Regular Article

CLINICAL TRIALS AND OBSERVATIONS

Genome-wide study of methotrexate clearance replicates *SLCO1B1*

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Key Points

- A genome-wide study of the association of over 5 million SNPs with methotrexate clearance in 1279 patients treated with HDMTX in multicenter COG trials 9904 and 9905.
- We replicated the finding that inherited variations in *SLCO1B1* are the most important genetic variations influencing methotrexate clearance.

Methotrexate clearance can influence the cure of and toxicity in children with acute lymphoblastic leukemia (ALL). We estimated methotrexate plasma clearance for 1279 patients with ALL treated with methotrexate (24-hour infusion of a 1 g/m² dose or 4-hour infusion of a 2 g/m² dose) on the Children's Oncology Group P9904 and P9905 protocols. Methotrexate clearance was lower in older children ($P = 7 \times 10^{-7}$), girls ($P = 2.7 \times 10^{-4}$), and those who received a delayed-intensification phase ($P = .0022$). A genome-wide analysis showed that methotrexate clearance was associated with polymorphisms in the organic anion transporter gene *SLCO1B1* ($P = 2.1 \times 10^{-11}$). This replicates findings using different schedules of high-dose methotrexate in St Jude ALL treatment protocols; a combined meta-analysis yields a *P* value of 5.7×10^{-19} for the association of methotrexate clearance with *SLCO1B1* SNP rs4149056. Validation of this variant with 5 different treatment regimens of methotrexate solidifies the robustness of this pharmacogenomic determinant of methotrexate clearance. This study is registered at http://www.clinicaltrials.gov as NCT00005585 and NCT00005596. (*Blood*. 2013;121(6): 898-904)

Introduction

High-dose methotrexate (HDMTX) is an important element of chemotherapy in the treatment of acute lymphoblastic leukemia (ALL) and other malignancies.[1-4] Mikkelsen et al[5] showed that a 1 g/m² dose given over 24 hours resulted in significantly greater active methotrexate polyglutamates in leukemic cells than the same dose given over 4 hours. In a randomized clinical trial, investigators from the Children's Oncology Group (COG) tested the efficacy and toxicity of a 2 g/m² dose over 4 hours versus a 1 g/m² dose over 24 hours.[6,7] The systemic exposure to methotrexate (ie, plasma concentration over time) is related to cure and toxicity in children with ALL.[8,9] In a group of children with ALL who were treated and monitored at a single institution (St Jude Children's Research Hospital), with extensive prospective therapeutic drug monitoring, even including pharmacokinetically guided dosage adjustments, we identified variants in the *SLCO1B1* gene that were associated with methotrexate clearance in a genome-wide association study (GWAS).[10,11] Herein, we sought to test whether we could replicate these GWAS findings in a large cohort of patients treated with alternative HDMTX schedules on the COG multi-institutional trials P9904 and P9905.

Methods

This study was approved by the institutional review boards of all participating institutions, and informed consent was obtained in accordance with the Declaration of Helsinki. Patients in P9904 included National Cancer Institute (NCI) standard-risk (age 1.00-9.99 years and initial white blood cell count [WBC]< 50 000/μL) patients with an *ETV6-RUNX1* translocation or simultaneous trisomies of chromosomes 4 and 10, whereas patients in P9905 included a mixture of NCI standard-risk patients without favorable genetic lesions, NCI high-risk (age ≥ 10 years and/or initial WBC ≥ 50 000/μL) patients with favorable genetic changes, and other NCI high-risk patients who did not meet age-, WBC-, and sex-specific criteria for especially high-risk disease originally described by Borowitz et al[6] and Shuster et al.[12] Patients were randomized in a 2 × 2 manner to 1 of 4 arms for consolidation: (A) 24-hour methotrexate infusion (1 g/m² given as a 200 mg/m² bolus over 20 minutes, followed by 800 mg/m² over 23.6 hours) and no delayed intensification (DI) phase; (B) 4-hour methotrexate infusion (2 g/m²) with no DI; (C) 24-hour methotrexate infusion with DI; and (D) 4-hour methotrexate infusion with DI. Leucovorin at 10 mg/m² was given every 6 hours (3 doses), beginning at 42 hours after the start of the infusion and continued until the plasma methotrexate level was less than
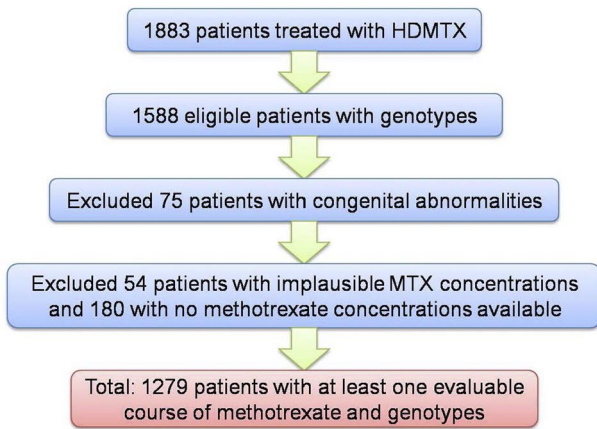

Figure 1. Flow chart of patients included in this analysis. A total of 1883 patients were treated with HDMTX on P9904 and P9905, and 1588 were eligible and had germline DNA evaluable. Patients who had congenital abnormalities (usually Down syndrome) were excluded because they may not have received the full methotrexate dose. Patients with implausible or no evaluable methotrexate plasma concentrations were excluded (see "Methods" for details).

$0.2\mu M$. Plasma methotrexate concentrations were requested to be drawn at the end of infusion (4 or 24 hours) and 24 hours later (postinfusion), and leucovorin was increased based on plasma methotrexate concentration (details in supplemental Methods, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). All patients with t(1;19)(q23;p13) were in P9905 as a separate stratum—all were assigned to receive a DI phase and randomized to arms C or D for the methotrexate infusion. Patients with simultaneous trisomies of chromosomes 4 and 10 all were assigned to receive no DI and randomized to arms A or B for the methotrexate infusion. Patients who did not respond to induction chemotherapy were not eligible.

Data were retrieved from the COG Statistical and Data Center research databases. Of the 1883 patients treated with HDMTX on P9904 and P9905, 1588 were eligible and had germline DNA evaluable.[6] Patients who had congenital abnormalities (usually Down syndrome, which is known to affect methotrexate clearance)[13-15] or who did not have any courses that passed methotrexate quality control were excluded (Figure 1). Demographic information on the patients included can be found in supplemental

Table 1. Although participating institutions in COG P9904/9905 were asked to submit data on plasma methotrexate concentrations, the data had not previously undergone any analysis or quality control. Therefore, it was necessary to establish a quality control approach to exclude courses with implausible data; for example, courses with undetectable plasma concentrations of methotrexate at the end of infusion were considered implausible and likely due to errors in drawing samples or in data entry. Courses with only 1 sample were excluded, as were courses for which the concentration of the postinfusion sample (28 or 48 hours) was greater than the end of infusion sample (4 or 24 hours). In addition, samples were removed that fell outside the expected 1-99th percentile range of methotrexate concentrations predicted with the use of the methotrexate pharmacokinetic parameters observed in nearly 500 prospectively monitored patients from St Jude's Total XV study (supplemental Table 2).[10,16]

In practice, this step resulted in the following: for the 24-hour infusion arms, end of infusion samples less than $2\mu M$ or greater than $38.5\mu M$ were excluded, and 48-hour samples greater than $7.5\mu M$ were excluded; for the 4-hour infusion arms, end of infusion samples less than $25\mu M$ or greater than $310\mu M$ were excluded, and 28-hour samples greater than $25\mu M$ were excluded. Low postinfusion samples were not excluded because they were considered plausible, whereas undetectable concentrations at the end of infusion (Figure 2) must be related to sampling or data entry errors. Thus, 252 of 4205 24-hour infusion courses (15 of 730 patients) and 653 of 4277 4-hour infusion courses (39 of 749 patients) were excluded on the basis of implausible pharmacokinetic data (likely due to data entry or sample acquisition error; Figure 2). Overall, the median clearance for all courses after excluding the outliers was similar to the median clearance prior to excluding the outliers (144.6 vs 141.2 mL/min/m² for the 4-hour and 162.5 vs 162.3 mL/min/m² for the 24-hour infusions).

Nonlinear mixed-effects modeling (Monolix 3.1; Lixoft) was used to determine population and individual posthoc methotrexate pharmacokinetic parameters. Specifically, a 2-compartment pharmacokinetic model with first-order elimination was fit to the data. The distribution of the parameters (both interoccasion and interindividual) was assumed log-normal. A proportional residual error model was used assuming normal distribution of the residuals. Furthermore, because of the sparse nature of the sampling in the current study, we included as a prior distribution the methotrexate pharmacokinetics from the St Jude Total XV study (supplemental Table 2). For the genome-wide analysis, we used the average methotrexate clearance value per patient.[10]

Figure 2. Concentration versus time plots. (A) Twenty-four-hour methotrexate infusion of 1 g/m². Concentrations shown were scheduled to be drawn at 24 hours (end of infusion) and at 48 hours (data are jittered [horizontal offset added] for better visualization). The pink lines represent the cut-offs (based on the range of the St Jude pharmacokinetic simulation) outside of which samples were excluded. There is no lower threshold for exclusion (pink line) for postinfusion samples. (B) Plasma concentrations were estimated for St Jude Total XV patients on the basis of a simulated dose of 1 g/m² methotrexate over 24 hours (solid black line) and COG patients (dotted black line) receiving the same dose. The 1-99th percentile range is shown with shading for the St Jude Total XV patients (blue) and COG patients (red). (C) 4-hour methotrexate infusion of 2 g/m². Concentrations shown were scheduled to be drawn at 4 hours (end of infusion) and at 28 hours (data are jittered for better visualization). (D) Plasma concentrations were estimated for St Jude Total XV patients based on a simulated dose of 2 g/m² methotrexate over 4 hours (solid black line) and for COG patients (dotted black line) receiving the same dose. The 1-99th percentile range is shown with shading for the St Jude Total XV patients (blue) and COG patients (red).
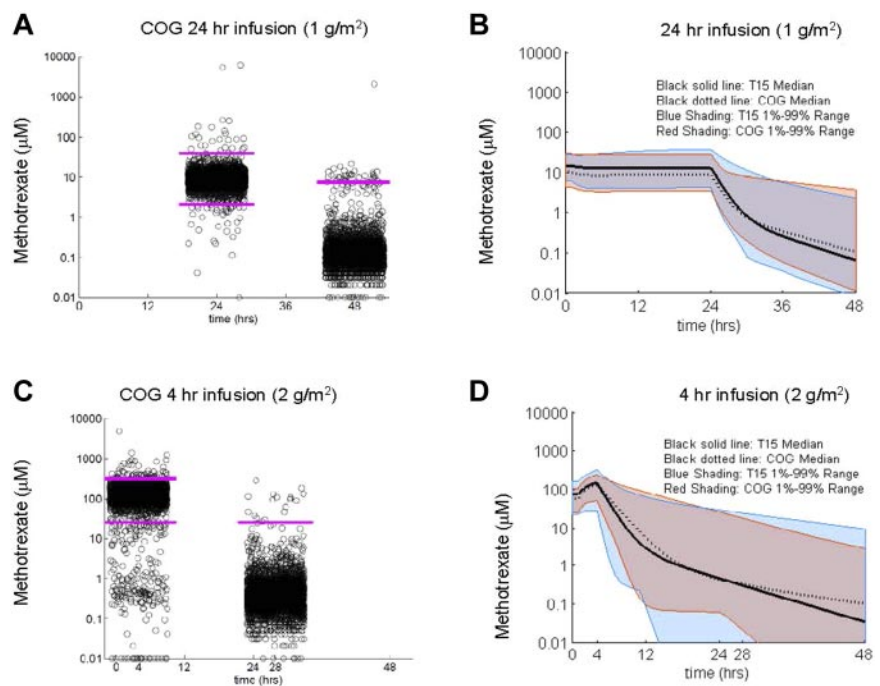
Table 1. Covariates related to methotrexate clearance in a multivariate general linear model

Factor	Direction	P^*	$R^2{}^*$	P†	R^2†
24-hour infusion (vs 4 hour)	24-hour higher	$< 2 \times 10^{-16}$	38%	$< 2 \times 10^{-16}$	38%
DI (vs no DI)	DI lower	.0010	0.5%	.0022	0.4%
Age	Older lower	9×10^{-7}	1.1%	7×10^{-7}	1.2%
Sex	Boys greater	.00028	0.6%	.00027	0.5%
rs4149056 T > C	C lower			2.1×10^{-11}	2%

DI indicates delayed intensification; direction, direction of association with clearance, ie, those receiving the 24-hour infusion had greater clearance than those receiving the 4-hour infusion; P, association of the factor with average methotrexate clearance based on a multivariate analysis; and R^2, the amount of interindividual variation explained by each factor.

*P and R^2 values for the multivariate model, including infusion length, use of DI, age, and sex.

†P and R^2 value for the multivariate model and also including the rs4149056 genotype.

Germline DNA was isolated at the time of remission from peripheral blood. DNA was extracted and genotyped using the Genome-Wide Human SNP Array 6.0 as described (Affymetrix, Santa Clara, CA).[17] DNA also was genotyped by the Illumina GoldenGate platform for 7 single-nucleotide polymorphisms (SNP; SNP Center, Johns Hopkins University). We did not perform sequencing or genotype for any rare variants. Genotype calls were coded as 0, 1, or 2 for the number of B alleles (AA, AB, BB). SNPs with a minor allele frequency less than 1% or a call rate less than 95% were excluded. Imputation of SNPs in the 1000 Genomes dataset not represented on the Affymetrix SNP 6.0 array was performed on each ancestral group independently using MACH1.0 (see supplemental Methods).[18]

A GWAS between germline SNP genotypes and log2-transformed methotrexate clearance was performed with the use of a general linear model assuming an additive effect of allelic dosage, with all models including age, genetic ancestry, sex, and treatment regimen as covariates, as we previously described.[10,11] Genetic ancestry was estimated with STRUC-TURE as previously described (see supplemental Methods).[17] Age was coded in years and treated as a continuous variable. Treatment regimen was coded as a categorical variable. Adjusted methotrexate clearance refers to the residual from a linear regression including the clinical covariates. A meta-analysis was performed by combining the statistics from the current GWAS with those obtained from the GWAS of methotrexate clearance in St Jude patients.[10] We calculated the combined P values from the meta-analysis using the Stouffer method.[19,20]

Results

We initially tested covariates for their association with methotrexate clearance in a multivariate general linear model (Table 1). Receipt of the 4-hour (rather than the 24-hour infusion; $P < 2 \times 10^{-16}$; Figure 3A) treatment arm, including a DI phase ($P = .0010$; supplemental Figure 1), older age ($P = 9 \times 10^{-7}$; supplemental Figure 2, consistent with previous reports),[10] and female sex ($P = .000\,28$, supplemental Figure 3) were all associated with lower clearance. Methotrexate clearance was not related to genetically defined patient ancestry in this study (supplemental Figure 4). In sum, we were able to explain 42.1% of the interindividual variability in methotrexate clearance with the clinical covariates and 1 SNP in *SLCO1B1*, rs4149056 (Table 1), in the same range as in our previous study.[11]

When we adjusted for age, sex, and treatment arm, methotrexate clearance displayed a unimodal population distribution (Figure 3B). A GWAS was performed for SNP-genotype associations with clearance, including age, sex, genetic ancestry, and treatment arm as covariates. The GWAS showed a strong association between methotrexate clearance and SNPs in *SLCO1B1* (Figure 4A; Table 2). There were 4 typed SNPs that reached a genome-wide significance threshold of $P < 5 \times 10^{-8}$ (rs4149081, rs4149056, rs11045879, and rs11045821). rs4149056 is a nonsynonymous coding SNP that has been shown to have reduced transport of

methotrexate in vitro.[11] Infusion length, treatment including a DI, age, and sex remained significantly associated with clearance in a multivariate model, including the rs4149056 genotype (Table 1); it is unclear why there was an association of DI with methotrexate clearance because it persisted after we adjusted for the sex, race, and age factors that might have confounded the association. The other 3 significant SNPs are in linkage disequilibrium (LD) with rs4149056. With each copy of the C allele at rs4149056, the adjusted methotrexate clearance was reduced by 12 mL/min/m^2 (Table 2; supplemental Figure 5); thus, clearance is (on average) approximately 13% lower in patients with CC versus TT genotypes at rs4149056, a magnitude of difference in exposure that could have significance for efficacy or adverse events.[9]
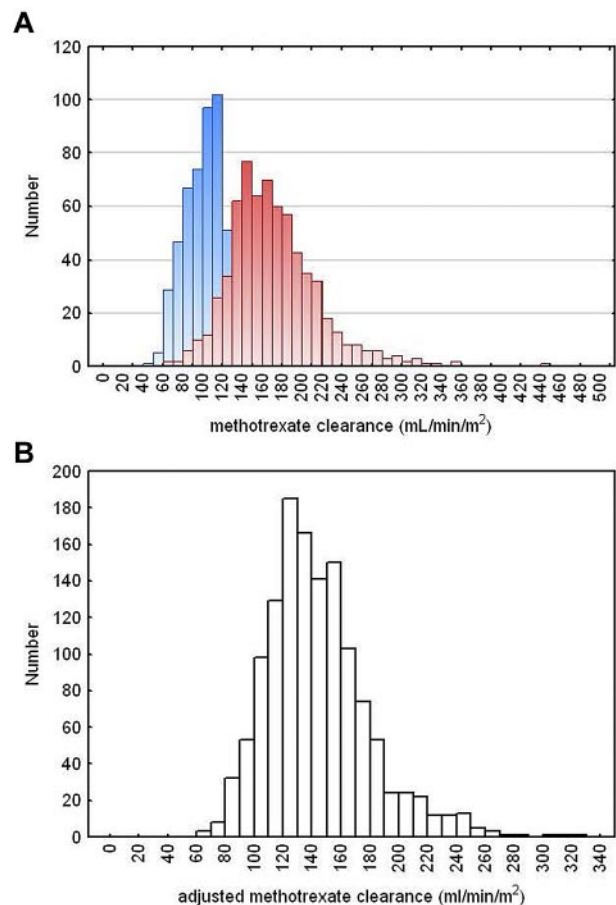

Figure 3. Histogram of each patient's average methotrexate clearance. (A) Unadjusted methotrexate clearance for the 655 COG patients receiving the 4-hour infusion (blue), and the 624 receiving the 24-hour infusion (red). (B) Methotrexate clearance in all COG patients (n = 1279), adjusted for age, sex, race, and treatment arm (residual from a linear regression model).
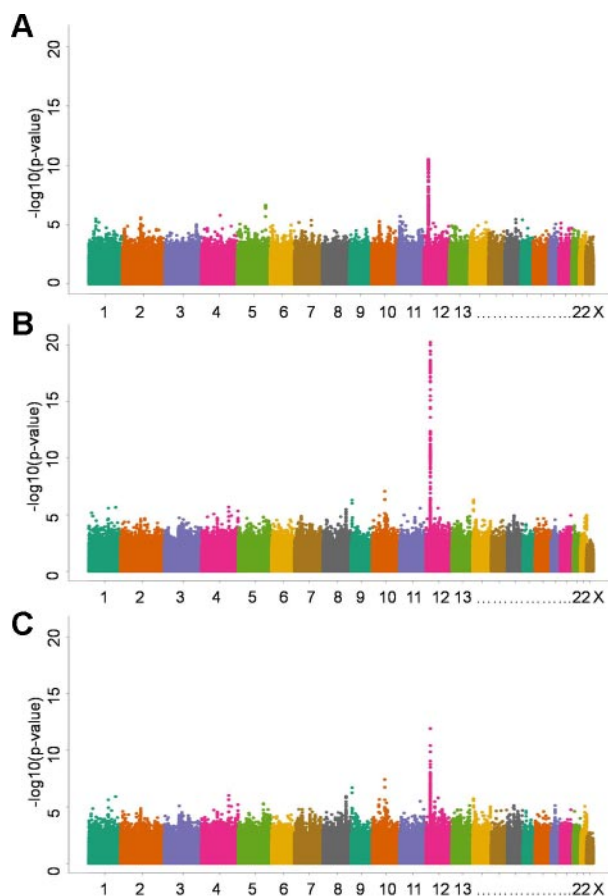
Figure 4. Manhattan plot of *P* values for a genome-wide association with methotrexate clearance. Each chromosome is plotted along the x-axis, whereas the y-axis plots the negative $\log_{10}$ of the *P* value for the association of each SNP with methotrexate clearance. Higher points on the graph indicate a stronger association with methotrexate clearance. Typed and imputed SNPs are shown in all panels (5.2 million SNPs). Methotrexate clearance is adjusted for age, sex, race, and treatment arm. (A) Association of each SNP with methotrexate clearance in COG patients (n = 1279). (B) Meta-analysis of St Jude (n = 699) and COG patients (n = 1279). (C) Meta-analysis of St Jude and COG patients, after we adjusted for rs4149056.

In a meta-analysis in which we combined patients treated with the COG and St Jude protocols, *SLCO1B1* remained significantly related to methotrexate clearance (Figure 4B). Of the 103 typed or imputed SNPs that reached a Bonferroni-corrected genome-wide significance threshold of $P < 9.62 \times 10^{-9}$ (supplemental Table 3), 101 were in *SLCO1B1*. The meta-analysis *P* value for the top SNP in *SLCO1B1*, rs4149080, is 5.61×10^{-21} (supplemental Table 3). This SNP is in LD ($r^2 = 0.897$) with rs4149056 in European patients and was imputed in both cohorts. The SNP typed in both the COG and St Jude cohorts that had the smallest *P* value was rs11045879, which is also in LD with rs4149056 ($r^2 = 0.865$), and had a *P* value of 3.13×10^{-19} in the meta-analysis. Two SNPs in *SLCO1A2* also were related to methotrexate clearance at the genome-wide level (7.6×10^{-10} and 1.9×10^{-9}); however, this is most likely because they are very near *SLCO1B1* on chromosome 12 and in LD with the rs4149056 SNP in *SLCO1B1* (Figure 5).[21,22] They are not in LD with the rs11045891 and rs4148981 SNPs that are *SLCO1A2* enhancers.[23] SLCO1B3 is a closely related family member that also transports methotrexate.[24,25] Although they did not reach genome-wide significance, 2 SNPs in *SLCO1B3* were related to methotrexate clearance ($P < 3 \times 10^{-6}$), and the association remained after we adjusted for SNPs in *SLCO1B1*. Four SNPs

in *SLC19A1* (the reduced folate carrier) were interrogated in this study on the Affymetrix array (2 are in LD with functional polymorphisms rs1051266 and rs1131596, $r^2 > 0.9$), but none reached genome-wide significance for an association with methotrexate clearance.

The nonsynonymous rs4149056 (T521C) SNP has been extensively characterized in relation to other phenotypes[26-28] and shown to reduce methotrexate transport in vitro.[11] After we adjusted for the rs4149056 genotype, a significant association remained in *SLCO1B1* at the genome-wide significance level (Figure 4C). The SNP with the lowest *P* value after adjustment for rs4149056 was an intronic SNP, rs4149040 ($P = 1.2 \times 10^{-12}$, supplemental Table 4), which is in LD with the nonsynonymous SNP rs2306283. Together, rs2306283 and rs4149056 define the most common haplotypes of *SLCO1B1* (*1a, *1b, *5, and *15)[29] and are in partial LD ($D' = 0.91, r^2 = 0.25$). As we previously demonstrated, the relationship between methotrexate clearance and rs2306283 genotype is evident only in an analysis stratified for rs4149056 genotype (supplemental Figure 6, $P = 4 \times 10^{-11}$),[11] indicating a highly reproducible SNP-by-SNP interaction. Patients with at least 1 C allele at rs4149056 have lower clearance than those with 2 T alleles (supplemental Figure 5); however, within each rs4149056 genotypic group, the A allele at rs2306283 was associated with even lower clearance, whereas the variant G allele was associated with increased clearance (supplemental Figure 6). We also replicated that the rs11045872 variant G allele was associated with greater methotrexate clearance ($P = 3.8 \times 10^{-7}$ in COG patients, supplemental Table 3; supplemental Figure 7).

Discussion

We have replicated the association between *SLCO1B1* and methotrexate clearance[10,11] with 2 additional schedules of intravenous methotrexate administration in the setting of a cooperative group trial. We estimated methotrexate clearance using 2 plasma methotrexate concentrations per course via a Bayesian pharmacokinetic modeling approach. The mean and range of clearances that we estimated from only 2 plasma concentrations was similar to that estimated from an entirely different group of patients with more samples per course (St Jude Total XV participants).[10,11] Further external validity of methotrexate clearance estimates from these large multicenter trials is provided by the fact that we replicated the association of methotrexate clearance with age, sex, and treatment regimen. We previously found that black patients had the greatest methotrexate clearance[10]; although this association was not replicated here, there were relatively few black patients (5%) in this COG cohort.

SLCO1B1 is an organic anion transporter that is known to transport methotrexate; minor alleles for SNPs in the *SLCO1B1* gene are associated with both increased and decreased transporter function and span multiple functional domains in the gene.[11] We found SNPs associated with both increased and decreased methotrexate clearance in the COG cohort, replicating our previous finding in the St Jude cohort (supplemental Figures 5-7).[11] We improved our power to detect significant associations by performing a meta-analysis combining the COG and St Jude cohorts. The meta-analysis showed that only SNPs on chromosome 12 near *SLCO1B1* were related to methotrexate clearance in these patients, many of which were not significant at the genome-wide threshold in either cohort alone. The nonsynonymous rs4149056 (T521C) SNP has been the subject of multiple clinical studies of statins and other agents.[26,30-34] However, even after adjustment for the

Table 2. Highest ranked SNPs associated with methotrexate clearance in a meta-analysis (typed in 1279 COG patients, typed or imputed in 699 SJ patients)

rsID	Meta P	A/B alleles	Gene	COG P	COG effect size	COG source	COG B allele freq	SJ P	SJ effect size	SJ source	SJ B allele freq
rs11045879	3.13×10^{-19}	T/C	*SLCO1B1*	8.31×10^{-10}	−11.69	SNPCHIP	0.17	6.23×10^{-11}	−11.27	SNPCHIP	0.16
rs4149056	5.72×10^{-19}	T/C	*SLCO1B1*	2.01×10^{-10}	−13.29	SNPCHIP	0.14	4.90×10^{-10}	−12.00	GoldenGate	0.12
rs4149081	9.74×10^{-19}	G/A	*SLCO1B1*	1.58×10^{-10}	−12.19	SNPCHIP	0.18	1.05×10^{-9}	−10.61	SNPCHIP	0.16
rs11045813	4.04×10^{-13}	A/G	*SLCO1B1*	2.95×10^{-7}	−12.89	GoldenGate	0.88	2.85×10^{-7}	−9.65	GoldenGate	0.87
rs11045818	1.69×10^{-12}	A/G	*SLCO1B1*	1.35×10^{-6}	−11.66	SNPCHIP	0.88	2.63×10^{-7}	−9.88	SNPCHIP	0.87
rs11045872	2.42×10^{-12}	A/G	*SLCO1B1*	3.77×10^{-7}	11.49	SNPCHIP	0.15	1.36×10^{-6}	8.70	SNPCHIP	0.15
rs11045821	5.59×10^{-12}	G/A	*SLCO1B1*	3.33×10^{-8}	13.49	SNPCHIP	0.13	2.44×10^{-5}	8.46	Imputed	0.14
rs11045870	3.81×10^{-11}	A/G	*SLCO1B1*	9.17×10^{-6}	−10.51	GoldenGate	0.86	8.94×10^{-7}	-8.74	GoldenGate	0.85
rs10841753	5.31×10^{-11}	T/C	*SLCO1B1*	6.32×10^{-6}	8.83	SNPCHIP	0.21	1.89×10^{-6}	7.87	SNPCHIP	0.18
rs11045892	6.60×10^{-11}	A/G	*SLCO1B1*	3.27×10^{-5}	10.64	GoldenGate	0.14	3.76×10^{-7}	8.98	GoldenGate	0.15
rs11045825	7.80×10^{-11}	T/C	*SLCO1B1*	9.10×10^{-8}	12.94	SNPCHIP	0.13	1.16×10^{-4}	8.12	Imputed	0.16
rs2169969	1.67×10^{-10}	A/G	*SLCO1B1*	4.35×10^{-7}	11.75	SNPCHIP	0.15	6.84×10^{-5}	8.23	Imputed	0.17
rs16923647	7.64×10^{-10}	T/C	*SLCO1A2*	1.31×10^{-4}	8.98	SNPCHIP	0.88	1.09×10^{-6}	10.18	SNPCHIP	0.90

A/B alleles indicates first allele shown is A, second is B; COG B-allele freq, B-allele frequency in the COG cohort; COG effect size, the change in methotrexate clearance (mL/min/m²) with each copy of the B allele in COG cohort; COG P, P-value for COG cohort; COG source, source of SNP genotype data in COG cohort—SNPCHIP (Affymetrix SNP 6.0), GoldenGate (Illumina), or imputed (details in supplemental Methods); Meta P, combined P-values (Stouffer method) from 1279 COG and 699 SJ patients; rsID, dbSNP ID; SJ B allele freq, B-allele frequency in the St Jude cohort; SJ effect size: the change in methotrexate clearance (mL/min/m²) with each copy of the B allele in St Jude cohort; SJ P, P-value for St Jude cohort; SJ source, source of SNP genotype data in SJ cohort—SNPCHIP (Affymetrix 500K), GoldenGate (Illumina), or imputed (details in supplemental Methods); and SNP, single-nucleotide polymorphism.

All SNPs significantly related to methotrexate clearance (n = 103, typed and imputed) can be found in supplemental Table 3.

rs4149056 SNP, other SNPs in *SLCO1B1* remained significantly related to methotrexate clearance, indicating that there are multiple variants in *SLCO1B1* that can influence methotrexate clearance. In this independent cohort, we replicated our previous finding, that is, that the rs2306283 G allele was associated with greater clearance after we adjusted for the rs4149056 genotype.[11]
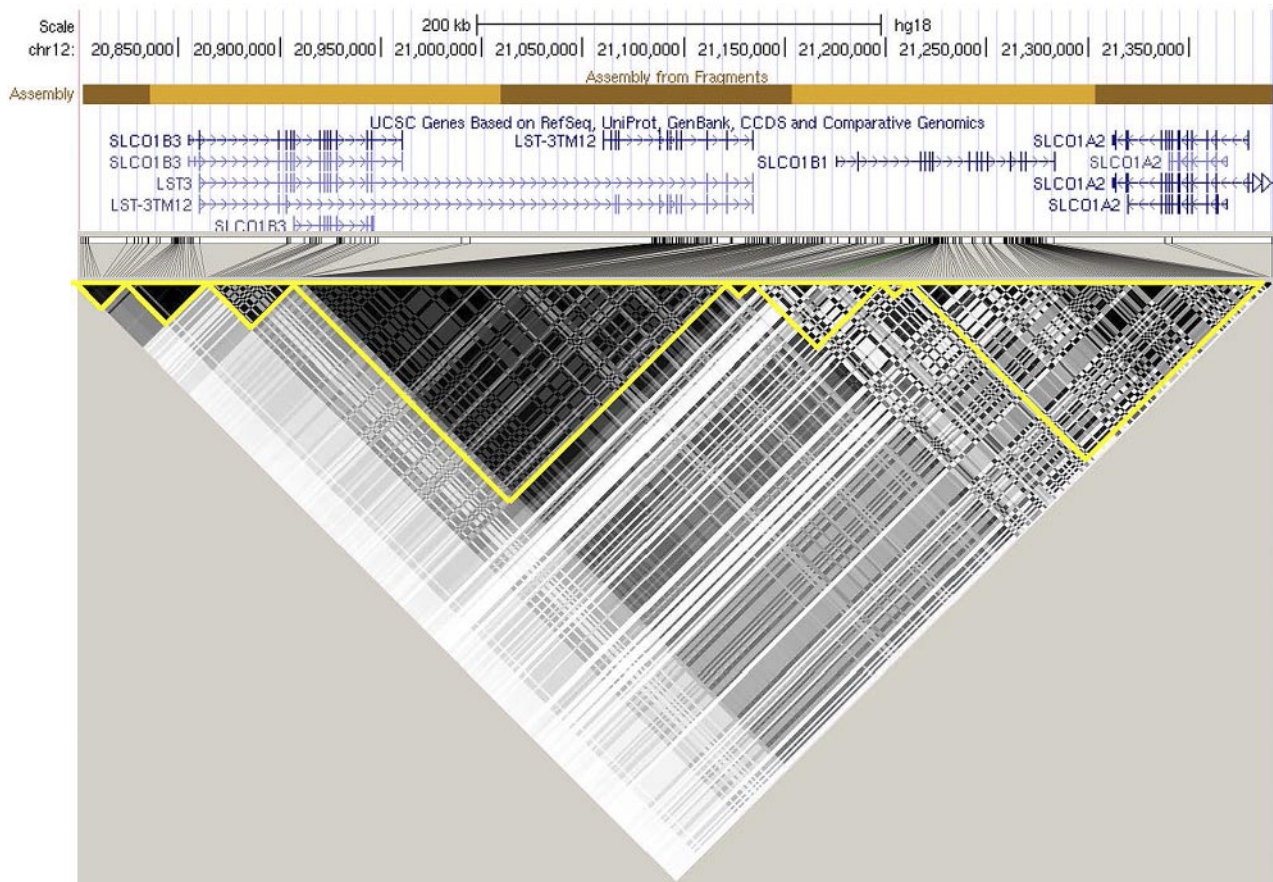

Figure 5. LD of SNPs with $P < 10^{-5}$ in meta-analysis spanning *SLCO1B3*, *LST-3TM12*, *SLCO1B1*, and *SLCO1A2* generated with the use of Haploview software[21] in European-ancestry patients only (COG n = 806 and SJ n = 459). The darkness of each box represents the association between 2 SNPs (R²), with white being R² = 0, black being R² = 1, and 0 < R² < 1 being shades of gray. LD blocks, highlighted with the yellow lines, were defined by confidence intervals.[22] Assembly hg18 from http://genome.ucsc.edu used for display.

Variants in *SLCO1B1* could be clinically useful in identifying patients at risk of low methotrexate clearance, particularly in settings in which plasma methotrexate concentrations are not readily available. Toxicity associated with 1 g/m² and 2 g/m² doses has been reported.[35,36] One possible intervention would be to increase IV hydration and/or alkalinization in the subset of patients with a "low-clearance" *SLCO1B1* genotype to force greater clearance of methotrexate, but the effects of this intervention on toxicity are not known. Tools for aggregation of *SLCO1B1* variants into clinically important haplotypes are available (http://www.p-harmgkb.org/gene/PA134865839), and such haplotypes are already used for dosing decisions for other drugs, such as statins.[37] In addition, it is likely that the coadministration of HDMTX with other SLCO1B1 substrates or inhibitors will result in increased plasma methotrexate concentrations.[38-40]

In summary, we have replicated the finding that *SLCO1B1* is related to methotrexate clearance. This validates our previous GWAS[10] in a separate group of patients who received different dosages of HDMTX in a multi-institutional clinical trial, solidifying the robustness of this pharmacogenomic determinant of methotrexate clearance.

Acknowledgments

Authorship

Contribution: L.B.R., C-H.P., W.E.E., and M.V.R. designed research, analyzed and interpreted data, and drafted the manuscript; J.C.P., C.S., W.Y., Y.F., C.C., and M.D. analyzed and interpreted data and performed statistical analysis; and N.J.W., P.L.M., S.P.H., and M.L. collected, analyzed and interpreted data.

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