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

Kooloos, W.M.

Chapter 11:

Summary

Rheumatoid arthritis (RA) is prevalent in approximately one percent of all types of populations. Characteristically, this immune-mediated disease is related with symmetrically inflammation, destruction of the joints leading to overall functional impairment and (serious) comorbidity.
Regardless of the increasing comprehension on the etiology and pathogenesis, a therapy resulting in remedy of the disease is not achieved to date. Consequently, in order to gain optimal benefit treatment is aimed at remission of disease by opposing the immune response with disease-modifying antirheumatic drugs (DMARDs). However, in practice suboptimal results are achieved with the use of DMARDs including methotrexate (MTX) and Tumor Necrosis Factor (TNF) inhibitors. Namely, highly differential response rates in overall clinical efficacy and/or toxicity have been observed in clinical trials with MTX and TNF inhibitors. Partly, pharmacogenetics is responsible for this variance in response. Therefore, the primary focus of this thesis is to assess the role of pharmacogenetics in the variation of treatment outcome in patients diagnosed with rheumatoid arthritis and treated with the DMARDS MTX and adalimumab.

Methotrexate

Initially, in the first part of this thesis an overview was presented of previously performed studies concerning genetic variability contributing to differences in response to MTX in RA treatment (**chapter 2**). Most pharmacogenetic studies have an insufficient sample size (power) to detect true associations with treatment response. In addition, other factors, like nongenetic factors, ethnicity and clear endpoints, influence treatment outcome. Therefore, definitive conclusions about the role of genetic prognostic factors in treatment outcome to MTX cannot be drawn from this literature study.
As it is generally accepted that MTX may act in RA through inhibition of folate pathway enzymes, other reports indicate that efficacy may also be related to the release of endogenous antiinflammatory adenosine. With this hypothesis, the relationship between SNPs in genes related to adenosine release and MTX treatment outcome in patients with recent-onset RA was explored in **chapter 3**. Results of this analysis did show an association between allelic variants in the adenosine monophosphate deaminase (*AMPD*), 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (*ATIC*), and inosine triphosphate pyrophosphatase (*ITPA*) genes and clinical response to MTX therapy in patients with recent-onset RA. Patients carrying the *AMPD1* T allele, the *ATIC* CC genotype, or the *ITPA* CC genotype were 2–3 times more likely to have a good clinical response, defined by a disease activity score (DAS) of ≤2.4, following 6 months of MTX therapy. Additionally, the rate of good clinical response increased substantially in patients carrying the 3 favorable genotypes. With regard to the occurrence of adverse drug events, the only association found was with the *ATIC* G allele. However, this association was not significant after adjustment for multiple testing. No associations between SNPs in the genes methionine synthase or methionine synthase reductase and MTX efficacy or toxicity were found.
So far, most genetic variants are selected for analysis based upon their hypothetical relation to the mechanism of MTX or inflammatory process in RA (**chapters 2 and 3**). Ideally, functional genetic variants are chosen because the alteration in protein function is thought to influence drug action and thus may explain interindividual differences in drug response. **Chapter 4** assessed the role of SNPs in genes with proven functional consequences on efficacy and toxicity of MTX in the BeSt cohort. It was observed that toxicity was potentially associated with *ABCB1* 3435C/T and *TLR4* +896A/G. However, none of these associations remained significant after (Bonferroni) correction for multiple testing. No significant associations of *DHFR* 829C/T, *ABCB1* 3435C/T, *ITPA IVS2* +21A/C, *HLA-G*

-/+14bp, *IMPDH2* +787C/T, *TGFB1* +869T/C and *TLR4* +896A/G with MTX efficacy were found. Additionally in this chapter, results from previous research according to reported endpoints were replicated in our cohort. Particularly, replication analyses are important, since pharmacogenetic studies have the potential to result in reporting false positive findings. However, no significant results were detected with these analyses in our cohort.

Previously, a clinical pharmacogenetic predictive model was developed for predicting the efficacy of MTX monotherapy in patients with recent-onset RA comprising the Dutch BeSt Cohort. The model consists of non-genetic factors sex, rheumatoid factor and smoking status, Disease Activity Score (DAS) before starting MTX and 4 genetic polymorphisms (*MTHFD1* 1958G>A, *AMPD1* 34C>T, *ITPA* 94A>C and *ATIC* 347C>G). With this model, a true positive predictive vale of 95%, true negative predictive value of 86% and categorization of 60% of the patients was achieved. In **chapters 5 and 6** the performance of the predicitve model was validated in a second Dutch cohort (chapter 5) and in a Swedish cohort (chapter 6).
In **chapter 5,** it was demonstrated that the clinical pharmacogenetic model to predict MTX monotherapy efficacy in patients with established RA does not perform as good as in DMARD naïve patients with recent-onset RA (BeSt cohort). Namely, it was found that the model had lower true positive and negative predictive values (47% and 81%, respectively) compared with the true positive and negative predictive values reported in the BeSt cohort (95% and 86%, respectively). Several explanations may be responsible for these findings: variation in RA disease duration and history of DMARD use; lack of (pharmacogenetic) association with components of the model in the second cohort; the presence of differences in tight control strategy of RA and, consequently, the variance in dosage between the two cohorts leading to different response rates.
In **chapter 6**, a model for predicting the efficacy of MTX monotherapy was validated in a cohort of DMARD naïve and early RA patients originated from the Swefot trial. Similarly as in **chapter 5**, both true predictive values observed in patients of the validation cohort were significantly lower compared to the values found in the original BeSt cohort (95% and 86%, respectively), since application of this pharmacogenetic model resulted in a true positive predictive value of 70% and a true negative predictive value of 68%. However, for the predictive values accuracy, number of patients classified and discriminative ability (AUC) the results were comparable between the Swefot cohort and BeSt cohort (for accuracy 48% vs. 53%: p=0.572; for number of patients classified 70% vs 60%: p=0.182 and for AUC 75% vs. 85%: p=0.111, respectively).
Overall, these validation data imply that efficacy of a substantial part of early RA patients treated with MTX could be predicted by this clinical pharmacogenetic model, but that this model may exclusively be applicable in DMARD naïve RA patients with short duration of disease. Additional replication and (ideally) performance of prospectively designed studies with this model in large cohorts is warranted to demonstrate the legitimate predictive value in rheumatology practice.

Chapter 7 evaluates the role of the haplotypes comprising the SNPs MTHFR 1298A>C and MTHFR 677C>T in treatment outcome to MTX in RA. Specifically, in this chapter optimalization of a previously designed pharmacogenetic model was aimed with addition of the number of haplotypes comprising MTHFR 1298A-677C alleles as additional criterion. Furthermore, the predictive value of the most predictive number haplotype is compared with the SNPs *AMPD1* 34C>T, *ITPA* 94C>A, *ATIC* 347C>G and *MTHFD1* 1958G>A involved in predicting MTX efficacy. It was observed that the predictive performance of the pharmacogenetic model to predict the efficacy of MTX therapy in this group of early RA patients was not improved when the *MTHFR* haplotype was included in the model. Moreover, the discriminative effect for the prediction of MTX efficacy including 1 or 2 or (solely) 2 copies of the 1298A and 677C haplotype was significantly smaller compared with the four SNPs

AMPD1 34C>T, *ITPA* 94C>A, *ATIC* 347C>G and *MTHFD1* 1958G>A. These results suggest that a (leading) role for the *MTHFR* 1298A and 677C haplotype with regard to predicting efficacy of MTX monotherapy in early RA patients seems unlikely. Future research is necessary to elucidate the exact pharmacogenetic and biological role of *MTHFR* 1298A>C and *MTHFR* 677C>T and their haplotypes in the efficacy of MTX in RA.

Adalimumab

At the start of this second part of the thesis an overview was presented of reports on associations between genetic variants and the drug efficacy of TNF inhibitors in RA (**chapter 8**). Similar to reports concerning pharmacogenetics of MTX in RA, the majority of pharmacogenetic studies were underpowered to detect accurate associations.
In **chapter 9**, SNP selection for pharmacogenetic association studies was discussed. Additionally, a pharmacogenetic pathway approach is presented together with proposed criteria for systematic selection of SNPs. These comprise the following genetic characteristics of SNPs: heterozygosity, validation, ethnicity, functionality, linkage disequilibrium and Tag SNPs. With the application of these criteria, an objective selection can be achieved: 186 SNPs in 111 genes out of 51,793 SNPs in 124 genes were included. Specifically, this method was applied for the selection of potential interesting SNPs within genes related involved in the mechanism of action of adalimumab and/or inflammatory process of RA. This approach has several advantages over either the candidate gene approach or the genome wide SNP analysis. First, because the rate limiting step in the described pathway is unknown, this systems pharmacology approach provides a solution: variability in the entire pathway is explored. In fact, the relative contribution of the different SNPs in the pathway to the explanation of variability to drug response can be assessed. Secondly, an important statistical advantage is present: the chance of false-positive results is lower compared to the genome-wide method, because of decreased multiple testing.

Chapter 10 put the presented systematically selection of SNPs in chapter 9 into practice: efficacy of treatment with adalimumab was associated with genetic variants selected by a pharmacogenetic pathway approach using a custom made anti-TNFα SNP array. Additionally, in this chapter SNPs from previous research on pharmacogenetics of TNF inhibitors in RA and susceptibility to RA were included for analysis. Results elucidated 19 SNPs, 11 SNPs and 8 SNPs, which were significantly associated with EULAR good response, EULAR remission and relative change in DAS28, respectively ($p<0.05$). Four SNPs, rs1126535 in *CD40LG*, rs6828477 in *KDR*, rs1267067 in *TANK* and rs25648 in *VEGFA* demonstrated the most evidence for potentiality in determining adalimumab therapy outcome, since these SNPs were significantly associated according to all three primary and secondary endpoints ($P<0.05$). Notably, p-values of this explorative study were presented without (Bonferroni) adjustments to make the results accessible for clear interpretation. Ideally, replication in a second comparable cohort of patients would be optimal, but not always feasible. Nevertheless, results from this explorative study provide new insights regarding the potential of pharmacogenetics of adalimumab in RA.