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Genome-wide Association Study of Methotrexate-Induced Liver Injury in Rheumatoid Arthritis Patients

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Hepatotoxicity is a serious adverse drug reaction related to methotrexate (MTX). However, the cause of drug-induced liver injury (DILI) is still unclear and unpredictable. Genetic risk factors may predispose for MTX-DILI. Therefore, we conducted a nested case-control genome-wide association study to explore genetic risk factors associated with MTX-DILI. Seven international groups contributed blood samples and data of patients with rheumatoid arthritis who used MTX. MTX-DILI was defined as an alanine aminotransferase (ALT) level of at least three times the upper limit of normal (ULN), to increase contrast controls ALT levels did not raise above two times the ULN. Per study site, control subjects and patients with MTX-DILI (ratio 3:1) were matched for age, gender, and duration of MTX use. Patients were genotyped using Illumina GSA MD-24v1-0 and data were imputed using the 1000 Genomes reference panel. Single-nucleotide polymorphisms (SNPs) were analyzed using an additive genetic model, corrected for sex, country, and age. A *P*-value of $\leq 5 \times 10^{-8}$ was considered significant, whereas a *P*-value of $\leq 5 \times 10^{-6}$ was considered suggestive. A total of 108 MTX-DILI cases and 311 controls were included for association analysis. None of the SNPs were significantly associated with MTX-DILI. However, we found seven suggestive genetic variants associated with MTX-DILI (*P*-values 7.43×10^{-8} to 4.86×10^{-6}). Of those, five SNPs were in the intronic protein-coding regions of *FTCDNL1*, *BCOR*, *FGF14*, *RBMS3*, and *PFDN4/DOK5*. Investigation of candidates *SPATA9* (rs72783407), *PLCG2* (rs60427389), *RAVER2* (rs72675408), *JAK1* (rs72675451), *PTPN2* (rs2476601), *MTHFR* C677T (rs1801133), and into the HLA region did not show significant findings. No genetic variants associated with MTX-DILI were found, whereas suggestive SNPs need further investigation.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Hepatotoxicity is a serious adverse drug reaction related to methotrexate (MTX). However, the etiology of drug-induced liver injury (DILI) is unknown. Genetic risk factors, such as those that have previously been linked in other research, may predispose to MTX-DILI.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Which genetic polymorphisms may be associated with MTX-DILI?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Using a genome-wide association study with 108 cases of MTX DILI and 311 controls, 7 novel genetic variants—in rs12693892 (*LINC01877* and *FTCDNL1*), rs4827191 (*BCOR*), rs75805413 (*FGF14*), rs73044680 (*RBMS3*), rs7447381 (*LINC01170*), rs12693889 (*LINC01877*), and rs67738640 (*PFDN4/DOK5*)—suggestive (*P*-value $\leq 5 \times 10^{-6}$) for association with MTX-DILI were identified. No significant associations were found between MTX-DILI and previously associated variants in *SPATA9* (rs72783407), *PLCG2* (rs60427389), *RAVER2* (rs72675408), *JAK1* (rs72675451), and *PTPN2* (rs2476601).

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ This study contributes to the genetic knowledge of how MTX-DILI may develop. Additionally, earlier genetic findings have been scrutinized to see whether they play a pivotal role in the etiology of drug-induced liver damage.

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Low-dose methotrexate (MTX) is the cornerstone of antirheumatic drug treatment. Although MTX is an effective and safe antirheumatic drug, adverse drug reactions (ADRs) are the reasons for discontinuation in about 16% of patients.[1,2] Common ADRs of MTX include bone marrow suppression resulting in leuco- or thrombocytopenia, gastrointestinal complaints, and hepatotoxicity. Drug-induced liver injury (DILI) is one of the most potentially serious ADRs, but the mechanism of liver damage due to the use of MTX is not yet completely clear.[3] One potential cause of DILI is folate depletion and accumulation of MTX polyglutamates in the liver.[4] Another possible mechanism is the release of adenosine, which stimulates the matrix proteins by fibrogenic activation of stellate cells in the liver and results in the formation of fibrosis.[5] Risk factors for MTX-induced hepatotoxicity are concomitant use of nonsteroidal anti-inflammatory drugs (NSAIDs), obesity, and (excessive) alcohol consumption.[3,6–8]

Elevated levels of the hepatic transaminase enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) occur in 7.5 to 26% of the patients with rheumatoid arthritis (RA) treated with MTX, and the incidence of high-level enzyme elevation (at least 2–3 times the upper limit of the normal range (ULN)) has been found in 3.5% of patients.[9,10] Persistent elevated levels correlate with histopathological abnormalities and changes in fibrosis assessed by liver biopsy samples, although the relationship with the clinical outcome, clinical liver cirrhosis, has not been well-established. As a result, monitoring of the transaminase levels in MTX-treated patients with RA and adapting the dose accordingly is important (American College of Rheumatology Guideline for the Management of Rheumatoid Arthritis). According to this guideline, it is recommended to initially monitor transaminases every 2–4 weeks, then every 12 weeks in patients on stable therapeutic MTX doses.[11] MTX therapy should be discontinued if two subsequent ALT/AST levels are higher than three times the ULN. Once liver enzyme levels are normalized, MTX treatment can be resumed at a lower dose.

Prior studies have found potential genetic variants related to MTX-induced liver toxicity. For instance, a large meta-study by Owen *et al.*[12] showed that the *MTHFR C677T* polymorphism is a potential genetic variant predictive of increased risk of MTX-induced liver toxicity. Patients with RA treated with MTX who carry the C677T variant showed an increased risk of hepatotoxicity (odds ratio (OR): 1.71, 95% confidence interval: 1.32–2.21, $P < 0.001$) compared with patients with the wildtype genotype. Furthermore, prior studies have found associations between (MTX) DILI and *SPATA9* (rs72783407), *PLCG2* (rs60427389), *RAVER2* (rs72675408), *JAK1* (rs72675451), *PTPN2* (rs2476601), or *MTHFR C677T* (rs1801133).[13–15] Interestingly, genetic variants in the human leukocyte antigen (*HLA*) gene have been related to DILI for a variety of drugs, albeit not with MTX-induced DILI.[16] For example, HLA-B*14:01 is associated with trimethoprim-sulfamethoxazole-induced liver injury in European Americans, and HLA-B*5701 is associated with flucloxacillin-induced DILI.[17,18]

We hypothesize that genetic polymorphisms could be associated with MTX-induced DILI, and therefore we performed a genome-wide association study (GWAS) in a case–control design to explore genetic risk factors associated with MTXDILI in patients with RA.

MATERIALS AND METHODS

Study population

From the literature, publications were sought on biomarker studies in patients with RA on MTX monotherapy and principal investigators were invited to participate in this GWAS MTXDILI study. MTX DILI cases were defined as patients with RA using MTX having a single event of an ALT level of > 3× ULN at any time during follow-up, whereas controls had an ALT always ≤ 2× ULN.

Seven international research groups from Poland, the United Kingdom, Slovenia, and The Netherlands provided blood or DNA samples and clinical data for the GWAS. The included patients ($n = 430$) were from previously described clinical cohorts: RAMS study (acronym for Rheumatoid Arthritis Medication Study, Manchester, United Kingdom, $n = 24$),[19] BeSt study (Dutch acronym for Behandel-Strategieën, "treatment strategies," Leiden, The Netherlands, $n = 114$),[20] tREACH trial (acronym for treatment in the Rotterdam Early Arthritis Cohort, Rotterdam, The Netherlands, $n = 48$),[21] DREAM registry (acronym for Dutch Rheumatoid Arthritis Monitoring, Nijmegen, the Netherlands, $n = 84$),[22] or were derived from routine clinical practice in Slovenia (University Medical Centre Ljubljana, Ljubljana, Slovenia, $n = 76$), The Netherlands (Reade, Amsterdam, The Netherlands, $n = 48$), or Poland (Pomeranian Medical University, Szczecin, Poland, $n = 36$).

All included patients were diagnosed with RA according to the 1987 American College of Rheumatology (ACR) and/or 2010 ACR/European League Against Rheumatism (EULAR) criteria and used MTX with folic acid for at least 3 months. Controls were matched per study site to cases (at a 3:1 ratio) for age (±5 years), gender, and the duration of MTX use (±50 days). Data collected for each patient were baseline laboratory measurements at the start of MTX, age, gender, and concomitant drug treatment (NSAIDs, corticosteroids, and other disease-modifying antirheumatic drugs (DMARDs)). According to the study protocol or the standard treatment procedure, regular laboratory measurements were included during the follow-up time (until ALT > 3 ULN of the included cases or the related control).

At each site, investigators obtained written informed consent from the patients. Every participating study group provided consent for the use of these samples for the current study. In addition, each study site obtained approval from the local ethics committee or institutional research ethics board.

Sample size

A *post hoc* power analysis was performed as to estimate the power given the number of cases and controls. Logistic regression of the binary response variable (ALT > 3× ULN) on a binary independent variable (genotype of the individual single-nucleotide polymorphism (SNP) with a sample size of 840 alleles and baseline risk of 25% (ratio case/control groups of 3:1) and a minor allele frequency (MAF) of 50% achieves 80% power at a 5×10^{-8} significance level to detect a minimal OR of 2.5. For an MAF of 10% and a baseline risk of 20%, the minimal OR is 4.2 (see **Table S1**).

Genotyping and quality control

Full blood samples or germline DNA were collected at each site and sent to the Leiden University Medical Center (LUMC) for preparation according to the manufacturer's recommended protocol. DNA concentrations were prepared to a concentration of 4 µg (50 ng/µL) and were assessed by a spectrophotometer (Nanodrop, Wilmington, DE). GWAS genotyping was conducted with the Illumina GSA Beadchip Illumina GSA MD-24v1-0 in the Human Genotyping Facility Genetic Laboratory at the Erasmus MC, Rotterdam, The Netherlands. This array contains 693,931 SNPs.

The RAMS study had previously performed a GWAS using GRCh37/hg19 imputed with the 1000 Genomes V3 reference panel. For the analysis, raw data from the SNPs corresponding to the GSA Beadchip were extracted from the RAMS GWAS and merged with the GWAS data of the genotyped samples of our cohort.[19]

Quality control (QC) checks were performed using R software version 3.5.0[23] and PLINK software, version 1.07. Patients were excluded from analyses based on an individual genotype call rate < 97%, gender mismatch between reported and estimated sex based on genotypes of the X-chromosome (using PLINK), or excess of heterozygous genotypes as measured by the inbreeding coefficient. The inbreeding *F*-statistic was used to detect an excess of heterozygosity based on outlier detection. For QC steps, genetic markers were filtered at an MAF of 0.5%. Genetic markers were excluded based on an SNP call rate < 80% and a *P*-value ≤ 10^{-7} for the Hardy–Weinberg equilibrium (HWE) goodness-of-fit test. HWE testing was performed in the total sample.[24,25]

After the exclusion of patients and markers in these marginal QCs, the remaining set was used for integrative QC assessment. To evaluate the possibility of population stratification or outliers, multidimensional scaling (MDS) analysis was performed using PLINK. SNPs were pruned using PLINK prior to performing the MDS. Standard parameters were used (windowSize = 50, windowShift = 5, and thresholdVIF = 2). Additionally, pairwise identity by state (IBS) statistics were calculated to identify potential duplicates. MDS and IBS were computed using PLINK. Patients who were identified as outliers based on IBS clustering were excluded from the analysis. MDS coordinates were extracted and used as covariates in the association analysis. SNP imputation was performed using the programs "shapeit" and "impute2" with default parameters using the reference panel 1000 Genomes build version 3 with the total, "cosmopolitan," set of individuals.[26] An MDS plot was used to compare the self-reported ethnicity and genetic concept of ancestry.

Data analysis

Association analysis of individual SNPs with MTX DILI was performed using an additive genetic model, with sex, country, and age as covariates. In addition, *SPATA9* (rs72783407), *PLCG2* (rs60427389), *RAVER2* (rs72675408), *JAK1* (rs72675451), or *PTPN2* (rs2476601), *MTHFR C677T* (rs1801133), and the *HLA* gene region were explored to investigate associations between MTX DILI and these SNPs, using the additive genetic model, with covariates sex, country, and age.

A model replacing the country with the two MDS coordinates was also fitted (see **Figure S1**). SNPs on the X-chromosome were analyzed using an additive model that treats male patients as homozygous for haploid alleles (coding the genotypes as either 0 or 2). The X-chromosome was not imputed and models were not evaluated on the X-chromosome for the imputed data.

Statistical analyses were performed in R statistics version 3.5.0. Associations with a *P*-value ≤ 5×10^{-8} were considered statistically genomewide significant and associations with a *P*-value between 5×10^{-8} and 5×10^{-6} were considered suggestive for association.[24] The *HLA*-region consisted of 28,902 SNPs, and consequently the significance level for the *P*-value was set to 1.73×10^{-6}. Post-association QC was performed by visual inspection of Quantile-Quantile plots of *P*-values of association tests and computation of the inflation factor (IF).[25] To improve readability,

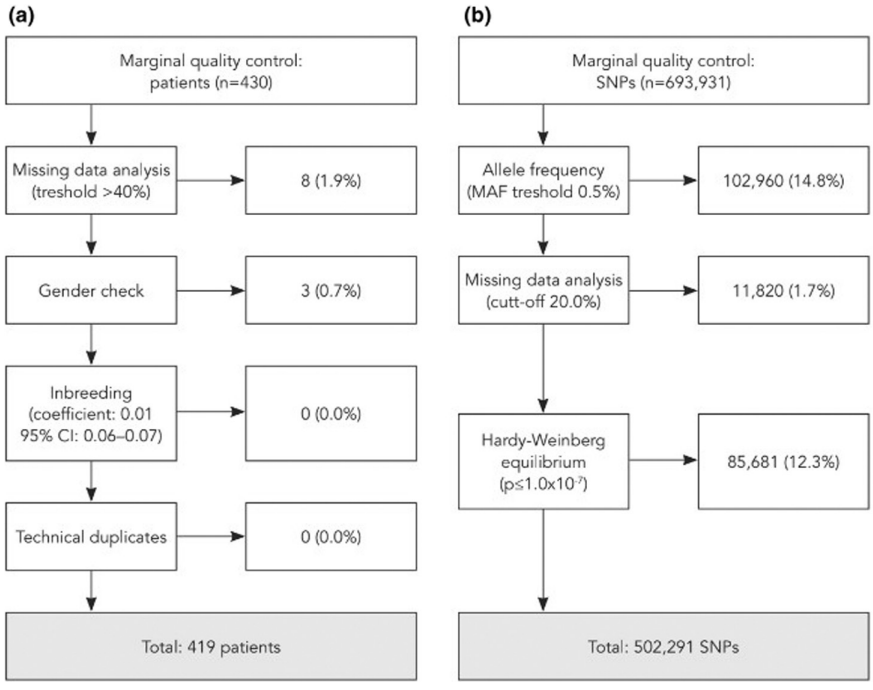

Figure 1 Flow chart of the QCs for individuals (**a**) and SNPs (**b**). CI, confidence interval; MAF, minor allele frequency; SNPs, single-nucleotide polymorphisms; QCs, quality controls.

and account for the high correlation between neighboring SNPs, the list of top SNPs according to *P*-values only contain the best association in a window of 100 kb.

RESULTS

Quality control

Eleven patients were excluded from the analysis based on QC criteria. Seven patients were excluded based on low call rates (< 97%; **Figure 1a**), three patients were excluded due to a failing sex-check and one patient was deemed to be an outlier based on MDS plots. There were 191,640 SNPs that were excluded due to low allele frequency, low call rate, or not meeting the HWE criterion (**Figure 1b**, including 8,821 SNPs with exclusion overlap). No outliers were detected by the IBS analysis. After applying the QC criteria, a total of 502,291 SNPs in 419 patients with RA, 108 cases, and 311 controls, were available for association analysis.

Study population

The demographic and clinical characteristics of the study population are shown in **Table 1**. The mean dose of MTX was 18.8 mg/week (SD: 6.0, range: 7.5–30 mg/week), mean age was 54.8 years (SD: 13.0, range: 20–87 years), with disease duration (and follow-up time) of 34.3 ± 84.2 weeks. All patients used folic acid. MTX DILI occurred after cases used MTX for at least 104 days, with an average of 1,169 days.

GWAS results

After merging with the RAMS GWAS and applying QC criteria, 502,291 SNPs were analyzed for association with MTX-DILI. None of the SNPs reached genome-wide significance (P-value $\leq 5 \times 10^{-8}$) in the multivariate analysis corrected for gender, age, and country (**Figure 2**). The IF for this analysis was 1.04. The model with MDS coordinates instead of the country showed almost identical results (IF: 1.04, top-SNPS identical, data not shown). Therefore, we restricted our further analyses to the model correcting for ethnicity and country. Seven SNPs were found to meet the threshold of P-value $\leq 5 \times 10^{-6}$ and were therefore considered suggestive of an association with MTX DILI representing 6 independent signals (**Table 2**). From those, 5 SNPs were related to genes in *FTCDNL1* rs12693892, *BCOR* rs4827191,

Table 1 Demographic and clinical characteristics of the included patients (*N* = 419)

Characteristic	Cases (*n* = 108)	Controls (*n* = 311)	Total (*n* = 419)
Age, years, mean	56.1±11.1	54.4±13.6	54.9±13.0
Female gender, *n* (%)	84 (77.8%)	241 (77.5%)	325 (77.8%)
Smoker, *n* (%)[a]	23 (27.1%)	73 (34.0%)	96(32%)
Alcohol consumption, *n* (%)[a,b]	38 (50.0%)	90 (50.3%)	128 (50.2%)
Disease duration, weeks	41.2±86.3	31.8±80.9	34.3±84.2
DAS28 start of MTX	4.7±1.4	4.6±1.4	4.6±1.4
Rheumatoid factor positive, *n* (%)[a]	67 (77.0%)	166 (69.2%)	233 (71.3%)
C-reactive protein, mean	21.8±28.1	20.6±22.3	20.9±23.9
MTX maintenance doses, mg/week	18.4±5.9	18.9±6.1	18.8±6.0
Folic acid doses, mg/week	6.3±2.9	6.5±3.3	6.5±3.2
Other DMARD use, *n* (%)[a]	16 (30.8%)	56 (37.1%)	72 (35.5%)
NSAID use, *n* (%)[a]	10 (37.0%)	22 (27.8%)	32 (30.2%)
Corticosteroid use, *n* (%)[a]	26 (81.2%)	71 (74.0%)	128 (75.8%)
Laboratory values at start MTX			
AST (U/L)	26.3±7.3	26.0±28.2	26.1±24.4
ALT (U/L)	25.6±14.7	24.8±18.1	25.0±17.3
Creatinine (μmol/L)	65.7±22.4	71.9±23.0	56.7±36.4
Hemoglobin (mmol/L)	7.9±0.8	8.3±2.5	8.2±2.2
Gamma-GT (U/L)	34.5±18.5	31.6±20.2	32.4±19.7
Laboratory values at DILI			
AST (U/L)	105.9±80.2		
ALT (U/L)	179.70±111.6		
Creatinine (μmol/L)	74.3±18.3		
Hemoglobin (mmol/L)	8.2±0.6		
Bilirubin (μmol/L)	13.0±13.8		

AST, aspartate transaminase; ALT, alanine transaminase; DILI, drug-induced liver injury; DAS28, Disease Activity Score of 28 joints; DMARD, disease-modifying anti-rheumatic drugs; MTX, methotrexate; NSAID, nonsteroidal anti-inflammatory drug.
Continuous values as mean ± standard deviation.
[a]Not all data were available.
[b]Alcohol consumption is defined as at least three alcoholic units per week.

FGF14 rs75805413, *RBMS3* rs73044680, and *PFDN4/DOK5* rs67738640. In addition, imputation of genetic markers using the 1000 Genomes dataset led to 9,328,966 SNPs but did not lead to hits after Bonferroni correction (P-value > 5×10^{-8}).

Additionally, there were no significant associations between MTX-DILI and the imputed genetic variants of *MTHFR* C677T (rs1801133, P-value = 0.4), *SPATA9* (rs72783407, P-value = 0.6), *PLCG2* (rs60427389, P-value = 0.3), *RAVER2* (rs72675408, P-value = 0.6), *JAK1* (rs72675451, P-value = 0.4), and *PTPN2* (rs2476601, P-value = 0.9). In addition, the result of the SNPs in the HLA region (chromosome 6: 29,941,260 to 33,143,325 base pairs) found no significant associations (**Figure 3**; P-values > 10^{-4}).

DISCUSSION

This study investigates for genome-wide genetic variants associated with MTX-DILI. In this exploratory study, we identified seven suggestive novel variants (P-value ≤ 5×10^{-6}) in rs12693892 (*LINC01877* and *FTCDNL1*), rs4827191 (*BCOR*), rs75805413 (*FGF14*), rs73044680 (*RBMS3*), rs7447381 (*LINC01170*), rs12693889 (*LINC01877*) and rs67738640 (*PFDN4/DOK5*). Although no SNPs with genome-wide significance (P-value < 5.0×10^{-8}) were found, the most promising SNP was *LINC01877* rs12693892 with a P-value of 7.43×10^{-8}.

Our results indicate a gene, coding for a protein associated with the mechanism of action of MTX, that might play a role in MTX-DILI. Albeit *LINC01877* rs12693892 is a non-protein-coding RNA sequence, it is close to the *FTCDNL1* gene (156,200 base pair difference). Protein expression of *FTCDNL1* was mainly found in the brain and liver tissue, whereas *FTCDNL1* provides a transferase and binding activity of folic acid.[27] The use of MTX and a deficiency of *FTCDNL1* could lead to a reduction of the protective effects of folic acid and therefore could be related to DILI. No other studies found an association of liver injury with rs12693892, whereas 2 other SNPs in *FTCDNL1* (rs10203122 and rs7605378) were previously associated with osteoporosis.[28]

The MTHFR enzyme is important in folate metabolism, an integral process for cell metabolism in the DNA, RNA, and protein methylation.[29] Previous studies showed that *MTHFR C677T* (rs1801133) was associated with MTX DILI.[12] Analysis of the MTHFR C677T in our study revealed no association with MTX DILI (P-value = 0.4, imputation quality = 1), supporting previous findings that no consistent association was found across studies.[8] In addition, significant SNPs related to ALT elevations or MTX-DILI were found in three prior GWAS. These relationships involved *SPATA9* (rs72783407), *PLCG2* (rs60427389), *RAVER2* (rs72675408), *JAK1* (rs72675451), and *PTPN2* (rs2476601).

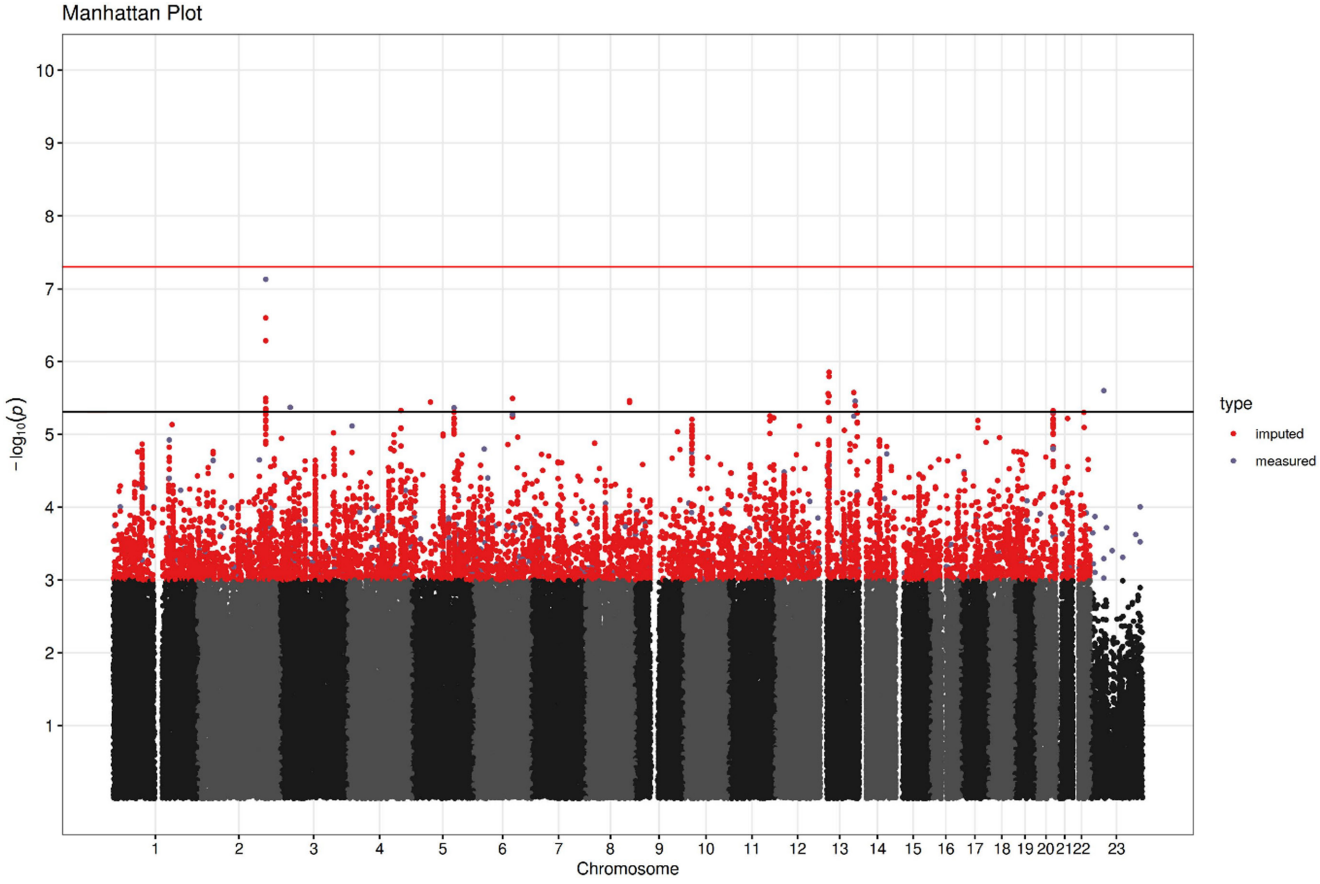

Figure 2 Manhattan plot for the model MTX DILI correction with gender, age, and country. The significance level (red horizontal line) is set to 5.0×10^{-8} and the suggestive significant level (black horizontal line) is set to 5.0×10^{-6}. The red dots are the imputed data and the dark blue dots are the measured data. DILI, drug-induced liver injury; MTX, methotrexate.

Table 2 Suggestive genetic variants related to MTX toxicity using the model corrected for sex, country, age, and SNP (*P*-value $< 5.0 \times 10^{-6}$)

Marker	Type of variant	Gene	Allele	Chromosome (GRCh37.p13)	MAF	1000 Genome MAF	*P*-value
rs12693892	Intergenic	LINC01877 FTCDNL1	A>G	2:200483842	0.4964	0.3764	7.43×10^{-8}
rs4827191	Regulatory region	BCOR	A>C	23:39901078	0.1646	0.1891	2.51×10^{-6}
rs75805413	Intergenic	FGF14	T>C	13:102917882	0.0152	0.0042	3.50×10^{-6}
rs73044680	Intergenic	RBMS3	A>G	3:295756660	0.0426	0.0176	4.26×10^{-6}
rs7447381	Intergenic	LINC01170	C>T	5:123401074	0.4839	0.4820	4.33×10^{-6}
rs12693889	Intronic	LINC01877	T>A,C	2:200473658	0.1975	0.2456	4.53×10^{-6}
rs67738640	Intergenic	PFDN4 DOK5	T>C	20:52931326	0.0589	0.1336	4.86×10^{-6}

MAF, minor allele frequency; MTX, methotrexate.

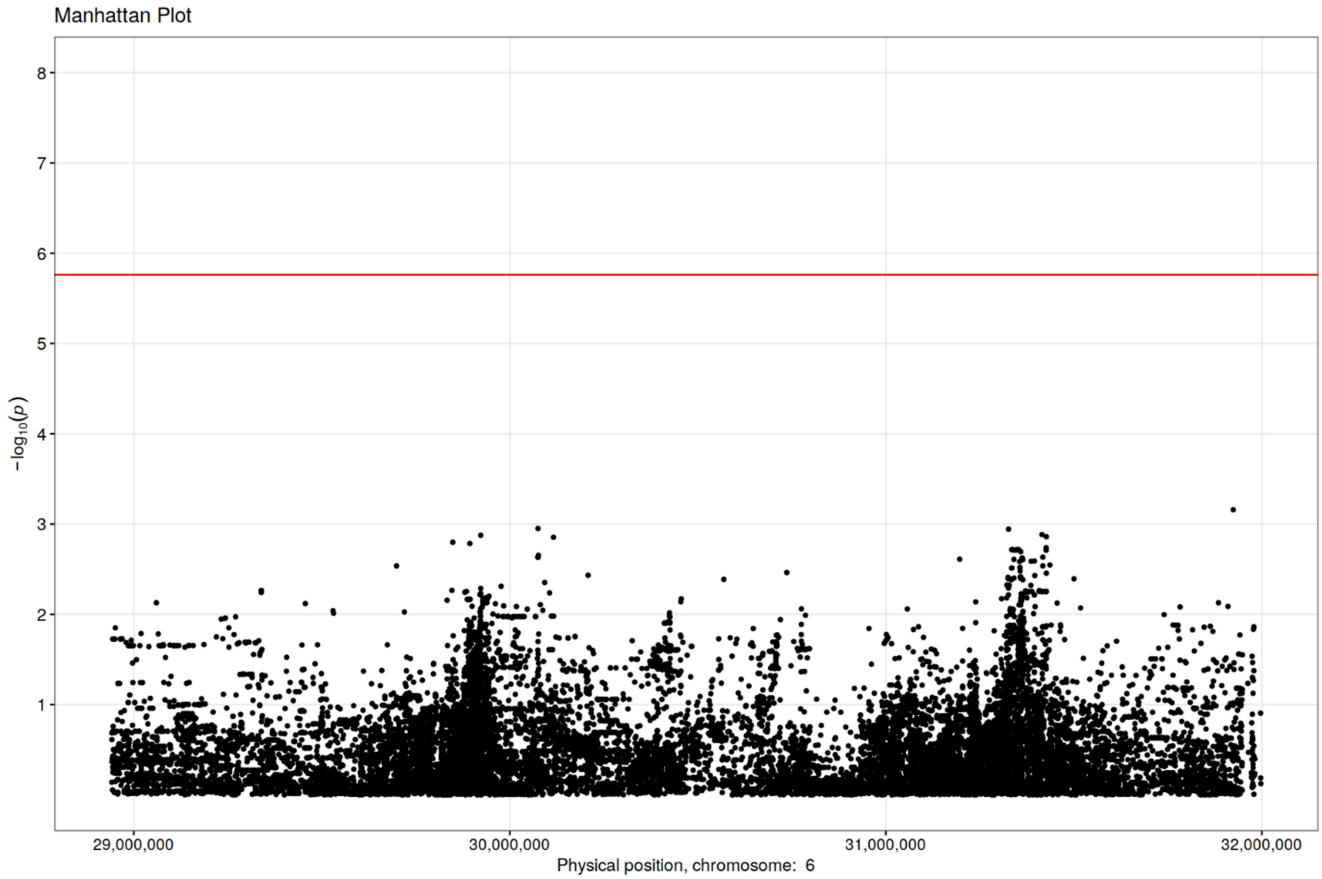

Figure 3 Manhattan plot of the HLA region for the model MTX-DILI corrected with gender, age, and country. The significance level (red horizontal line) is set to the Bonferroni boundary for the HLA region (*P*-value = 1.73×10^{-6}). DILI, drug-induced liver injury.

Nevertheless, after examining those SNPs, these variants were not replicated in our study (*P*-value > 0.05).

Previous studies have shown associations of drug-induced liver toxicity with SNPs in the HLA region, but this has not been investigated for MTX-DILI. HLA is responsible for regulating the immune system and can (fail to) protect against external pathogens. It has also been related to several autoimmune illnesses, such as RA and celiac disease. In our study, we found no evidence of any association between the HLA SNPs and MTX-DILI. Previous studies have demonstrated associations of idiosyncratic drug-induced liver toxicity to SNPs in the HLA region, but such signals could not be replicated in our study, although we explicitly analyzed this region.

In this study, we defined the phenotype "MTX induced liver injury" as a single ALT elevation of more than three times the

ULN. Because we used a collection of retrospective cohorts, expert adjudication of liver injury was not possible as it relies on several laboratory values which were not available throughout. Most studies use a definition of two to three times the ULN to define liver injury. As a surrogate, we applied a more stringent cutoff of more than three times the ULN. This may reduce the potential risk of dilution of the phenotype by lack of expert adjudication and the use of a single instead of multiple ALT measurements. ALT elevation is also not specific to DILI as other factors may also cause ALT rise.[30–35] For example, obesity can be associated with more severe inflammation due to high or reduced levels of secretory adipocytes products, such as resistin and adipocytokine leptin. These inflammatory processes may exacerbate chemical-induced hepatotoxicity.[36]

Causality of ALT elevation and MTX use was not explicitly assessed in our study. Indeed, several factors besides MTX use, such as alcohol consumption or obesity, may potentially induce DILI. However, the time relationship of MTX use and ALT elevation, which is a key component of the study design, is one of the most important factors in causality assessment tools such as the Naranjo scale and the Liverpool Causality Assessment Tool. Other elements of these assessment tools, such as rechallenge and therapeutic drug monitoring, cannot be addressed in a retrospective study design but we are convinced that our surrogate outcome is highly correlated with the outcome determined using full causality assessment.

In addition to body mass index, other potential risk factors for MTX-DILI are the lack of folate supplementation, weekly dose of MTX, exposure to MTX (MTX cumulative dose), the length of disease duration, gender, and age.[37,38] In our study, all patients used folic acid, and other risk factors, such as gender, age, and MTX-dosage, and disease duration, were matched to the case–control design or were corrected in our data analysis.

In this study, we tested if common SNPs were associated with MTX-DILI. However, SNPs with low prevalence may be the one with significant effects and could be involved in the development of DILI, although these would be thus rare and therefore not clinically relevant. Therefore, we cannot entirely exclude genetic risk factors for MTX-DILI. Furthermore, our study was carried out in patients of European ancestry. These results could have a disparity in the analysis of associations with other ancestries, like African or Asian populations.

In conclusion, we identified no novel genetic risk factors related to MTX-DILI in patients with RA of European ancestry. However, we found some suggestive SNPs that need replication to be verified.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

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AUTHOR CONTRIBITIONS

F.E., J.J.S., A.A.dB., J.M.W.H., F.A.S.K., S.M.M.V., N.N., A.P., M.T.N., V.D., S.B., C.F.A., and H.-J.G. wrote the manuscript. F.E., J.J.S., M.T.N., V.D., C.F.A., and H.-J.G. designed the research. F.E., J.J.S., A.A.dB., J.M.W.H., F.A.S.K., S.M.M.V., N.N., and H.-J.G. performed the research. F.E., S.B., N.N., and S.M.M.V. analyzed the data.

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