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Chapter 8

Pharmacogenetics of TNF inhibitors in rheumatoid arthritis

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Abstract

Etanercept, infliximab and adalimumab have shown clinical benefit in immune-mediated inflammatory diseases; however, the outcome of treatment with these tumour-necrosis factor inhibitors remains insufficient in 40–60% of individuals with rheumatoid arthritis. Moreover, their use is accompanied by adverse events and unintentional immune suppression. Pharmacogenetics has the potential to increase efficacy and ameliorate adverse events and immune suppression, and its application might be of clinical benefit for patients with rheumatoid arthritis. Pharmacogenetic studies have shown associations between single nucleotide polymorphisms in genes encoding enzymes related to the pharmacodynamics and pharmacokinetics of these drugs and treatment outcome. As we discuss here, replication and prospective validation are warranted before pharmacogenetics can be used in clinical practice.

Introduction

Although the pathogenesis of many autoimmune diseases including rheumatoid arthritis remains unknown, studies have shown that tumour-necrosis factor- α (TNF α) has a key role in the inflammatory process of these immune-mediated disorders. TNF α is known to play a leading role in stimulating cytokine (including its own) and chemokine production, in enhancing neutrophil, chondrocyte and osteoclast activation, expression of adhesion molecules. Also, it facilitates also as a co-stimulator of T-cell activation and antibody production by B cells (1;2).

Consequently, TNF α has emerged as an important target in novel therapeutic strategies used to treat rheumatoid arthritis. The anti-TNF targeting drugs currently used in daily clinical practice are etanercept (Enbrel®), infliximab (Remicade®) and adalimumab (Humira®). Etanercept is a human, soluble, dimeric TNF type II receptor linked to an IgG1 Fc half that binds to and inactivates TNF α . The chimaeric IgG1 monoclonal antibody infliximab and the complete humanized IgG1 monoclonal antibody adalimumab bind to TNF α with high affinity and

thereby inactivate it. The therapeutic effect of these biological agents is achieved by blocking the potential interaction of TNF α with the accessory TNF cell-surface receptors (3;4). In vivo and (more in) in vitro studies have demonstrated that this effects in neutralization and blockage (5;6); interaction with Fc receptor (cross-linkage) (7); initiation of reverse signalling, leading to blockage, increased apoptosis or growth arrest (8;9); reduction of inflammatory cytokine production and angiogenic factor expression (6;10-12); Mediation of complement-dependent cytotoxicity and antibody-dependent cytotoxicity (5;9); down-regulation or discontinuation of bone and cartilage destruction (13;14). In this manner, an expanding array of drug therapy options for the treatment of rheumatoid arthritis in the clinic has been established over the past decade (15;16).

However, high costs, adverse drug events and unintentional concomitant immune suppression, leading to serious (opportunistic) infections, present limitations that might prevent the prescription of these biological drugs (17;18). For example, Bongartz et al. (19) have provided evidence for a higher risk of serious infections (odds ratio (OR) 2.0) and a dose-dependent, increased risk of malignancies (OR 3.3) in patients with rheumatoid arthritis who are treated with anti-TNF antibody therapy. Another limitation is that the treatment outcome of the TNF inhibitors remains insufficient in 40–60% of patients with rheumatoid arthritis (20-22).

Pharmacogenetics has the potential to increase drug efficacy and to ameliorate adverse events and immune suppression. Its application might be of great clinical benefit for individuals affected with rheumatoid arthritis. Studies have shown associations between single nucleotide polymorphisms in genes encoding enzymes related to the pharmacodynamics of the anti-TNF drugs used to treat this disease and treatment outcome. The ultimate aim of using pharmacogenetic markers is to predict the probability of a wanted or unwanted drug response in individual patients (23). Replication and prospective validation are warranted before pharmacogenetics can be used in clinical practice (24).

Here, we review the potential of pharmacogenetics and its impact on anti-TNF therapy outcome in individuals with rheumatoid arthritis.

Diagnostics and therapeutics of rheumatoid arthritis

Treatment of rheumatoid arthritis usually follows a stepwise approach that is based on the evaluation of disease activity and radiological progression of joint damage (21). The most commonly used measure to evaluate disease activity is the '28-joint disease activity score' (DAS28), which includes

an assessment of 28 joints for swelling and tenderness, the erythrocyte sedimentation rate and a general health assessment using a visual analogue scale (25). To assess disease activity in clinical trials, specific improvement and response criteria have been developed (26). These American College of Rheumatology (ACR) improvement criteria are based on a perceptual improvement (20, 50, 70 and 90%) in disease symptoms (termed ACR20, ACR50, ACR70 and ACR90, respectively). The ACR criteria measure only change (dichotomous outcome), whereas the DAS28 measures both change and the extent of rheumatoid arthritis (continuous outcome). The validity of both methods for describing the results of randomized clinical trials is comparable (25;27). In this way, data integration is being performed by presenting both criteria in clinical trials. Radiological damage is evaluated using the Sharp–van der Heijde score, which assesses erosions and joint space narrowing of joints of hands and feet in rheumatoid arthritis (28).

In daily clinical practice, the design of a therapy plan would ideally be based on monitoring disease activity and strict treatment scheduling to prevent functional disability (29). First, patients would be treated with disease-modifying antirheumatic drugs (DMARDs), of which methotrexate is the first drug of choice (30;31). Should there be an unfavourable response, side-effects and/or drug toxicity, therapy would then be escalated to include biological agents, such as TNF inhibitors, either alone or in combination with DMARDs, and modifications of the dosing regimen (20;22;32). After the onset of disease, tightly scheduled management of treatment is required to maintain efficacy (33). Because high and variable disease activity results in joint damage, effective intervention with TNF inhibitors, either as a monotherapy or in combination with DMARDs, can halt the progression of radiological damage, which consequently translates into a slowing or cessation of functional decline (34;35).

Pharmacogenetics of TNF inhibitors

Pharmacogenetics holds the promise not only to explain interindividual variability in drug response, but also to predict efficacy and adverse drug events in different patients (23). Importantly, several studies have revealed that failure to respond to TNF blocking drugs is not a class effect, but instead is related to the individual drug. For example, the response rates to adalimumab have been evaluated in patients who were unresponsive to etanercept or infliximab (36). Remarkably, the response rates measured were similar to those in patients not previously exposed to TNF blocking agents, which makes a class effect of these agents unlikely (20). The results of several studies in which anti-TNF treatment has been switched underline the pharmacogenomic independency of these drugs (37;38). Therefore, pharmacogenetic results are presented in this review with annotation of the specific TNF inhibitor.

Studies have gathered considerable information on drug interaction with, and mediation of, the cytokine TNF α (2). The fact that these drugs target TNF α has led to interest in TNF α itself as a candidate gene for pharmacogenetic association studies. In recent years, many polymorphisms in genes encoding proteins related to TNF α have been identified that might be associated with treatment outcome. Such candidate gene polymorphisms have been investigated for their ability to predict treatment outcome in patients with rheumatoid arthritis receiving anti-TNF drugs.

The polymorphism in the promoter region of *TNF* SNP –308A>G is one of the most studied variation in *TNF* gene (Table 1). However, results considering an association of infliximab, etanercept or adalimumab efficacy with this polymorphism are inconsistent. For etanercept, six studies reported an association with this SNP (39–44) with half of these studies finding an association with clinical response. Specifically, patients genotyped for *TNF* –308 GG were likely to obtain a better response compared to patients with an A-allele genotype (40;42;44). This was further elucidated by a meta-

analysis performed by Lee et al in order to compare the results concerning the *TNF* -308A>G. After inclusion and analysis of six studies, it was seen that patients carrying an A allele have a poorer response to anti-TNF therapy than those with the G allele (45). This *TNF* -308A>G polymorphism was also studied in relation with treatment outcome and infliximab. The majority of these studies also found a positive association of the GG genotype with infliximab efficacy (42;44;46-49).

However, regarding treatment with adalimumab, an association was found with the G-allele, as part of a single *TNF* haplotype (238G/-308G/-857C) and inefficacy (50). Additionally for the SNP -857 C>T in the *TNF* gene it was reported that T-allele carriers were associated with a positive response to etanercept therapy (41).

Interestingly, it is hypothesized that altered binding capacities of the two TNF α receptors (TNFRSF1A and TNFRSF1B) for TNF α is due to genetic variation in these receptors (51). Therefore, SNPs in genes encoding these receptors are linked to treatment outcome in several reports. Initially in one study, three SNPs within the *TNFRSF1A* gene were explored (at positions -609, -580, -383), but no associations with response to etanercept was seen (39).

More data are available about the SNP +676 T>G in the *TNFRSF1B* gene. Specifically, three reports demonstrated a link with the G-allele and inefficacy to infliximab and etanercept, whereas other studies reported no effect of this SNP on treatment outcome to all three TNF inhibitors (39;52-55).

Besides anti-TNF α neutralizing properties, TNF inhibitors may involve in effects via their IgG1 Fc fragments, for example by complement activation and binding to cellular Fc-gamma receptors (FcR) (56). Hypothetically, ligation of the low-affinity FcR type IIIA can induce apoptosis in synovial macrophages. However, significant effects of *FcR IIIA* -158V>F on the efficacy of infliximab and etanercept were not found (39;54;56). In contrast, increased efficacy was found with the -158 FF genotype in two smaller cohorts of patients treated with either of the three TNF inhibitors (57;58). Regarding the SNP -131R>H within *FcGR IIA* gene, an association was found with a positive clinical response and patients with the homozygous wild-type genotype (58). However the whole genetic region of the Fc-gamma receptors is characterized by extensive gene duplication and the presence of insertions and deletion. This all lead to a highly polymorphic locus and no studies have been published that the full variability of the locus into account.

It is thought that the pro-inflammatory cytokine IL-1 enhances the inflammatory response in RA (2). This is also seen in clinical trials, in which RA patients were successfully treated with the IL1-receptor antagonist anakinra (59). Therefore, genetic variation within genes coding for IL1 or its receptor could potentially influence treatment outcome to TNF inhibitors. In three studies, SNPs within the genes *IL-1B* and *ILRN* were assessed (43;60;61). Only one association was found in a small cohort of patients: carriage of the number of tandem repeats within the *IL1RN* gene was associated with obtaining a better clinical response to infliximab therapy (61).

Notably, anti-inflammatory cytokines, such as IL-10, could also influence TNF-inhibitor therapy outcome. Indeed, it is demonstrated that the SNP *IL-10* -1087G>A is associated with an increased IL10 production and hereby anti-inflammatory response (62). However, this result was not detected in an association study with a clinical endpoint to measure efficacy in etanercept treatment (43). One other study focused on two *IL-10* promoter microsatellite polymorphisms, *IL10.R* and *IL10.G*, which have been shown to be related with IL-10 secretion (63). A positive response was associated with carriage of the R3 allele or R3-G9 haplotype, whereas the allele G13 and the haplotype R2-G13 were present in patients with moderate or no response (64).

For many years it is known that *HLA-DRB1* shared epitope (SE) is associated with susceptibility to RA. More recently, other SNPs, for example in the *PTPN22* gene, have been associated with susceptibility to RA. Patients with mutations in these genes are likely to have a more severe disease activity state and at baseline compared with patients not having mutations in these disease-susceptibility genes. In contrast,

an association between *HLA-DRB1* (SE) and treatment outcome was only reported in one of seven studies (39;41;50;60;65-67). In this study, two *HLA-DRB1* alleles encoding the SE were related to a positive clinical response to etanercept treatment (39).

Only recently, a genome-wide association study using a 300K-SNP array was performed to analyze response in anti-TNF treatment (etanercept, infliximab and adalimumab). Four SNPs were significantly associated with treatment outcome in this genome-wide analysis in loci *MAFB*, *IFN γ* , *PON1* and *IL10* genes. However, replications of these SNPs in independent and larger data sets are required due to the small sample size used in this study (68) (not displayed in table 1).

Beside its potential to reduce disease activity leading to the reduction of inflammation and joint damage, the use of biologic DMARDs in RA has raised concern about the risk of serious and opportunistic infections. Specifically, Bongartz *et al.* (19) have provided evidence for a higher risk of e.g. serious infections (odds ratio of 2.0) in patients with RA who are treated with anti-TNF antibody therapy. Yet, common infections, such as upper respiratory infections and urinary tract infections, have not been studied in large prospective clinical trials. Only, one group performed an association study concerning this last topic (69). In this study, the SNPs *TNF* -238G>A, *LTA* +365G>C and *FCGR3A* +176F/V were significantly associated with experiencing a urinary tract infection during MTX and etanercept treatment. Additionally, the number of risk alleles (*TNF* -238 A-allele, *LTA* +365 C-allele and *FCGR3A* +176 F-allele) was correlated with an increased risk to this type of infection, demonstrating an additive effect (69).

Gene	Function	Genetic polymorphism(s)	Clinical effect on:	
			Toxicity	Efficacy
TNF	TNF α production and regulation	-308 G>A	A-allele associated with increased TNF α levels after INF (49); No association with toxicity ETN (69)	GG associated with efficacy ETN and INF (40,42,44,47,48); In hp G-allele effect on inefficacy ADA (50); A-allele associated with inefficacy (45); No association with efficacy ETN, INF and ADA (39,41-43,53,60)
		-857C>T	*	T-allele associated with efficacy ETA (41); In hp C-allele effect on inefficacy ADA (50)
		-238G>A	A-allele effect on toxicity ETA (69)	G-allele associated with inefficacy INF (42); In hp G-allele effect on inefficacy ADA (50); No association with efficacy INF (60)
		-1031T>C, -863 C>A, +488, +2018	No effect on efficacy or toxicity ETA, INF and ADA (39,41,42,50,69)	
TNF microsatellites	Linked to TNF α -308 A>G polymorphism	a,b,c,d,e	*	TNF α 11 and β 4 haplotype associated with efficacy INF (67)
TNFRSF1A	TNF α soluble receptor type 1	-609, -580, -383	*	No effect on efficacy ETA (39)
TNFRSF1B	TNF α soluble receptor type 2	676T>G	*	No effect on efficacy ETA, INF and ADA (39,55); GG associated with inefficacy ETA and INF (73) G-allele associate with inefficacy INF (53,54)
Lymphotoxin α (LTA)	Mediation of inflammatory actions	+319C>A, +177A>G, +249, +720	No effect on efficacy or toxicity ETA (39,41,69)	
		+365	C-allele effect on toxicity ETA (69)	No effect on efficacy ETA (39)
HLA DRB1, DRQ1 alleles (SE)	Antigen presenting molecules	See references	*	No effect on efficacy ETA, INF ADA (41,50,60,65-67); HLA-DRB1 associated with efficacy ETA (39);
PTPN22	Involved in T-cell receptor signaling pathway	1858 C>T	*	No effect on efficacy ETA, INF and ADA (65)
FCGR (I,II,III)	Influence cell activation, apoptosis. Indirect target anti-TNF	131H/R, NA1/NA2, 212V/F	No effect on efficacy or toxicity ETA and INF (39,54,69) 131 RR associated with efficacy INF (58)	
		-158V/F	*	No effect on efficacy ETA and INF (56); FF associated with efficacy ETN, INF, ADA (57,58)
		176F/V	F-allele effect on toxicity ETA (69)	No effect on efficacy ETA (39)
Interleukin-10 (IL-10)	Anti-inflammatory cytokine	-1087G>A	*	No effect on efficacy ETA (43)
		Several microsatellites, see reference	*	IL-10- R3 and haplotype IL-10 R3-R9 associated with efficacy ETA (64)
Interleukin-1 (IL-1)	Pro-inflammatory cytokine	IL-1 β +3954C>T	*	No effect on efficacy INF (60,61)

IL-1 receptor antagonist	Inhibits action of interleukine 1	IL-1 RN +2018 T>C	*	C-allele associated with inefficacy INF (60)
		IL-1 RN VNTR intron 2	*	IL1RN*2 allele associated with efficacy INF (61)
MIF	Pro-inflammatory cytokine, Modulation of macrophage and T-cell function	-173 C>C, CATT (7) repeat	*	No effect on efficacy INF (74)

Table 1. Pharmacogenetic association studies of TNF inhibitors 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

Conclusion

TNF inhibitors have been demonstrated to be effective in the treatment of rheumatoid arthritis. Nevertheless, several patients fail to achieve a good response, develop serious side-effects and/or experience drug toxicity, which precludes further treatment with the drug. Unfortunately, interindividual differences in drug response cannot be predicted in patients and (genetic) markers are warranted to individualize and optimize drug treatment. Here, we have discussed mainly reports of associations between genetic polymorphisms in candidate genes and drug efficacy of TNF inhibitor treatment in rheumatoid arthritis, because clear data on associations between toxicity and TNF-inhibiting therapy and associations between genetic characteristics and discontinuation of TNF-inhibiting treatment are limited.

Most pharmacogenetic studies performed so far have an insufficient sample size (power) to detect expected differences in genotype frequencies between responders and non-responders.

Replication and validation in larger comparable cohorts are required before definitive conclusions can be drawn (70). From the studies that have been published, no conclusions can be made on the potential utility of genotyping for *TNF* -308 A/G, the *HLA-DRB1* shared epitope or *TNF* microsatellite haplotypes to predict treatment outcome in rheumatoid arthritis patients who are treated with infliximab. Similarly, on the basis of the levels of significance, the clinical use of genotyping rheumatoid arthritis patients who are treated with etanercept cannot be implemented as yet.

Several difficulties exist in interpreting and comparing the results in pharmacogenetic studies. For example, difficulties arise when genetic variations are known to be disease related, such as the *HLA-DRB1* shared epitope gene in rheumatoid arthritis (71). Patients with mutations in these genes are likely to have a more severe disease and thus a higher state of disease activity at baseline, as compared with patients lacking such mutations. Owing to regression to the mean, patients with high disease activity, in contrast to those with low disease activity, might show a higher response. Predicting a positive or negative treatment outcome is thus hampered by higher disease activity at baseline, rather than referring to an effect of variance in genotype.

In addition, owing to their mechanism of action, the dose of anti-TNF drugs should be considered when interpreting and comparing treatment outcome in pharmacogenetic studies. In theory, the cellular amount of TNF α and, thus, the amount available for inhibition by anti-TNF drugs, might depend on the genotype. The clinical consequence of the genotype might thus be dependent on drug dose, because increasing or decreasing dosage could have a similar net effect. In pharmacogenetic studies, therefore, it is important that baseline characteristics (disease activity state) and drug dosages between cohorts are kept at similar level to estimate adequately associations between genetic polymorphisms and treatment outcome. To avoid genetic variation in a population itself as a predictor for clinical response, the prevalence of a candidate gene in responders and non-responders and in controls must be compared in pharmacogenetic studies. In this way, a genuine gene-dose effect becomes visible (24).

Furthermore, the problem of potential functionality of a candidate gene, tested in vitro, remains because any functionality determined can have no relevance to the in vivo mechanism of drug action. Such genes can be in linkage with other loci, which have a true influence on the pharmacology of the drug (72). Lastly, the location of SNPs on chromosomes and the frequency of SNPs vary to a great extent between different populations; in the interpretation of any associations presented, the genetic variation between racial and ethnic groups has to be considered.

We conclude that pharmacogenetics of anti-TNF drugs in the treatment of patients with rheumatoid arthritis has the potential to optimize therapy and clinical outcome. In general, however, the current studies are too small and subsequent findings in larger studies often fail to replicate the original

data. Continued large-scale studies are essential before a pharmacogenetic approach will be applicable in daily clinical practice.

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