Published in final edited form as:
Pharmacogenet Genomics. 2011 October ; 21(10): 679–686. doi:10.1097/FPC.0b013e328343dd93.

PharmGKB summary: methotrexate pathway

Torben S. Mikkelsen[a,b], **Caroline F. Thorn**[c], **Jun J. Yang**[b], **Cornelia M. Ulrich**[h], **Deborah French**[b], **Gianluigi Zaza**[b], **Henry M. Dunnenberger**[b], **Sharon Marsh**[i], **Howard L. McLeod**[f], **Kathy Giacomini**[e], **Mara L. Becker**[g], **Roger Gaedigk**[g], **James Steven Leeder**[g], **Leo Kager**[j], **Mary V. Relling**[b], **William Evans**[b], **Teri E. Klein**[c], and **Russ B. Altman**[c,d]

[a]Department of Pediatric Oncology and Hematology, Aarhus University Hospital, Skejby, Denmark

[b]St Jude Children's Research Hospital, Memphis, Tennessee

[c]Department of Genetics, Stanford University Medical Center, Stanford

[d]Department of Bioengineering, Stanford University Medical Center, Stanford

[e]University of California, San Francisco, California

[f]Institute for Pharmacogenomics and Individualized Therapy, University of North Carolina, Chapel Hill, North Carolina

[g]Division of Clinical Pharmacology and Medical Toxicology, Children's Mercy Hospitals and Clinics, Kansas City, Missouri, USA

[h]German Cancer Research Center and National Center for Tumor Diseases, Heidelberg, Germany

[i]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada

[j]St Anna Children's Hospital, Vienna, Austria

Keywords

methotrexate; 5,10-methylenetetrahydrofolate reductase; pathway; pharmacogenomic; SLC19A1; thymidylate synthetase

Methotrexate is a folate analog that is used in the treatment of cancers (e.g. acute lymphoblastic leukemia, non-Hodgkin lymphoma, osteosarcoma, and colon cancer) and autoimmune diseases (e.g. rheumatoid arthritis, Crohn's disease, and psoriasis). In the treatment of autoimmune diseases, methotrexate is usually administrated orally or subcutaneously, whereas in the cancer treatment, it can be given orally, intramuscularly, as intrathecal injections, or as intravenous infusions (up to 12 g/m^2) [1-3]. The pharmacokinetics and pharmacodynamics of methotrexate show large interpatient variability regardless of the route of administration or disease being treated [4-6]. The goal of this study is to provide an introduction to methotrexate pharmacogenomics, showing the candidate genes in the PharmGKB methotrexate pathway (Fig. 1), important variants (Tables 1 and 2), discussing key knowledge, and pointing to more in-depth resources.

Correspondence to Teri E. Klein, PhD, Department of Genetics, Stanford University Medical Center, 300 Pasteur D. Lane L301, Mail Code 5120, Stanford, CA 94305-5120, USA, Tel: + 1 650 725 0659; fax: + 1 650 725 3863; feedback@pharmgkb.org.

The interindividual variability in methotrexate pharmacokinetics can be explained partially by genetic variations in membrane transporter proteins with an affinity for methotrexate [4,89]. In the gastrointestinal tract, methotrexate is absorbed through active transport mediated by the reduced folate carrier (*SLC19A1*) and possibly also by the proton-coupled folate transporter *SLC46A1* (*HCP1, PCFT*) at the apical membrane of enterocytes [90]. Furthermore, the bioavailability of methotrexate after oral dosing may be affected by ABC transporters, which can move methotrexate out of the enterocytes and back into the intestinal tract (*ABCC2, ABCB1*, and *ABCG2*) or into the blood (*ABCC1* and *ABCC3*) [91-93]. Systemic clearance of methotrexate happens primarily through renal glomerular filtration and active secretion over the proximal tubular cells. Several renal transporter proteins have an affinity for methotrexate (SLC22A6, SLC22A8, SLC19A1, ABCG2, ABCC2, and ABCC4), and single nucleotide polymorphisms (SNPs) in *ABCC2* have been associated with delayed methotrexate clearance [53,89,94-98]. Hepatic uptake of methotrexate involves the *SLCO1B1* and *SLCO1B3* transporters, in which SNPs have recently been found to explain up to 10% of the interpatient variability in the clearance of high-dose methotrexate [4,99-101]. Most of the methotrexate in hepatocytes reenters the blood circulation by transporter proteins (*ABCC3* and *ABCC4*) in the basolateral membrane, and only a small portion of the methotrexate is excreted into the bile duct by the *ABCC2* and *ABCB1* transporters [102-105]. Inside the hepatocytes, aldehyde oxidase can convert methotrexate to 7-hydroxymethotrexate, a metabolite that is eliminated by the same route as methotrexate. At the blood–brain barrier, ABC transporters with affinities for methotrexate are located in endothelial cells, but the effect of facilitated transport of methotrexate out of the cerebrospinal fluid remains to be unclear [106].

The pharmacodynamic profile of methotrexate can, to a large extent, be explained by its interactions with enzymes in the folate pathway. The effects on methotrexate response of variations in these genes have been extensively studied in cancer treatments [107]. At extracellular methotrexate concentrations below 20 μmol/l, methotrexate enters cancer cells primarily through the reduced folate carrier (*SLC19A1*) [108,109], whereas efflux across the cell membrane is mediated by various ABC transporters; variations in these genes are known mechanisms of drug resistance in cancer cells [110]. Inside the cells, methotrexate is converted to active methotrexate polyglutamates (MTXPGs) by folylpolyglutamate synthetase, which adds glutamate residues to methotrexate [111]. The primary action of methotrexate is inhibition of the enzyme dihydrofolate reductase (*DHFR*), which converts dihydrofolate to tetrahydrofolate (THF)[112]. Tetrahydrofolate is essential for *de novo* purine synthesis, and in the biologically active form, 5-methyl-tetrahydrofolate, is an important cofactor in one-carbon metabolism. The effect of methotrexate depends on the function and expression of several other enzymes in the folate pathway, including methylenetetrahydrofolate dehydrogenase (*MTHFD1*), 5,10-methylenetetrahydrofolate reductase (*MTHFR*), and thymidylate synthetase (*TYMS*). Compared with methotrexate, the active metabolites, MTXPGs induce stronger inhibition of the target enzymes (i.e. *TYMS* and *DHFR*) and further inhibit key enzymes such as phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (*ATIC*) in the *de novo* purine synthesis pathway. MTXPG inhibition results in decreased protein and DNA methylation in addition to impaired DNA formation and repair. MTXPG levels are sustained inside the cells for a longer time than those of methotrexate; degradation of MTXPGs to methotrexate depends on the activity of the lysosomal enzyme γ-glutamyl hydrolase, which catalyzes the removal of polyglutamates [113-115]. MTXPGs have been investigated in relation to clinical outcomes in rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA). Specifically, higher concentrations of long-chain MTXPGs have been associated with favorable outcomes in RA [116] and risk of gastrointestinal and hepatic toxicity in JIA [117].

Gene expression and genetic variation in candidate genes have been studied extensively in relation to many methotrexate response measures, including MTXPG accumulation (γ-glutamyl hydrolase, folylpolyglutamate synthetase, and *SLC19A1*) [108,118,119], reduction in tumor size (*SLC19A1* and *DHFR*) [120], toxicity (*MTHFR*, and *TYMS*) [11,15], and relapse (*DHFR*, *TYMS*, *MTHFR*, *DHFR*, and *SLC19A1*) [29,37,40,45,54,87,121]. Conflicting results among studies suggest that the effects of genetic variation are therapy dependent and probably reflect the route of administration, dose, and duration of methotrexate treatment [37,40]. Although these studies have contributed to our understanding of the effects of Methotrexate and the molecular mechanisms involved in drug resistance, no genetic variant has yet been prospectively evaluated as a predictor of an outcome in a clinical trial.

Genome-wide studies have linked genes outside the folate pathway to the pharmacokinetics and effects of methotrexate; many of these genes have not been analyzed earlier in studies using the candidate approach. A recent study analyzed the association between MTXPG accumulation and genetic variations such as leukemic cell gene expression, somatic copy number variation, and SNPs [122]. Six genes on chromosome 18 (*FHOD3*, *IMPA2*, *ME2*, *SLC39A6*, *SMAD2*, and *SMAD4*) and one on chromosome 10 (*RASSF4*) were found to be associated with in-vivo intracellular accumulation of MTXPG in leukemic cells in all three categories of genetic variation. In another genome-wide study of patients with acute lymphoblastic leukemia, in-vivo response to MTX was found to be significantly associated with the expression of genes in the nucleotide pathway (e.g. *TYMS*), but also with genes involved in cell proliferation and apoptosis, and DNA repair and replication in the leukemic cells [123]. Finally, a genome-wide association study that assessed the link between inherited genomic variation and initial treatment response in patients with acute lymphoblastic leukemia showed 14 SNPs significantly associated with both treatment response and methotrexate clearance or MTXPG accumulation in leukemic cells [124]; early treatment response assessed by eradication of leukemic cells is strongly associated with cure rates and is therefore considered an important clinical phenotype.

No genome-wide association studies have yet been carried out in patients with rheumatoid arthritis, but inherited variations in most genes from the folate pathway have been examined in relation to methotrexate treatment response and toxicity [12,35]. However, to see a clinically relevant effect of genetic variants in the folate, purine, and pyrimidine pathways, it seems crucial to study gene–gene interactions; it has been suggested that the effects of individual SNPs are enhanced when they occur in combination with other common SNPs in these pathways [1]. Combinations of SNPs in the genes *ATIC* and adenosine receptor 2a have been associated with differential MTXPG concentrations in JIA [117]. The anti-inflammatory effect of methotrexate is further thought to be mediated through interaction with the adenosine biosynthesis pathway [125]. MTXPGs inhibit the enzyme *ATIC*, which after a cascade of events leads to the accumulation of the anti-inflammatory molecule adenosine; SNPs in genes from the adenosine biosynthesis pathway (i.e. *ATIC*, inosine triphosphatase, and adenosine monophosphate deaminase 1) have been found to predict the efficacy of methotrexate treatment for RA and JIA [1,62,76,126].

Regardless of disease, it seems clear that future studies should continue to examine the combined effect of variations in multiple genes to characterize the extent of genomic determinants on variation in the pharmacokinetics and pharmacodynamics of methotrexate.

Acknowledgments

The authors thank Fen Liu for assistance with the graphics. This work is supported by the NIH/NIGMS (U01GM61374).

References

1. Dervieux T, Wessels JA, Van der ST, Penrod N, Moore JH, Guchelaar HJ, et al. Gene–gene interactions in folate and adenosine biosynthesis pathways affect methotrexate efficacy and tolerability in rheumatoid arthritis. Pharmacogenet Genomics. 2009; 19:935–944.
2. Pui CH, Campana D, Pei D, Bowman WP, Sandlund JT, Kaste SC, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Engl J Med. 2009; 360:2730–2741. [PubMed: 19553647]
3. Widemann BC, Balis FM, Kempf-Bielack B, Bielack S, Pratt CB, Ferrari S, et al. High-dose methotrexate-induced nephrotoxicity in patients with osteosarcoma. Cancer. 2004; 100:2222–2232. [PubMed: 15139068]
4. Trevino LR, Shimasaki N, Yang W, Panetta JC, Cheng C, Pei D, et al. Germ line genetic variation in an organic anion transporter polypeptide associated with methotrexate pharmacokinetics and clinical effects. J Clin Oncol. 2009; 27:5972–5978. [PubMed: 19901119]
5. Buitenkamp TD, Mathot RAA, de Haas V, Pieters R, Zwaan CM. Methotrexate-induced side effects are not due to differences in pharmacokinetics in children with Down syndrome and acute lymphoblastic leukemia. Haematologica. 2010; 95:1106–1113. [PubMed: 20418240]
6. Tetef ML, Margolin KA, Doroshow JH, Akman S, Leong LA, Morgan RJ Jr, et al. Pharmacokinetics and toxicity of high-dose intravenous methotrexate in the treatment of leptomeningeal carcinomatosis. Cancer Chemother Pharmacol. 2000; 46:19–26. [PubMed: 10912573]
7. Costea I, Moghrabi A, Laverdiere C, Graziani A, Krajinovic M. Folate cycle gene variants and chemotherapy toxicity in pediatric patients with acute lymphoblastic leukemia. Haematologica. 2006; 91:1113–1116. [PubMed: 16870553]
8. Robien K, Bigler J, Yasui Y, Potter JD, Martin P, Storb R, et al. Methylenetetrahydrofolate reductase and thymidylate synthase genotypes and risk of acute graft-versus-host disease following hematopoietic cell transplantation for chronic myelogenous leukemia. Biol Blood Marrow Transplant. 2006; 12:973–980. [PubMed: 16920564]
9. Chiusolo P, Reddiconto G, Casorelli I, Laurenti L, Sora F, Mele L, et al. Preponderance of methylenetetrahydrofolate reductase C677T homozygosity among leukemia patients intolerant to methotrexate. Ann Oncol. 2002; 13:1915–1918. [PubMed: 12453860]
10. Gemmati D, Ongaro A, Tognazzo S, Catozzi L, Federici F, Mauro E, et al. Methylenetetrahydrofolate reductase C677T and A1298C gene variants in adult non-Hodgkin's lymphoma patients: association with toxicity and survival. Haematologica. 2007; 92:478–485. [PubMed: 17488658]
11. Ulrich CM, Yasui Y, Storb R, Schubert MM, Wagner JL, Bigler J, et al. Pharmacogenetics of methotrexate: toxicity among marrow transplantation patients varies with the methylenetetrahydrofolate reductase C677T polymorphism. Blood. 2001; 98:231–234. [PubMed: 11418485]
12. Ranganathan P, Culverhouse R, Marsh S, Mody A, Scott-Horton TJ, Brasington R, et al. Methotrexate (MTX) pathway gene polymorphisms and their effects on MTX toxicity in Caucasian and African American patients with rheumatoid arthritis. J Rheumatol. 2008; 35:572–579. [PubMed: 18381794]
13. Chandran V, Siannis F, Rahman P, Pellett FJ, Farewell VT, Gladman DD. Folate pathway enzyme gene polymorphisms and the efficacy and toxicity of methotrexate in psoriatic arthritis. J Rheumatol. 2010; 37:1508–1512. [PubMed: 20472929]
14. Chiusolo P, Reddiconto G, Farina G, Mannocci A, Fiorini A, Palladino M, et al. MTHFR polymorphisms' influence on outcome and toxicity in acute lymphoblastic leukemia patients. Leuk Res. 2007; 31:1669–1674. [PubMed: 17512587]
15. Robien K, Schubert MM, Chay T, Bigler J, Storb R, Yasui Y, et al. Methylenetetrahydrofolate reductase and thymidylate synthase genotypes modify oral mucositis severity following hematopoietic stem cell transplantation. Bone Marrow Transplant. 2006; 37:799–800. [PubMed: 16501586]

16. Robien K, Schubert MM, Bruemmer B, Lloid ME, Potter JD, Ulrich CM. Predictors of oral mucositis in patients receiving hematopoietic cell transplants for chronic myelogenous leukemia. J Clin Oncol. 2004; 22:1268–1275. [PubMed: 15051775]

17. Van Ede AE, Laan RF, Blom HJ, Huizinga TW, Haagsma CJ, Giesendorf BA, et al. The C677T mutation in the methylenetetrahydrofolate reductase gene: a genetic risk factor for methotrexate-related elevation of liver enzymes in rheumatoid arthritis patients. Arthritis Rheum. 2001; 44:2525–2530. [PubMed: 11710708]

18. Xiao H, Xu J, Zhou X, Stankovich J, Pan F, Zhang Z, et al. Associations between the genetic polymorphisms of MTHFR and outcomes of methotrexate treatment in rheumatoid arthritis. Clin Exp Rheumatol. 2010; 28:728–733. [PubMed: 20863444]

19. Tukova J, Chladek J, Hroch M, Nemcova D, Hoza J, Dolezalova P. 677TT genotype is associated with elevated risk of methotrexate (MTX) toxicity in juvenile idiopathic arthritis: treatment outcome, erythrocyte concentrations of MTX and folates, and MTHFR polymorphisms. J Rheumatol. 2010; 37:2180–2186. [PubMed: 20595278]

20. Shimasaki N, Mori T, Torii C, Sato R, Shimada H, Tanigawara Y, et al. Influence of MTHFR and RFC1 polymorphisms on toxicities during maintenance chemotherapy for childhood acute lymphoblastic leukemia or lymphoma. J Pediatr Hematol Oncol. 2008; 30:347–352. [PubMed: 18458567]

21. Krajinovic M, Lemieux-Blanchard E, Chiasson S, Primeau M, Costea I, Moghrabi A. Role of polymorphisms in *MTHFR* and *MTHFD1* genes in the outcome of childhood acute lymphoblastic leukemia. Pharmacogenomics J. 2004; 4:66–72. [PubMed: 14647408]

22. Aplenc R, Thompson J, Han P, La M, Zhao H, Lange B, et al. Methylenetetrahydrofolate reductase polymorphisms and therapy response in pediatric acute lymphoblastic leukemia. Cancer Res. 2005; 65:2482–2487. [PubMed: 15781665]

23. Nuckel H, Frey UH, Durig J, Duhrsen U, Siffert W. Methylenetetrahydrofolate reductase (*MTHFR*) gene 677C>T and 1298A>C polymorphisms are associated with differential apoptosis of leukemic B cells *in vitro* and disease progression in chronic lymphocytic leukemia. Leukemia. 2004; 18:1816–1823. [PubMed: 15385937]

24. Kishi S, Griener J, Cheng C, Das S, Cook EH, Pei D, et al. Homocysteine, pharmacogenetics, and neurotoxicity in children with leukemia. J Clin Oncol. 2003; 21:3084–3091. [PubMed: 12915598]

25. Hughes LB, Beasley TM, Patel H, Tiwari HK, Morgan SL, Baggott JE, et al. Racial or ethnic differences in allele frequencies of single-nucleotide polymorphisms in the methylenetetrahydrofolate reductase gene and their influence on response to methotrexate in rheumatoid arthritis. Ann Rheum Dis. 2006; 65:1213–1218. [PubMed: 16439441]

26. Warren RB, Smith RL, Campalani E, Eyre S, Smith CH, Barker JN, et al. Outcomes of methotrexate therapy for psoriasis and relationship to genetic polymorphisms. Br J Dermatol. 2009; 160:438–441. [PubMed: 19016697]

27. Mena JP, Salazar-Paramo M, Gonzalez-Lopez L, Gamez-Nava JI, Sandoval-Ramirez L, Sanchez JD, et al. Polymorphisms C677T and A1298C in the *MTHFR* gene in Mexican patients with rheumatoid arthritis treated with methotrexate: implication with elevation of transaminases. Pharmacogenomics J. 2010 Epub ahead of print.

28. Huang L, Tissing WJ, de Jonge R, Van Zelst BD, Pieters R. Polymorphisms in folate-related genes: association with side effects of high-dose methotrexate in childhood acute lymphoblastic leukemia. Leukemia. 2008; 22:1798–1800. [PubMed: 18368069]

29. Rocha JC, Cheng C, Liu W, Kishi S, Das S, Cook EH, et al. Pharmacogenetics of outcome in children with acute lymphoblastic leukemia. Blood. 2005; 105:4752–4758. [PubMed: 15713801]

30. Shimasaki N, Mori T, Samejima H, Sato R, Shimada H, Yahagi N, et al. Effects of methylenetetrahydrofolate reductase and reduced folate carrier 1 polymorphisms on high-dose methotrexate-induced toxicities in children with acute lymphoblastic leukemia or lymphoma. J Pediatr Hematol Oncol. 2006; 28:64–68. [PubMed: 16462575]

31. Seidemann K, Book M, Zimmermann M, Meyer U, Welte K, Stanulla M, et al. MTHFR 677 (C–>T) polymorphism is not relevant for prognosis or therapy-associated toxicity in pediatric NHL: results from 484 patients of multicenter trial NHL-BFM 95. Ann Hematol. 2006; 85:291–300. [PubMed: 16463153]

32. Hayashi H, Fujimaki C, Daimon T, Tsuboi S, Matsuyama T, Itoh K. Genetic polymorphisms in folate pathway enzymes as a possible marker for predicting the outcome of methotrexate therapy in Japanese patients with rheumatoid arthritis. J Clin Pharm Ther. 2009; 34:355–361. [PubMed: 19827168]
33. Jazbec J, Kitanovski L, Aplenc R, Debeljak M, Dolzan V. No evidence of association of methylenetetrahydrofolate reductase polymorphism with occurrence of second neoplasms after treatment of childhood leukemia. Leuk Lymphoma. 2005; 46:893–897. [PubMed: 16019535]
34. Pakakasama S, Kanchanakamhaeng K, Kajanachumpol S, Udomsubpayakul U, Sirachainan N, Thithapandha A, et al. Genetic polymorphisms of folate metabolic enzymes and toxicities of high-dose methotrexate in children with acute lymphoblastic leukemia. Ann Hematol. 2007; 86:609–611. [PubMed: 17323057]
35. Wessels JAM, de Vries-Bouwstra JK, Heijmans BT, Slagboom PE, Goekoop-Ruiterman YPM, Allaart CF, et al. Efficacy and toxicity of methotrexate in early rheumatoid arthritis are associated with single-nucleotide polymorphisms in genes coding for folate pathway enzymes. Arthritis Rheum. 2006; 54:1087–1095. [PubMed: 16572443]
36. Robien K, Ulrich CM, Bigler J, Yasui Y, Gooley T, Bruemmer B, et al. Methylenetetrahydrofolate reductase genotype affects risk of relapse after hematopoietic cell transplantation for chronic myelogenous leukemia. Clin Cancer Res. 2004; 10:7592–7598. [PubMed: 15569990]
37. Laverdiere C, Chiasson S, Costea I, Moghrabi A, Krajinovic M. Polymorphism G80A in the reduced folate carrier gene and its relationship to methotrexate plasma levels and outcome of childhood acute lymphoblastic leukemia. Blood. 2002; 100:3832–3834. [PubMed: 12411325]
38. Kishi S, Cheng C, French D, Pei D, Das S, Cook EH, et al. Ancestry and pharmacogenetics of antileukemic drug toxicity. Blood. 2007; 109:4151–4157. [PubMed: 17264302]
39. Imanishi H, Okamura N, Yagi M, Noro Y, Moriya Y, Nakamura T, et al. Genetic polymorphisms associated with adverse events and elimination of methotrexate in childhood acute lymphoblastic leukemia and malignant lymphoma. J Hum Genet. 2007; 52:166–171. [PubMed: 17180579]
40. Gregers J, Christensen IJ, Dalhoff K, Lausen B, Schroeder H, Rosthoej S, et al. The association of reduced folate carrier 80G>A polymorphism to outcome in childhood acute lymphoblastic leukemia interacts with chromosome 21 copy number. Blood. 2010; 115:4671–4677. [PubMed: 20335220]
41. Drozdzik M, Rudas T, Pawlik A, Gornik W, Kurzawski M, Herczynska M. Reduced folate carrier-1 80G>A polymorphism affects methotrexate treatment outcome in rheumatoid arthritis. Pharmacogenomics J. 2007; 7:404–407. [PubMed: 17325736]
42. James HM, Gillis D, Hissaria P, Lester S, Somogyi AA, Cleland LG, et al. Common polymorphisms in the folate pathway predict efficacy of combination regimens containing methotrexate and sulfasalazine in early rheumatoid arthritis. J Rheumatol. 2008; 35:562–571. [PubMed: 18322994]
43. Krajinovic M, Costea I, Chiasson S. Polymorphism of the thymidylate synthase gene and outcome of acute lymphoblastic leukaemia. Lancet. 2002; 359:1033–1034. [PubMed: 11937185]
44. Costea I, Moghrabi A, Krajinovic M. The influence of cyclin D1 (CCND1) 870A>G polymorphism and CCND1-thymidylate synthase (TS) gene–gene interaction on the outcome of childhood acute lymphoblastic leukaemia. Pharmacogenetics. 2003; 13:577–580. [PubMed: 12972956]
45. Dulucq S, St-Onge G, Gagne V, Ansari M, Sinnett D, Labuda D, et al. DNA variants in the dihydrofolate reductase gene and outcome in childhood ALL. Blood. 2008; 111:3692–3700. [PubMed: 18096764]
46. Stamp LK, Chapman PT, O'Donnell JL, Zhang M, James J, Frampton C, et al. Polymorphisms within the folate pathway predict folate concentrations but are not associated with disease activity in rheumatoid arthritis patients on methotrexate. Pharmacogenet Genomics. 2010; 20:367–376. [PubMed: 20386493]
47. Lauten M, Asgedom G, Welte K, Schrappe M, Stanulla M. Thymidylate synthase gene polymorphism and its association with relapse in childhood B-cell precursor acute lymphoblastic leukemia. Haematologica. 2003; 88:353–354. [PubMed: 12651279]

48. Sharma S, Das M, Kumar A, Marwaha V, Shankar S, Aneja R, et al. Interaction of genes from influx-metabolism-efflux pathway and their influence on methotrexate efficacy in rheumatoid arthritis patients among Indians. Pharmacogenet Genomics. 2008; 18:1041–1049. [PubMed: 19093297]
49. Kooloos WM, Wessels JA, Van der Straaten T, Allaart CF, Huizinga TW, Guchelaar HJ. Functional polymorphisms and methotrexate treatment outcome in recent-onset rheumatoid arthritis. Pharmacogenomics. 2010; 11:163–175. [PubMed: 20136356]
50. Letourneau IJ, Deeley RG, Cole SP. Functional characterization of nonsynonymous single nucleotide polymorphisms in the gene encoding human multidrug resistance protein 1 (MRP1/ABCC1). Pharmacogenet Genomics. 2005; 15:647–657. [PubMed: 16041243]
51. Warren RB, Smith RL, Campalani E, Eyre S, Smith CH, Barker JN, et al. Genetic variation in efflux transporters influences outcome to methotrexate therapy in patients with psoriasis. J Invest Dermatol. 2008; 128:1925–1929. [PubMed: 18256692]
52. Zeng H, Chen ZS, Belinsky MG, Rea PA, Kruh GD. Transport of methotrexate (MTX) and folates by multidrug resistance protein (MRP) 3 and MRP1: effect of polyglutamylation on MTX transport. Cancer Res. 2001; 61:7225–7232. [PubMed: 11585759]
53. Vlaming ML, Van Esch A, Pala Z, Wagenaar E, Van de Wetering K, Van Tellingen O, et al. Abcc2 (Mrp2), Abcc3 (Mrp3), and Abcg2 (Bcrp1) are the main determinants for rapid elimination of methotrexate and its toxic metabolite 7-hydroxymethotrexate *in vivo*. Mol Cancer Ther. 2009; 8:3350–3359. [PubMed: 19996279]
54. Ansari M, Sauty G, Labuda M, Gagne V, Laverdiere C, Moghrabi A, et al. Polymorphisms in multidrug resistance-associated protein gene 4 is associated with outcome in childhood acute lymphoblastic leukemia. Blood. 2009; 114:1383–1386. [PubMed: 19515727]
55. Volk EL, Farley KM, Wu Y, Li F, Robey RW, Schneider E. Overexpression of wild-type breast cancer resistance protein mediates methotrexate resistance. Cancer Res. 2002; 62:5035–5040. [PubMed: 12208758]
56. Chen ZS, Robey RW, Belinsky MG, Shchaveleva I, Ren XQ, Sugimoto Y, et al. Transport of methotrexate, methotrexate polyglutamates, and 17beta-estradiol 17-(beta-D-glucuronide) by ABCG2: effects of acquired mutations at R482 on methotrexate transport. Cancer Res. 2003; 63:4048–4054. [PubMed: 12874005]
57. Sharma S, Das M, Kumar A, Marwaha V, Shankar S, Singh P, et al. Purine biosynthetic pathway genes and methotrexate response in rheumatoid arthritis patients among north Indians. Pharmacogenet Genomics. 2009; 19:823–828. [PubMed: 19902562]
58. Merrill JT, Shen C, Schreibman D, Coffey D, Zakharenko O, Fisher R, et al. Adenosine A1 receptor promotion of multinucleated giant cell formation by human monocytes: a mechanism for methotrexate-induced nodulosis in rheumatoid arthritis. Arthritis Rheum. 1997; 40:1308–1315. [PubMed: 9214432]
59. Hider SL, Thomson W, Mack LF, Armstrong DJ, Shadforth M, Bruce IN. Polymorphisms within the adenosine receptor 2a gene are associated with adverse events in RA patients treated with MTX. Rheumatology (Oxford). 2008; 47:1156–1159. [PubMed: 18539621]
60. Varani K, Massara A, Vincenzi F, Tosi A, Padovan M, Trotta F, et al. Normalization of A2A and A3 adenosine receptor upregulation in rheumatoid arthritis patients by treatment with anti-tumor necrosis factor alpha but not methotrexate. Arthritis Rheum. 2009; 60:2880–2891. [PubMed: 19790066]
61. Bar-Yehuda S, Silverman MH, Kerns WD, Ochaion A, Cohen S, Fishman P. The anti-inflammatory effect of A3 adenosine receptor agonists: a novel targeted therapy for rheumatoid arthritis. Expert Opin Investig Drugs. 2007; 16:1601–1613.
62. Wessels JAM, Kooloos WM, De Jonge R, de Vries-Bouwstra JK, Allaart CF, Linssen A, et al. Relationship between genetic variants in the adenosine pathway and outcome of methotrexate treatment in patients with recent-onset rheumatoid arthritis. Arthritis Rheum. 2006; 54:2830–2839. [PubMed: 16947783]
63. Lee YC, Cui J, Costenbader KH, Shadick NA, Weinblatt ME, Karlson EW. Investigation of candidate polymorphisms and disease activity in rheumatoid arthritis patients on methotrexate. Rheumatology (Oxford). 2009; 48:613–617. [PubMed: 19193698]

64. Krajinovic M, Robaey P, Chiasson S, Lemieux-Blanchard E, Rouillard M, Primeau M, et al. Polymorphisms of genes controlling homocysteine levels and IQ score following the treatment for childhood ALL. Pharmacogenomics. 2005; 6:293–302. [PubMed: 16013960]
65. Al-Shakfa F, Dulucq S, Brukner I, Milacic I, Ansari M, Beaulieu P, et al. DNA variants in region for noncoding interfering transcript of dihydrofolate reductase gene and outcome in childhood acute lymphoblastic leukemia. Clin Cancer Res. 2009; 15:6931–6938. [PubMed: 19861437]
66. Lievers KJ, Kluijtmans LA, Boers GH, Verhoef P, den Heijer M, Trijbels FJ, et al. Influence of a glutamate carboxypeptidase II (GCPII) polymorphism (1561C–>T) on plasma homocysteine, folate and vitamin B(12) levels and its relationship to cardiovascular disease risk. Atherosclerosis. 2002; 164:269–273. [PubMed: 12204797]
67. Sanchez-del-Campo L, Montenegro MF, Cabezas-Herrera J, Rodriguez-Lopez JN. The critical role of alpha-folate receptor in the resistance of melanoma to methotrexate. Pigment Cell Melanoma Res. 2009; 22:588–600. [PubMed: 19493312]
68. Zaza G, Yang W, Kager L, Cheok M, Downing J, Pui CH, et al. Acute lymphoblastic leukemia with TEL-AML1 fusion has lower expression of genes involved in purine metabolism and lower de novo purine synthesis. Blood. 2004; 104:1435–1441. [PubMed: 15142881]
69. Garcia-Bournissen F, Moghrabi A, Krajinovic M. Therapeutic responses in childhood acute lymphoblastic leukemia (ALL) and haplotypes of gamma glutamyl hydrolase (*GGH*) gene. Leuk Res. 2007; 31:1023–1025. [PubMed: 16999998]
70. Dervieux T, Kremer J, Lein DO, Capps R, Barham R, Meyer G, et al. Contribution of common polymorphisms in reduced folate carrier and gamma-glutamylhydrolase to methotrexate polyglutamate levels in patients with rheumatoid arthritis. Pharmacogenetics. 2004; 14:733–739. [PubMed: 15564880]
71. Van der Straaten RJ, Wessels JA, de Vries-Bouwstra JK, Goekoop-Ruiterman YP, Allaart CF, Bogaartz J, et al. Exploratory analysis of four polymorphisms in human *GGH* and *FPGS* genes and their effect in methotrexate-treated rheumatoid arthritis patients. Pharmacogenomics. 2007; 8:141–150. [PubMed: 17286537]
72. Van Ede AE, Laan RF, De Abreu RA, Stegeman AB, Van de Putte LB. Purine enzymes in patients with rheumatoid arthritis treated with methotrexate. Ann Rheum Dis. 2002; 61:1060–1064. [PubMed: 12429535]
73. Rizzo R, Rubini M, Govoni M, Padovan M, Melchiorri L, Stignani M, et al. HLA-G 14-bp polymorphism regulates the methotrexate response in rheumatoid arthritis. Pharmacogenet Genomics. 2006; 16:615–623. [PubMed: 16906016]
74. Baricordi OR, Govoni M, Rizzo R, Trotta F. In rheumatoid arthritis, a polymorphism in the *HLA-G* gene concurs in the clinical response to methotrexate treatment. Ann Rheum Dis. 2007; 66:1125–1126. [PubMed: 17626975]
75. Stamp LK, O'Donnell JL, Chapman PT, Barclay ML, Kennedy MA, Frampton CM, et al. Lack of association between HLA-G 14 bp insertion/deletion polymorphism and response to long-term therapy with methotrexate response in rheumatoid arthritis. Ann Rheum Dis. 2009; 68:154–155. [PubMed: 19088262]
76. Wessels JAM, Van der Kooij SM, le Cessie S, Kievit W, Barerra P, Allaart CF, et al. A clinical pharmacogenetic model to predict the efficacy of methotrexate monotherapy in recent-onset rheumatoid arthritis. Arthritis Rheum. 2007; 56:1765–1775. [PubMed: 17530705]
77. De Jonge R, Hooijberg JH, Van Zelst BD, Jansen G, Van Zantwijk CH, Kaspers GJ, et al. Effect of polymorphisms in folate-related genes on in-vitro methotrexate sensitivity in pediatric acute lymphoblastic leukemia. Blood. 2005; 106:717–720. [PubMed: 15797993]
78. Bertrand R, MacKenzie RE, Jolivet J. Human liver methenyltetrahydrofolate synthetase: improved purification and increased affinity for folate polyglutamate substrates. Biochim Biophys Acta. 1987; 911:154–161. [PubMed: 3801490]
79. Patino-Garcia A, Zalacain M, Marrodan L, San-Julian M, Sierrasesumaga L. Methotrexate in pediatric osteosarcoma: response and toxicity in relation to genetic polymorphisms and dihydrofolate reductase and reduced folate carrier 1 expression. J Pediatr. 2009; 154:688–693. [PubMed: 19159907]

80. Linnebank M, Malessa S, Moskau S, Semmler A, Pels H, Klockgether T, et al. Acute methotrexate-induced encephalopathy–causal relation to homozygous allelic state for MTR c. 2756A>G (D919G)? J Chemother. 2007; 19:455–457. [PubMed: 17855192]
81. Berkun Y, Abou Atta I, Rubinow A, Orbach H, Levartovsky D, Aamar S, et al. 2756GG genotype of methionine synthase reductase gene is more prevalent in rheumatoid arthritis patients treated with methotrexate and is associated with methotrexate-induced nodulosis. J Rheumatol. 2007; 34:1664–1669. [PubMed: 17611986]
82. McGuire JJ. Anticancer antifolates: current status and future directions. Curr Pharm Des. 2003; 9:2593–2613. [PubMed: 14529544]
83. Sun W, Wu RR, Van Poelje PD, Erion MD. Isolation of a family of organic anion transporters from human liver and kidney. Biochem Biophys Res Commun. 2001; 283:417–422. [PubMed: 11327718]
84. Uwai Y, Iwamoto K. Transport of aminopterin by human organic anion transporters hOAT1 and hOAT3: comparison with methotrexate. Drug Metab Pharmacokinet. 2010; 25:163–169. [PubMed: 20460822]
85. Unal ES, Zhao R, Goldman ID. Role of the glutamate 185 residue in proton translocation mediated by the proton-coupled folate transporter SLC46A1. Am J Physiol Cell Physiol. 2009; 297:C66–C74. [PubMed: 19403800]
86. Badagnani I, Castro RA, Taylor TR, Brett CM, Huang CC, Stryke D, et al. Interaction of methotrexate with organic anion transporting polypeptide 1A2 and its genetic variants. J Pharmacol Exp Ther. 2006; 318:521–529. [PubMed: 16702441]
87. Krajinovic M, Costea I, Primeau M, Dulucq S, Moghrabi A. Combining several polymorphisms of thymidylate synthase gene for pharmacogenetic analysis. Pharmacogenomics J. 2005; 5:374–380. [PubMed: 16130010]
88. Goode EL, Potter JD, Bigler J, Ulrich CM. Methionine synthase D919G polymorphism, folate metabolism, and colorectal adenoma risk. Cancer Epidemiol Biomarkers Prev. 2004; 13:157–162. [PubMed: 14744749]
89. Rau T, Erney B, Gores R, Eschenhagen T, Beck J, Langer T. High-dose methotrexate in pediatric acute lymphoblastic leukemia: impact of ABCC2 polymorphisms on plasma concentrations. Clin Pharmacol Ther. 2006; 80:468–476. [PubMed: 17112803]
90. Qiu A, Jansen M, Sakaris A, Min SH, Chattopadhyay S, Tsai E, et al. Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. Cell. 2006; 127:917–928. [PubMed: 17129779]
91. Gradhand U, Kim RB. Pharmacogenomics of MRP transporters (ABCC1-5) and BCRP (ABCG2). Drug Metab Rev. 2008; 40:317–354. [PubMed: 18464048]
92. Kruh GD, Belinsky MG, Gallo JM, Lee K. Physiological and pharmacological functions of Mrp2, Mrp3 and Mrp4 as determined from recent studies on gene-disrupted mice. Cancer Metastasis Rev. 2007; 26:5–14. [PubMed: 17273943]
93. Chan L, Lowes S, Hirst BH. The ABCs of drug transport in intestine and liver: efflux proteins limiting drug absorption and bioavailability. Eur J Pharm Sci. 2004; 21:25–51. [PubMed: 14706810]
94. El-Sheikh AA, Van den Heuvel JJ, Koenderink JB, Russel FG. Interaction of nonsteroidal anti-inflammatory drugs with multidrug resistance protein (MRP) 2/A. J Pharmacol Exp Ther. 2007; 320:229–235. [PubMed: 17005917]
95. Hulot JS, Villard E, Maguy A, Morel V, Mir L, Tostivint I, et al. A mutation in the drug transporter gene ABCC2 associated with impaired methotrexate elimination. Pharmacogenet Genomics. 2005; 15:277–285. [PubMed: 15864128]
96. Uwai Y, Taniguchi R, Motohashi H, Saito H, Okuda M, Inui K. Methotrexate-loxoprofen interaction: involvement of human organic anion transporters hOAT1 and hOAT3. Drug Metab Pharmacokinet. 2004; 19:369–374. [PubMed: 15548848]
97. Van Aubel RA, Smeets PH, Peters JG, Bindels RJ, Russel FG. The *MRP4/ABCC4* gene encodes a novel apical organic anion transporter in human kidney proximal tubules: putative efflux pump for urinary cAMP and cGMP. J Am Soc Nephrol. 2002; 13:595–603. [PubMed: 11856762]

98. Vanwert AL, Sweet DH. Impaired clearance of methotrexate in organic anion transporter 3 (Slc22a8) knockout mice: a gender-specific impact of reduced folates. Pharm Res. 2008; 25:453–462. [PubMed: 17660957]

99. Abe T, Kakyo M, Tokui T, Nakagomi R, Nishio T, Nakai D, et al. Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. J Biol Chem. 1999; 274:17159–17163. [PubMed: 10358072]

100. Abe T, Unno M, Onogawa T, Tokui T, Kondo TN, Nakagomi R, et al. LST-2, a human liver-specific organic anion transporter, determines methotrexate sensitivity in gastrointestinal cancers. Gastroenterology. 2001; 120:1689–1699. [PubMed: 11375950]

101. Van de SE, Van der Kruijssen CM, Wagenaar E, Burggraaff JE, Mesman E, Kenworthy KE, et al. Methotrexate pharmacokinetics in transgenic mice with liver-specific expression of human OATP1B1 (SLCO1B1). Drug Metab Dispos. 2009; 37:277–281. [PubMed: 19022939]

102. Kitamura Y, Hirouchi M, Kusuhara H, Schuetz JD, Sugiyama Y. Increasing systemic exposure of methotrexate by active efflux mediated by multidrug resistance-associated protein 3 (Mrp3/Abcc3). J Pharmacol Exp Ther. 2008; 27:465–473. [PubMed: 18719291]

103. Vlaming ML, Mohrmann K, Wagenaar E, de Waart DR, Elferink RP, Lagas JS, et al. Carcinogen and anticancer drug transport by Mrp2 *in vivo*: studies using Mrp2 (Abcc2) knockout mice. J Pharmacol Exp Ther. 2006; 318:319–327. [PubMed: 16611851]

104. Vlaming ML, Pala Z, Van EA, Wagenaar E, Van TO, de Waart DR, et al. Impact of Abcc2 (Mrp2) and Abcc3 (Mrp3) on the in-vivo elimination of methotrexate and its main toxic metabolite 7-hydroxymethotrexate. Clin Cancer Res. 2008; 14:8152–8160. [PubMed: 19088030]

105. Vlaming ML, Pala Z, Van EA, Wagenaar E, de Waart DR, Van de WK, et al. Functionally overlapping roles of Abcg2 (Bcrp1) and Abcc2 (Mrp2) in the elimination of methotrexate and its main toxic metabolite 7-hydroxymethotrexate *in vivo*. Clin Cancer Res. 2009; 15:3084–3093. [PubMed: 19383815]

106. Lîscher W, Potschka H. Role of drug efflux transporters in the brain for drug disposition and treatment of brain diseases. Prog Neurobiol. 2005; 76:22–76. [PubMed: 16011870]

107. Schmiegelow K. Advances in individual prediction of methotrexate toxicity: a review. Br J Haematol. 2009; 146:489–503. [PubMed: 19538530]

108. Belkov VM, Krynetski EY, Schuetz JD, Yanishevski Y, Masson E, Mathew S, et al. Reduced folate carrier expression in acute lymphoblastic leukemia: a mechanism for ploidy but not lineage differences in methotrexate accumulation. Blood. 1999; 93:1643–1650. [PubMed: 10029593]

109. Zhang L, Taub JW, Williamson M, Wong SC, Hukku B, Pullen J, et al. Reduced folate carrier gene expression in childhood acute lymphoblastic leukemia: relationship to immunophenotype and ploidy. Clin Cancer Res. 1998; 4:2169–2177. [PubMed: 9748136]

110. Hooijberg JH, de Vries NA, Kaspers GJ, Pieters R, Jansen G, Peters GJ. Multidrug resistance proteins and folate supplementation: therapeutic implications for antifolates and other classes of drugs in cancer treatment. Cancer Chemother Pharmacol. 2006; 58:1–12. [PubMed: 16362298]

111. Barredo JC, Synold TW, Laver J, Relling MV, Pui CH, Priest DG, et al. Differences in constitutive and post-methotrexate folylpolyglutamate synthetase activity in B-lineage and T-lineage leukemia. Blood. 1994; 84:564–569. [PubMed: 7517720]

112. Gorlick R, Goker E, Trippett T, Waltham M, Banerjee D, Bertino JR. Intrinsic and acquired resistance to methotrexate in acute leukemia. New Eng J Med. 1996; 335:1041. [PubMed: 8793930]

113. Panetta JC, Wall A, Pui CH, Relling MV, Evans WE. Methotrexate intracellular disposition in acute lymphoblastic leukemia: a mathematical model of gamma-glutamyl hydrolase activity. Clin Cancer Res. 2002; 8:2423–2429. [PubMed: 12114448]

114. Cheng Q, Yang W, Raimondi SC, Pui CH, Relling MV, Evans WE. Karyotypic abnormalities create discordance of germ line genotype and cancer cell phenotypes. Nat Genet. 2005; 37:878–882. [PubMed: 16041371]

115. Cheng Q, Cheng C, Crews KR, Ribeiro RC, Pui CH, Relling MV, et al. Epigenetic regulation of human gamma-glutamyl hydrolase activity in acute lymphoblastic leukemia cells. Am J Hum Genet. 2006; 79:264–274. [PubMed: 16826517]

116. Dervieux T, Furst D, Lein DO, Capps R, Smith K, Walsh M, et al. Polyglutamation of methotrexate with common polymorphisms in reduced folate carrier, aminoimidazole carboxamide ribonucleotide transformylase, and thymidylate synthase are associated with methotrexate effects in rheumatoid arthritis. Arthritis Rheum. 2004; 50:2766–2774. [PubMed: 15457444]

117. Becker ML, Gaedigk R, van Haandel L, Thomas B, Lasky A, Hoeltzel M, et al. The effect of genotype on methotrexate polyglutamate variability in juvenile idiopathic arthritis and association with drug response. Arthritis Rheum. 2010 Epub ahead of print.

118. Cheng Q, Wu B, Kager L, Panetta JC, Zheng J, Pui CH, et al. A substrate specific functional polymorphism of human [gamma]-glutamyl hydrolase alters catalytic activity and methotrexate polyglutamate accumulation in acute lymphoblastic leukaemia cells. Pharmacogenet Genomics. 2004; 14:557.

119. Kager L, Cheok M, Yang W, Zaza G, Cheng Q, Panetta JC, et al. Folate pathway gene expression differs in subtypes of acute lymphoblastic leukemia and influences methotrexate pharmacodynamics. J Clin Invest. 2005; 115:110–117. [PubMed: 15630450]

120. Guo W, Healey JH, Meyers PA, Ladanyi M, Huvos AG, Bertino JR, et al. Mechanisms of methotrexate resistance in osteosarcoma. Clin Cancer Res. 1999; 5:621. [PubMed: 10100715]

121. Krajinovic M, Lemieux-Blanchard E, Chiasson S, Primeau M, Costea I, Moghrabi A. Role of polymorphisms in *MTHFR* and *MTHFD1* genes in the outcome of childhood acute lymphoblastic leukemia. Pharmacogenomics J. 2003; 4:66–72. [PubMed: 14647408]

122. French D, Yang W, Cheng C, Raimondi SC, Mullighan CG, Downing JR, et al. Acquired variation outweighs inherited variation in whole genome analysis of methotrexate polyglutamate accumulation in leukemia. Blood. 2009; 113:4512–4520. [PubMed: 19066393]

123. Sorich MJ, Pottier N, Pei D, Yang W, Kager L, Stocco G, et al. In-vivo response to methotrexate forecasts outcome of acute lymphoblastic leukemia and has a distinct gene expression profile. PloS Med. 2008; 5:e83. [PubMed: 18416598]

124. Yang JJ, Cheng C, Yang W, Pei D, Cao X, Fan Y, et al. Genome-wide interrogation of germ line genetic variation associated with treatment response in childhood acute lymphoblastic leukemia. JAMA. 2009; 301:393–403. [PubMed: 19176441]

125. Chan ES, Cronstein BN. Molecular action of methotrexate in inflammatory diseases. Arthritis Res. 2002; 4:266–273. [PubMed: 12106498]

126. Hinks A, Moncrieffe H, Martin P, Lal SD, Ursu S, Kassoumeri L, et al. Genetic polymorphisms in key methotrexate pathway genes associated with response to MTX treatment in juvenile idiopathic arthritis. Arthritis Rheum. 2009; 60(10 Suppl):S233.

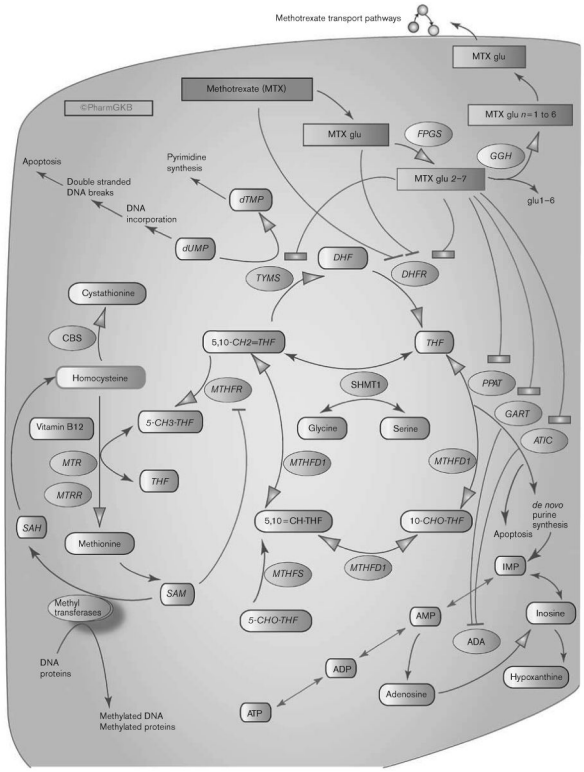

Fig. 1.

Stylized cell showing candidate genes involved in pharmacokinetics and pharmacodynamics of methotrexate. A fully interactive version is available online at http://www.pharmgkb.org/do/serve?objId=PA2039&objCls=Pathway, and includes additional views that show transport and action in different cell types.

Table 1

Well-studied genes and variants involved in methotrexate pharmacogenomics

Gene	Variant (rs#)	Phenotype (population) (PMID)	No association (population) (PMID)
MTHFR	677C > T (rs1801133)	C toxicity/adverse events (pediatric ALL, CML) (16870553 [7]) (16920564 [8]) T toxicity/adverse events (pediatric ALL; NHL; CML; RA, Black; PsA; RA, White; RA, Asian; JIA, White; pediatric ALL, Asian) (12453860 [9], 17488658 [10], 11418485 [11], 18381794 [12], 20472929 [13], 17512587 [14], 16501586 [15], 15051775 [16], 11710708 [17], 20863444 [18], 20595278 [19], 18458567 [20]) C efficacy (pediatric ALL; NHL; CML) (14647408 [21]; 15781665 [22], 17488658 [10], 17512587 [14], 15385937 [23])	Toxicity (pediatric ALL; pediatric ALL, Asian; RA, Black, White; RA, Hispanic; Psoriasis; NHL) (12915598 [24], 16439441 [25], 19016697 [26], 20514079 [27], 18368069 [28], 15713801 [29], 16462575 [30], 15781665 [22], 16463153 [31]) Efficacy (psoriasis; NHL; pediatric ALL) (19016697 [26], 16463153 [31], 19827168 [32], 16019535 [33])
MTHFR	1298A>C (rs1801131)	A toxicity (RA, White; pediatric ALL)(16439441 [25], 18368069 [28], 17323057 [34]) C toxicity/adverse events (NHL; CML; RA; RA Hispanic; CML) (17488658 [10], 16501586 [15], 16572443 [35], 20514079 [27], 16920564 [8]) A efficacy (RA, White; CML, White) (16572443 [35], 15569990 [36]) C efficacy (RA, Asian; pediatric ALL; CML) (20863444 [18], 14647408 [21], 15385937 [23])	Toxicity (RA, Black; RA, White, Black; Psoriasis, PsA, pediatric ALL) (16439441 [25], 18381794 [12], 19016697 [26], 20472929 [13], 16870553 [7], 15713801 [29], 15781665 [22], 17512587 [14]) Efficacy (Psoriasis; pediatric ALL) (19016697 [26], 15781665 [22], 17512587 [14], 16019535 [33])
SLC19A1	80G>A (rs1051266)	A toxicity/adverse events (pediatric ALL; ALL; pediatric ALL, Asian) (12411325 [37], (17264302 [38], 17180579 [39], 20335220 [40]) G toxicity (pediatric ALL, Asian) (16462575 [30]) A efficacy (RA; RA, Asian; ALL) (17325736 [41], 18322994 [42], 20335220 [40], 19827168 [32]) G efficacy (pediatric ALL) (12411325 [37])	Toxicity (pediatric ALL, PsA) (12915598 [24], 20472929 [13], 18368069 [28], 16870553 [7], 15713801 [29])
TYMS	28 bp repeat (rs34743033)	3R/2R toxicity (CML) (16920564 [8]) 3R/3R reduced efficiacy (pediatric ALL) (11937185 [43], 12972956 [44], 15713801 [29], 14647408 [21], 18096764 [45])	Efficacy (RA, White) (20386493 [46], 12651279 [47]) Toxicity (pediatric ALL, CML) (16870553 [7], 16501586 [15])

ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; NHL, non-Hodgkin's lymphoma; PMID, PubMed identifier; PsA psoriatic arthritis; RA, rheumatoid arthritis.

Table 2

Additional candidate genes and variants for methotrexate pharmacogenomics

Gene	Studied variants (rs)	PMIDs
ABCB1	3435C>T (rs1045642), 1236 C>T (rs1128503)	19093297 [48], 20386493[a][46], 18381794 [12], 20136356 [49]
ABCC1	Arg723Gln (rs4148356), rs3784862, Ala1337Thr (rs28364006), 4002 G>A (rs2230671), IVS 14+115C>T, IVS 18-30C>G (rs2074087)	16041243 [50], 18381794[a][12], 20386493 [46], 18256692 [51]
ABCC2	1249 G>A (rs2273697), IVS23+56C>T (rs4148396)	18381794 [12], 20386493 [46]
ABCC3		11585759[b][52], 19996279[b][53]
ABCC4	934A>C (rs2274407), −1393T>C	19515727 [54]
ABCG2	914C>A (rs2231142), rs17731538	20386493 [46], 12208758[b][55], 12874005[b][56]
ADA	Val178Val (rs244076), IVS 2 C/G (rs1799880)	19902562 [57]
ADORA1	rs16851020, rs3766553, rs3766554, rs17530497, rs4423076, rs1845466, rs3753475, rs10800901, rs10920574, rs7549561, rs6427993, rs6701725, rs12123037, rs1494487, rs3898276	9214432[b][58], 18256692[a][51]
ADORA2A	1976T>C (rs5751876), rs5760410, rs2298383, rs3761422, rs2267076, rs2236624, rs9624472, rs4822489	18539621 [59], 19902562 [57]
ADORA3		19790066 [60], 17922624 [61]

Gene	Studied variants (rs)	PMIDs
AMPD1	34C>T (rs17602729)	16947783 [62], 19902562[a][57], 20386493 [46]
ATIC	347C>G (rs2372536) rs4673993	19902562[a][57], 16947783 [62], 19016697[a][26], 20386493[a][46], 19193698 [63]
CBS	844ins68	14647408[a][21], 16013960 [64]
CCND1	870A>G (rs9344)	12972956 [44], 16870553 [7], 18096764 [45]
DHFR	35289A>G (rs1232027), −473T>C (rs1650697), 308G > A (rs1105525), rs7387	20472929 [13], 19861437 [65], 18096764 [45], 19902562 [57], 20136356[a][49]
FOLH1	1561C>T	12204797[a][66]
FOLR1		19493312[b][67], 19093297[a][48]
FPGS	rs1544105	19902562 [57], 19093297[a][48]
GART		15142881[b][68]
GGH	452C>T (rs11545078) −401T>C (rs3758149) 16T>C (rs1800909)	20386493[a][46], 16999998 [69], 19827168 [32], 15564880 [70], 17286537 [71]
GSTM1	Null	17180579 [39], 15713801 [29]
HPRT1		12429535[b][72]
HLA-G	14bp in/del	16906016 [73], 17626975 [74], 19088262[a][75], 20136356[a][49]
IMPDH2	+787C>T	20136356[a][49]
ITPA	94C>A (rs41320251), IVS2 +21A>C (rs7270101)	16947783 [62], 17530705 [76], 19193698[a][63], 20136356[a][49]
MTHFD1	1958G>A (rs2236225)	14647408 [21], 15797993[a][77], 17530705 [76], 20386493[a][46]
MTHFR	1793G>A (rs2274976), rs2066462	20863444 [18]
MTHFS		3801490[b][78]
MTR	2756A>G (rs1805087)	20386493[a][46], 19159907 [79], 18322994 [42], 17855192 [80], 17611986 [81]
MTRR	66A>G (rs1801394)	20386493[a][46], 15797993 [77],

Gene	Studied variants (rs)	PMIDs
		18368069 [28]
NOS3	894G>T (rs1799983)	14647408 [21], 16013960 [64]
NP		12429535[b] [72]
PPAT		14529544[b] [82]
SHMT1	1420C>T (rs1979277)	20386493 [46], 18368069 [28], 15797993 [77]
SLC22A11		11327718[b] [83]
SLC22A6		20460822[b] [84]
SLC22A8		20460822[b] [84]
SLC46A1		19403800[b] [85]
SLCO1B1	rs11045879, rs4149081	19901119 [4]
SLCO1A2	Glu172Asp (rs11568563), Arg168Cys (rs11568564), Asn277DEL (rs72559749)	16702441[b] [86]
TGFB1	869T>C (rs1982073)	20136356[a] [49]
TLR4	896A>G (rs4986790)	20136356 [49]
TYMS	1494del (TTAAAG)	18381794 [12], 16130010 [87]

Annotations are available in full at http://www.pharmgkb.org/do/serve?objCls=Drug&objId=PA450428&tabType=tabGenetics#tabview=tab2.

PMID, PubMed identifier.

[a] No association.

[b] In-vitro study, (a) folate only, not methotrexate.