Acta Med. Okayama, 2020
Vol. 74, No. 6, pp. 545–550

Acta Medica Okayama
http://escholarship.lib.okayama-u.ac.jp/amo/

Case Report

Delayed Methotrexate Elimination after Administration of a Medium Dose of Methotrexate in a Patient with Genetic Variants Associated with Methotrexate Clearance

Yasuhisa Tatebe[a, c], Kiichiro Kanamitsu[b]*, Hirotaka Kanzaki[a], Hisashi Ishida[b], Kaori Fujiwara[b], Kana Washio[b], Yoshihisa Kitamura[a], Toshiaki Sendo[a], Akira Shimada[b], and Hirokazu Tsukahara[b]

Departments of [a]Pharmacy, [b]Pediatrics, Okayama University Hospital, [c]Department of Medicinal Pharmacology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan

Polymorphisms in methotrexate transporter pathways have been associated with methotrexate toxicities and clearance. Recent genome-wide association studies have revealed that the *SLCO1B1* T521C variant is associated with methotrexate elimination. We present a case of a pediatric patient with acute lymphoblastic leukemia who suffered from persistently high plasma methotrexate concentrations and acute kidney injuries after the administration of a medium dose of methotrexate. Subsequent genetic analysis showed that he was a carrier of dysfunctional genetic variants associated with methotrexate clearance. This case highlights that polymorphisms of methotrexate transporter pathways can adversely affect methotrexate elimination in a clinically significant manner.

Key words: methotrexate, polymorphism, drug elimination, acute kidney injury, acute lymphoblastic leukemia

Methotrexate (MTX) interferes with the metabolism of folic acid and blocks the synthesis of DNA. MTX plays an important role in the treatment of acute lymphoblastic leukemia (ALL), both as a prophylaxis against and a treatment for central nervous system (CNS) involvement, due to its ability to penetrate the brain-blood barrier. MTX is administered at various doses ranging from higher than 500 mg/m^2 intravenously to as low as 12 mg intrathecally [1]. While acute kidney injury (AKI) can develop as a consequence of the precipitation of MTX and its metabolites in the renal tubules following the administration of MTX at doses exceeding 500 mg/m^2, AKI has rarely been reported at lower doses [1-3].

Recent studies have reported that polymorphisms within several MTX pathway genes are suspected to exacerbate MTX toxicities and increase MTX plasma levels [4-6]. Moreover, genome-wide association studies (GWAS) have revealed that plasma MTX elimination is associated with polymorphisms in the Solute Carrier Organic Anion Transporter Family Member 1B1 (*SLCO1B1*) gene [7-9]. However, the clinical significance of such polymorphisms remains to be elucidated.

Herein, we report the case of a pediatric patient with ALL who suffered from delayed MTX elimination following the intravenous administration of MTX at a dose of 150 mg/m^2 during maintenance therapy. We analyzed his germline polymorphisms of candidate genes for MTX toxicities and clearance, and determined that he was a carrier of dysfunctional variants.

Case Report

The patient was a 7-year-old boy who had com-

Received February 10, 2020; accepted July 22, 2020.
*Corresponding author. Phone: +81-86-235-7249; Fax: +81-86-221-4745
E-mail: kanami-k@cc.okayama-u.ac.jp (K. Kanamitsu)

Conflict of Interest Disclosures: No potential conflict of interest relevant to this article was reported.

plained of fever and lower leg pain for 2 months. Blood examination revealed an elevated white blood cell count of 17,000/μl, platelet count of 71,000/μl, and lactate dehydrogenase of 6,230 U/l. Bone marrow aspiration revealed myeloperoxidase-negative lymphoblasts exceeding 90% of all nucleated cells. Flow cytometry analysis of the blasts showed that they were positive for CD10, CD19 and CD79a. He was diagnosed with B-cell precursor ALL (BCP-ALL). G-band analysis of bone marrow cells did not reveal chromosomal abnormalities, but the *TCF3-PBX1* chimeric gene was detected by polymerase chain reaction (PCR). No evidence of CNS involvement was found. The patient received prednisolone for 7 days, but the count of peripheral lymphoblasts exceeded 1,000/μl on the next day, demonstrating his poor response to prednisolone. The patient was enrolled in an extremely high-risk group of the Japan Association of Childhood Leukemia Study (JACLS) ALL-02 study [10]. The treatment protocol for the extremely high-risk group of the JACLS ALL-02 consisted of Induction Therapy (Weeks 1-5), Early Intensification (Weeks 6-9), Consolidation Therapy A1, A2, B1 and B2 (Weeks 10-25), Re-induction Therapy (Weeks 26-29) and Maintenance Therapy (Weeks 30-108). Anti-leukemia agents included in the protocol were prednisolone, dexamethasone, cyclophosphamide, L-asparaginase, pirarubicin, vincristine, methotrexate, cytosine arabinoside, etoposide and 6-mercaptopurine. Bone marrow aspiration performed on day 15 of the induction phase showed hematological remission, and *TCF3-PBX1* was not detected in bone marrow cells by PCR.

As a prophylaxis against CNS involvement, he received 3 g/m^2 of MTX twice during consolidation therapy. In order to promote MTX clearance and prevent renal impairment, alkaline hydration at a rate of more than 2,500 ml/m^2/24 h was initiated with acetazolamide prior to MTX infusion and continued until the plasma MTX level was sufficiently decreased. Moreover, medicines which can delay MTX clearance, such as trimethoprim-sulfamethoxazole (TMP-SMX), were withdrawn and avoided. Each dose of MTX was followed by leucovorin (LV) at a dose of 15 mg/m^2 every 6 h from 42 h after the start of the MTX infusion. The plasma MTX levels fell to 0.1 μmol/l within 72 h after the start of the MTX infusion. Non-hematological adverse events of Common Terminology Criteria for Adverse Events (CTCAE) grade 3 or worse were limited to abnormal liver function tests. Although the patient had received agents with potential renal toxicities (cyclophosphamide: total dose 5,700 mg/m^2; methotrexate: total dose 6,000 mg/m^2) during inpatient treatment, his creatinine clearance was not impaired (166.2 ml/min/1.73 m^2) and his renal function was normal.

The maintenance therapy was initiated on an outpatient basis with TMP-SMX-based prophylaxis against Pneumocystis jiroveci pneumonia. In week 104 of the chemotherapy, MTX was intravenously administered at a dose of 150 mg/m^2 together with daily oral 6-mercaptopurine at a dose of 50 mg/m^2. Before MTX administration, it was confirmed that the patient's physical examination findings and laboratory parameters were not remarkable. The patient visited the hospital 2 days later complaining of a poor appetite and vomiting. He had a low-grade fever of 37.3°C. He had lost 1 kg of weight compared with his last hospital visit, and his oral mucosa was dry. A diagnostic work-up for gastrointestinal symptoms and fever was performed. Rapid antigen detection tests for influenza virus, respiratory syncytial virus and group A *Streptococcus* were negative. Urine, stool and blood cultures were obtained, but their results turned out to be negative several days later. Chest and abdominal X-ray were not remarkable.

Ultrasonography showed significant respiratory variation in inferior vena cava diameter. A blood examination revealed elevated serum creatinine (Cr), urea nitrogen (UN), and C-reactive protein (CRP) levels (Fig. 1). A marked reduction in the estimated glomerular filtration rate (25 ml/min/1.73 m^2) was observed [11]. A urine test revealed aciduria (pH 5.5) and albuminuria (0.3 g/l). Significant dehydration and the development of AKI (CTCAE grade 3) were suspected.

At approximately 48 h after MTX administration, the plasma MTX level was 0.73 μmol/l, which was indicative of delayed MTX elimination. To improve the MTX clearance, a large-volume intravenous fluid infusion and urinary alkalization with sodium bicarbonate were initiated. Moreover, TMP-SMX was discontinued because it could impair MTX clearance. LV rescue was started at a high dose (60 mg/m^2) to minimize adverse events caused by prolonged MTX exposure [12]. On day 6 after MTX administration, a blood examination showed that the plasma MTX concentration had fallen below 0.1 μmol/l, and the Cr, UN, and CRP levels had improved (Fig. 1). The patient was transferred to

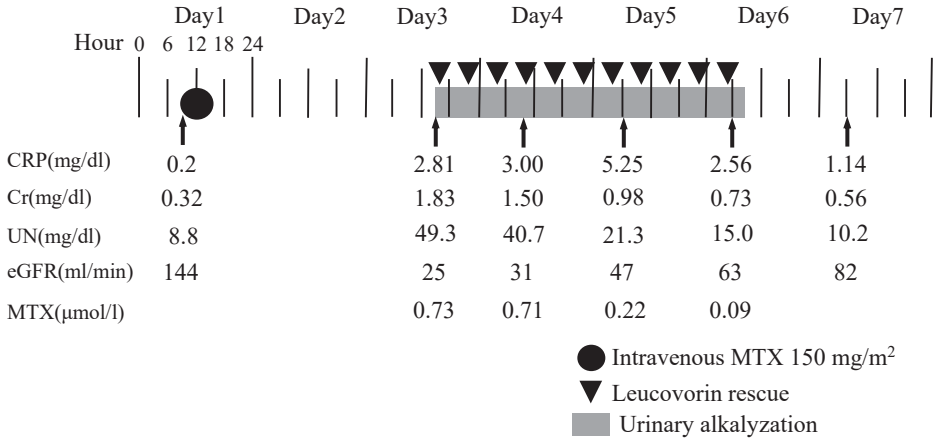

Fig. 1 Timeline of the patient's renal function indices and serum CRP and MTX concentrations after the administration of MTX. The supportive care provided to promote MTX excretion is indicated at the top. Cr, serum creatinine; CRP, serum C-reactive protein; eGFR, estimated glomerular filtration rate; MTX, methotrexate; UN, serum urea nitrogen.

another hospital for further follow-up on day 5 of hospitalization. Maintenance therapy was discontinued because its schedule was close to an end. Creatinine clearance evaluated three months after discharge was 225.6 ml/min/1.73 m^2.

Due to the scarcity of AKI and delayed MTX elimination after the administration of medium doses of MTX, we analyzed the patient for single nucleotide polymorphisms (SNP) of candidate genes associated with MTX pharmacokinetics and toxicities. The candidate genes consisted of *SLCO1B1*, solute carrier family 19 member 1 (*SLC19A1*), solute carrier family 22 member 8 (*SLC22A8*), methylene tetrahydrofolate reductase (*MTHFR*), ATP binding cassette subfamily B member 1 (*ABCB1*), ATP binding cassette subfamily C member 2 (*ABCC2*) and AT-rich interaction domain 5B (*ARID5B*) [2,5-9,13-15]. Genomic DNA was isolated from peripheral blood mononuclear cells using a QIAamp DNA Blood Mini Kit. The DNA was amplified by PCR with individual primer sets, and direct sequencing was performed to determine the genotype of each SNP of the target genes. The analyzed SNPs and the sequences of the primer sets for each SNP are listed in Table 1.

The direct sequencing analysis showed that the patient was homozygous for the minor allele of the *SLCO1B1* rs4149056 (Fig. 2A), which has been proved to adversely affect MTX clearance by GWAS [7-9]. He was also homozygous for the minor alleles of *SLCO1B1* rs4149081 and rs11045879, which are in linkage disequilibrium with rs4149056 [7]. In addition, he carried heterozygous minor alleles of *MTHFR* rs1801133 (Fig. 2B), *ARID5B* rs4948496, rs4948487 and *SLC22A8* rs4149183 (Table 1).

Discussion

MTX clearance affects the toxicity and efficacy of MTX treatment in ALL patients. Many clinical parameters, including renal function, the existence of third-space fluid collection, urinary pH, and the concurrent use of drugs that inhibit MTX excretion, are known to interfere with MTX excretion, and hence, the pharmacokinetics of MTX [1,16].

The *SLCO1B1* gene encodes an organic anion transporter and is known to affect the therapeutic as well as the toxic effects of many different drugs [17]. It also plays a crucial role in the hepatic uptake and clearance of MTX [4]. An *in vitro* analysis showed that MTX uptake was reduced in cells with damaging *SLCO1B1* variants [15]. Recently, GWAS revealed that rs4149056, a non-synonymous SNP of *SLCO1B1* (T521C), is associated with decreased MTX clearance. Specifically, it was found that MTX clearance was reduced by 13% in patients with the CC genotype compared with that seen in patients with the TT genotype [7,8].

The patient also carried heterozygous minor alleles of *MTHFR* rs1801133, *ARID5B* rs1801133/rs4948487, and *SLC22A8* rs4149183. A previous study showed that

Table 1 The list of analyzed polymorphisms of the candidate genes associated with MTX toxicities and plasma levels

Gene	rsID	Molecular change	Major/Minor alleles	Patient genotype	Primer set
SLCO1B1	rs4149056	T521C	T/C	CC	Forward: CAGCCATGAGGAACTATGAGTCCAT Reverse: AGCCCCAATGGTACTATGGGAGTC
SLCO1B1	rs11045879	intron variant	T/C	CC	Forward: CTGGAGACCACTGTGCTTTTTACTG Reverse: GCAATTATTGCAAGGTTTCAGGACA
SLCO1B1	rs4149081	intron variant	G/A	AA	Forward: CTGACTTTGCATGCAGTATGGTATCA Reverse: AATTGACATATGACCAGAGCCCC
MTHFR	rs1801133	C677T	C/T	CT	Forward: CAGAAGCATATCAGTCATGAGCCC Reverse: ATGCCTTCACAAAGCGGAAGAAT
MTHFR	rs1801131	A1298C	A/C	AA	Forward: AGTTTGCATGCTTGTGGTTGAC Reverse: CAGCATCACTCACTTTGTGACCA
SLCO19A1	rs1051266	G80A	G/A	GG	Forward: GACCATCTTCCAAGGTGCCCT Reverse: GAAGCTCTCCCCTGGCCGTAT
ABCB1	rs1045642	C3435T	C/T	CC	Forward: CTGGTCCTGAAGTTGATCTGTGAAC Reverse: AAATCAAACTATAGGCCAGAGAGGCTG
ABCC2	rs717620	5'-UTR	C/T	CC	Forward: GGTGACCACCCTAAGTTAACTAACTACCAC Reverse: TAGTCACATGTCCATCCACTGTTTCA
ARID5B	rs4948502	intron variant	T/C	TT	Forward: CCATGTAACTCCAGTTCAGGAAAGC Reverse: GAGAGTTGTTTCTTAGGCCCACTGA
ARID5B	rs4948496	intron variant	C/T	CT	Forward: GCCAGAGAATTGATATCCTCAGAGG Reverse: ACTTGTTGGTAGGGACTGGAATCAG
ARID5B	rs4948487	intron variant	C/A	CA	Forward: CAGCAGTCAACCCATAAAAAGTCAGG Reverse: CACTAGATGCCACTATGAACAATGGC
SLC22A8	rs4149183	intron variant	T/C	CT	Forward: GAGTCTATGGTGCTACAGGCCTGAT Reverse: GGTTCAACATGTTGGTAATGAGCTCTC

heterozygous carriers of *MTHFR* rs1801133 minor alleles had higher levels of plasma MTX than homozygous carriers of major alleles [5]. On the other hand, heterozygous carriers of minor alleles of *ARID5B* rs1801133/rs4948487 or *SLC22A8* rs4149183 did not exhibit exacerbated MTX toxicity or decreased MTX clearance compared to homozygous carriers of major alleles [6]. According to the GWAS, none of these polymorphisms were associated with impaired MTX clearance [7].

AKI are not uncommon after the administration of MTX at doses exceeding 500 mg/m^2. MTX-induced renal toxicity usually results in crystal-induced AKI. After the administration of MTX, most of the drug and its metabolites are excreted in urine, but they can precipitate within the renal tubules in patients with increased urinary concentrations of MTX or acidic urine [3, 16]. In rare cases, such renal impairment can develop after the administration of lower doses of MTX [2].

In the present case, direct sequencing of the candidate genes for delayed MTX clearance revealed that the patient was homozygous for dysfunctional alleles of *SLCO1B1* rs4149056 and heterozygous for a dysfunctional allele of *MTHFR* rs1801133. However, neither nephrotoxicity nor delayed MTX elimination was observed when higher doses of MTX were administered during Consolidation Therapy, probably because renal impairment was prevented by massive hydration of more than 2,500 ml/m^2/day, the discontinuation of drugs preventing methotrexate elimination such as TMP-SMX and urine alkalization with acetazolamide and sodium bicarbonate. This suggests that, in addition to the dysfunctional minor alleles associated with MTX toxicities and clearance, other risk factors, such as volume depletion, acidic urine and the concurrent use of drugs that interfere with MTX clearance, are required for renal impairment and delayed MTX elimination.

It is possible that the patient was suffering from gastroenteritis of unknown etiology when the intravenous MTX was administered and that he subsequently became dehydrated because of decreased oral intake due to nausea and vomiting. We speculate that the significant decrease in MTX clearance was at least partly due to loss-of-function polymorphism in *SLCO1B1*

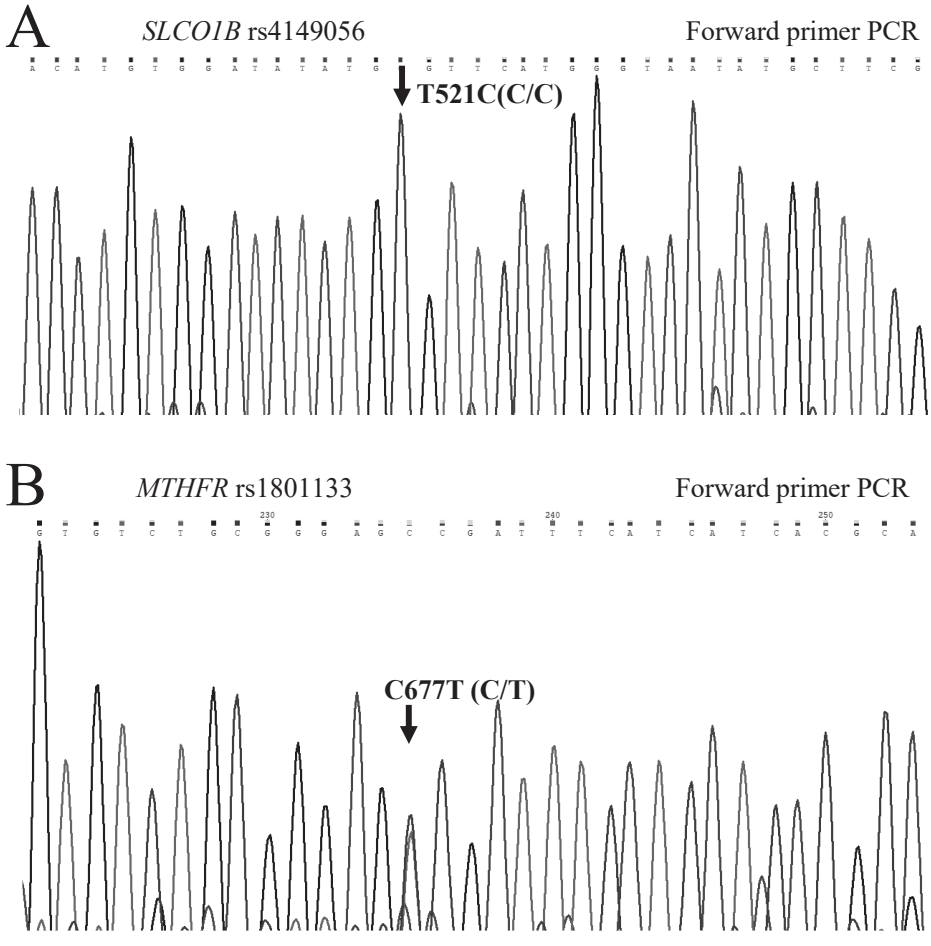

Fig. 2 DNA sequence patterns of the *SLCO1B1* single nucleotide polymorphism rs4149056 (c.T521C). The homozygous genotype (CC) is indicated by an arrow.

(rs4149056) and *MTHFR* (rs1801133), as well as other risk factors such as aciduria, decrease in urine output and the concurrent use of TMP-SMX. Finally, AKI may have developed due to intratubular crystal formation.

This case suggests that renal function should be monitored using the estimated glomerular filtration rate and/or creatinine clearance throughout the course of chemotherapy that includes MTX, and investigation of *SLCO1B1* and *MTHFR* gene polymorphisms should be carried out if any signs indicating kidney impairment or delayed MTX clearance are observed. If any of the gene polymorphisms affecting MTX toxicities and clearance are positive, it is prudent to closely monitor the MTX concentration and perform renal-protective measures such as intravenous hydration even when MTX is administered at a medium or low dose.

References

1. Howard SC, McCormick J, Ching-Hon Pui, Buddington RK and Harvey RD: Preventing and Managing Toxicities of High-Dose Methotrexate. Oncologist (2016) 21: 1471–1482.
2. Kivity S, Zafrir Y, Loebstein R, Pauzner R, Mouallem M and Mayan H: Clinical characteristics and risk factors for low dose methotrexate toxicity: A cohort of 28 patients. Autoimmun Rev (2014) 13: 1109–1113.
3. Garneau AP, Riopel J and Isenring P: Acute Methotrexate-Induced Crystal Nephropathy. N Engl J Med (2015) 373: 2691–2693.
4. van de Steeg E, van der Kruijssen CM, Wagenaar E, Burggraaff JE, Mesman E, Kenworthy KE and Schinkel AH: Methotrexate Pharmacokinetics in Transgenic Mice with Liver-Specific Expression of Human Organic Anion-Transporting Polypeptide 1B1 (SLCO1B1). Drug Metab Dispos (2009) 37: 277–281.
5. Suthandiram S, Gan GG, Zain SM, Bee PC, Lian LH, Chang KM, Ong TC and Mohamed Z: Effect of polymorphisms within methotrexate pathway genes on methotrexate toxicity and plasma levels in adults with hematological malignancies. Pharmacogenomics

(2014) 15: 1479–1494.
6. Csordas K, Lautner-Csorba O, Semsei AF, Harnos A, Hegyi M, Erdelyi DJ, Eipel OT, Szalai C and Kovacs GT: Associations of novel genetic variations in the folate-related and ARID5B genes with the pharmacokinetics and toxicity of high-dose methotrexate in paediatric acute lymphoblastic leukaemia. Br J Haematol (2014) 166: 410–420.
7. Ramsey LB, Panetta JC, Smith C, Yang W, Fan Y, Winick NJ, Martin PL, Cheng C, Devidas M, Pui CH, Evans WE, Hunger SP, Loh M and Relling MV: Genome-wide study of methotrexate clearance replicates SLCO1B1. Blood (2013) 121: 898–904.
8. Radtke S, Zolk O, Renner B, Paulides M, Zimmermann M, Möricke A, Stanulla M, Schrappe M and Langer T: Germline genetic variations in methotrexate candidate genes are associated with pharmacokinetics, toxicity, and outcome in childhood acute lymphoblastic leukemia. Blood (2013) 121: 5145–5153.
9. Treviño LR, Shimasaki N, Yang W, Panetta JC, Cheng C, Pei D, Chan D, Sparreboom A, Giacomini KM, Pui CH, Evans WE and Relling MV: Germline GV in an Organic Anion Transporter Polypeptide Associated With Methotrexate Pharmacokinetics and Clinical Effects. J Clin Oncol (2009) 27: 5972–5978.
10. Hasegawa D, Imamura T, Yumura-Yagi Keiko, Takahashi Y, Usami I, Suenobu So-Ichi, Nishimura S, Suzuki N, Hashii Y, Deguchi T, Moriya-Saito A, Kato K, Kosaka Y, Hirayama M, Iguchi A, Kawasaki H, Hori H, Sato A, Kudoh T, Nakahata T, Oda M, Hara J and Horibe K: Risk-adjusted therapy for pediatric non-T cell ALL improves outcomes for standard risk patients: results of JACLS ALL-02. Blood Cancer J (2020) 10: 23.
11. Nagai T, Uemura O, Ishikura K, Ito S, Hataya H, Gotoh Y, Fujita N, Akioka Y, Kaneko T and Honda M: Creatinine-based equations to estimate glomerular filtration rate in Japanese children aged between 2 and 11 years old with chronic kidney disease. Clin Exp Nephrol (2013) 17: 877–881.
12. Stoller RG, Hande KR, Jacobs SA, Rosenberg SA and Chabner BA: Use of Plasma Pharmacokinetics to Predict and Prevent Methotrexate Toxicity. N Engl J Med (1977) 297: 630–634.
13. Zhang HN, He XL, Wang C, Wang Y, Chen YJ, Li JX, Niu CHe and Gao P: Impact of SLCO1B1 521T>C variant on leucovorin rescue and risk of relapse in childhood acute lymphoblastic leukemia treated with high-dose methotrexate. Pediatr Blood Cancer (2014) 61: 2203–2207.
14. Lopez-Lopez E, Martin-Guerrero I, Ballesteros J, Piñan MA, Garcia-Miguel P, Navajas A and Garcia-Orad A: Polymorphisms of the SLCO1B1 gene predict methotrexate-related toxicity in childhood acute lymphoblastic leukemia. Pediatr Blood Cancer (2011) 57: 612–619.
15. Ramsey LB, Bruun GH, Yang W, Trevino LR, Vattathil S, Scheet P, Cheng C, Rosner GL, Giacomini KM, Fan Y, Sparreboom A, Mikkelsen TS, Corydon TJ, Pui CH, Evans WE and Relling MV: Rare versus common variants in pharmacogenetics: SLCO1B1 variation and methotrexate disposition. Genome Res (2012) 22: 1–8.
16. Widemann BC and Adamson PC: Understanding and Managing Methotrexate Nephrotoxicity. Oncologist (2006) 11: 694–703.
17. van de Steeg E, Wagenaar E, van der Kruijssen CM, Burggraaff Johanna EC, de Waart Dirk R, Elferink Ronald PJ Oude, Kenworthy Kathryn E and Schinkel Alfred H: Organic anion transporting polypeptide 1a/1b-knockout mice provide insights into hepatic handling of bilirubin, bile acids, and drugs. J Clin Invest (2010) 120: 2942–2952.