Potential role of pharmacogenetics for optimalization of drug therapy in rheumatoid arthritis

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Chapter 2:

Pharmacogenetics of methotrexate in rheumatoid arthritis

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Abstract

Over the last decades important progress is being made regarding disease modifying anti-rheumatic drugs (DMARDs), like methotrexate (MTX), in the treatment of rheumatoid arthritis (RA). Nevertheless, a substantial part of the patients fail to achieve a good response and/or experience toxicity, which limits further treatment leading to progression of inflammation and destruction of joints. These high interindividual differences in drug response gave rise to the need for prognostic markers in order to individualize and optimize therapy with these anti-rheumatic agents. Besides demographic and clinical factors, studies in the research field of pharmacogenetics have reported potential markers associated with clinical response on treatment with MTX. However, publicized conflicting results and underlying interpretation difficulties inhibit drawing definitive conclusions. Presently, clinical implementation of pharmacogenetics as an important step for individualizing drug therapy in RA is not feasible yet. Replication and prospective validation in large patient cohorts are required before pharmacogenetics can be used in clinical practice. This review provides the current state of art in genotyping RA patients as a potential guide for clinical decision making.

Introduction

Rheumatoid arthritis has a prevalence of ~1% in the Western population (1). This autoimmune disease is characterized by a chronic inflammatory process within the synovial joints, progressive (radiological) joint damage and significant functional impairment (2). In the last decades patients have been treated with traditional disease modifying anti-rheumatic drugs (DMARDs) including methotrexate (MTX), sulphasalazine and leflunomide, or a combination of DMARDs. More recently, growing evidence for the central role of tumour-necrosis factor alpha (TNFα) in the pathogenesis of RA has led to the introduction of TNF inhibitors, such as etanercept, infliximab and adalimumab (3). These biological DMARDs have proven to play an important role in the treatment of persistent RA in patients, who achieve an incomplete response or develop adverse drug events to traditional DMARDs (4-6). In addition, biologicals with alternate mechanisms of actions such as rituximab, abatacept and tocilizumab have recently been developed, (7-9). To date, the place in RA therapy of these new agents is less established.

Ideally, RA therapy is based on strict monitoring of disease activity and tight control treatment in order to prevent progression of joint damage and functional disability (10). Namely, it is established that high and variable disease activity is related to increasing joint damage and that effective intervention stops this progression (11;12). In current clinical practice, newly diagnosed RA patients are treated with traditional DMARDs, in which methotrexate (MTX) is the drug of first choice (13;14). In case of unfavourable response, side effects and/or drug toxicity, alteration of dose regimen or drug therapy towards a combination of DMARDs and/or biologicals is recommended.(4;15;16).

Still, different response rates are seen in RA patients treated with MTX. Substantial percentages of 30-40% of RA patients fail to achieve a satisfactory response. Moreover, 15-30% of the patients develop adverse drug events (16-18). These different responses lead to studies identifying influence of demographic, clinical and immunological variables on treatment outcome with MTX (19). Next to these factors, genetic influences have also been explored in the last decade. Generally, pharmacogenetics has the potential to increase drug efficacy and to ameliorate adverse events (20;21). Therefore, its application might be of great clinical benefit for individuals affected with RA. Studies have reported associations between single nucleotide polymorphisms (SNPs) in genes encoding enzymes related to the pharmacokinetics and pharmacodynamics of MTX and treatment outcome (22-25). The ultimate aim of using pharmacogenetic markers is to predict the probability of a wanted or unwanted drug response in individual patients (20;21).

This review presents an overview of genetic variability contributing to differences in response to MTX in RA treatment.

Pharmacogenetics of methotrexate

Although the exact mechanism of action of MTX is unclear, numerous enzymes have demonstrated to be important for its anti-proliferative and immunosuppressive effects (26;27). Before MTX is being metabolized inside the cell, MTX enters the cell e.g. by the transporter-enzyme reduced folate carrier (RFC). Efflux from the cell is facilitated by ATP-binding cassette (ABC) transporters, e.g. ABCC1 to 5 and ABCG2 and (less proven by) ABCB1 (28;29). If MTX enters the cell, the drug is polyglutamated, meaning that groups of glutamic acid are added to MTX. This process is catalyzed by the enzyme folylpolyglutamate synthetase (FPGS) and reversed by gammaglutamyl hydrolase (GGH), respectively.

Polyglutamated MTX (MTXPGs) inhibits several enzymes directly such as thymidylate synthase (TYMS), dehydrofolate reductase (DHFR), whereas indirect inhibition occurs on methylenetetrahydrofolate reductase (MTHFR), a key enzyme in the folate pathway (26). MTXPGs also inhibit the conversion of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) to formyl-AICAR, which is facilitated by the enzyme AICAR transformylase (ATIC). Accumulation of AICAR has a direct inhibitory effect on other enzymes, like adenosine monophosphate deaminase (AMPD1). This accumulation may lead to the release of adenosine, a potential anti-inflammatory agent (30;31).
To date, SNP selection for pharmacogenetic association studies concerning MTX is done within genes encoding enzymes in these hypothetical pathways regarding MTX's mechanism of action. However, the association of polymorphisms in these pathway genes have yielded mixed results. Table 1 presents the pharmacogenetic data of RA patients treated with MTX monotherapy.
Regarding transport enzymes, association studies of MTX treatment outcome to genetic polymorphisms in the genes *ABCB1*, *RFC* and *ABCC2* have been performed (25;32-40). It has been found that SNPs in the transporters ABCB1 and RFC associate with MTX efficacy and toxicity. However, conflicting data were seen. For example, studies with the *ABCB1* 3435 C>T have reported that the genotype TT was associated with efficacy (34) and inefficacy (36). In addition, one study detected an association of the TT genotype with toxicity (40). For *ABCC2*, no associations of SNPs with toxicity were found (25).
The best-studied SNPs concerning MTX treatment outcome are at the positions 677C>T and 1298A>C within the gene coding for the folate key-enzyme MTHFR (24;25;37;39-47). This enzyme catalyzes the conversion of homocysteine to methionine for a variety of metabolic reactions (30). Functional studies have elucidated that these two polymorphisms are associated with diminished enzyme activity of MTHFR leading to homocysteinemia (48). In fact, it is demonstrated that a decrease in activity could lead to homocysteinemia and eventually could be related to toxicity, e.g. influencing the gastrointestinal tract in RA patients on MTX therapy (48). As a consequence, several reports studied the association of *MTHFR* 677C>T and 1298A>C with toxicity. Regarding *MTHFR* 677C>T, seven studies found no association with overall MTX-induced toxicity (37;41-43;47-49), whereas other studies found associations with GI toxicity for the CT genotype (48), increased MTX discontinuation due to increased liver enzyme levels for 677 T-allele carriers (24), alopecia in Afro-Americans (25), and overall toxicity (24;45;46). In other studies, *MTHFR* 1298 A-allele carriers were related to side effects in two reports (41;42), whereas two groups found no association (43;45) and two groups detected an association between 1298 C-allele carriers with overall toxicity and gastrointestinal toxicity (37;49).
Additionally, associations with MTX efficacy were assessed in most of the studies, involving *MTHFR* genetics. Of the seven studies performed, only three studies found that patients genotyped for *MTHFR* 677CC were more likely to achieve a good response, defined as a decrease or an obtained absolute value of disease activity score (DAS) (37;44;49). Also, reports on *MTHFR* 1298A>C provide inconclusive results. One report found associations with efficacy of the 1298AA genotype and a decrease in DAS (37). In contrast, three studies reported an association between C-allele carriers and disease improvement as defined as the likelihood to be treated with a higher dose, a tendency for remission or decrease in ESR and/or CRP level (44-46). Still, five reports did not find associations of *MTHFR* 1298A>C with efficacy (40-43;49). The enzymes methylenetetrahydrofolate dehydrogenase (MTHFD1), methylenetetrahydrofolate (SHMT1), and thymidylate synthase enhancer region (TSER) are indirectly influenced by MTXPGs (26). Regarding efficacy, one SNP in the *MTHFD1* gene could be related with inefficacy to MTX treatment (39). Yet, for *SHMT1* and *TSER* an association with a single genetic polymorphism within each gene and developing side effects and alopecia in specific was demonstrated (47) (table 1).

Two direct targets of MTX are the enzymes DHFR and TYMS (26;30). Regarding *DHFR*, one study was performed in which no associations with efficacy or toxicity were found (37). For *TYMS*, four studies were performed (36;39;40;43). Only, one study reported an association of a polymorphism, 6 basepair (bp) deletion, within the 3'UTR region of the gene and achieving good response, defined as likelihood to be treated with a higher dose or decrease in CRP level (43).
Recently an association between MTX and HLA-G antigens, defined as nonclassical major histocompatibility complex class Ib molecules important for maintaining anti-inflammatory conditions, was found in an in vitro study (50). The *HLA-G* 14 bp deletion is thought to increase *HLA-G* mRNA and protein stability, possibly leading to prolonged anti-inflammatory actions. Therefore, MTX may act synergistic with this deletion. It was shown that MTX induces soluble HLA-G, whereas a homozygous deletion of 14bp in this *HLA*-gene was more frequently detected in patients with response to MTX. However, the role of the *HLA-G* 14 bp polymorphism in vivo in clinical response to MTX remains conflicting (50-52).
Generally, regarding MTX-induced toxicity, no associations between polymorphisms in the pathway enzymes including, *TYMS*, *DHFR*, *AMPD1*, *ITPA* genes and the occurrence of side effects in RA patients exist (36;37;40;53) (Table 1).
Direct involved in MTX's polyglutamation are the enzymes FPGS and GGH. Two SNPs, 114G>A and 1994A>G, in the *FPGS* gene were not reported to be related to efficacy or toxicity in RA patients (54). Concerning *GGH*, in three studies no significant effect of three SNPs, -401C>T, 452C>T and 16C>T, with efficacy was demonstrated (39;49;54). However, an association of *GGH* -401C>T with toxicity was seen in one study (49).

Gene	Function	Genetic polymorphism(s)	Clinical effect on:	
			Toxicity	**Efficacy**
MTHFR	Catalyzes methylene THF to methyl-THF; indirect target MTX	677C>T	- Effect on GI toxicity (48); T-allele associated with toxicity and increased liver enzyme levels (24;45;46); No association with toxicity (37;48;40-43;47;49)	-No association with efficacy (24;25;40;42;43); Association with efficacy (37;44;49)
		1298 A>C	A-allele associated with toxicity (41;42); C-allele associated with toxicity and GI toxicity (37;40;49); No association with toxicity (43;45)	No association with efficacy (40;42;43;45;49); Association with efficacy (37;46); May affect efficacy (44)
ATIC	Conversion of AICAR to 10-formyl-AICAR; target of polyglutamated MTX	347C>G	GG associated with toxicity and GI toxicity (47;49;53); No effect on toxicity (36)	Association with efficacy (39;53;47); No association with efficacy (36;49)
DHFR	Reduction of DHF to THF; target of MTX	-473G>A, 35389G>A	No effect on efficacy or toxicity (37)	
MTHFD1	catalyzes interconversion of 1-carbon derivatives of THF; indirect target MTX	1958G>A	*	AA associated with inefficacy (39)
SHMT1	catalyzes conversion of serine and THF to glycine and methylene-THF: indirect target MTX	1420C>T	No association with toxicity (47;49); CC associated with alopecia and CNS side effects (47)	No association with efficacy (39); CC associated with efficacy (49);
TSER	Enhancer region of TYMS; indirect target of MTX	'5 UTR 28bp repeat	No association with toxicity (43;49); Association with toxicity and alopecia (47)	No association with efficacy (39;43;49)
TYMS	Conversion of dUMP to dTMP; target of MTX	'3 UTR 6bp deletion	No effect on toxicity (36;40)	May affect MTX efficacy (as defined by MTX dose and CRP level) (43); No effect on efficacy (as defined by MTX dose) (36;40)
AMPD1	Conversion of AMP to ADP and ATP; indirect target MTX	34C>T	No association with toxicity (53)	T-allele associated with efficacy (39;53)
MTR	Methylation of homocysteine to methionine; indirect target MTX	2756A>G	No association with toxicity (40;53); AA associated with toxicity (49)	No association with efficacy (40;49;53)
MTRR	Methylation of cofactors required for MTR action; indirect target MTX	66A>G	No association with toxicity (40;53); GG associated with toxicity (49)	No effect on efficacy (40;49;53)
ITPA	Conversion IMP to ITP; indirect target MTX	94C>A	No association with toxicity (53)	CC associated with efficacy (39;53)
ADORA2A	Adenosine A2a receptor	5 SNPs (4 in intron+ 1 in downstream)	All SNPs associated with Toxicity (55); Two SNPs with GI toxicity (55)	*
FPGS	Adding polyglutamates to MTX; prolonging cellular retention MTX	1994A>G, 114G>A	No effect on efficacy or toxicity (54)	
GGH	Conversion of long chain polyglutamated MTX into short chain by removing polyglutamates	452C>T, 16C>T	No effect on toxicity (54)	May affect efficacy (54); No association with efficacy (39)
		- 401C>T	CC associated with toxicity (49)	No association with efficacy (49);

ABCB1	Efflux transporter on cells; efflux of MTX	3435C>T	ABCB1 3435 TT associated with toxicity (40); No association with toxicity (36)	No association with efficacy (40); TT associated with efficacy (34); TT associated with inefficacy (36)
		+1236C>T, 2677G>T	No effect on efficacy or toxicity (25;40)	
RFC	Folate entry in the cell	-43T>C, 696C>T	*	No effect on efficacy (32)
		80G>A	RFC1 80GG associated with toxicity (40); No association with toxicity (36;49;53)	No effect on efficacy (32;36;37;40;49); RFC 80A-allele associated with efficacy (35)
ABCC2	Efflux transporter on cells of MTX	1249 G>A, 1058 G>A, IVS23 +56 T>C	No effect on toxicity (25)	*
HLA-G	Persistence of anti-inflammatory conditions	14bp deletion	*	-14/ -14 bp associated with efficacy (50;51) . No effect on efficacy (52)

Table 1. Pharmacogenetic association studies of methotrexate with treatment outcome in rheumatoid arthritis

* = No information on association(s) with specific efficacy or toxicity was present regarding this SNP under study
Abbreviations and accessory full names of formal genes can be relocated in the NCBI gene database

Because, it is thought that MTX has an influence on adenosine pathway, SNPs within genes coding for *AMPD1*, *ATIC*, *MTR*, *MTRR*, *ITPA* were correlated with treatment outcome in several studies (36;40;47;53). Our group identified significant associations with clinical response, defined as an absolute value of DAS of less than 2.4, and the SNPs *AMPD1* 34C>T, *ATIC* 347C>G, *ITPA* 94C>A. In the toxicity analysis, only *ATIC* GG was associated with toxicity (53).
In general, several studies demonstrated no effect of *MTR* and *MTRR* on MTX efficacy (40;49;53). Regarding toxicity, in only one study (49) a relation between *MTR* 2756A>G and *MTRR* 66A>G and toxicity in a small group of patients was seen. However in two previously performed studies this was not reported (40;53). Since, the anti-inflammatory effects of adenosine are mediated by adenosine receptors, one group studied polymorphisms in genes coding for the adenosine receptor (*ADORA2A*) in relation with MTX therapy outcome. Five SNPs, were reported to be associated with adverse events on MTX. Specifically, two SNPs were related with gastrointestinal side effects (55) (Table 1).
Several nongenetic factors have been reported to influence efficacy of MTX treatment over the last years. These factors include demographic, life-style and clinical determinants such as disease activity at baseline, gender and smoking. Still, associations of these factors have not been translated into clinical tools in order to guide MTX treatment in RA patients. However, recently, a pharmacogenetic model in combination with clinical factors to predict MTX efficacy in recent-onset RA was developed (39). In this study it was reported that the clinical factors gender, rheumatoid factor combined with smoking status and disease activity at baseline were predictive for MTX response. The included genetic factors were the SNPs *AMPD1* C>T, *ATIC* 347C>G, *ITPA* 94 A>C and *MTHFD* 1958G>A. The prediction resulted in the classification of 60% of the RA patients into MTX responders and nonresponders, with 95% and 86% as true positive and negative response rates, respectively. Evaluation of this predictive model in a second group of 38 RA patients supported our results (39). Still, this model needs further prospective validation before its implementation in clinical practice.

Conclusion

MTX has been demonstrated to be effective drugs in the treatment of RA. Still, various percentages in efficacy and toxicity are seen. Unfortunately, these interindividual differences cannot be predicted in individual patients and markers such as polymorphisms, are necessary to individualize and optimize drug treatment. Yet, most pharmacogenetic studies performed have an insufficient sample size (power) to detect true associations with treatment response. In addition, other factors, like non-genetic factors, ethnicity and clear endpoints, influence treatment outcome. Particularly, disease activity score (DAS) at baseline determines to a large extend the response of RA patients treated with DMARD therapy as was demonstrated from previous studies. Also regarding clear endpoints, various use of disease activity parameters and/or cutoff levels for the definition of response, e.g. elevated liver enzyme levels in the case of side effects and an absolute value of DAS in the case of efficacy, may contribute to different results. In order to optimally compare studies or perform meta-analyses, criteria regarding efficacy and toxicity should be standardized. Finally, opposite or alternative results found may be explained by differences in SNP allele frequencies between various ethnic populations, which makes these association studies unlikely to compare.
Therefore, definitive conclusions about the role of genetic prognostic factors in treatment outcome to MTX cannot be drawn. Large randomized prospective studies are required to effectively replicate and validate these findings, before a pharmacogenetic approach is applicable in daily clinical practice.

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