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CASE REPORT

Is Pharmacogenetic Panel Testing Applicable to Low-Dose Methotrexate in Rheumatoid Arthritis? – A Case Report

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Purpose: Pharmacogenetic (PGx) panel testing could help to determine the heritable component of a rheumatoid arthritis (RA) patient's susceptibility for therapy failure and/or adverse drug reactions (ADRs) from methotrexate (MTX). Considering the literature mentioning the potential applicability of PGx panel testing within MTX regimens, we discuss the case of a patient who was treated with MTX, suffered from ADRs, and obtained a reactive PGx panel testing.

Genotyping: We used a commercial PGx panel test involving the ABC-transporters P-glycoprotein (P-gp; gene: *ABCB1*), and breast cancer resistance protein (BCRP; gene: *ABCG2*), the solute carriers reduced folate carrier 1 (RFC1; gene: *SLC19A1*), and organic anion transporting polypeptide 1B1 (OATP1B1; gene: *SLCO1B1*), and the enzymes inosine triphosphatase (ITPA), and glutathione transferase P1 (GSTP1). In addition, we genotyped the patient for the enzymes 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase (AICAR)/inosine monophosphate (IMP) cyclohydrolase (gene name: *ATIC*), gamma-glutamyl hydrolase (gene name: *GGH*) and methylenetetrahydrofolate reductase (gene name: *MTHFR*).

Results: The PGx profile of the patient revealed genetic variants in SLC19A1, ABCB1, and MTHFR, which may explain the ADRs experienced during the treatment with MTX and a potentially lower efficacy of MTX. Based on our interpretation of the PGx profile, we recommended the patient to avoid MTX in the future.

Conclusion: The MTX pathway is complex, which makes the interpretation of genetic variants affecting metabolism challenging. A reactive PGx panel test was applicable to explain ADRs experienced during MTX treatment for a patient with RA. However, the clinical utility of PGx-guided MTX treatment in a primary care setting is still limited. In order to base a recommendation for MTX on PGx data, we need genome-wide association studies, large prospective multicenter studies and PGx studies, which analyze different multi-gene haplotypes and gene-drug-drug interactions for MTX.

Keywords: pharmacogenetics, PGx, ABCB1, SLC19A1, MTHFR, rheumatoid arthritis, methotrexate, MTX

Introduction and Background

Rheumatoid arthritis (RA) is a common chronic disease with a prevalence of 0.5% to 1.1%,[1] where a suitable pharmacotherapy can prevent irreversible joint deformation, and thereby increase the quality of life. Here, it is assumed that an early diagnosis with adequate treatment is crucial as early treatments have been linked to better response rates,[2] thereby leading to less joint damage in the long term.

Methotrexate (MTX) is a folic acid antagonist with anti-inflammatory and immune-modulating effects. Low-dose MTX regimens are indicated in the treatment of arthritis.[3] In this context, MTX is considered as a "disease-modifying anti-rheumatic drug" (DMARD) and is considered first choice,[4] where it can be combined with other DMARDs including small molecules (eg, JAK-inhibitors) or biologicals (eg, TNF-alpha-inhibitors) in patients exhibiting insufficient MTX-response.[3] However, nearly 30% of the patients treated with MTX experience inefficacy or adverse drug reactions (ADRs).[4,5]

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Interindividual differences in MTX response may find its explanation in its complex metabolism.[6–8] In the following, we will describe various enzymes and transporters, which are part of the MTX metabolism (see Figure 1), and we will highlight those, where genetic variants have been linked to changes in MTX efficacy or safety (see "evidence for polymorphism" in Tables 1 and 2). We are also reporting on genes, which are not mentioned in the Swiss drug label (summarized at https://www.pharmgkb.org/labelAnnotations and[9]), and which are not assessed for genetic variability in our patient.

In the context of oral application, MTX has to pass enterocytes. Here, entry is assumed to be primarily governed by the reduced folate carrier (RFC1, gene name: *SLC19A1*), and the proton-coupled folate transporter (PCFT, gene name: *SLC46A1*).[10] Early findings linked the G80A (rs1051266) variant in RFC1 to changes in folate status,[11] which is the physiologic substrate of this particular transporter. There are multiple reports linking the G allele to an increased risk of drug-induced toxicity, especially gastrointestinal toxicity,[10,12] hepatotoxicity,[12,13] and alopecia.[13] However, other reports associated the A allele with an enhanced efficacy of MTX and also with higher intracellular levels of methotrexate polyglutamate (MTX-PG), an active metabolite of MTX.[14]

While *SLC19A1* and *SLC46A1* are facilitating cellular entry, there are members of the ATP-binding cassette transporter family, namely *ABCB1*,[15] *ABCC2* and *ABCG2*, that are assumed to limit methotrexate bioavailability by active efflux of the molecule.[16] MTX response depends on the expression of *ABCG2* (breast cancer resistance protein, BCRP)[17] and it has been shown that genetic variants influenced MTX plasma levels in pediatric patients.[18] Moreover, ABCG2 was associated with MTX discontinuation in a clinical PGx model.[19] For ABCB1 (P-glycoprotein, P-gp), there are reports testing the association of genetic variants with efficacy or safety of MTX. The T allele in the C3435T (rs1045642) polymorphism was associated with a higher risk for ADRs,[20] non-response,[21] and low disease activity.[22] However, the C allele has been associated with increased toxicity[12,23] Therefore, we only know that there might be a certain effect; however, it is impossible to precisely define the impact of ABCB1 on the MTX pathway.

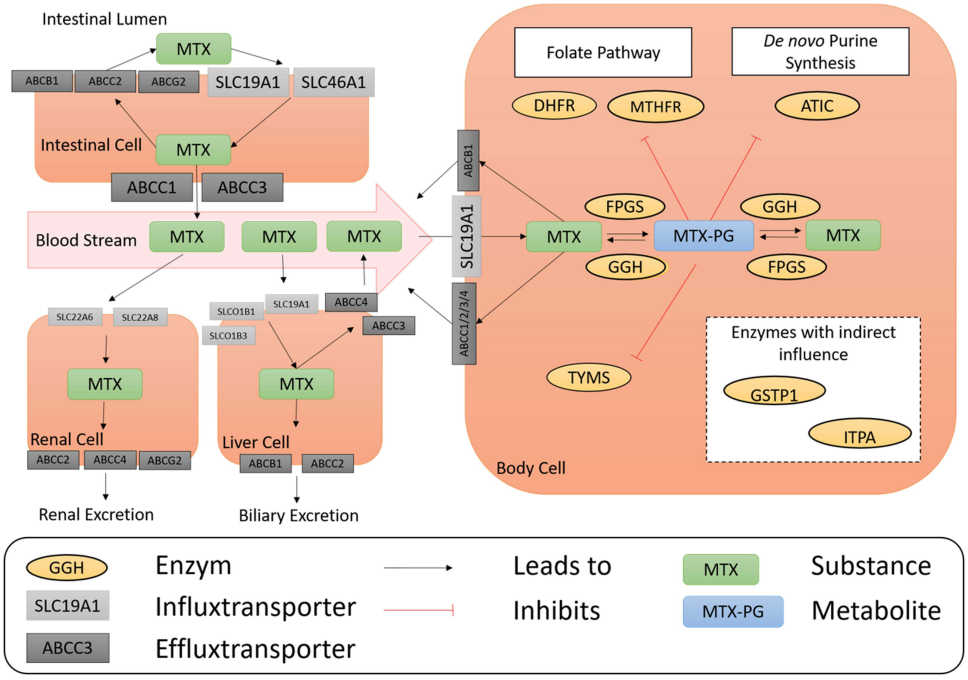

Figure 1 Overview of the current understanding on enzymes and transporters involved in the pharmacokinetics and pharmacodynamics of methotrexate (MTX).
Abbreviations: ABCB1, ATP-binding cassette transporter B1 (aka P-glycoprotein); ABCC1–4, ATP-binding cassette transporter C1-C4 (aka multidrug resistance protein) (MRP1 to MRP4); ABCG2, ATP-binding cassette transporter G2 (aka breast cancer resistance protein BCRP); ATIC, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase (AICAR)/inosine monophosphate (IMP) cyclohydrolase; DHFR, dihydrofolate reductase; FPGS, folylpolyglutamyl synthase; GGH, gamma-glutamyl hydrolase; GSTP1, glutathione transferase P1; ITPA, inosine triphosphate pyrophosphatase; MTHFR, methylenetetrahydrofolate reductase; MTX-PG, methotrexate polyglutamate; SLC19A1, solute carrier 19A1 (aka reduced folate carrier 1, RFC1); SLCO1B1/1B3, organic anion transporting polypeptide (OATP) 1B1 and OATP1B3; SLC22A6/A8, organic anion transporter (OAT)1 and OAT3; SLC46A1, proton-coupled folate transporter (PCFT); TYMS, thymidylate synthase.

Table 1 Summary of the Genetic Variants of the Patient Determined with a Commercial PGx Panel Test for MTX

Gene, NE (Nucleotide Exchange); AE (Amino Acid Exchange)	Genotype of the Patient	Function in Pathway	Evidence for Polymorphism
ATP-binding cassette transporter B1 (ABCB1) aka P-glycoprotein, Efflux transporter →altered transport			
ABCB1, g.2685+49T>C	rs2032583 CT	Active cellular efflux[16]	No studies in relation to low-dose MTX
ABCB1 c.3435T>C; p.I1145I	rs1045642 CC		T allele: a higher risk for ADRs,[20] non-response,[21] low disease activity;[22] C allele: increased toxicity[12,23]
ABCB1 c.1236T>C; p.G412G	rs1128503 CC		Conflicting evidence[19,24]
ABCB1 c.2677G>A; p.A893T	rs2032582 GG		No studies in relation to low-dose MTX
ABCB1 c.2677G>T; p. A893S	rs2032582 GG		No studies in relation to low-dose MTX
ATP-binding cassette transporter G2 (ABCG2) aka Breast cancer resistance protein (BCRP), Efflux transporter →no alternation in transport			
ABCG2 c.421C>A; p.Q141K	rs2231142 CC	Active cellular efflux[16]	A allele: adverse events[47]
ABCG2 g.1194+928A>G,	rs13120400 GG		G allele: reduced improvement or reduced severity[48]
ABCG2 g.89055379G>A,	rs17731538 GG		A allele: reduced improvement or reduced severity[48]
Glutathione transferase P1 (GSTP1), Detoxification of organic substances and protection of the organism from oxidative stress →no risk			
GSTP1 c.313A>G; p.I105V	rs1695 AG	Detoxification of drugs	Conflicting evidence[38,39]
Inosine triphosphate pyrophosphatase (ITPA), Adenosine pathway →no risk			
ITPA c.94C>A; p. P32T	rs1127354 CC	Conversion of inosine triphosphate (ITP) to inosine monophosphate (IMP)	C allele: decreased risk of gastrointestinal toxicities and increased response to MTX[32,37]
Organic anion transporting polypeptide 1B1 (SLCO1B1), Uptake transporter →no alteration in transport			
SLCO1B1 c.521T>C; p.V174A	rs4149056 TT	Hepatic MTX excretion[26]	No studies in relation to low-dose MTX
SLCO1B1 c.463C>A; p.P155T	rs11045819 CC		No studies in relation to low-dose MTX
SLCO1B1 c.388A>G; p.N130D	rs2306283 AA		No studies in relation to low-dose MTX
SLCO1B1 c.-910G>A or g.4195G>A	rs4149015 GG		No studies in relation to low-dose MTX
Solute carrier 19A1 (SLC19A1), Uptake transporter → higher risk for toxic side effects; lower chance for remission			
SLC19A1 (RFC1) c. 80G>A; p. H27R	rs1051266 GG	Entry in enterocytes[10]	G allele: gastrointestinal toxicity,[10,12] hepatotoxicity,[12,13] and alopecia;[13] A allele: enhanced efficacy of MTX[14]

Notes: The subtitle consists of the gene name, the abbreviation, the pathway and the evaluation of the commercial PGx panel test for MTX.

MTX is primarily excreted through renal glomerular filtration, however in the tubular system there are multiple transporters expressed that are known to interact with MTX including the aforementioned *SLC19A1, ABCG2, ABCB1, ABCC2*. However, *ABCG2, ABCB1, and ABCC2* are also expressed in the canalicular membrane of hepatocytes, thereby influencing biliary excretion of MTX involving the efflux transporters *ABCC2, ABCB1* and *ABCG2*, while the sinusoidal

Table 2 Summary of the Observed Genotypes Detected in Additional Genes Involved in the MTX Pathway

Gene, NE (Nucleotide Exchange); AE (Amino Acid Exchange)	Genotype of the Patient	Function in Pathway	Evidence for Polymorphism
5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase (AICAR)/ inosine monophosphate (IMP) cyclohydrolase (ATIC), De novo purine synthesis pathway →no risk			
ATIC c.347C>G; p. Thr116Ser	rs2372536 CC	Conversion of AICAR into formyl aminoimidazole carboxamide ribonucleotide (FAICAR).[36]	G allele: increased risk of toxicity and better response.[21,36,37]
Gamma-glutamyl hydrolase (GGH), Polyglutamation →no risk			
GGH−401 C>T	rs3758149 CC	Conversion of MTX-PG to MTX depends on the gamma-glutamyl hydrolase.[49]	T allele: decreased conversion of MTX to active metabolite and decreased MTX response[28,49]
Methylenetetrahydrofolate reductase (MTHFR), Folate pathway → risk of higher toxicity and lower MTX response			
MTHFR c.1298 A>C; p. Glu429Ala	rs1801131 AC	Conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate	C allele: central nervous system toxicity,[32] hepatotoxicity,[33] gastrointestinal ADRs[34] or overall toxicity;[25] A allele: better MTX response[25,35]
MTHFR c.67765 C>T; p. p. Ala222Val	rs1801133 CT		T allele: increased risk for gastrointestinal ADRs,[29,30] hepatotoxicity,[13,30] and neurotoxicity[31]

Notes: The subtitle consists of the gene name, the abbreviation, the pathway and the evaluation for MTX.

transporters *ABCC3* and *ABCC4* extrude MTX back into the circulation.[6,10] Finally, the hepatocellular entry of MTX is assumed to involve the sinusoidal organic anion transporting polypeptides (OATP) OATP1B1 and OATP1B3, which are encoded by *SLCO1B1* and *SLCO1B3*, respectively. Even if only a small part of the MTX dose is excreted via the bile, there are data obtained in a transgenic mouse model supporting OATP1B1 as a determinant of hepatic MTX excretion.[26] In particular, for OATP1B1 we know that there are genetic variants influencing its transport function.[27]

In addition to transmembrane transport, MTX also undergoes conversion catalyzed by multiple enzymes. One of these conversions is the polyglutamation process catalyzed by the folylpolyglutamate synthetase (*FPGS*) resulting in the biologically more active methotrexate polyglutamate (MTX-PG). Both MTX and MTX-PG interact with enzymes in the folate pathway, thereby exerting the pharmacological function. The conversion of MTX-PG to MTX depends on the gamma-glutamyl hydrolase (*GGH*),[6] where the C401T (rs3758149) polymorphism has been tested for its influence on MTX-PG levels, drug response and the risk for ADRs.[13,28] The homozygous genotype TT was associated with higher MTX-PG-levels and altered MTX-response, whereas the wild-type genotype CC was associated with a higher risk of overall ADRs.[13,28]

Intracellular MTX and its polyglutamated metabolite (MTX-PG) inhibit the dihydrofolate reductase (DHFR), which catalyzes the reduction of dihydrofolate (DHF) to tetrahydrofolate (THF), thereby leading to less THF, which is important for the *de novo* purine synthesis and the production of biologically active folate cofactors.[6] Indeed, the reduction in THF is assumed to impact enzymes of the folate pathway, eg, the methylenetetrahydrofolate reductase (*MTHFR*). For *MTHFR*, the two polymorphisms A1298C (rs1801131) and C677T (rs1801133) have been extensively investigated. The T allele within the C677T polymorphism was associated with an increased risk for toxicity, especially gastrointestinal ADRs,[29,30] hepatotoxicity,[13,30] and neurotoxicity.[31] The C allele within the A1298C polymorphism of *MTFHR* was linked to a higher risk for ADRs, such as central nervous system toxicity,[32] hepatotoxicity,[33] gastrointestinal ADRs[34] or overall toxicity,[25] whereas the A allele was linked to a higher probability of response measured as a percentage of improvement in the JADAS-71 score[35] at 3 and 6 months after treatment start and at the last follow-up visit.[25]

In addition to the folate pathway, MTX-PG inhibits the bifunctional purine biosynthesis protein (PURH), also known as inosine monophosphate (IMP) cyclohydrolase (gene name: *ATIC*), which converts aminoimidazole carboxamide

ribonucleotide (AICAR) into formyl aminoimidazole carboxamide ribonucleotide (FAICAR). The C347G (rs2372536) variant in *ATIC* has been associated with decreased *ATIC* activity leading to an accumulation of AICAR and adenosine. The increased concentration of adenosine in the extracellular space is thought to be part of the mechanism of MTX action as adenosine exerts anti-inflammatory functions and antiproliferative effects.[36] The G allele in the C347G polymorphism is extensively discussed as a factor impacting the risk for toxicity and improved response to MTX at the same time.[21,37] Also involved in the purine homeostasis is the inosine triphosphate pyrophosphatase (*ITPA*) converting inosine triphosphate (ITP) to inosine monophosphate (IMP). In *ITPA*, the C allele in the C94A (rs1127354) variant has been linked to a decreased risk of gastrointestinal toxicities as well as an increased response to MTX.[32,37] The last enzyme of the MTX pathway we want to mention is the glutathione transferase P1 (*GSTP1*). Glutathione transferases are involved in the detoxification of drugs, and their variants have been associated with increased drug toxicity. The G allele in the A313G (rs1695) variant in *GSTP1* has been associated with central nervous system toxicity;[38] however, in another study, no association with hepatotoxicity[39] was found.

Taken together, the MTX pathway has to be rated as rather complex, where various genetic variants have been associated with changes in pharmacokinetics and/or drug response and safety. Already in 2006, Ranganathan et al[36] suggested that Pharmacogenetics (PGx), the study of genetic variations related to drug response,[40] could help individualize the treatment with MTX. Consequently, pharmacogenetic (PGx) testing could be applied to determine and/or predict the heritable component of a RA patient's susceptibility to experience therapy failure and/or ADRs from MTX treatment in a reactive and/or pre-emptive PGx test setting.[7,8,41]

Considering the literature mentioning the potential applicability of PGx panel testing within MTX regimens, we would like to discuss the case of a patient with RA, who was treated with MTX, suffered from ADRs, and obtained a reactive PGx panel testing within the setting of a case series called "Pharmacogenetic Testing of Patients with Adverse Drug Reactions or Therapy Failure" (Clinicaltrials.gov: NCT04154553).

Case Presentation

A male patient of 87 kg, born 1980, presented his case in a community pharmacy offering a commercial PGx panel test. The patient was diagnosed with rheumatic arthritis in 2014 by the physician (CCP 911 kU/L (<7), Rheuma factors 304 kU/L (<14), CRP 1.5 mg/l (<10)). One year later, a therapy with MTX was started, increasing from 12.5 mg subcutaneously weekly, by 2.5 mg at a time up to the target dose of 20 mg. This dosage is in agreement with the S2 guidelines published by the German Society of Rheumatology[42] and the guidelines of the American College of Rheumatology.[4] To bridge the gap until the effectiveness of MTX could be expected (about six to eight weeks), the patient received prednisone 20 mg for one week, then 10 mg and 7.5 mg for 2 weeks each until a provisional maintenance dose of 5 mg was reached. In addition, an intake of 5 mg folic acid was recommended 12 to 24 hours after MTX application to reduce ADRs. After four months of treatment, the MTX therapy was discontinued due to various ADRs, such as nausea, headache, and sore muscles. According to information from the treating rheumatologist, the monthly measured lab values (CRP, transaminases, blood count) did not show any abnormalities. The patient's symptoms stopped as soon as he discontinued drug intake. He then started taking low-dose steroids for about six months. Later on, he switched to biological DMARDs, namely baricitinib and tofacitinib. The latter was used for the longest period of time with a dose regimen of 5 mg twice a day. The reason for switching from baricitinib to tofacitinib was symptoms of influenza which he suffered from every now and then. At the time of counselling, the patient was taking tofacitinib and prednisone as anti-rheumatics, supplemented with NSAIDs and the proton pump inhibitor dexlansoprazole (summarized in Table 3). Even if the patient is currently on a treatment with biologics, which are hitherto not known to be linked in efficacy or safety to genetic variants in the pharmacokinetically relevant genes typically screened for genetic variability in PGx testings, a PGx panel test was issued on a reactive basis in order to determine whether his PGx profile would be applicable to explain the ADRs he experienced during intake of MTX.

Table 3 Medication of the Patient at the Time of Pharmacogenetic Panel Testing

Substance	Dosage	Indication
Tofacitinib 5 mg	1-0-1	Rheumatoid arthritis
Prednisone 5 mg	1-0-0	Rheumatoid arthritis
Diclofenac 150 mg	0-1-0	Influenza symptoms
Ibuprofen 200 mg	1-0-1 as necessary	Influenza symptoms
Dexlansoprazole 30 mg	0-1-0	as long as Ibuprofen
Vitamin D3 4500 IE/ml, 10ml	2 bottles per month	Vitamin D3 deficiency

Analyses of Single Nucleotide Polymorphisms in the MTX Pathway

Following the protocol as approved by the ethics committee northwestern and central Switzerland (EKNZ-2019-01452), the patient signed an informed consent. We used the commercial PGx panel test called Stratipharm issued by humatrix AG (Pfungstadt, Germany). It consists of a laboratory analysis of approximately 100 pharmacologically relevant polymorphisms in over 30 different genes, which code for transport proteins, metabolizing enzymes, or drug targets. Polymorphisms are detected applying real-time PCR using the automated Life Technologies QuantStudio 12 k flex (Thermo Fisher, Waltham, MA, USA) with the respective chemistry. Humatrix AG provides not only the results of genetic testing but also the prediction of a drug-specific phenotype (PGx profile). In addition to the commercial PGx panel test we genotyped two variants of the methylenetetrahydrofolate reductase (*MTHFR*), one variant of the gamma-glutamyl hydrolase (*GGH*) and one variant of the 5-aminoimidazole-4-carboxamide ribonucleotide formyl-transferase (AICAR)/inosine monophosphate (IMP) cyclohydrolase (gene name: *ATIC*). These variants were selected as important determinants in the MTX pathway based on previous reports on MTX efficacy and safety. The additional genotyping was performed after DNA extraction from blood samples using the QIAcube and respective chemistry (qiagen, Hilden, Germany) and followed by real-time PCR based genotyping using commercially available TaqMan probe/primer mixes and genotyping chemistry (Applied Biosystems, Thermo Fisher, Waltham, MA, USA).

For MTX, the commercial PGx panel test considers genetic variants in the ATP-binding cassette (ABC)-transporter P-glycoprotein (P-gp, gene: *ABCB1*) and breast cancer resistance protein (BCRP; gene: *ABCG2*), and the solute carriers reduced folate carrier 1 (RFC1; gene: *SLC19A1*), and organic anion transporting polypeptide 1B1 (OATP1B1; gene: *SLCO1B1*). Furthermore, the commercial PGx panel test evaluates genetic variants of the enzymes inosine triphosphatase (*ITPA*), and the glutathione transferase P1 (*GSTP1*). The genetic profile of the patient for these genes and the predicted MTX phenotype are summarized in Table 1. In detail, based on the heterozygosity in ABCB1 phenotypically associated with "altered transport activity", the heterozygosity in GSTP1 associated with "no elevated risk", and the homozygosity in SLC19A1 associated with an "increased risk for toxic ADRs and lower chance for remission", the overall interpretation by the commercial PGx panel test was as follows:

> The genetic profile of the patient may result in a reduced response and an increased risk of adverse drug effects. The therapy with MTX can be continued, but should be monitored for that.

Moreover, we have determined the genetic make-up of the patient for the enzymes 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase (AICAR)/inosine monophosphate (IMP) cyclohydrolase (gene name: *ATIC*), gamma-glutamyl hydrolase (gene name: *GGH*) and methylenetetrahydrofolate reductase (gene name: *MTHFR*). The results of the genotyping of selected variants and the respective function within the MTX pathway is summarized in Table 2. Here, our patient is heterozygous carrier of both variants assessed within the MTHFR gene locus.

Finally, we also had a look at the genetic constellation of our patient evaluating his co-medication consisting of diclofenac, ibuprofen, prednisone, dexlansoprazole and vitamin D3 (see Table 3). For the non-steroidal anti-inflammatory

drugs (NSAR), the CYP2C9 intermediate metabolizer status was detected. No further PGx warnings appeared for other co-medications. For vitamin D3, no PGx information was available.

Interpretation and Recommendation

Interpretation

As illustrated in the introduction and shown in Figure 1 the MTX pathway is complex, which makes the interpretation of genetic variants affecting metabolism challenging. The PGx profile of the patient revealed genetic variants in SLC19A1, ABCB1, and MTHFR, which may explain the ADRs experienced during the treatment with MTX and a potentially lower efficacy of MTX.

SLC19A1 codes for the reduced folate carrier and facilitates cellular entry, making it a crucial step in the MTX pathway. Our patient is homozygous carrier of the risk allele (G) in SLC19A1 G80A (rs1051266). There are multiple reports linking the G allele with ADRs, such as gastrointestinal toxicity,[10,12] hepatotoxicity,[12,13] and alopecia,[13] supporting our conclusion that the genetic constellation of the *SLC19A1* of the patient leads to a high probability of ADRs. Moreover, as the 80G allele has been linked to a lower chance for remission,[43] a treatment with MTX of a patient with this genotype appears unfavorable.

The patient is heterozygous in ABCB1 T49C (rs2032583), and homozygous to the C allele in ABCB1 C3435T (rs1045642). Both variants are located within the gene locus *ABCB1*, which encodes for the efflux transporter P-glycoprotein: The observed genotype led to the indication of "altered transport" in the results of the commercial PGx panel test. We are not aware of studies investigating the influence of T49C polymorphism on MTX efficacy and safety. Studies on C3435T polymorphism had diverging results. The 3435C allele was associated with increased toxicity,[17] while the 3435T allele has been associated with high disease activity and increased ADRs,[20] non-response,[21] and with a low disease activity score[22] and PharmGKB[44] cites that carriers of the 3435T allele might have an increased risk of toxicity. With these inconclusive findings reported in the literature, it is difficult to decide on the impact the variants in *ABCB1* would have on MTX transport, thereby making a predictive decision impossible.

In addition to the variants identified with the commercial PGx panel test, we observed the patient's heterozygosity for the variants A1298C (rs1801131) and C677T (rs1801133) in the *MTHFR* coding for methyltransferase folate reductase. There are many studies in patients with low-dose MTX linking these two variants to ADRs or even toxicities. In summary, the findings of these studies suggest that the 677T allele is a risk factor for gastrointestinal ADRs,[29,30] hepatotoxicity,[13,30] and neurotoxicity[31] and that the 1298C allele is linked to an increased risk for central nervous system toxicity,[32] hepatotoxicity,[33] gastrointestinal ADRs[34] or overall toxicity.[25] Here, a part of the patients' ADRs (nausea) might be explained. However, a study[34] also described a better response in patients carrying 1298AA or 677CC, supporting the assumption that our patient might have had insufficient response to MTX.

In addition to transport and polyglutamation, the pharmacology of MTX also involves pathways such as the adenosine pathway, folate pathway, methionine pathway, and *de novo* purine synthesis. So far, 120 variants in more than 30 genes implicated in the MTX pathway have been investigated.[45] For example, a systematic review of genetic biomarkers for the efficacy of MTX described *SLC19A1* rs1051266, *ATIC* rs7563206, dihydrofolate reductase (*DHFR*) rs836788, thymidylate synthase (*TYMS*) rs2244500, rs2847153, and rs3786362 as relevant genes.[7] With respect to the amount of the literature and the level of evidence, the 19 variants in nine genes assessed within our patient can only be considered candidate genes. Overall 15 variants in 6 genes (mostly transporters) were tested through commercial PGx panel test to which in-house genotyping of 4 variants in 3 genes was added. As the patient was heterozygous to some of the variants, they could have explained the ADRs seen during the treatment.

Recommendation

Based on our interpretation, we concluded that the ADRs previously experienced by the patient were possibly linked to his PGx profile. We therefore recommended the patient to avoid MTX in the future and to stay on his current treatment (tofacitinib, 5 mg, 2 times daily). There were no alerts for this tofacitinib in the PGx profile. The small molecule is metabolized by CYP3A4. Even though this enzyme exhibits high interindividual variability, it is currently assumed that the CYP3A-phenotype is not well predicted by genetic variants.[46] Moreover, the patient also wanted to know if

biological DMARDs, such as abatacept or rituximab would be an option for his future treatment of RA. However, for biologics (as well as for janus kinase inhibitors), there is still little to no evidence for the impact of genetic variants involved in pharmacokinetics as summarized in the herein applied PGx panel. As the recommendations provided by the commercial PGx panel test are based on published evidence, both biologics and janus kinase inhibitors appear as uncritical ("no warning") in the PGx profile by the commercial panel test.

By effecting a PGx panel test, we had the opportunity to analyze the impact of the patient's genetic make-up on his comedication. Accordingly, we were able to provide additional recommendations related to PGx. In detail, due to his CYP2C9 intermediate metabolizer status, treatment with non-steroidal anti-inflammatory drugs (NSAR) should only be applied at the lowest possible dose over the shortest possible period. Moreover, the interaction of the NSAR with prednisone increases the risk of gastrointestinal bleeding. Therefore, we recommended to continue the intake of a proton pump inhibitor, which was already prescribed (dexlansoprazole). All other substances on his current prescription could be continued as described.

Discussion

We presented a case of PGx panel testing for a patient experiencing ADR during MTX treatment. As MTX is still the first-choice drug[4,42] in RA and counts as one of the most effective treatments, the patient was curious about the ADRs, which he experienced under MTX and wanted to know if they had a genetic basis. Our patient learned that his genetic constellation is unfavorable to a treatment with MTX and as he had made the experience of these various described ADRs, he then regained confidence in his current treatment.

Although we were able to link the ADRs to genetic variability in this reactive setting, the question remains whether pre-emptive PGx panel testing in the primary care setting can be used to guide low-dose MTX treatment. Our recommendation to avoid use of MTX in the present case should be nuanced as firstly, PGx results are probabilistic and patients can behave differently from what is expected based on the PGx test and secondly, many individuals may carry these variations and if a general avoidance is adopted, many of them will be deprived of a useful and cost-effective drug. At this point, we argue that due to the complexity of the MTX pathway involving various polymorphic genes, the current evidence for pre-emptive PGx panel testing is insufficient, and it is difficult to translate the prevailing evidence as a whole into clinical recommendations.

When considering all relevant genes, a PGx panel testing for MTX will almost always identify one or more variants potentially affecting efficacy or toxicity. Under the current circumstance, this would lead us to the recommendation to avoid MTX in most cases as evidence-based dosing recommendations are missing. We would argue that the clinical applicability for preemptive PGx panel testing for MTX is currently lacking.

Conclusion

A reactive PGx panel test was applicable to explain ADRs experienced during MTX treatment for a patient with RA. At the moment, the clinical utility of PGx-guided MTX treatment in a primary care setting is limited. In order to base a recommendation for MTX on PGx data, we need genome-wide association studies, large prospective multicenter studies and PGx studies, which analyze different multi-gene haplotypes and gene-drug-drug interactions for MTX. For now, PGx panel testing for MTX appears to be limited to experts which have the possibility of in-depth pharmacological investigations, including therapeutic drug monitoring. In the future, we need a PGx panel text for MTX with clear weight given to each genetic variant. For this, the established and clinically evaluated algorithms augmented by artificial intelligence that are considering the relevance of all tested variants together with other relevant data at the same time in one patient, as well as the inclusion of PGx data into an electronic medical health record, will enable PGx-guided MTX therapy in a primary care setting.

Ethics

The patient has provided informed consent for the use of her data as well as for the publishing of the case details for research purposes. The case was collected in the framework of the observational study "Pharmacogenetic Testing of Patients with unwanted Adverse Drug Reactions or Therapy Failure" (Clinicaltrials.gov: NCT04154553) approved by the

ethics committee northwestern and central Switzerland (Ethikkommission Nordwest- und Zentralschweiz, Hebelstrasse 53, 4056 Basel, eknz@bs.ch) (EKNZ-2019-01452) on 31.10.2019.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal *Pharmacogenomics and Personalized Medicine*; and agree to be accountable for all aspects of work.

Disclosure

The authors report no conflicts of interest in this work.

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