Pharmacogenetics: implications for therapy in rheumatic diseases

Lesley Davila and Prabha Ranganathan

Abstract | DMARDs not only improve the joint pain and swelling associated with rheumatoid arthritis (RA), but also slow down the joint damage associated with the disease. The efficacy of biologic therapies, introduced in the past decade for the treatment of RA, has been unequivocally established. Similarly, in addition to traditional drugs such as hydroxychloroquine, new biologic agents such as rituximab have been introduced for systemic lupus erythematosus in recent years. However, considerable variability occurs in the responses of patients to these therapies. Pharmacogenetics, the study of variations in genes encoding drug transporters, drug-metabolizing enzymes and drug targets, and their translation to differential responses to drugs, is a rapidly progressing field in rheumatology. Pharmacogenetic applications, particularly to the old vanguard DMARD, methotrexate, and the newer, more expensive biologic agents, might make personalized therapy in rheumatic diseases possible. The pharmacogenetics of commonly used DMARDs and of biologic therapies are described in this Review.

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Introduction

We have made tremendous strides in the past decade in our understanding of the genetics of susceptibility to rheumatic diseases,[1] as well as the genetics of response to therapeutics. Genetic variations that influence responses to drugs include polymorphisms in genes encoding drug metabolizing enzymes, drug transporters, and drug targets.[2] Pharmacogenetics is the study of such polymorphisms and their effects on drug response.[3] A 'polymorphic' gene is a gene that has allelic variants, which can affect the activity and/or quantity of the encoded protein. Pharmacogenetics has the potential to explain differences between individuals in their responses to drug therapies, and more importantly, to help optimize treatments for individual patients. Molecular sequencing and high-throughput technologies make it possible for the human genome to be rapidly scanned for several hundred genetic polymorphisms—such as single-nucleotide polymorphisms (SNPs)—that might regulate clinically important inter-individual differences in responses to pharmacologic treatments.

In the past decade, biologic agents have dramatically changed the landscape of treatment for diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Although biologic therapies are highly effective, concerns remain about the high costs of these medications, and about the spectrum of adverse events associated with their use. Prospective screening of the genomes of individual patients to identify those at highest risk of adverse events or suboptimal response will make it possible to tailor treatments to individual patients with a rheumatic disease. Pharmacogenetics offers the promise of such personalized therapeutics.

In this article we highlight some of the important advances in the field of pharmacogenetics as they pertain to RA and SLE, the two most common rheumatic diseases, and the future applications and directions of this emerging field. We consider the pharmacogenetics of each medication in turn, discussing the implications of the existing data as they apply to one or both of these diseases.

Pharmacogenetics of traditional DMARDs

Many polymorphisms and other genetic variations are implicated in the pharmacogenetics of myriad DMARDs. Nevertheless, despite more than a decade of research, and a multitude of markers studied to date, only one pharmacogenetic assay is in use in clinical rheumatology practice at the present time, and none has yet been validated by the appropriate regulatory body in the United States, the FDA's Center for Devices and Radiological Health (FDA-CDRH), for technical and application robustness. The thiopurine *S*-methyltransferase (*TPMT*) genotyping assay, which we will discuss, is in the package insert for azathioprine. Besides *TPMT* in clinical rheumatology practice, *HER2* and *KRAS* are pharmacogenetic markers used in clinical oncology practice, and all three have never been put through a rigorous validation process. That these tests are used, reimbursed, and trusted shows (in the absence of a formal validation process) that the key features for a pharmacogenetic marker to be brought into clinical practice are: demonstration of marker reliability by replication of findings, data that will alter practice (changing the medicine or dose prescribed), and availability of an assay that will not be prohibitively hard to use (in terms,

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Key points

- Responses to therapies used in rheumatic diseases vary considerably between individual patients
- Pharmacogenetics—how drug efficacy and toxicity are affected by variations in genes encoding drug metabolizing enzymes, transporters and targets—is a nascent, promising area of research in rheumatology
- Pharmacogenetic applications, both for traditional agents such as methotrexate, and for biologic agents, might facilitate individualized therapy in rheumatoid arthritis and systemic lupus erythematosus
- The importance of a few genetic variants has been established by reproducibility, notably 677C>T polymorphism of methylene tetrahydrofolate reductase, and thiopurine S-methyltransferase allelic variants—markers of methotrexate and azathioprine toxicity, respectively
- Although more research is needed to replicate preliminary findings, and to formally validate established markers, several exploratory, promising new markers are showing the future potential of this exciting field

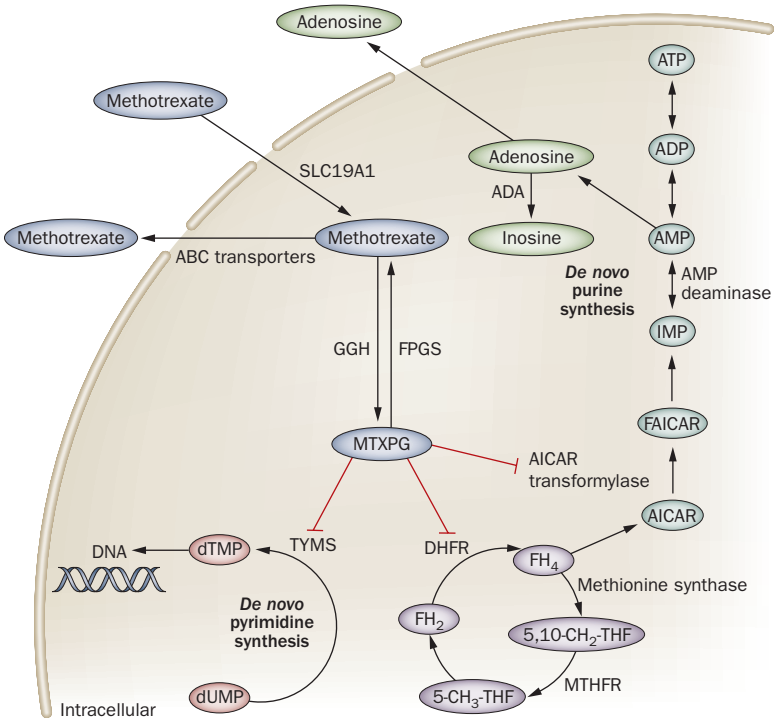

Figure 1 | Cellular pathway of methotrexate—transport, conversion to polyglutamate forms, and downstream effects. Cellular uptake of methotrexate follows the folate pathway; its efflux is by ABC transporters. Within the cell, GGH converts the drug to MTXPGs (whose cellular retention is greater than that of methotrexate). MTXPGs impede generation of bioactive forms of folate, inhibit *de novo* pyrimidine synthesis and cause accumulation of AICAR in the *de novo* purine synthesis pathway. AICAR inhibits ADA and AMP deaminase, causing accumulation of adenosine, which has anti-inflammatory activity. Polymorphisms in genes encoding many of the enzymes in these pathways are thought to modulate methotrexate efficacy and toxicity. Abbreviations: 5-CH_3-THF, 5-methyl tetrahydrofolate; 5,10-CH_2-THF, 5,10-methylene tetrahydrofolate; ADA, adenosine deaminase; AICAR, aminoimidazole carboxamide adenosine ribonucleotide; DHFR, dihydrofolate reductase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; FAICAR, 10-formyl AICAR; FH_2, dihydrofolate; FH_4, tetrahydrofolate; FPGS, folylpolyglutamate synthase; GGH, γ-glutamyl hydrolase; IMP, inosine monophosphate; MTHFR, methylene tetrahydrofolate reductase; MTXPG, methotrexate polyglutamate; TYMS, thymidylate synthase. Reproduced from Ranganathan *et al.* Methotrexate pharmacogenetics. *Arthritis Rheum*. **54**, 1366–1377 (2006) by permission of John Wiley & Sons, Inc.

for example, of turnaround time and complexity of interpretation). Except for *TPMT*, all other markers we discuss in this article should be considered developmental and exploratory, and not yet ready for clinical use.

Methotrexate

Methotrexate has been the first-line therapy for RA for well over two decades, with excellent safety and efficacy.[4,5] The cellular effects of methotrexate on the folate and nucleotide pathways (Figure 1) are central to its benefits in RA.

Effects on folate and nucleotide pathways

Methotrexate is transported into the cell by folate transporter 1 (also known as solute carrier family 19, member 1 [SLC19A1]) (Figure 1). Efflux of the drug from the cell is controlled by members of the ATP-binding cassette (ABC) family of transporters, also known as multidrug-resistant proteins.[6,7] Within the cell, methotrexate is converted into a range of polyglutamate forms known as MTXPGs by the enzyme folylpolyglutamate synthase (FPGS), in a process that can be reversed by the enzyme γ-glutamyl hydrolase. Polyglutamation increases retention of methotrexate within the cell.[8] MTXPGs inhibit dihydrofolate reductase, the enzyme that reduces dihydrofolate to tetrahydrofolate. Conversion of tetrahydrofolate into a 5-methyl form (by methionine synthase, encoded by *MTR*) involves synthesis of the intermediate metabolite 5,10-methylene tetrahydrofolate by serine hydroxymethyltransferase (SHMT1) (Figure 1). The product, 5-methyl tetrahydrofolate, is a biologically important moiety that acts as a carbon donor for several cellular reactions, including the conversion of homocysteine to methionine.[9]

Besides inhibiting the folate pathway, MTXPGs also influence *de novo* pyrimidine synthesis by inhibiting thymidylate synthetase (TYMS), which converts deoxyuridylate to deoxythymidylate.[10] Methotrexate and its metabolites have effects on purine synthesis too. MTXPGs inhibit the enzyme AICAR transformylase (also known as bifunctional purine biosynthesis protein, PURH, which is encoded by *ATIC*) leading to intracellular accumulation of aminoimidazole carboxamide adenosine (AICA) ribonucleotide. This product and its metabolites inhibit two enzymes that are important in adenosine metabolism, adenosine deaminase and AMP deaminase, causing intracellular accumulation of adenosine nucleotides (Figure 1). When these nucleotides are dephosphorylated, there is accumulation of extracellular adenosine, which is a powerful anti-inflammatory agent.[11]

Genetic variations in folate and nucleotide pathways

Several genetic variations in components of the folate and nucleotide pathways have been studied in an attempt to predict methotrexate efficacy and toxicity (Table 1). Two polymorphisms of the gene encoding methylene tetrahydrofolate reductase (MTHFR), 677C>T and 1298A>C are in linkage disequilibrium and lead to reduced levels, and thus activity, of MTHFR. Although these variants seem to affect the efficacy and toxicity of methotrexate, their influence has not been unequivocally established.[12–15] One meta-analysis found an association between the

Table 1 | Pharmacogenetics of methotrexate—known variants and putative clinical effects

Gene, product and role	Variant	Cellular effects of variant	Studies, designs and participants	Reported clinical effects
Folate pathway variants				
SLC19A1, FOLT, transports folate (and methotrexate) into cell	80G>A	Results in higher intracellular levels of MTXPGs	Takatori (2006),[26] retrospective, *n*=124; all patients with RA	No effect on efficacy
			Dervieux (2004),[30] cross-sectional, *n*=108; all patients with RA	Associated with increased efficacy
MTHFR, MTHFR, generation of 5-methyl tetrahydrofolate	677C>T	Produces a thermolabile MTHFR variant; decreases enzyme levels	Van Ede *et al.* (2001),[13] prospective, *n*=236; all patients with RA	Associated with discontinuation of methotrexate due to increased toxicity
			Urano *et al.* (2002),[15] retrospective, *n*=106; all patients with RA	Associated with increased toxicity
			Fisher & Cronstein (2009),[16] meta-analysis, *n*=1,400	Associated with increased toxicity
			Lee & Song (2010),[17] meta-analysis, *n*=1,514	No effect on toxicity or efficacy
			Weisman *et al.* (2006),[31] cross-sectional, *n*=214; all patients with RA	Associated with increased toxicity
	1298A>C	Decreases MTHFR enzymatic activity	Berkun *et al.* (2004),[14] cross-sectional, *n*=93; all patients with RA	Associated with reduced toxicity
			Urano *et al.* (2002),[15] retrospective, *n*=106; all patients with RA	Associated with increased efficacy
			Fisher & Cronstein (2009),[16] meta-analysis, *n*=660	No effect on toxicity
			Lee (2010),[17] meta-analysis, *n*=1,514	No effect on toxicity or efficacy
SHMT1, SHMT, generation of 5,10-methylene tetrahydrofolate	1420C>T	Alters enzyme activity	Weisman *et al.* (2006),[31] cross-sectional, *n*=214; all patients with RA	Associated with increased toxicity
Drug transporter variants				
ABCB1, MDR1, efflux pump	3435C>T	Increases efflux of methotrexate	Takatori *et al.* (2006),[26] retrospective, *n*=124; all patients with RA	Associated with decreased efficacy
Nucleotide synthesis variants				
TYMS, TYMS, conversion of dUMP to dTMP in *de novo* pyrmidine synthesis (inhibited by MTXPGs)	5'-UTR repeat element	Triple repeat allele associated with increased TYMS activity	Kumagai *et al.* (2003),[24] retrospective, *n*=167; 115 patients with RA treated with methotrexate, 52 controls	Triple repeat allele associated with decreased efficacy
			Dervieux *et al.* (2004),[30] cross-sectional, *n*=108; all patients with RA	Triple repeat allele associated with decreased efficacy
			Weisman *et al.* (2006),[31] cross-sectional, *n*=214; all patients with RA	Double repeat allele associated with toxicity
	3'-UTR deletion	Decreases mRNA stability and expression	Kumagai *et al.* (2003),[24] retrospective, *n*=167; 115 patients with RA treated with methotrexate, 52 controls	Associated with improved efficacy
ATIC, PURH, formylation of AICAR during *de novo* purine synthesis (inhibited by MTXPGs)	347C>G	Alters enzyme activity and increases intracellular AICAR levels	Wessels *et al.* (2006),[25] prospective, *n*=205; all patients with RA	Associated with improved efficacy and increased toxicity
			Takatori *et al.* (2006),[26] retrospective, *n*=124; all patients with RA	No effect on efficacy
			Dervieux *et al.* (2004),[30] cross-sectional, *n*=108; all patients with RA	Associated with improved efficacy
			Weisman *et al.* (2006),[31] cross-sectional, *n*=214; all patients with RA	Associated with increased toxicity
Cytokine pathway variants				
IL1RN, IL-1Ra, blocks induction of inflammation by IL-1 (the ratio of IL-1:IL-1Ra is thought to be affected by methotrexate)	IL-1RN*3	Modulates IL-1 cytokine synthesis	Tolusso *et al.* (2006),[29] cross-sectional, *n*=304; 126 patients with RA, 178 healthy controls	Associated with decreased efficacy

Abbreviations: AICAR, AICA ribonucleotide; FOLT, folate transporter 1; IL-1Ra, interleukin 1 receptor antagonist; MDR1, multidrug resistance protein 1; MTHFR, methylene tetrahydrofolate reductase; MTXPGs, polyglutamate forms of methotrexate; PURH, bifunctional purine biosynthesis protein PURH; RA, rheumatoid arthritis; SHMT, serine hydroxymethyltransferase; UTR, untranslated region.

677C>T polymorphism and methotrexate toxicity, but no such association for the 1298A>C variant.[16] However, another meta-analysis (which included 1,514 patients with RA) found no association between either of these polymorphisms and methotrexate toxicity and efficacy.[17]

A polymorphic tandem repeat sequence can occur in the 5'-untranslated region (5'-UTR) of *TYMS*, with a variable number of 28 bp repeat elements.[18] These repeat sequences can enhance *TYMS* mRNA expression and TYMS enzyme activity *in vitro*.[18–20] Individuals who are homozygous for the triple repeat allele reveal higher *TYMS* mRNA expression than those homozygous for the double repeat allele.[20,21] Conversely, a 6 bp deletion polymorphism, 1494–1499delTTAAAG, in the 3'-UTR of *TYMS*[22] leads to decreased mRNA stability and reduced expression of TYMS protein.[23] These *TYMS* polymorphisms have been reported to affect the efficacy of methotrexate in patients with RA.[24] In addition, polymorphisms in the adenosine pathway, including those in *ATIC*[25] and the transporter genes *SLC19A1* and *ABCB1*, are reported to influence responses to methotrexate in patients with RA (Table 1, Figure 1).[26]

Genetic variations in cytokine pathways

The actions of methotrexate are incompletely understood, and are not limited to effects on the folate and adenosine pathways. IL-1, a pivotal proinflammatory cytokine in RA, has a naturally occurring antagonist called IL-1 receptor antagonist (IL-1Ra). Methotrexate is thought to both inhibit production of IL-1, and also to induce a higher ratio of IL-1Ra:IL-1, in peripheral blood mononuclear cells of patients with RA,[27,28] which might account for some of its anti-inflammatory effects. To find out whether genetic variations in the IL-1 pathway can affect methotrexate efficacy, 126 patients with RA were assessed for polymorphisms in *IL1B* and *IL1RN* (both of which encode IL-1 cytokines), and response to methotrexate.[29] The *IL-1RN*3* allele was identified in this study as a marker of resistance to methotrexate treatment.[29]

Composite genotype risk models

Some researchers have attempted to build pharmacogenetic models for the efficacy and toxicity of methotrexate in patients with RA using composite risk genotypes. A pharmacogenetic index made up of the sum of homozygous variant genotypes in *SLC19A1*, *ATIC*, and *TYMS*, and erythrocyte concentrations of long-chain MTXPGs were examined in one study.[30] Being homozygous for at least one variant genoytype (and/or, accordingly, having a higher pharmacogenetic index than people with non-variant genotypes) correlated with increased MTXPG levels and increased response to methotrexate in this study.[30] Similarly, *MTHFR* genotypes in concert with variants in *TYMS*, *ATIC*, and *SHMT1* have been used in toxicogenetic indices to predict methotrexate toxicity:[31] variants in *MTHFR* (677C>T), *SHMT1*, *TYMS*, and *ATIC*, both individually and as a composite index, were all associated with toxicity.[31] In another study, 17 polymorphisms in 13 genes encoding enzymes in the methotrexate cellular pathway were combined with clinical parameters to build a model to predict methotrexate efficacy.[32] A model comprising rheumatoid factor status, smoking status, gender, disease activity, 3 polymorphisms in adenosine pathway genes, and one polymorphism in a folate pathway gene, was predictive of methotrexate efficacy. By contrast, a study that examined polymorphisms in methotrexate pathway genes and in genes involved in immune tolerance and susceptibility to RA, such as *HLAG, TLR4* and *TGFB1*, was unable to predict outcomes in terms of methotrexate efficacy and toxicity in patients with RA, after correction for multiple testing.[33]

A multitude of studies, therefore, have examined polymorphisms in the transporter, folate and adenosine pathways, in IL-1 genes, and in immune tolerance and disease susceptibility genes to predict outcomes of methotrexate treatment in RA (Table 1). Among the genotype–response associations reported, the association of the *MTHFR* 677C>T variant with methotrexate toxicity seems to be the most robust, having been reproduced in more than one study. Unfortunately, the validity and reproducibility of other associations are questionable. Thus, sadly, despite more than a decade of research in this area, strong pharmacogenetic data to guide methotrexate therapy in RA are currently lacking.

Azathioprine

Azathioprine retains an important place in the repertoire of treatments for SLE, despite the advent of newer biological agents such as rituximab. Figure 2 shows the metabolism of azathioprine; the prodrug is converted into 6-mercaptopurine *in vivo*. 6-mercaptopurine is activated by hypoxanthine–guanine phosphoribosyl transferase to thio-inosine monophosphate, which is then converted to cytotoxic thioguanine nucleotides or methylmercaptopurine nucleotides. Thioguanine nucleotides are inactivated by TPMT (producing methylmercaptopurine) or by oxidation by the enzyme xanthine dehydrogenase/oxidase, to thiouric acid (Figure 2). Inosine monophosphate is phosphorylated to inosine triphosphate (ITP) in a process that is reversed by inosine triphosphate pyrophosphatase (ITPase, encoded by *ITPA*), which prevents accumulation of ITP. ITPase-deficient patients with inflammatory bowel disease treated with azathioprine can develop azathioprine toxicity because of accumulation of thio-ITP.[34]

TPMT activity in erythrocytes varies between individuals: approximately 90% of the population have high activity, 10% have intermediate activity, and 0.3% have low or no activity.[35] Three allelic variants of *TPMT*, *TPMT*2*, *TPMT*3A*, and *TPMT*3C* account for 80–95% of people with low or intermediate TPMT activity.[36–38] Individuals with low TPMT activity exposed to standard doses of azathioprine can develop severe, even fatal, hematopoietic toxicity; such patients require substantial dose reduction to avoid toxicity.[39] Patients with rheumatic diseases heterozygous for the *TPMT*3A* allele and treated with azathioprine have a higher risk of hematopoietic and gastrointestinal toxicity from the drug than those with the wild-type alleles.[40,41] *TPMT* variants have been

strongly associated with myelosuppression in patients with SLE, sometimes leading to fatality.[42] A recent meta-analysis concluded that patients who are homozygous or heterozygous for any of the *TPMT* variant alleles that lead to absent or intermediate TPMT activity, are at high risk for drug-induced myelosuppression.[43]

Other studies suggest that *TPMT* genotyping alone might not be sufficient to predict azathioprine toxicity,[44,45] and that *ITPA* variants might be instrumental as well. Polymorphisms in *ITPA* are associated with azathioprine toxicity—particularly gastrointestinal adverse events—in patients with inflammatory bowel disease, but have not been studied in rheumatic diseases to date.[34]

Thus, *TPMT* genotyping (but not yet *ITPA* genotyping) has been proven in several studies to be helpful in predicting azathioprine toxicity (Table 2). In fact, as we have mentioned, centers are now using a commercially available *TPMT* genotyping assay to guide azathioprine therapy in clinical practice.

Sulfasalazine

Sulfasalazine is frequently prescribed to patients with RA. After ingestion, the drug is split by intestinal bacteria into 5-amino salicyclic acid and sulfapyridine (Figure 3). Sulfapyridine is metabolized in the liver by acetylation, hydroxylation, and ultimately glucuronidation. Arylamine N-acetyltransferase 2 (NAT-2) acetylates sulfapyridine, and its gene, *NAT2,* is polymorphic. 40–70% of individuals are homozygous or compound heterozygous for *NAT2* polymorphisms,[46] which influence the 'acetylator' status of individuals, making them slow or rapid acetylators. Slow acetylators might experience more sulfasalazine toxicity, such as headache, nausea, abdominal discomfort and rash, compared with rapid acetylators.[47,48]

NAT2 variants that confer slow acetylator status influence hematologic adverse events such as agranulocytosis, and other events such as fever and rash, that can be severe enough to require hospitalization of patients with RA taking sulfasalazine.[49,50] The preliminary results of Tanaka *et al.*[50] were successfully validated in an independent sample of 186 patients with RA.[51] Besides RA, the rapid acetylator status might also be a predictor of sulfasalazine efficacy in patients with discoid lupus (a form of chronic cutaneous lupus), whereas the slow acetylator phenotype is a predictor of adverse events such as leucopenia and rash.[52] Despite these preliminary findings (which are summarized in Table 3), studies of the effects of *NAT2* variants on sulfasalazine toxicity are, so far, plagued by the same small-sample-size limitations as existing pharmacogenetic studies of methotrexate.

Hydroxychloroquine

Antimalarials such as hydroxychloroquine are effective treatments for SLE. After oral administration, hydroxychloroquine is rapidly absorbed from the gut and metabolized in the liver by cytochrome P450 (CYP) enzymes to its active metabolite, N-desethylhydroxychloroquine (Figure 4). N-desethylhydroxychloroquine is a weak base that accumulates in acidic vesicles, such as cellular

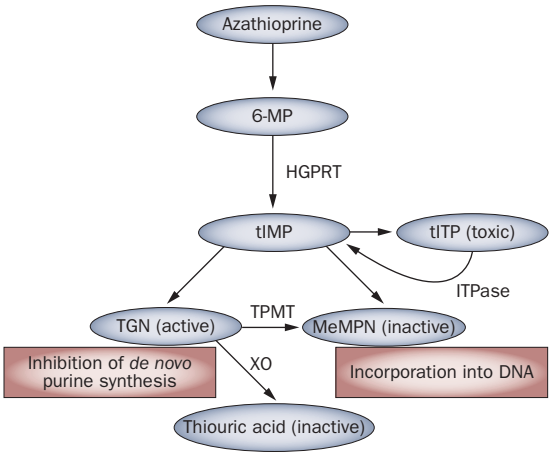

Figure 2 | Metabolism of azathioprine. Ingested azathioprine (a prodrug and purine analog) is converted to 6-MP, which is then activated to tIMP by HGPRT. tIMP is converted to TGN, MeMPN, or tITP. tITP is toxic and is converted back to tIMP by ITPase; lack of ITPase can cause azathioprine toxicity. TGN (which inhibit *de novo* purine synthesis) are inactivated by TPMT to MeMPN, or are oxidized to inactive thiouric acid by XO. Variants of *TPMT* are associated with adverse events; a commercial *TPMT* genotyping assay is used to predict azathioprine toxicity. Abbreviations: 6-MP, 6 mercaptopurine; HGPRT. hypoxanthine–guanine phosphoribosyl transferase; MeMPN, methylmercaptopurine nucleotides; TGN, thioguanine nucleotides; tIMP, thio-inosine monophosphate; tITP, thio-inosine triphosphate; TPMT, thiopurine methyltransferase; XO, xanthine oxidase.

endosomes and lysosomes, increasing the pH of these compartments.[53] Consequently, its presence inhibits the action of acidic proteases involved in multiple cellular functions, such as Toll-like receptor signaling, antigen presentation, and production of proinflammatory and anti-inflammatory cytokines such as tumor necrosis factor (TNF) and IL-10.[54,55]

No data yet demonstrate that variants in the genes encoding the CYP enzymes that metabolize hydroxychloroquine influence either efficacy or toxicity of the drug. However, polymorphisms in cytokine genes such as *TNF* and *IL10*, have been studied for their ability to predict responses to hydroxychloroquine in patients with SLE. An *IL10* –1082 A>G polymorphism, along with two others (*IL10* –819 C>T and –592 C>A), all in the *IL10* promoter region, influence basal and induced IL-10 production, with the GCC/GCC haplotype conferring the highest level of IL-10 production.[56–58] Similarly, the *TNF* –308G/G genotype confers the phenotype of high serum TNF levels (the TNF –308A>G variant is discussed further in the section on biologic agents). In a case–control study serum TNF levels were measured in 171 patients with SLE and 215 healthy controls, alongside genotyping for the *IL10* –1082A>G and TNF –308A>G promoter gene polymorphisms in 192 patients with SLE and 343 matched healthy controls.[59] Patients with SLE had higher serum TNF levels compared with healthy controls, but patients with SLE treated with antimalarials had lower serum TNF levels. Patients who carried the genotype that translated into low IL-10 and high TNF

Table 2 | Pharmacogenetics of azathioprine—known variants and putative clinical effects

Gene, product and role	Variant	Cellular effects of variant	Studies, designs and participants	Reported clinical effects
TPMT, TPMT, inactivates thioguanine nucleotides	*TPMT*2* *TPMT*3A* *TPMT*3C*	Each variant allele results in decreased enzyme activity	Kerstens *et al.* (1995),[40] cross-sectional, *n*=3; all patients with RA	Associated with increased toxicity
			Stolk *et al.* (1998),[41] prospective, *n*=99; 33 patients with established RA, 24 with early RA, 42 healthy controls	Associated with increased toxicity
			Higgs *et al.* (2010),[43] meta-analysis of 67 studies	Associated with increased toxicity
ITPA, ITPase, prevents accumulation of thio-ITP	94C>A	Accumulation of thio-ITP	Marinaki *et al.* (2004),[34] cross-sectional, *n*=130; 62 patients with IBD, 68 healthy controls	Associated with increased toxicity

*Although not formally validated by the FDA, the TPMT genotyping assay is in routine clinical use. Abbreviations: IBD, inflammatory bowel disease; ITP, inosine triphosphate; ITPase, ITP pyrophosphatase; RA, rheumatoid arthritis; TPMT, thiopurine S-methyltransferase.

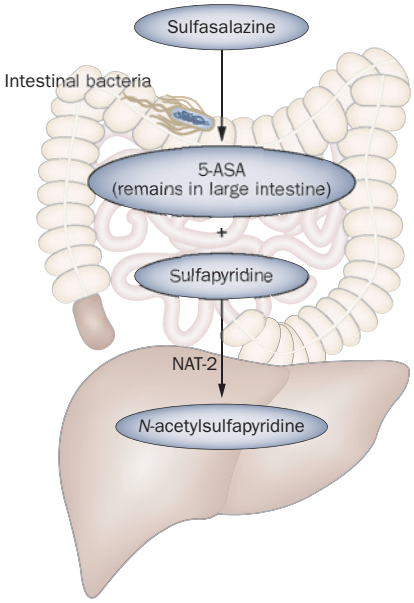

Figure 3 | Metabolism of sulfasalazine. Sulfasalazine is converted to 5-ASA and sulfapyridine by intestinal bacteria. In the liver, sulfapyridine is converted to N-acetylsulfapyridine, in a process that involves acetylation by NAT-2. Variants of *NAT2* affect the acetylation rate; slow acetylation is thought to lead to therapy-related adverse events. Abbreviations: 5-ASA, 5-aminosalicylic acid; NAT-2, N-acetyltransferase 2.

production (that is, *IL10* 1082 A/A and *TNF* –308G/G), had the best response to hydroxychloroquine, suggesting that hydroxychloroquine-mediated downregulation of TNF was influenced by these polymorphisms (Table 3).[59] Nevertheless, pharmacogenetic data that could guide the use of hydroxychloroquine are currently sparse, despite it being the most widely-used drug in patients with SLE.

Leflunomide

Leflunomide is an isoxazole derivative used as a DMARD for the treatment of RA. Its metabolism, in plasma and in intestinal mucosa, produces an active, open-ring metabolite, A77 1726, that causes noncompetitive and reversible inhibition of dihydroorotate dehydrogenase (DHODH).[60] This enzyme is key for *de novo* pyrimidine synthesis,[61] and the effect of leflunomide treatment is a decrease in lymphocyte proliferation (Figure 5). Several polymorphisms that seem to affect responses to leflunomide are discussed in this section, and summarized in Table 4.

The human *DHODH* sequence frequently contains a missense polymorphism in the first exon (19A>C), which leads to a lysine to glutamine amino acid substitution in the N-terminal region of the polypeptide. This segment controls the insertion of the polypeptide into the mitochondrial inner membrane, and thus the cellular effects of the enzyme. The *DHODH* 19A>C polymorphism has been studied as a predictor of both efficacy and toxicity of leflunomide: Pawlik *et al.*[62] examined responses to leflunomide monotherapy in 147 patients with RA and found that remission was more frequent in patients who carried the C rather than the A allele, with no significant association between either of the alleles and the frequency of adverse events.[62] However, in a retrospective study of 105 patients with RA, this polymorphism resulted in a 6.8-fold increased risk of overall adverse events from leflunomide, including gastrointestinal, mucosal, and hepatic toxicity.[63]

Polymorphisms in genes encoding the estrogen receptor have been studied, by Dziedziejko *et al.*,[64] for association with responses to leflunomide, because *in vitro* studies suggest that estrogen interferes with the suppression of cytokine production by the drug.[65,66] Two estrogen receptors—α and β—are known, and the genes *ESR1* and *ESR2* that encode them are responsible for transducing extracellular signals into transcriptional responses. Several *ESR1* and *ESR2* polymorphisms have been identified previously, and in a prospective study of 115 patients with RA, the *ESR1* rs9340799 AA and rs2234693 TT genotypes were associated with response to treatment with leflunomide after 12 months of therapy.[64] One possible mechanism for such an association is that these polymorphisms influence estrogen receptor expression levels.

Polymorphisms, therefore, influence the downstream effects of leflunomide's active metabolite, but genetic variations also influence generation of that metabolite. The cytochrome P450 (CYP) system, particularly the enzyme encoded by *CYP1A2*, activates leflunomide,[67] and a prospective study of 106 patients with RA identified a potential association of the *CYP1A2*1F* polymorphism

Table 3 | Pharmacogenetics of various DMARDs—known variants and putative clinical effects

Gene, product and role	Variant	Cellular effects of variant	Studies, designs and participants	Reported clinical effects
Sulfasalazine				
NAT2, arylamine *N*-acetyltransferase 2, acetylation of sulfapyridine	*NAT2*4* haplotype	Lack of *NAT2*4* predicts slow acetylation	Wadelius (2000),[49] case–control, *n*=562; 114 patients with inflammatory arthritis and bowel disease, 448 controls	No effect on toxicity
			Tanaka (2002),[50] retrospective, *n*=144; all patients with RA	Increased toxicity in slow acetylators
			Taniguchi (2007),[51] retrospective, *n*=186; all patients with RA	Increased toxicity in slow acetylators
			Sabbagh (1997),[52] prospective, *n*=11; all patients with discoid lupus erythematosus	Increased toxicity in slow acetylators and increased efficacy in rapid acetylators
Hydroxychloroquine				
IL10, IL-10, proinflammatory cytokine	1082 A>G 819 C>T 592 C>A	Alter *IL10* promoter region, influencing basal and induced IL-10 production	Lopez (2006),[59] case–control, *n*=386; 171 patients with SLE; 215 healthy controls	Associated with increased efficacy
TNF, TNF, proinflammatory cytokine	308 A>G	Alters *TNF* promoter activity and serum TNF levels	Lopez (2006),[59] case–control, *n*=386; 171 patients with SLE; 215 healthy controls	Associated with increased efficacy
Cyclophosphamide				
CYP2C, CYP enzyme, metabolism of cyclophosphamide	*CYP2C19*2* *CYP2B6*5*	Alter metabolism of cyclophosphamide into active and inactive compounds	Takada (2004),[69] prospective, *n*=62; all patients with lupus nephritis	*CYP2C19*2* homozygotes and heterozygotes at reduced risk of premature ovarian failure; *CYP2C19*2* or *CYP2B6*5* homozygotes with poor response at increased risk of end-stage renal disease
GSTP1, GSTP, conjugating enzyme responsible for detoxification of cyclophosphamide	GSTP Ile105Val	Decreases catalytic activity and thermal stability of enzyme	Zhong (2006),[70] prospective, *n*=102; all patients with SLE	Increased risk of myelotoxicity and gastrointestinal toxicity

Abbreviations: CYP, cytochrome P450; GSTP, glutathione *S*-transferase P; SLE, systemic lupus erythematosus; TNF, tumor necrosis factor.

with leflunomide toxicity.[68] The *CYP1A2*1F* CC genotype seems to accelerate conversion of leflunomide to A77 1726, relative to other *CYP1A2*1F* genotypes, leading to higher concentrations of this metabolite, and subsequent toxicity.

Cyclophosphamide

Cyclophosphamide, a DNA alkylating agent, is used in the treatment of many autoimmune diseases, including SLE and particularly, glomerulonephritis secondary to SLE. Upon administration, it enters the liver and is metabolized to both active and inactive compounds (Figure 6). CYP enzymes in the liver have a substantial role in its metabolism, and *CYP* polymorphisms might have an impact on the response to cyclophosphamide treatment. Indeed, Takada *et al.*[69] demonstrated that homozygous or heterozygous carriers of the *CYP2C19*2* allele were protected from premature ovarian failure (which is an adverse event associated with the use of this cytotoxic agent), but people homozygous for *CYP2C19*2* or *CYP2B6*5* (another CYP polymorphism) had a higher likelihood than those with wild-type alleles, or heterozygous carriers of a variant allele, of developing end-stage renal disease when treated with cyclophosphamide for lupus nephritis, indicating a suboptimal response to the drug in patients with these genotypes.

Polymorphisms that can alter the rate of metabolism comprise only part of the potential genetic influence on cyclophosphamide's effects; clearance of its metabolites seems, likewise, to be subject to variation. Glutathione *S*-transferases (GSTs), a superfamily of conjugating enzymes, are involved in detoxifying various compounds in the liver, including cyclophosphamide. The enzyme encoded by *GSTP1* has substantial affinity for the metabolites of cyclophosphamide, and in a prospective study of 102 patients with lupus nephritis, polymorphisms in this gene were evaluated for their potential to alter the efficacy and toxicity of the drug.[70] A polymorphism in codon 105 of *GSTP1* gene (causing an isoleucine to valine amino acid substitution) decreases substrate specific catalytic activity and thermal stability of the encoded GST protein,[71] and carriers of this polymorphism treated with cyclophosphamide for lupus nephritis were at increased risk of myelotoxicity and gastrointestinal toxicity.[70]

Pharmacogenetics of biologic agents

TNF antagonists

Pharmacogenetic studies of anti-TNF therapies have focused on the 'TNF locus', as well as on genes encoding TNF receptors. Nevertheless, as with pharmacogenetic studies of traditional DMARDs, it should be emphasized that none of the variants that have been associated

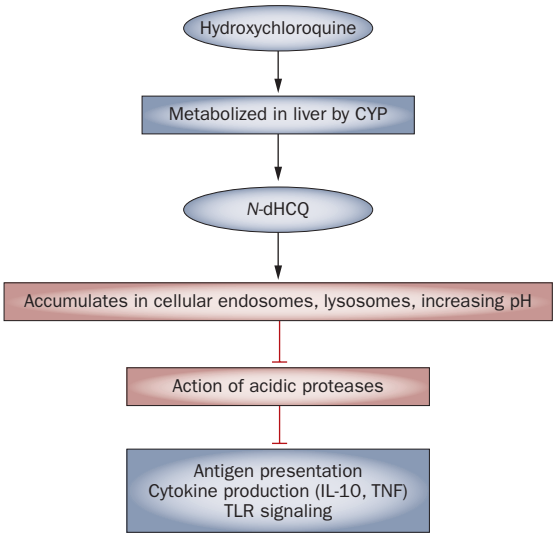

Figure 4 | Metabolism of hydroxychloroquine. Rapid intestinal absorption of hydroxychloroquine is followed by hepatic CYP enzyme-mediated metabolism, producing *N*-dHCQ, the active metabolite. This weak base accumulates in acidic compartments and increases their pH, inhibiting the activity of acidic proteases. Downstream functions, including TLR signaling and cytokine production, are thus inhibited. No polymorphisms in CYP genes are known to affect hydroxychloroquine efficacy or safety, but *TNF* and *IL10* polymorphisms might be predictive of response to the drug.[56–59] Abbreviations: CYP, cytochrome P450; *N*-dHCQ, *N*-desethylhydroxychloroquine; TLR, Toll-like receptor; TNF tumor necrosis factor.

with response to TNF antagonists have yet been validated as clinical markers of response to therapy. Existing pharmacogenetic data for biologic agents are summarized in Table 5.

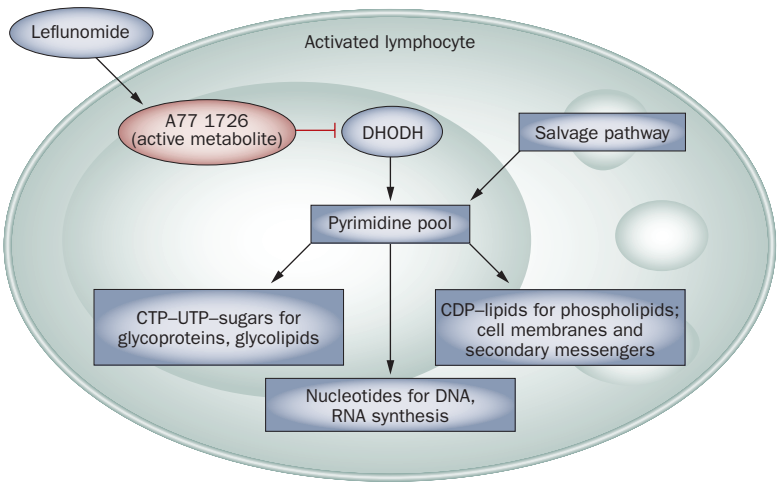

Figure 5 | Metabolism of leflunomide. The active metabolite of leflunomide (A77 1726), whose CYP enzyme-mediated production occurs in plasma and intestinal mucosa, is taken up by activated lymphocytes, wherein it inhibits the action of DHODH. *De novo* pyrimidine synthesis is thus inhibited, leading to decreased lymphocyte proliferation. Polymorphisms in *DHODH* and CYP enzyme genes are thought to affect leflunomide toxicity. Abbreviations: CYP, cytochrome P450; DHODH; dihydroorotate dehydrogenase.

TNF and TNF receptor gene variants

LTA, *TNF* and *LTB* (encoding three members of the TNF [ligand] superfamily) are located in tandem in the MHC Class III region on the short arm of chromosome 6, close to the HLA B locus and the MHC Class II DR genes (Figure 7). These regions influence susceptibility to several rheumatic diseases, including *HLA-B27* for ankylosing spondylitis, and the HLA shared epitope alleles for RA.[72]

Several genes in the TNF locus have known polymorphisms, including SNPs at –308 and –238 in the *TNF* promoter, and an intronic SNP at +489.[73] Although promoter SNPs can affect gene expression levels, the functions of these *TNF* promoter SNPs have not been clearly defined,[74] with some studies suggesting the –308 SNP influences circulating TNF levels,[75–77] whereas other studies show found no such effect.[78–80] The influence of these SNPs on responses to anti-TNF therapies are discussed below. Although it does not affect TNF transcription, the intronic +489 SNP seems to be a marker of severe RA, with the 489AA genotype being protective from severe disease in a study in 163 patients with RA and 67 healthy controls.[81]

TNF binds to TNF receptor superfamily members TNF-R1 (encoded by *TNFRSF1A*) and TNF-R2 (encoded by *TNFRSF1B*). A SNP in exon 6 of *TNFRSF1B* at codon 196 causes substitution of methionine for arginine.[82] The 196Arg allele not only influences circulating TNF levels, by affecting membrane receptor shedding and ligand binding, but also increases IL-6 production (according to a study in 105 Japanese patients with SLE).[82]

DNA microsatellites—repeat sequences of the bases A and T—are found in the intronic portions of DNA, where they can affect DNA folding and conformation, and thus influence gene transcription. Being highly polymorphic, DNA microsatellites can function as genetic markers, when they occur in linkage disequilibrium with a functional variant. The TNF locus has five such microsatellites, TNFa through TNFe, further classified on the basis of the number of repeat sequences (for example, TNFa1–13).[83] TNF microsatellites might influence TNF production: *in vitro*, TNFd and TNFa2 microsatellites are associated with high, and TNFa6 with low, levels of TNF.[83]

Fcγ receptor variants

Anti-TNF therapies are antibodies, and their Fcγ immunoglobulin component is recognized by the Fcγ receptor (FcγR); drug-mediated antibody-dependent cellular cytotoxicity (ADCC) is influenced by FcγR polymorphisms. A SNP in *FCGR3A*, encoding a Val158Phe variant FcγR IIIA, influences IgG1 Fc binding affinity, and thus ADCC and apoptosis: the Val158 version has greater affinity for IgG1 and promotes increased ADCC in comparison with the Phe158 variant of the receptor (in natural killer cells and monocytes isolated from homozygous healthy donors).[84] Some data suggest that besides influencing toxicity, this polymorphism might also influence susceptibility to and severity of RA,[85,86] but these associations remain controversial.[87,88] Nevertheless, that the *FCGR3A* genotype might influence the biological

Table 4 | Pharmacogenetics of leflunomide—known variants and putative clinical effects

Gene, product and role	Variant	Cellular effects of variants	Studies, designs and participants	Reported clinical effects
DHODH, DHODH, key enzyme of *de novo* pyrimidine synthesis	19 C>A	Alters N-terminal region of DHODH, affecting insertion into mitochondrial membrane	Pawlik (2009),[62] prospective, $n=147$; all patients with RA	Associated with increased efficacy
			Grabar (2009),[63] retrospective, $n=105$; all patients with RA	Associated with increased toxicity
ESR1, estrogen receptor, interferes with downmodulation of cytokines	rs9340799 AA rs2234693 TT	Alters estrogen receptor expression	Dziedziejko (2010),[64] prospective, $n=115$; all women with RA	Associated with increased efficacy
CYP1A2, CYP enzyme, involved in activation of leflunomide	CYP1A2*1F CC	Increases activation, leading to higher drug levels	Grabar (2008),[68] prospective, $n=106$; all patients with RA	Associated with increased toxicity

Abbreviations: CYP, cytochrome P450; DHODH, dihydroorotate dehydrogenase; RA, rheumatoid arthritis.

activity of TNF antagonists is supported by studies in Crohn's disease, wherein homozygosity for the Val158 genotype was associated with a greater anti-inflammatory effect of infliximab.[89] However, in a study of 282 Swedish patients with RA the *FCGR3A* genotype did not influence response to etanercept or infliximab, perhaps because avid ligation of FcγR IIIA requires the exposure of two Fc receptor molecules, and this does not happen when TNF is bound by etanercept and infliximab.[90]

In a study of patients with RA, psoriatic arthritis or ankylosing spondylitis ($n=54$, 10, and 22, respectively), the *TNF* promoter –308 G allele was identified as a marker of response to the three TNF antagonists, etanercept, infliximab and adalimumab.[91] Furthermore, it was predictive of response to adalimumab in 81 patients with RA in another study.[92] However, a meta-analysis of *TNF* promoter polymorphisms, shared epitope alleles, and response to the TNF antagonists in patients with RA (which included these data) revealed a marginal association between the *TNF* –238 SNP and response to infliximab, but no link between response and the –308 SNP or the shared epitope alleles.[93] Similarly, the TT genotype of the *TNFRSF1B* 196 T>G polymorphism was predictive of a greater response to etanercept and infliximab in a study of 175 patients with RA,[94] whereas another study that examined SNPs spanning the *TNFRSF1B* and *ADAM17* (encoding the TNF-converting enzyme ADAM 17) genes in a large UK cohort of patients with RA found no significant association between common SNPs in these genes and response to TNF antagonists.[95] With respect to the *TNF* microsatellites, one prospective study, in 78 infliximab-treated patients with RA, found that although individual TNFa and TNFb microsatellite alleles did not correlate with response to infliximab, the TNFa11;b4 microsatellite haplotype was found with increased frequency in people classified as responders.[96] In a prospective study in 457 patients with early RA, none of the five *TNF* microsatellite alleles were markers of response to etanercept; however, specific haplotypes spanning the *HLA-DRB1* region and SNPs in the *LTA–TNF* region were associated with response to etanercept.[97]

RA susceptibility genes

Besides examining those genes that are known to be involved in the production and reception of TNF, another approach to identifying predictors of response to TNF antagonists is to study variations in susceptibility genes, and genes in pathological pathways, of rheumatic diseases. To this end, 31 SNPs in genes associated with susceptibility to RA were examined in two large cohorts, totaling 1,283 patients with RA, who were receiving etanercept, infliximab or adalimumab. Of these SNPs, one located in *PTPRC* was associated with a better response to the three TNF antagonists, and there was a suggestive trend of this effect being more marked in patients positive for autoantibodies (against rheumatoid factor or citrullinated peptides).[98] The product of *PTPRC*, receptor-type tyrosine-protein phosphatase C, is a transmembrane receptor-like molecule expressed on the surface of nucleated hematopoietic cells that regulates not only B-cell-receptor and T-cell-receptor signaling, but also TNF secretion by monocytes.[99,100]

Mitogen-activated protein kinases (MAPKs) are crucial in several inflammatory pathways in RA, especially in the

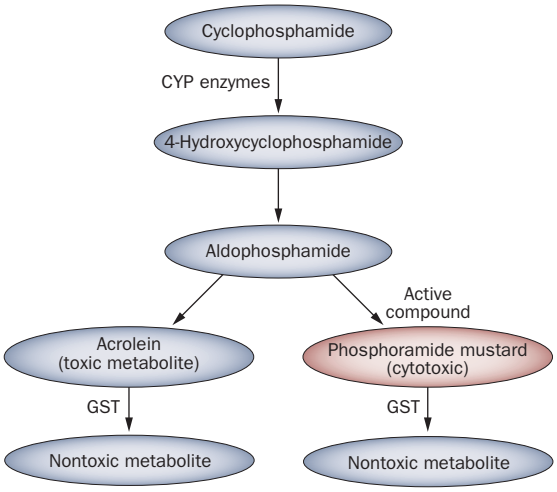

Figure 6 | Metabolism of cyclophosphamide. Cyclophosphamide is metabolized in the liver by CYP enzymes, leading to the production of active and inactive metabolites. CYP enzyme gene polymorphisms are implicated in suboptimal responses to cyclophosphamide. The toxic metabolites of cyclophosphamide are detoxified by GST enzymes; *GST* variant alleles have been associated with cyclophosphamide toxicity. Abbreviations: CYP, cytochrome P450; GST, glutathione *S*-transferase.

Table 5 | Pharmacogenetics of biologic agents

Gene, product and role	Variant	Cellular effects of variant	Studies, designs and participants	Reported clinical effects
TNF blockers				
TNF locus, TNF and its receptors	–308 G>A	Influences TNF production	Seitz (2007),[91] prospective, *n*=86; 54 patients with RA, 10 with PsA, 22 with AS	Associated with increased efficacy
			Cuchacovich (2006),[92] prospective, *n*=81; all patients with RA	Associated with increased efficacy
			Lee (2010),[93] meta-analysis (13 studies), 1,817 patients with RA	No effect on efficacy
	–238A>G	Influences TNF production	Lee (2010),[93] meta-analysis (13 studies), 1,817 patients with RA	Associated with increased efficacy
	TNFRSF1B 196 T>G	Influences receptor shedding and ligand binding; might increase IL-6 production	Fabris (2002),[94] retrospective, *n*=175; all patients with RA	Associated with decreased efficacy
			Potter (2010),[95] retrospective, *n*=602, all patients with RA	No effect on efficacy
	TNF microsatellites a–e	Affect DNA folding and conformation	Martinez (2004),[96] prospective, *n*=420; 78 patients with RA, 342 healthy controls	No effect on efficacy with individual microsatellites; *TNFa11;b4* haplotype more frequent in responders to infliximab
			Criswell (2004),[97] prospective, *n*=457; all patients with RA	No effect on efficacy
FCGR genes, FcγR, receptors for IgG	FcγR IIIA Val158Phe	Influences FcγR affinity for IgG1; affects ADCC	Kastbom (2007),[90] prospective, *n*=282; all patients with RA	No effect on efficacy
PTPRC, receptor-type tyrosine-protein phosphatase C (CD45), regulates BCR and TCR signaling, and secretion of TNF by monocytes	rs10919563	Influences the secretion of cytokines including TNF	Cui (2010),[98] prospective, *n*=1,283; all patients with RA	Associated with increased efficacy
MAPK14, MAPK 14, signaling molecule involved in production of proinflammatory cytokines and MMPs	SNPs in *MAPK14* and in genes encoding proteins upstream and downstream in signaling pathway	Influence MAPK signaling pathway, production of cytokines and MMPs	Coulthard (2011),[102] prospective, *n*=1,102; all patients with RA	Associated with increased efficacy of infliximab and adalimumab, but not etanercept
Rituximab				
FCGR genes, FcγR, (receptors for IgG)	FcγR IIIA Val158Phe	Influences FcγR affinity for IgG1, affects ADCC	Anolik (2003),[107] prospective, *n*=12; all patients with SLE	Associated with increased efficacy
			Pers (2007),[106] prospective, *n*=15; all patients with primary Sjögren's syndrome	No effect on efficacy

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; FcγR, Fcγ receptor; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SNP, single nucleotide polymorphism.

production of proinflammatory cytokines and matrix metalloproteinases.[101] In a large UK cohort of 1,102 patients with RA, seven SNPs in five genes encoding proteins in signaling pathways upstream and downstream of MAPKs, and a MAPK isoform, were nominally associated with a better response to infliximab and adalimumab, but not etanercept.[102]

Rituximab

Similar to anti-TNF agents, responses to rituximab—a monoclonal antibody to CD20 effective in the treatment of RA and SLE—might also be determined by FcγR polymorphisms. Rituximab causes cell lysis through complement-dependent cytotoxicity, antibody-dependent cytotoxicity, and induction of apoptosis.[103] Studies of FcγR IIA and IIIA polymorphisms as predictors of response to rituximab in the treatment of lymphomas and leukemias have yielded inconsistent results.[104,105] Similarly, in rheumatic diseases, the FcγR IIIA Val158Phe polymorphism did not predict the efficacy of rituximab in patients with Sjögren's syndrome,[106] but was helpful in predicting the drug's efficacy in patients with SLE (Table 5).[107]

Future directions

Rigorous interpretation of the various pharmacogenetic studies we have described is, at present, difficult and confounded by several factors. For example, the proximity of the HLA and TNF loci, and the profound known impact of these loci on the susceptibility to and severity of diseases such as RA and SLE, complicates the interpretation of pharmacogenetic studies of TNF antagonists. The results from such studies can be skewed, because the same genetic

variants that are potential predictors of response to therapy might also be markers of more severe disease, which might respond more robustly to anti-TNF treatments.

Other factors complicate how we construe the results of these studies. Diseases such as RA and SLE are complex, polygenic diseases, with considerable phenotypic and genetic heterogeneity that (so far) precludes neat categorization of patients by their predicted responses to specific drugs. Indeed, several mechanisms might be operational in these disease states, and focusing on only a few genes, and variations within, as predictors of drug response might not be fruitful. Similarly, several drugs used to treat rheumatic diseases affect a multitude of genes and pathways, and the few variants of such pathways that have been studied to date might be woefully inadequate. For this reason, unless the functional significance of a single gene variant is unequivocally established, determination of haplotypes and/or of multigene signatures in candidate regions (such as *TNF* and *MTHFR*) rather than of individual SNPs, might yield stronger predictive capability, although such approaches can be more costly in terms of time and money. There is also the issue of access to synovial tissue. Ideally, genetic studies should use synovial tissue—the 'target organ' of inflammatory arthritis—but unlike studies in oncology (where tumor tissue is almost always available), synovial tissue is, unfortunately, rarely obtained during diagnosis, and DNA from peripheral blood has to suffice. Finally, it is worth reiterating that most pharmacogenetic studies to date have been underpowered because of small sample sizes, and performed in racially homogeneous populations (the exceptions are few[108,109]), bringing the validity and reproducibility of these results in other populations into question. Large, prospective, multicenter, multiethnic studies are needed to overcome these problems.

Genome-wide association studies (GWAS) in pharmacogenetics are just emerging, and are fraught with challenges. One issue is overcoming the problem of multiple comparisons; that is, when 500,000 to 1,000,000 SNPs are analyzed, teasing out the SNPs with real effects on drug response can be difficult, especially when the effects are modest. Added to this problem is the multifactorial nature of most drug responses, making small genetic effects hard to detect in data from GWAS. When pharmacogenetic findings from one GWAS need to be replicated, choosing a replication population which is closely matched in demographics, drug administration, and the phenotype under study, can be a daunting task. Finally, small sample sizes and the lack of replication studies (the bane of most pharmacogenetic studies in rheumatic diseases, as has been pointed out in this Review) might limit the integration of GWAS into pharmacogenetics. On the other hand, highly efficient genotyping platforms that are now available might expedite the adoption of GWAS by pharmacogenetics.[110]

Whether pharmacogenetics is ready for 'prime time' (that is, integration into clinical practice) will also be determined by the cost-effectiveness of these approaches.[111] Drugs with a narrow therapeutic index and severe, expensive adverse events are the ideal candidates for pharmacogenetic testing, and its clinical application

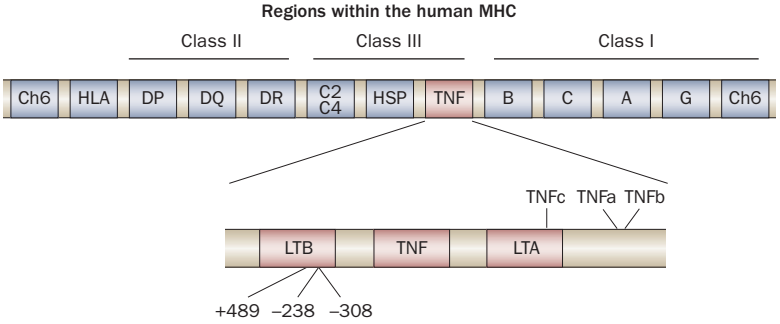

Figure 7 | The TNF locus, with some of the polymorphic sites that are thought to influence the outcome of anti-TNF therapy. *LTB*, *TNF* and *LTA* (which encode 3 members of the TNF [ligand] superfamily) make up the TNF locus. Intronic and promoter region polymorphisms of these genes are reported to modulate the effects of anti-TNF therapy. TNF microsatellites TNFa–TNFe, which contain variable numbers of repeat sequences, are also located in the TNF locus, and are reported to affect the production of TNF. Abbreviation: TNF, tumor necrosis factor. Reproduced from Ranganathan, P. Pharmacogenomics of tumor necrosis factor antagonists in rheumatoid arthritis. *Pharmacogenomics* **2**, 279–282 (2005), by permission of Future Medicine Ltd.

to the prescription of such drugs will result in substantial cost savings. An example (as we have discussed) is the use of *TPMT* genotyping before initiation of azathioprine, which is becoming standard of care in most clinical practices. This test has been shown to be a cost-effective approach, with the advantage of sparing patients from aggravating and sometimes fatal toxicities.[111,112]

Conclusions

A vast, growing body of literature describes the pharmacogenetics of drugs used in the treatment of rheumatic diseases. Gene variants in specific cellular pathways modified by oral agents, such as *MTHFR* in the folate pathway (for methotrexate), *TPMT* in the methylation pathway (for azathioprine), and *NAT2* in the acetylation pathway (for sulfasalazine), have been the targets of pharmacogenetic studies, and do seem to influence patients' responses to these drugs. The era of biologic agents such as TNF antagonists (which, although highly effective treatments for inflammatory arthritides, are expensive, are not consistently efficacious in all patients, and are associated with adverse events including an increased risk of infection and malignancy), has only intensified the need to predict which patients will be helped by specific drugs. Numerous studies have focused on variations in genes such as *TNF* (including *TNF* microsatellites), *TNFRSF1A* and *TNFRSF1B*, FcγR genes, MAPK signaling pathway genes, and genes that influence RA susceptibility, as a means of pre-selecting patients with the greatest likelihood of response to these agents. Nevertheless, with the exception of the *TPMT* genotyping assay as a predictor of azathioprine toxicity, no pharmacogenetic tests are currently in clinical use; even the *TPMT* genotyping assay has not been formally validated by the FDA.

The ultimate goal of pharmacogenetics in rheumatology is to define genetically distinct subsets of patients who have differential responses to the various therapies used to treat rheumatic diseases. The ideal pharmacogenetic

assay would quickly, accurately and inexpensively provide composite genotypes for an individual patient, to enable selection of the most suitable drug for that patient. Although it should be acknowledged that such an assay is currently unavailable, the commitment of major funding agencies to pharmacogenetic research is evident through the establishment of the International HapMap Consortium[113] and the Pharmacogenetics Research Network[114] by the National Institutes of Health. Continued research in this burgeoning field is sure to fulfill the promise of individualized drug therapy in the rheumatic diseases in the near future.

Review criteria

Published articles for inclusion in this Review were chosen from the senior author's (P. Ranganathan) repertoire of papers on pharmacogenetics in rheumatoid arthritis and systemic lupus erythematosus, collected between 1999 and 2011, and from PubMed searches using terms "pharmacogenetics", "rheumatoid arthritis", "systemic lupus erythematosus", "methotrexate", "azathioprine", "sulfasalazine", "hydroxychloroquine", "leflunomide", "cyclophosphamide", "TNF antagonists", and "rituximab". Only full-text papers published in English were included.

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Author contributions
Both authors contributed equally to all aspects of the preparation of this manuscript.