Future treatment of rheumatic diseases: the role of pharmacogenetics

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'The identification of single nucleotide polymorphisms for relevant genes relating to drug targets and metabolic pathways has provided a natural set of predictive biomarkers to be explored in pharmacogenetic research.'

In the past decade, the development and governmental agency approval of numerous targeted biologic agents for the treatment of rheumatic diseases has provided an unprecedented array of highly efficacious therapeutic agents. In addition, recent advances in our understanding of metabolic pathways relating to synthetic DMARDs, including methotrexate (MTX) and azathioprine, have provided a rational basis for exploring interindividual variation in drug response and toxicity.

At the same time, the completion of the human genome sequencing project has been a major advance in the field of human genetics. The identification of single nucleotide polymorphisms (SNPs) for relevant genes relating to drug targets and metabolic pathways has provided a natural set of predictive biomarkers to be explored in pharmacogenetic research. Moreover, the development of rapid, high-throughput methods to genotype these SNPs has provided researchers with unprecedented tools for these studies. As the technology advances, the diminishing costs of performing these studies has led to the emergence of pharmacogenetic studies in multiple disciplines, including the rheumatic diseases.

However, despite these advances and opportunities, the emerging pharmacogenetic data in the rheumatic diseases remain difficult to interpret. There are a number of likely explanations for the conflicting evidence, and it is likely that larger, well-designed studies will be required before most pharmacogenetic biomarkers of response and toxicity will be ready for utilization in clinical practice. Nevertheless, promising studies are emerging for agents used for the treatment of rheumatoid arthritis (RA) and other rheumatic diseases.

Methotrexate pharmacogenetics

The recent development of many new targeted therapies for RA has led to many new and effective treatments. However, virtually all of them are used in conjunction with MTX, a slower acting, oral DMARD. The initial clinical trials showing its efficacy were conducted in the mid-1980s [1–4]. There is marked variability in the efficacy and toxicity among RA patients treated with MTX, however, leading to an interest in the pharmacogenetics of the drug.

MTX is a structural analogue of folic acid; it enters the cell via the reduced folate carrier SLC19A1, and is then activated to MTX polyglutamates (MTXPGs) via a γ-glutamyl hydrolase (GGH). It blocks the action of dihydrofolate reductase (DHFR), inhibiting formation of thymidylate, inosinic acid and other purine metabolites; it also impairs protein synthesis by interfering with the conversion of amino acids. MTX is metabolized from a monoglutamate to a polyglutamate, which can inhibit other enzymes in addition to DHFR, including thymidylate synthetase (TYMS) and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase (also called ATIC). Other downstream proteins are also involved in the efficacy and toxicity of MTX, including methylenetetrahydrofolate reductase (MTHFR).

Several different genetic polymorphisms have been examined for both efficacy and toxicity. In 2002, Urano and colleagues studied 106 patients with RA and reported on two polymorphisms in the *MTHFR* gene, showing that the A1298C SNP was associated with greater efficacy (relative ratio [RR]: 2.18; confidence interval [CI]: 1.17–4.06) reflected in a lower average dose of MTX, and that the C677T SNP was associated with greater toxicity (RR: 1.25; CI: 1.05–1.49) [5]. It is difficult to extrapolate these results to broader practice, however, as a threshold of greater than 5 mg/week is not the benchmark used for defining lack of efficacy in clinical practice or clinical trials. The authors do give data demonstrating no significant changes in tender or swollen joints related to the SNPs studied.

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Other authors have examined these SNPs as well. Berkun and colleagues (n = 93 patients) found the A1298C SNP protective from MTX side effects, defined as the presence of any of gastrointestinal symptoms, oral ulcers, alanine aminotransferase (ALT) levels more than doubling, induced or aggravated skin nodules, or leukocyte count below 3500/mm^3 (odds ratio [OR] AA vs CC 5.24, CI 1.38–20) [6]. They found no correlation between the A1298C or the C677T SNPs with efficacy. Hughes *et al.* examined the relationship between these SNPs and ethnicity [7], and found among Caucasians the 1298AA genotype was associated with a significant increase in adverse events, defined similarly though not identical to Berkun's criteria (OR: 15.86; CI: 1.51–167.2). This is consistent with other authors' findings, and that another *MTHFR* gene mutation found in African–Americans, the rs4846051 C allele, was also associated with adverse events (OR not given). Aggarwal and colleagues examined 150 patients with RA, and found no correlation between the C677T polymorphism and efficacy or toxicity [8].

More recently, Wessels and colleagues studied these SNPs more rigorously, examining 205 patients over a period of 6 months, and allowing for titration of MTX [9]. They found the 1298AA genotype associated with good improvement (based on change in the European League Against Rheumatism Disease Activity Score, [EULAR DAS]) relative to 1298C (OR: 2.3; CI: 1.18–4.41); they also found that the 677CC genotype was associated with better improvement. They found that the 1298C allele carriers had more toxicity, again similarly defined as the presence of any of gastrointestinal events, liver adverse drug events, pneumonitis, skin and mucosal disorders or leukopenia, (OR: 2.5; CI: 1.32–4.72). Taniguchi and colleagues examined 159 patients looking at the same SNPs (at 1298 and 677) [10], and found the C677T SNP associated with greater adverse events, although the authors do not clearly define what these events are (RR: 24.6; CI: 2.37–254.43), and that patients with the A1298C mutation required lower doses of MTX (RR: 1.84; CI: 1.12–3.01). Of note, this is one of the few prospective pharmacogenomic studies. The contradictory results found by the different authors are most likely due to differences in allele frequencies between the different groups studied, also referred to as population stratification, different definitions of efficacy and toxicity and type I and II statistical errors.

In 2003, Kumagai and colleagues examined 219 RA patients, looking at *TYMS* and *MTHFR* gene polymorphisms [11]. They found that patients homozygous for a triple repeat (3R/3R) polymorphism in the *TYMS* gene promoter region required a higher dose of MTX to control their disease (cut-off, 6 mg/week), and that patients homozygous for a variant deletion (3′UTR) had a significantly greater C-reactive protein response to MTX therapy. They did not find any associations with toxicity for the TYMS SNPs studied, and they found no association between the MTHFR polymorphisms C677T or A1298C for either efficacy or toxicity.

Dervieux and colleagues determined (n = 226) whether a G80A SNP in *SLC19A1* or a -401C/T promoter polymorphism in *GGH* would affect MTXPG levels [12], and found that patients with the SLC19A1 80AA genotype were 3.4-fold more likely to have elevated MTXPG levels compared with the group median than either the GG or GA genotype (CI: 1.4–8.4), and that the -401TT genotype was associated with lower levels of MTXPG compared with the group median (OR: 4.8; CI: 1.8–13.0). The same authors subsequently examined 108 patients looking at the SLC19A1 G80A, GGH -401C/T, and an ATIC C347G polymorphism to assess levels of MTXPG as well as patient response [13]. While the authors illustrate a relationship between homozygosity of all three SNPs correlating with higher MTXPG levels and greater efficacy, they did not describe the effects of individual polymorphisms. Wessels *et al.* also looked at the same ATIC C347G polymorphism [14], and found an association with greater toxicity of MTX (OR: 2.0; CI: 1.1–3.7). Drozkik and colleagues also examined the SLC19A1 G80A polymorphism [15], and found an association between the AA genotype and remission (OR: 3.32; CI: 1.26–8.79) and response (OR: 1.78; CI: 1.13–2.81).

'The studies on MTX pharmacogenomics are quite inconsistent.'

A number of other polymorphisms have been examined, including the *MDR1* gene 3435 C>T, showing an association with remission (OR: 4.65; CI: 1.66–13.05), the *AMPD1*, *ATIC* and *ITPA* genes collectively with 'good clinical response', (OR: 2.1: CI: 1.0–4.5, and OR: 2.7; CI: 1.1–8.1, respectively), the HLA-G 14bp polymorphism deletion (OR: 2.46; CI: 1.26–4.84),

and methionine synthase reductase (*MTR*) gene A2756G polymorphism, which was associated with MTX-induced nodulosis [14,16–18].

Other authors have found no association at all despite looking at multiple SNPs, including Takatori and colleagues, who examined the *SLC19A1* gene G80A, *MDR1* gene C3435T, and *ATIC* gene C347G polymorphisms [19].

The studies on MTX pharmacogenomics are quite inconsistent, most likely because the published studies have small sample sizes and are also likely to be ethnically different, impacting the penetration of the different polymorphisms in the different studies. In addition, it is unclear what negative and unpublished literature may exist, further challenging the goal of creating a pharmacogenomic profile. In addition, the studies on MTX polymorphisms also use different definitions for efficacy and toxicity, making it difficult to extrapolate from the literature what is truly significant. While some attempts have been made to address these difficulties, until universal definitions are adopted or larger studies are completed, the association between a given polymorphism and toxicity or efficacy will remain uncertain.

TNF-antagonist pharmacogenetics

The development of targeted biologic agents for the treatment of RA has changed the field of RA therapeutics, and consequently raised the question of whether genetic biomarkers can predict response. TNF antagonists have become the gold standard of biologics, against which newer biologic agents are compared for efficacy, safety and radiographic outcomes. Nevertheless, even patients treated with TNF-α antagonists fail to achieve the American College of Rheumatology (ACR) 20 responses in 25–30% of RA patients in randomized clinical trials [20–23]. Because of the specificity of the biologic target of TNF-α-antagonists, investigators have explored the role of TNF and TNF receptor gene polymorphisms as predictors of failed response to TNF antagonists. However, the literature is somewhat conflicting, primarily because many of the published studies have relatively small sample sizes. Moreover, it is unclear the extent to which publication bias may further distort our knowledge from the literature, as negative studies would be less likely to be submitted for publication or accepted by journals.

Only the TNF-α -308 G/A SNP has been replicated in multiple studies, although conflicting studies exist as well. Mugnier and colleagues reported that the -308 G/A SNP of the *TNF* gene was associated with improvement in disease activity score (DAS)-28 in a study of 59 RA patients treated with infliximab [24]. Fonseca *et al.* also observed that the -308 G/A SNP of the *TNF* gene was associated with decrease in DAS-28 scores for patients treated with infliximab [25]. However, two other studies reported no association with response to infliximab for the -308 G/A SNP, including a study of 78 RA patients by Martinez and coworkers [26,27]. Finally, a study by Padyukov failed to observe an association of the -308 G/A SNP with response to etanercept, although they did observe an association of the -308 G/A SNP in combination with the -1087 GG SNP of the *IL10* gene predicted response.

> '…two extended haplotypes including HLA-DRB1 alleles and six SNPs in the lymphotoxin-α/TNF region were also associated with response to treatment.'

To date, the two largest pharmacogenetic studies of TNF-antagonists have been substudies of industry-sponsored randomized clinical trials, and therefore have included fewer than 500 patients. The first study was based on the etanercept early RA (ERA) randomized clinical trial of 457 patients [28]. Criswell and colleagues defined response using the ACR-50 response to define the phenotype of response. They reported that the presence of two HLA-DRB1 alleles encoding the shared epitope (SE) was associated with achieving an ACR50 (OR: 4.3; 95% CI: 1.8–10.3). Moreover, they observed that two extended haplotypes including HLA-DRB1 alleles and six SNPs in the lymphotoxin-α/TNF region were also associated with response to treatment.

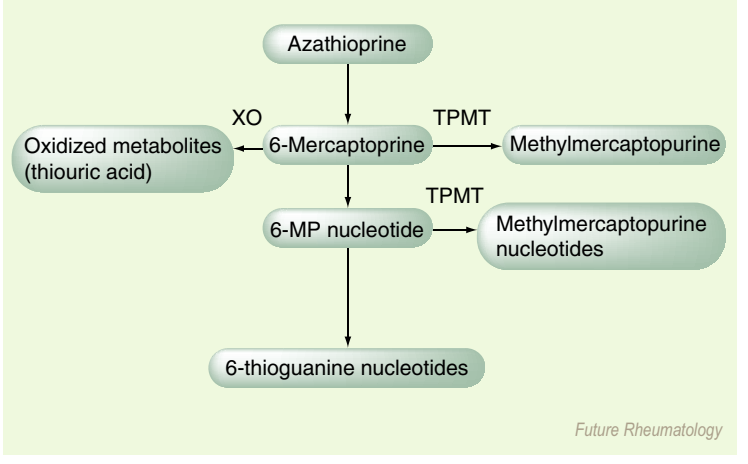
More recently, a pharmacogenetic substudy (n = 396) of the Research in Active Rheumatoid Arthritis (ReAct) randomized clinical trial of adalimumab was published [29]. Using ACR50 response as the primary outcome, the HLA-DRB1 shared epitope alleles were not associated with response. In addition, three SNPs of the *TNF* gene (-857C/T, -308A/G and -238A/G) were not individually associated with ACR50 response. However, a common haplotype combination of the three *TNF* gene SNPs was associated with a lower ACR50 response (34%) than other haplotypes (ranging from 47 to 71%), p = 0.004. Few of the other published studies have incorporated extended haplotype analysis into their work, thus precluding comparison of these study results with previously published studies.

The potential reason underlying the conflicting nature of the results of the TNF-antagonist pharmacogenetic studies include differences in TNF-antagonist agents (monoclonal antibodies to TNF vs a TNF soluble receptor that binds both TNF and lymphotoxin [LTA]), false negative results due to inadequate statistical power, as well as possible false positive results due to multiple testing. Finally, failure to account for possible population stratification may influence study results as well. Racial and ethnic differences in allele frequencies have been reported for a number of RA susceptibility genes, including those encoding SLC22A4, PTPN22 and TNF receptors, and thus population stratification may require novel statistical methods to account for these differences [30,31]. Systemic differences in ancestry between cases and controls can indeed lead to erroneous study conclusions [32,33]. Statistical approaches including genomic control using a panel of noncoding SNPs without any known association to the study phenotype can detect and adjust for these differences [33–35]. To date, none of the TNF-antagonist pharmacogenetic studies have incorporated population stratification adjustment. Finally, linkage disequilibrium between SNPs can also confound pharmacogenetic studies, and only a minority of the TNF-antagonist pharmacogenetic studies accounted for linkage disequilibrium and incorporated haplotype analyses.

Azathioprine pharmacogenetics

Azathioprine, commonly used in the treatment of systemic lupus erythematosus (SLE) and RA, is converted to 6-mercaptopurine in the liver which is further metabolized by xanthine oxidase or metabolized by thiopurine methyl transferase (TPMT) and the products excreted (**Figure 1**). The therapeutic effects of the drug result from the 6-thioguanine nucleotides that are incorporated into DNA by proliferating cells. Incorporation of 6-thioguanine nucleotides into DNA results in cytotoxicity. It is well known that accumulation of thiopurine metabolites is associated with severe marrow suppression, and within the past few years both genetically determined alterations in enzyme activity and the genetic polymorphisms associated with diminished TPMT activity have been elucidated [36].

Figure 1. Metabolism of azathioprine.



> '…susceptibility to severe marrow toxicity induced by standard doses of azathioprine can be predicted based on the genotype of the patient.'

In general, 10–11% of most Caucasian populations are heterozygous for the wild-type gene and a common variant (G460A and A719G). Homozygosity for the variant allele puts an individual at risk of developing catastrophic marrow suppression following standard doses of azathioprine. Heterozygous patients are at increased risk of developing marrow toxicity. Different genetic variants seem to occur with similar frequency in other ethnic and racial groups. Thus, susceptibility to severe marrow toxicity induced by standard doses of azathioprine can be predicted based on the genotype of the patient. Although use of azathioprine in the treatment of rheumatic diseases has diminished, it remains a potent and effective agent in the right circumstances. Genotyping of individuals can be carried out so as to avoid inducing disastrous marrow toxicity in susceptible individuals.

Conclusion

The development of genetic biomarkers that can predict response and toxicity to antirheumatic drugs represents a promising field of research. However, the absence of adequately powered, rigorous studies have led to somewhat conflicting evidence, particularly for TNF-antagonist pharmacogenetic studies. Ultimately, pharmacogenetic biomarkers will have to meet standard criteria of evidence-based medicine for laboratory tests prior to their widespread use by clinicians.

Financial & competing interests disclosure

Bruce N Cronstein holds patents on use of adenosine A2A receptor agonists to promote wound healing and use of A2A receptor antagonists to inhibit fibrosis. Patent on use of adenosine A1 receptor antagonists to treat osteoporosis and other diseases of bone. Within the past 2 years, he has undertaken consultation work for King Pharmaceuticals (licensee of patents above), CanFite Biopharmaceuticals, Bristol-Myers Squibb, Cellzome, Tap Pharmaceuticals, Prometheus Laboratories, Regeneron (Westat, DSMB), Sepracor, Amgen, Endocyte, Protalex, Allos, Inc., Combinatorx and Kyowa Hakka; is on the Honoraria/Speakers' Bureaus for Tap Pharmaceuticals and Amgen; holds stock in CanFite Biopharmaceuticals (received for membership in Scientific Advisory Board) and received grants from King Pharmaceuticals and the NIH.

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