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# Potential role of pharmacogenetics in anti-TNF treatment of rheumatoid arthritis and Crohn's disease

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**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% and ~25–40% of individuals with rheumatoid arthritis and Crohn's disease, respectively. 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 and Crohn's disease. Pharmacogenetic studies have shown associations between single nucleotide polymorphisms in genes encoding enzymes related to the pharmacodynamics of these drugs and treatment outcome. As we discuss here, replication and prospective validation are warranted before pharmacogenetics can be used in clinical practice.**

Although the pathogenesis of many autoimmune diseases including rheumatoid arthritis and Crohn's disease remains unknown, studies have shown that tumour-necrosis factor-α (TNFα) has a key role in the inflammatory process of these immune-mediated disorders. TNFα is known to be involved in stimulating cytokine (including its own) production, in enhancing the expression of adhesion molecules and in neutrophil activation, and is also a co-stimulator of T-cell activation and antibody production by B cells [1–4].

Consequently, TNFα has emerged as an important target in novel therapeutic strategies used to treat rheumatoid arthritis and Crohn's disease. 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 TNFα. The chimaeric IgG1 monoclonal antibody infliximab and the complete humanized IgG1 monoclonal antibody adalimumab bind to TNFα 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. In this manner, an expanding array of drug therapy options for the treatment of rheumatoid arthritis and Crohn's disease in the clinic has been established over the past decade [5–7].

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 [8,9]. For example, Bongartz *et al.* [10] 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% and 25–40% of patients with rheumatoid arthritis and Crohn's disease, respectively [11–14].

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 and Crohn's disease. Studies have shown associations between single nucleotide polymorphisms in genes encoding enzymes related to the pharmacodynamics

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of the anti-TNF drugs used to treat these diseases 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 [15]. Replication and prospective validation are warranted before pharmacogenetics can be used in clinical practice.

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

## Pathology, diagnostics and therapeutics

### *Rheumatoid arthritis*

Rheumatoid arthritis is a chronic inflammatory disorder that affects synovial joints and has a prevalence of ~1% in the Western population. The inflammation is driven by overproduction, largely by macrophages, of the pro-inflammatory cytokines TNFα and interleukin 1β (IL1β). Autocrine and paracrine involvement of these cytokines within the joint space results in maintenance of the inflammatory response, leading to cartilage degradation and bone erosion [1,16].

Treatment of rheumatoid arthritis usually follows a stepwise approach that is based on the evaluation of disease activity and radiological progression of joint damage [12]. The most commonly used measure to evaluate disease activity is the '28-joint disease activity score' (DAS28) [17], 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. To assess disease activity in clinical trials, specific improvement and response criteria have been developed. 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) [18,19]. 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 [20]. 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 [21].

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 [22]. First, patients would be treated with disease-modifying antirheumatic drugs (DMARDs), of which methotrexate is the first drug of choice [23,24]. 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 [11,13,25]. After the onset of disease, tightly scheduled management of treatment is required to maintain efficacy [26]. 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 [27,28].

### *Crohn's disease*

Increased TNFα in inflamed intestinal tissues and a marked clinical response to TNF inhibitors suggest that TNFα is a key cytokine in the pathogenesis of Crohn's disease. As an inflammatory mediator, TNFα has an important role in initiating and propagating the disease process. In the disease, segments of the whole gastrointestinal tract might be involved, but the inflammation is manifest mainly as terminal ileitis or enterocolitis. Transmural inflammation, in which all layers of the bowel segments involved are infiltrated, can lead to fistulas, abscesses and occasionally perforation. The natural history of the disease is characterized by periods of active and more quiescent disease. Similar to the treatment of rheumatoid arthritis, conventional therapeutic options have not led to reduced disease progression in Crohn's disease [4,29].

Evaluation of drug efficacy is more cumbersome in Crohn's disease than in rheumatoid arthritis, and several indices have been explored. In most clinical trials, the Crohn's Disease Activity Index (CDAI) is used as an outcome measure of efficacy [30]. This is a validated index based on subjective aspects rated by the patient over a period of seven days (e.g. frequency of liquid or very soft stools, abdominal pain, and general well-being) and objective observations (e.g. number of extra-intestinal symptoms, need for anti-diarrhoeal drugs, presence of abdominal mass, body weight and haematocrit levels). Because the CDAI is not suitable for evaluating fistulas, separate indices have been developed [31].

Pharmacotherapy of Crohn's disease is aimed at achieving remission with respect to acute inflammatory exacerbations. Thereafter, maintenance of remission is the ultimate challenge. To achieve these goals, 5-aminosalicylates, glucocorticoids, antimetabolites or antibiotics can initially be prescribed. Owing to the slow onset of action, incomplete response, immune suppression and/or long-term adverse events associated with these anti-inflammatory drugs, however, there is a need for newer, more effective agents with improved safety profiles [5,4]. The TNF inhibitor infliximab is the first biological agent approved for treatment of Crohn's disease. Over the past decade, its efficacy has been well-documented for the induction and maintenance of remission in both luminal and fistulizing disease [32–34]. On the basis of this success, other TNF-inhibiting strategies have arisen as viable alternatives for Crohn's disease treatment. Because infliximab induces a high level of human anti-chimaeric antibodies, the fully human TNF inhibitor adalimumab might be an alternative to overcome intolerance or refractoriness to infliximab [35].

In contrast to its efficacy in rheumatoid arthritis, etanercept is not as effective as infliximab in the treatment of Crohn's disease [36,37]. Differences between infliximab and etanercept in structure (in the Fc portion), in TNF-binding characteristics and in functionality at the cellular level might help to explain this difference. Infliximab binds with higher avidity to TNFα and TNFβ (also known as Lymphotoxin) in the lamina propria, whereas etanercept binds with lower avidity and to only TNFα. Furthermore, in contrast to etanercept, infliximab is thought to function also by killing TNF-producing cells [38,39]. Owing to its ineffectiveness, etanercept is not approved for Crohn's disease treatment.

## Pharmacogenetics

Even though TNF inhibitors have proved to be effective in the treatment of individuals with rheumatoid arthritis and Crohn's

disease (except for adalimumab and etanercept in Crohn's disease), a substantial proportion of the patient population fail to achieve a satisfactory clinical result. From the results of large clinical trials, it seems that treatment outcome remains insufficient in ~40–60% and 25–40% of patients with rheumatoid arthritis and Crohn's disease, respectively. Moreover, determinants for drug efficacy and toxicity for individual patients are largely unknown; therefore, identifying those patients who will benefit from TNF-inhibiting drug treatment remains a lottery [11,13,25,40,41].

Pharmacogenetics holds the promise not only to explain inter-individual variability in drug response, but also to predict efficacy and adverse drug events in different patients [15]. 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 [42,43]. 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 [40,11]. The results of several studies in which anti-TNF treatment has been switched underline the pharmacogenomic independency of these drugs [44–46].

Studies have gathered considerable information on drug interaction with, and mediation of, the cytokine TNFα [1,3,4]. The fact that these drugs target TNFα has led to interest in TNFα itself as a candidate gene for pharmacogenetic association studies. In recent years, many polymorphisms in genes encoding proteins related to TNFα have been identified that might be associated with treatment outcome. Such candidate gene polymorphisms (Tables 1 and 2) have been investigated for their ability to predict treatment outcome in patients with rheumatoid arthritis and Crohn's disease receiving anti-TNF drugs.

### *Candidate genes and etanercept in rheumatoid arthritis*

Padyukov *et al.* [47] described candidate genes that could influence treatment outcome to etanercept. None of the chosen alleles, encoding TNFα, IL10, interleukin-1 receptor antagonist (IL1RN) and transforming growth factor B1 (TGFB1), were found to be solely associated with response to treatment. Further analysis demonstrated, however, a significant association of a combination of genotypes from different cytokines with drug response ($P < 0.05$; Table 2). Of 23 patients with the genotype TNFα −308 GG and IL10 −1087 GG, 22 (96%) responded well according to ACR20 and DAS28 criteria. The other combination, consisting of alleles influencing the production of IL1RN (two repeats of the A-allele in intron 2) and TGFB1 (a rare C-allele in codon 25), was associated with a poor treatment outcome.

Kang *et al.* [48] selected several genetic polymorphisms within the TNF and LTα gene region in 70 Korean patients and analysed whether these polymorphisms were associated with treatment outcome. Only the −857 C/T single nucleotide polymorphism (SNP), in the promotor region of the TNFα gene, was significantly associated with response (Table 2). Most of the patients carrying the T-allele (96%), and a smaller percentage of patients in the CC group (79%), were responders according to the ACR20 improvement criteria. In addition, the percentage of responders in T-allele carriers (39%), according to the ACR70 improvement criteria, was higher than that in the CC group (13%). These differences in carrier analysis were not significant. When the ACR20 non-responders (according to the ACR20 improvement criteria) were compared against only the ACR70 responders (according to the ACR70 improvement criteria), however, the genotypic association was significant ($P = 0.033$; Table 2).

A link between responsiveness to etanercept and variants of genes that are supposedly functional in IL10 production [49] was demonstrated by Schotte *et al.* [50]. The IL10 microsatellites IL10.R

**TABLE 1**

**Functionality of candidate genetic polymorphisms in rheumatoid arthritis and Crohn's disease[a]**

| Gene/polymorphism | (Hypothetical) functionality of cytokine/receptor | Refs |
|---|---|---|
| TNFα −308 | Transcription/production of TNFα | [75] |
| TNFα −857 | Transcription/production of TNFα | [76] |
| TNF microsatellites a–e | Linkage to/influence on TNFα −308 | [77] |
| IL1RN | Regulation of IL1 antagonist production | [78] |
| IL10 −1087 | In linkage with functional SNPs in the regulation of IL10 production | [79] |
| IL10 R2, R3, G9 and G13 | In linkage with functional SNPs in the regulation of IL10 production | [49] |
| TGFB1 | Regulation of TGFβ1 production | [80] |
| HLA-DRB1 (SE) | Elemental influence on antigen presenting cells; influence on severity and susceptibility to rheumatoid arthritis | [81,82] |
| TNFR-1 +36 | Signal transduction leading to, for example, apoptosis | [83] |
| TNFR-2 +587 | Signal transduction leading to, for example, TNFα production; regulation of apoptosis in $CD8^+$ cells | [83,84] |
| LTα 1–1-1–1 | Mediation of inflammatory actions | [85] |
| FcGR IIIa −158 | Binding to IgG leading to cell specific functions such as phagocytosis | [86] |
| IBD5 | Influence on transcription and transporter function of organic cation transportation; interaction with other susceptible genes to Crohn's disease such as CARD15 | [87] |
| CARD15 +702 | Signal transduction leading to production of TNF | [88] |
| CARD15 +908 | Signal transduction leading to production of TNF | [88] |
| CARD15 +1007 | Signal transduction leading to production of TNF | [88] |

[a] Abbreviations: CARD, caspase-activating recruitment domain; HLA, human leukocyte antigen; IBD, inflammatory bowel disease; SE, shared epitope.

**TABLE 2**

**Significant influence of candidate genes on TNF-inhibiting treatment outcome in rheumatoid arthritis and Crohn's disease[a,b]**

| Candidate gene | $N_{patients}$ | Efficacy criteria; time | Association of genotype with a positive or negative response in disease | Refs |
|---|---|---|---|---|
| **Etanercept[c]** | | | | |
| HLA-DRB1 (SE) | 255 | ACR 50; 12 mths | The copies *0404 and *0101 haplotype of shared epitope are associated with positive response in rheumatoid arthritis | [51] |
| TNFα −308 and IL10 −1087 haplotype | 123 | ACR20 and DAS28; 3 mths | TNFα −308 GG and IL10 −1087 GG haplotype is associated with positive response in rheumatoid arthritis | [47] |
| IL1RN and TGFB1 haplotype | 123 | ACR20 and DAS28; 3 mths | IL1RN (two repeats of A-allele in intron 2) and TGFB1 (rare C-allele in codon 25) haplotype is associated with negative response in rheumatoid arthritis | [47] |
| TNFα −857 | 70 | ACR70; 3 mths | TNFα −857 T-allele is associated with positive response in rheumatoid arthritis[e] | [48] |
| IL10.R3 | 50 | DAS28; 48 mths | IL10 promotor microsatellite R3 is associated with positive response in rheumatoid arthritis | [50] |
| IL10.G13 | 50 | DAS28; 48 mths | IL10 promotor microsatellite G13 is associated with a moderate or negative response in rheumatoid arthritis | [50] |
| IL10.R2-G13 haplotype | 50 | DAS28; 48 mths | IL10 promotor microsatellites R2 and G13 haplotype is associated with a moderate or negative response in rheumatoid arthritis | [50] |
| IL10. R3-G9 haplotype | 50 | DAS28; 48 mths | IL10 promotor microsatellites R3 and G9 haplotype is associated with positive response in rheumatoid arthritis | [50] |
| **Infliximab[d]** | | | | |
| FcGR IIIa −158 | 200 | CRP level; 3 mths | FcGR IIIa −158 VV is associated with positive response in Crohn's disease | [64] |
| TNFR-1 +36 | 166 | CRP level; 2 mths | TNFR-1 +36 G-allele is associated with negative response in Crohn's disease | [63] |
| TNFα -308 | 59 | DAS28; 5 mths | TNFα −308 GG is associated with positive response in rheumatoid arthritis | [54] |
| LTα 1–1-1–1 haplotype | 59 | CDAI; 1 mths | LTα 1–1-1–1 haplotype is associated with negative response in Crohn's disease | [60] |
| IBD5 (5q31) | 40 | CDAI; 3 mths | IBD 5 (5q31) TT is associated with negative response in Crohn's disease | [65] |
| TNFα −308 | 22 | DAS28; 12 mths | TNFα −308 GG is associated with positive response in rheumatoid arthritis | [55] |

[a] Abbreviations: HLA, human leukocyte antigen; IBD, inflammatory bowel disease; SE, shared epitope.
[b] Studies are hierarchically listed on the basis of sample size power, which identifies those pharmacogenetic studies that are most likely to influence treatment.
[c] Significant candidate polymorphisms, which are of influence on etanercept treatment in patients with rheumatoid arthritis.
[d] Significant candidate polymorphisms, which are of influence on infliximab treatment in patients with rheumatoid arthritis and Crohn's disease.
[e] In this analysis, the best ACR70 responders were compared with the worst ACR20 non-responders.

and IL10.G were genotyped in 50 patients. The IL10 promotor microsatellite allele IL10.R3 and the haplotype R3-G9 were shown to be significantly associated with good response (OR 5.5, 95% confidence intervals [95%CI] 1.6–18, and OR 5.1, 95%CI 1.5–18, respectively). The allele IL10.G13 and the haplotype R2-G13 were significantly more common among patients with moderate or no response (OR 0.18, 95%CI 0.05–0.61, and OR 0.14, 95%CI 0.04–0.50, respectively). These results indicate that IL10 promotor microsatellite polymorphisms are predictors of response to etanercept (Table 2).

Criswell *et al.* [51] proposed that genetic variation in the HLA-DRB1 and TNF–Lymphotoxin-α (TNF–LTα) regions are associated with etanercept therapy outcome in early rheumatoid arthritis. They found that patients who inherited two copies of the HLA-DRB1-encoding shared epitope (allele *0404 and allele *0101) were significantly more likely to respond to treatment with standard-dose etanercept than were patients with one or zero copies (OR 4.3, 95%CI 1.8–10; Table 2). Genes in the TNF–LTα region did not appear to be related to therapy outcome. In addition, extended haplotypes spanning the HLA-DRB1 and TNF–LTα regions were analysed. High response rates (61 and 76%) were associated with the copies *0404 and *0101 encoding the shared epitope. Significant associations between these copies and achieving ACR50 at 12 months were found when these results were validated in a logistic regression analysis, including covariates (OR 2.5, 95%CI 0.8–7.3, and OR 4.9, 95%CI = 1.5–16 for the *0404 and *0101, respectively). However, additional analyses involving the HLA-DRB1 shared epitope showed that the rest of these copies did not contribute to the result of these extended haplotypes. Hypothetically, other genes have an influence on treatment outcome. Indeed, previous studies have reported that HLA associations with rheumatoid arthritis susceptibility and disease outcome are difficult to interpret [52,53]. The precise mechanism of action and their influence on treatment outcome, therefore, remain to be defined.

### *Candidate genes and infliximab in rheumatoid arthritis*

In four separate studies, patients receiving infliximab therapy have been genotyped for the TNFα SNP −308 A/G. Mugnier *et al.* [54] and Fonseca *et al.* [55] both demonstrated a positive association of clinical effect with the TNFα GG genotype ($P = 0.0086$ and $P < 0.05$, respectively; Table 2. By contrast, Marotte *et al.* [56] and Cuchacovich *et al.* [57] found no significant difference in response rates between the TNFα A- and G-alleles. Because the study group was larger (198 patients) and thus more highly powered, one might expect the Marotte *et al.* [56] investigation to represent the more reliable study.

Marotte *et al.* [56] and Martinez *et al.* [58] also studied the possible role of the HLA-DRB1 shared epitope in predicting infliximab outcome; however, they did not find a significant difference between shared epitope carriers in responders and non-respon-

ders. In the study by Marotte *et al.* [56], ACR20 values were obtained in 64.9, 67.0 and 66.0% of the patients with, respectively, none, one and two copies of the shared epitope. The frequency of shared epitope carriers (patients carrying one or two copies of the HLA-DRB1 shared epitope) in responders and non-responders, as demonstrated by Martinez *et al.* [58], was 71 and 64%, respectively.

Martinez *et al.* [58] also analysed the influence of TNF microsatellite haplotypes. After Bonferroni correction [59] for multiple testing, none of these haplotypes remained significant. Nevertheless, the increased prevalence of the haplotype TNFα11;b4 in responders (41% versus 16% in non-responders) highlights the existence of genetic determinants of response to infliximab therapy.

### *Candidate genes and infliximab in Crohn's disease*

Several genetic variants have been studied in the TNF-LTα region. In recent years. Taylor *et al.* [60] determined genotypes and found significance for presence of the LTα haplotype 1−1-1−1 (LTA haplotype NcoI-TNFc-aa13L-aa26) in the group of non-responders ($P = 0.007$; Table 2).

Louis *et al.* [61] genotyped Crohn's disease patients for the TNFα −308 A/G polymorphism and compared response rates after infliximab treatment. No significant difference between response groups could be demonstrated. The same result was obtained in the study by Mascheretti *et al.* [62], in which two cohorts of 90 and 444 patients (from the ACCENT I study) with chronic active Crohn's disease were analyzed. SNPs in TNFα, TNF receptor 1 (TNFR-1) and TNFR-2 were tested for associations with therapy outcome. Only the homozygous mutant G-allele at position TNFR-2 +587 was identified to predict a worse treatment outcome to infliximab in the small cohort ($n = 90$), but no significance was found when this result was replicated in the large cohort ($n = 444$).

Approximately two years later, Pierik *et al.* [63] also selected this TNFR-2 SNP, as well as the TNFR-1 +36 polymorphism. Similar to the results of Mascheretti *et al.* [62], only the TNFR-1 +36 polymorphism was associated with a decreased biological response, as measured by C-reactive protein (CRP) levels before and after treatment (OR 0.47, 95%CI 0.23–0.95; Table 2). CDAI was not used as a clinical outcome measurement for efficacy in this study.

The study by Louis *et al.* [64] reported an association between the IgG Fc receptor IIIa −158 SNP and good treatment outcome when a decrease in CRP levels was taken as the endpoint (OR 1.4, 95%CI 1.3–1.6; Table 2). In addition, a possible association, albeit not significant, with clinical outcome (CDAI) was demonstrated.

In the small study of Urcelay *et al.* [65], an interesting association between a genetic polymorphism located in the 5q31 locus (containing the IBD5 gene) and a lack of response to infliximab therapy was observed (OR 3.9, 95%CI 1.2–12; Table 2). As well as this candidate gene, Vermeire *et al.* [66] and Mascheretti *et al.* [67] (ACCENT I study) studied CARD15 (also known as NOD2) genetic polymorphisms for association with infliximab outcome in Crohn's disease. Three SNPs (+702, +908 and +1007) in the CARD15 gene were selected in both studies. As expected, a strong relation to susceptibility to Crohn's disease was found in both studies; however, no significant association with therapy outcome was demonstrated.

Recently, Willot *et al.* [68] evaluated three genetic polymorphisms in the gene encoding CRP (−717 G/A, +1444 C/T and +4 A/G) for association with treatment outcome in patients given infliximab. With biological and clinical measurements used as parameters of therapy outcome, no significant relationships with these genotypes were found.

### *Candidate genes and adalimumab in rheumatoid arthritis and Crohn's disease*

Only a single, small study has been published on the association of genetic variants and adalimumab efficacy in rheumatoid arthritis. Tutuncu *et al.* [69] investigated the Fcγ receptor type IIIA −158 polymorphism in four patients. The results of this study are such that they do not permit statistical analysis and do not show representative results. No further association studies have been done with adalimumab treatment outcome in rheumatoid arthritis and Crohn's disease patients so far.

## Conclusion

TNF inhibitors have been demonstrated to be effective in the treatment of rheumatoid arthritis and Crohn's disease. 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 reports of associations between genetic polymorphisms in candidate genes and drug efficacy of TNF inhibitor treatment in rheumatoid arthritis and Crohn's disease, 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 TNFα −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. Current results do, however, potentially indicate interindividual responses to etanercept. In addition, genotyping for IBD5, TNFR-1, FcGR IIIA and LTα seems to be marker for predicting a (lack of) response to infliximab treatment in patients with Crohn's disease.

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 and the CARD15 gene in Crohn's disease [71,72]. 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 TNFα 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 [73].

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 [74]. 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 and Crohn's disease 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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