Hispanic patients.[39] Another study reported that Hispanic patients had a reduced median steady state MTX level compared to

non-Hispanic patients, although this finding was not associated with treatment outcomes.[40] Beyond associations with MTX toxicity, it has also been reported that patients of Hispanic ethnicity have inferior outcomes after leukemia therapy compared to non-Hispanic white patients.[41,42] Using NA as a correlate of Hispanic ethnicity, Yang and colleagues reported that the risk of relapse increased with increasing percentage of NA ancestry among leukemia patients treated on protocols that did not include a "delayed intensification" phase.[21]

In our study, NA ancestry correlated with Hispanic ethnicity and effect estimates similarly suggested an increased likelihood of requiring $CPDG_2$ with a PPA of 94%. This attenuated effect may be explained by the smaller sample size of the genomic cohort. When analyzed in the genomic cohort, the effect of Hispanic ethnicity was similar to the effect of NA ancestry (3.10 [0.– 11.65] compared to 2.81 [0.80 – 10.92] for 0 to 100% NA ancestry), suggesting that, similar to Hispanic ethnicity, NA ancestry would more precisely show a positive association with $CPDG_2$ use in a larger cohort.

We found older age was also strongly associated with risk for requiring $CPDG_2$. Our model reported an 80% increase in the odds of relapse for every 5-year increase in age. For example, while the model predicted a 5.6% expected chance of requiring $CPDG_2$ for a 9-year-old Hispanic patient, the probability increased to 17.5% (7.9% " 30.8%) for a 20-year-old Hispanic patient. Increasing age has been implicated in decreased MTX clearance,[43,44] risk for acute kidney injury[43,45] and liver toxicity,[43,46] and has been demonstrated in studies of adults as well.[7] While studies have correlated the effect of age and drug clearance with patient weight and body surface area (BSA),[44] we found that age had a strong relationship with risk for requiring $CPDG_2$ even after controlling for BMI.

None of the 43 patients who received 1000 $mg/m^2$ or the five patients with Down syndrome who received 2000 $mg/m^2$ required $CPDG_2$. A review of the literature yielded little information about rates of $CPDG_2$ use in these dose ranges. This information is useful for clinicians to assess subjective risks for patients receiving these doses of MTX; however, a larger cohort would better allow risk quantification.

The genomic analysis revealed multiple SNVs that may be associated with requiring $CPDG_2$. Of the 49 variants that were evaluated, seven demonstrated a PPA > 88%. Most noteworthy among the associations is rs7317112, an intronic variant (A>G) in an enhancer region of *ABCC4*. Each additional G allele demonstrated 250% increase in the odds of $CPDG_2$ use with a PPA of 99.5%. This association was demonstrated while controlling for Native American genetic ancestry, and, in a separate model, while controlling for Hispanic ethnicity and age, providing evidence that the observed genetic association is independent of genetic ancestry, ethnicity, or age (Table 5).

*ABCC4*, also known as *MRP4,* codes for a protein that plays an important role as an efflux transporter for organic anions on the cell membrane[47] and has specifically been identified with the transport of MTX out of renal cells for elimination.[48] In one study, there was suggestive evidence of an association between the G allele and higher 72-hour MTX concentrations.[49] Another study reported that the AA genotype was associated with mucositis when compared to the AG or GG genotypes.[50] In the present study, the G allele

was found to be the risk allele. Taking this biological evidence together with the statistical evidence from this study, rs7317112 may help explain some of the variation in $CPDG_2$ requirement, independent of the effects of age, ethnicity or genetic ancestry.

The T allele of rs10908521, an intronic variant in the genes *NTRK1* and *INSRR*, demonstrated a PPA of 97%. This variant was previously reported to have a possible association with decreased uric acid clearance in Hispanic patients.[23] The SNV rs1353327 in the genes *STT3B* and *THRAP3P1* demonstrated a PPA of 92% and has been associated with a decreased uric acid to urine creatinine ratio in Hispanic patients.[23] The G allele of rs9345389 (A>G) in *TSG3* demonstrated a PPA of 95% in our analysis and was reported to be associated with both increased MRD and increased MTX clearance.[12] The C allele of rs414056 in *SLCO1B1* has previously been associated both with decreased high-dose MTX clearance and a decreased tolerance to oral MTX during maintenance therapy.[19,51] This SNV demonstrated a PPA of 90%. The SNV rs3824662 in *GATA3*, a locus previously associated with risk for increased minimal residual disease and relapse in Ph-like ALL patients when treated on MTX-containing treatment regimens, demonstrated a PPA of 89%.[52] The C allele of rs10821936 (C>T) in *ARID5B* demonstrated a PPA of 89% in our analyses and was previously reported to be associated with increased MTX polyglutamate (a highly active intracellular metabolite of MTX) in patients with B-ALL.[13] Both from the statistical evidence presented here and biological evidence from the literature, these loci are promising candidates that warrant further investigation.

Our study must be considered in the light of some limitations. First, there are a small number of events in the dataset, which limits the inferences that can be made. The wide CIs demonstrated for variables such as the SNVs are evidence of influence of the prior distributions on the parameter estimates, suggesting insufficient information from the data for precise inference. Therefore, the lack of associations between variables and the outcome should not be interpreted as evidence of no association. Because the parameter estimates are sensitive to the prior distribution, specifying more skeptical priors for the coefficients decreased, but did not negate, both the PPA and the magnitude of the observed associations. Certain variables that may be associated with $CPDG_2$ requirement were not included in the analysis, such as baseline creatinine and a prior history of renal injury. As an observational study, the identified risk factors may be postulated to have a causal role in the observed effect only if certain conditions are met, which were not evaluated in this present study.[53] From these initial findings, future studies should evaluate an expanded list of predictor variable and develop a graphical causal model that may allow for theory-based confounding adjustment and causal analysis. Finally, while the scope of this analysis was limited to $CPDG_2$ requirement, it may be instructive in future studies to evaluate the co-occurrence of other MTX-induced toxicities such as mucositis, hepatotoxicity, and neurotoxicity to understand the full clinical consequences and potential protective effects of $CPDG_2$ use.

The primary strength of the study is the ethnically diverse population from which the sample was drawn, allowing analysis of a large cohort of Hispanic patients, a demographic relatively under-represented in the literature. The dataset included every patient at our center with ALL who received 1000 $mg/m^2$ of MTX or greater in the specified timeframe, allowing a comprehensive look at our center's treatment experience. Despite the small number of

events in the dataset, the Bayesian techniques employed in the analysis allowed for full use of the data, yielding fruitful inferential findings. Similarly, the prior distribution acts as a regularizing measure that reduces overfitting and controls for multiple testing, a problem that plagues standard frequentist analyses in the pharmacogenomic literature.

## CONCLUSION

This study is the first to explore clinical and genetic risk factors for requiring $CPDG_2$. We found that Hispanic ethnicity and increasing age are independent demographic risk factors. We also found that no patients who received 1000 or 2000 $mg/m^2$ of MTX required $CPDG_2$, and we identified multiple genomic loci that are highly likely to be associated with risk for $CPDG_2$ independent of the effects of genetic ancestry or Hispanic ethnicity. These findings give clinicians important information to better estimate the risk for requiring $CPDG_2$ in their patients. This study also supports the need to account for ethnicity when developing predictive models for $CPDG_2$ requirement. Genomic information may similarly augment predictive abilities and further explain the heterogeneous phenotype of MTX toxicity.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Glossary

| | |
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| **$CPDG_2$** | Carboxypeptidase $G_2$ |
| **ALL** | Acute lymphoblastic leukemia |
| **MTX** | Methotrexate |
| **GWAS** | Genome-wide association study |
| **OR** | Odds ratio |
| **CI** | Compatibility interval |
| **SNV** | Single nucleotide variant |

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**Novelty and Impact:**

Little is known about risk factors for requiring glucarpidase for methotrexate toxicity during pediatric acute lymphoblastic leukemia (ALL) treatment. This is the first study to look in depth at demographic, clinical and genetic risk factors for glucarpidase use. We report that Hispanic ethnicity and age are independent risk factors. Moreover, genetic ancestry and several single nucleotide variants previously associated with methotrexate toxicity are potential genetic risk factors. If validated in independent populations, this information could be leveraged to improve risk-stratification strategies for preventing methotrexate toxicity in children with ALL.

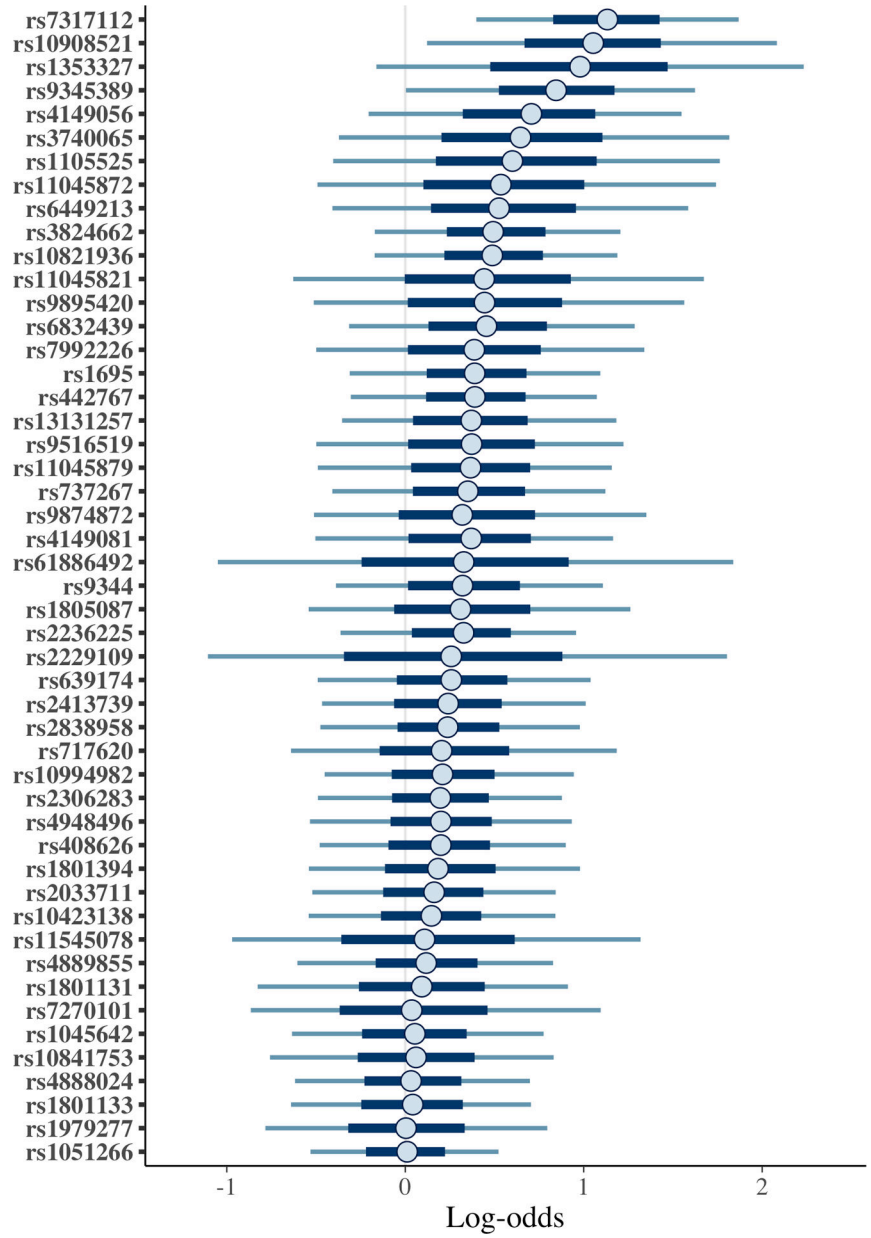


**FIGURE 1.**

Results of logistic regression models for the association of each SNV and $CPDG_2$ requirement. Each estimate is controlled for genetic ancestry and estimates are reported on the log-odds scale. All estimates are transformed to represent positive associations (i.e the allele associated with increased risk for $CPDG_2$ is the "risk" allele). Light blue points represent median values. Thick navy lines represent 50% compatibility intervals. Thin light blue lines represent 95% compatibility intervals.

**TABLE 1**

Descriptive statistics of the clinical and genomic cohorts

| Characteristic | | Clinical Cohort | | | Genomic Cohort | | |
|---|---|---|---|---|---|---|---|
| | | N | Did Not Receive $CPDG_2$, | Received $CPDG_2$, | N | Did Not Receive $CPDG_2$, | Received $CPDG_2$, |
| | | 375 | N = 357 (95%)[1] | N = 18 (4.8%)[1] | 154 | N = 143 (93%)[1] | N = 11 (7.1%)[1] |
| **Sex** | | | | | | | |
| | Female | | 135 (38%) | 8 (44%) | | 63 (44%) | 5 (45%) |
| | Male | | 222 (62%) | 10 (56%) | | 80 (56%) | 6 (55%) |
| **Age (standardized)** | | | 9.4(4.7, 13.6) | 12.7 (11.4, 15.2) | | 10.2 (6.4, 13.7) | 14.0 (11.2, 15.3) |
| **Ethnicity** | | | | | | | |
| | Non-Hispanic | | 156 (44%) | 1 (5.6%) | | 58 (41%) | 1 (9.1%) |
| | Hispanic | | 201 (56%) | 17 (94%) | | 85 (59%) | 10 (91%) |
| **Race** | | | | | | | |
| | Asian | | 25 (7.1%) | 0 (0%) | | 4 (2.9%) | 0 (0%) |
| | Black | | 37 (11%) | 0 (0%) | | 13 (9.3%) | 0 (0%) |
| | Native American | | 12 (3.4%) | 1 (5.6%) | | 8 (5.7%) | 0 (0%) |
| | White | | 276 (79%) | 17 (94%) | | 115 (82%) | 11 (100%) |
| | Unknown | | 7 (1.9%) | 0 (0%) | | 3 (1.9%) | 0 (0%) |
| **Methotrexate Dose** | | | | | | | |
| | 4000 $mg/m^4$ | | 15 (4.2%) | 1 (5.6%) | | 1 (0.7%) | 1 (9.1%) |
| | 5000 $mg/m^5$ | | 342 (96%) | 17 (94%) | | 142 (99%) | 10 (91%) |
| **Leukemia Type** | | | | | | | |
| | B-cell ALL | | 277 (78%) | 16 (89%) | | 114 (80%) | 10 (91%) |
| | T-cell ALL | | 66 (18%) | 1 (5.6%) | | 28 (20%) | 0 (0%) |
| | Infant ALL | | 14 (3.9%) | 1 (5.6%) | | 1 (0.7%) | 1 (9.1%) |
| **BMI (z-score)** | | | 0.22 (−0.71 1.34) | 1.16 (−0.81, 1.64) | | 0.13 (−0.69, 1.25) | 1.39 (0.10, 2.44) |

Carboxypeptidase $G_2$, $CPDG_2$.

[1]Statistics Presenting: n (%); median (inter-quartile range)

## TABLE 2

Results of the multivariable Bayesian logistic regression of clinical risk factors for requiring CPDG

| Variable | OR[1] | 95% CI[1] |
|---|---|---|
| **Ethnicity** | | |
| Non-Hispanic | - | - |
| Hispanic Ethnicity | 4.68 | 1.63–15.06 |
| **Age** | | |
| Age (standardized)[1] | 1.87 | 1.17– 3.17 |

[1]Odds ratio, OR; 95% compatibility interval, 95% CI.

[2]One standard deviation in age was 5.15 years

**TABLE 3**

Predictions of probability for requiring $CPDG_2$ from simulated data and the multivariable logistic regression model for age and ethnicity

| Simulated Data | Probability (%) | 95% CI (%) |
|---|---|---|
| 9-years-old, Non-Hispanic | 1.4 | 0.4 – 3.1 |
| 9-years-old, Hispanic | 5.6 | 2.9 – 9.1 |
| 19-years-old, Non-Hispanic | 4.8 | 1.1 – 12.0 |
| 19-years-old, Hispanic | 17.5 | 7.9 – 30.8 |

## TABLE 4

Results of the Bayesian logistic regression model of risk alleles for requiring CPDG.[1]

| SNV | OR | 95% CI | PPA[2] | Risk Allele | Reference Allele | Associated Gene | Previous Toxicity Association[3] |
|---|---|---|---|---|---|---|---|
| **rs7317112** | 3.10 | 1.12 – 6.75 | 99.5% | G | A | *ABCC4* | Genotype AA is associated with increased risk of mucositis when treated with methotrexate compared to genotypes AG + GG. |
| **rs10908521** | 2.87 | 0.53 – 8.05 | 97% | T | C | *INSRR/NTRK1* | Allele C is associated with uric acid clearance in Hispanic patients. |
| **rs1353327** | 2.66 | 0.35 – 9.41 | 92% | C | T | *STT3B/ THRAP3P1* | Allele T is associated with a decreased uric acid to urine creatinine ratio in Hispanic patients |
| **rs9345389** | 2.33 | 058 – 5.16 | 95% | G | A | *TSG3* | Allele G is associated with end-of-induction minimal residual disease (MRD) in childhood acute lymphoblastic leukemia (ALL) and is also associated with greater methotrexate clearance. when treated with methotrexate as compared to allele A. |
| **rs4149056** | 2.03 | 0.44–4.77 | 90% | C | T | *SLCO1B1* | Allele C has been associated with decreased high-dose methotrexate clearance and patients tolerating a lower dose of oral MTX. |
| **rs3824662** | 1.64 | 0.60 – 3.41 | 89% | A | C | *GATA3* | Allele A is associated with increased likelihood of relapse and increased minimal residual disease compared to allele C. |
| **rs10821936** | 1.63 | 0.60 – 3.36 | 89% | C | T | *ARID5B* | Allele C is associated with increased methotrexate polyglutamate accumulation when treated with methotrexate compared to allele T. |

Single nucleotide variant, SNV; odds ratio, OR; compatibility interval, CI.

[1] Analysis performed with each SNP and covariates for Hispanic, Asian and African genetic ancestry to control for correlation between ancestry and genotype.

[2] PPA = The probability of a simulated coefficient for each SNP to be greater than 1.

[3] All associations derived from www.PharmGKB.org except rs10908521, which is from Chittoor et al. 2017.

**Table 5**

Results of multivariable logistic regression analysis of factors associated with $CPDG_2$ requirement in the genomic cohort

| Covariate | Estimate[1] | 95% CI[1] |
|---|---|---|
| Age (standardized)[2] | 1.80 | 0.92 – 3.85 |
| Hispanic Ethnicity | 3.47 | 1.08 – 12.45 |
| rs7317112 | 3.07 | 1.21 – 8.03 |

[1]All values are odds ratios. CI, compatibility interval.

[2]One standard deviation in age was 5.15 years