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

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Chapter 12:

General discussion and future perspectives

Rheumatoid arthritis (RA) is a chronic autoimmune disease, which is characterized by inflammation leading to destruction and impairment of principally the joints. Treatment is aiming at limiting the progress of disease activity and involves the use of disease-modifying antirheumatic drugs (DMARDs) including methotrexate (MTX) and Tumor Necrosis Factor (TNF) inhibitors. Many clinical trials have demonstrated successful results with these classes of drugs underlining their suitability in the tight scheduled management of RA's disease progress. However, a considerable proportion of the patients do not experience a positive response. Especially, these patients are at risk of developing progressive and erosive RA. In the light of optimal management of individual patients, the idea of a priori prediction of drug response is considered an important achievement. It will enable physicians to readily select those patients sensitive to certain drug regimens and thereby minimizing irreversible joint dysfunction existing in severe untreated RA. Hereby, an important role is being played by pharmacogenetics as it is thought that drug response is, at least partly, a heritable trait.

Important progress has been made regarding pharmacogenetics in rheumatology in the last decade. Basically, explorative studies focusing on the pharmacokinetics and pharmacodynamics of antirheumatic agents have contributed to the introduction of new genetic markers. Still, consensus regarding their potential implication has not been reached. It is observed from previous research (**chapters 2 and 8**) and from the results presented in this thesis that definitive conclusions regarding the influence of genetics on treatment outcome to DMARDs can not be drawn. These differences in outcome are the result of a several factors, in which variance in study design plays an important role.

In this discussion a focus is placed on the interpretation and implication of potential pharmacogenetic findings. Hereby, answers are sought to the following questions: "What are the major points of concern in RA study design contributing to interpretation difficulties of pharmacogenetic results?" and "Which factors could be of influence in the implementation of proven genetic associations in rheumatology clinical practice?" Furthermore, an ideal study proposal for a prospective study concerning MTX and TNF inhibitors in RA is presented, in which strategy for therapy is guided by pharmacogenetics. In figure 1, a schematic overview of this chapter is provided.

Design and interpretation of pharmacogenetic studies

Power and sample size

In general, appropriate sample size is characterized by consideration of statistical power, which is the probability of correctly rejecting the null hypothesis in favor of the alternative hypothesis. Power analysis comprises selection of a sample size large enough to identify either a relation or effect size (1). Power calculation is optimally performed prior to analysis. In **chapter 10**, efficacy of treatment with adalimumab was associated with genetic variants related to the mechanism of action of TNF inhibitors and/or inflammatory process of RA. A power calculation analysis was performed to recognize the range of power in which the minimal allele frequencies (MAF) of the selected single nucleotide polymorphism (SNPs) would fall to find specific odds ratios (OR). In figure 2, this range is presented. With the appliance of a power calculation it was demonstrated that for a patient population of 325 patients, minimal allele frequencies (MAF) ranging from 10% to 50%, a response of 45% and a chosen type 1 error probability α=0.05, a power of >80% could be achieved to detect an odds ratio ranging from 1.4 (MAF of 50%- line A) to 2.0 (MAF of 10%- line B) (figure 2). Line C represents a MAF of 5% indicating the lower chance of finding a reasonable odds ratio with the achievement of a power of >80%. Notably, in more than 80% of the selected SNPs the MAF was higher than 30%. Results elucidated 19 SNPs, 11 SNPs and 8 SNPs in the TNF pathway, which were

significantly associated for adalimumab with EULAR good response, EULAR remission and relative change in DAS28, respectively ($p<0.05$). In the majority of the associated SNPs odds ratios and corresponding MAF was observed to achieve a power of >80% (tables 1,2 and 3 of **chapter 10**).

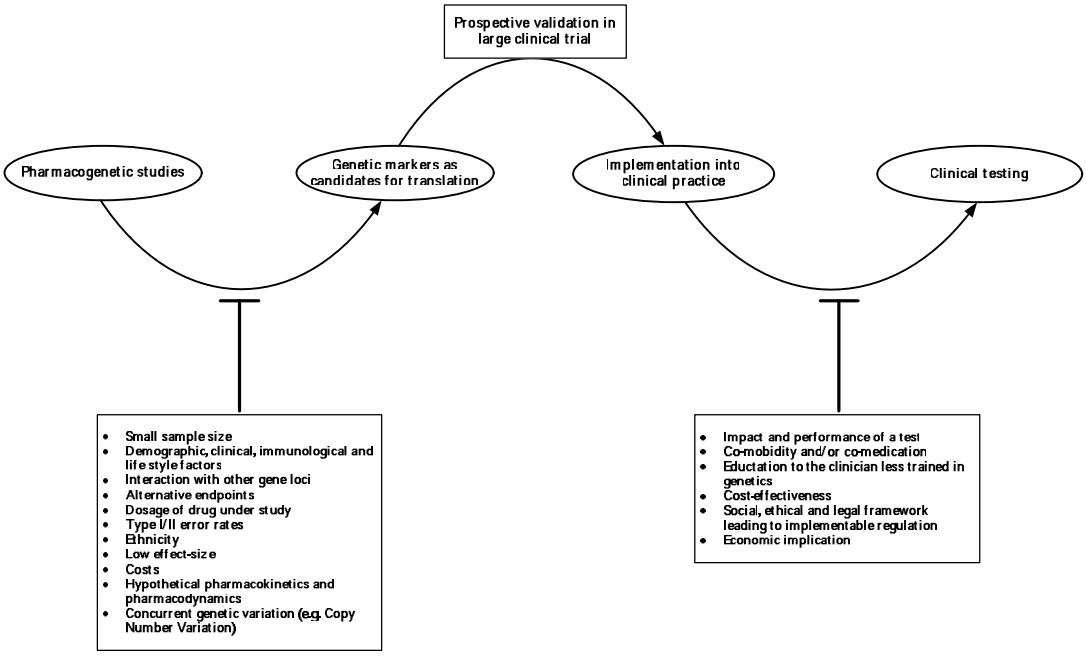

Figure 1. Stages towards implementation of pharmacogenetic markers into clinical practice

Stage 1: Before prospective validation in large studies of potential genetic polymorphisms can be performed, several aspects have to be noticed (see most left rectangle)
Stage 2: Next to general factors comprising cost-effective, regulatory and ethical aspects, several challenging factors appear before implementation of a pharmacogenetic marker as a tool in rheumatological clinical practice can take place (see most right rectangle)

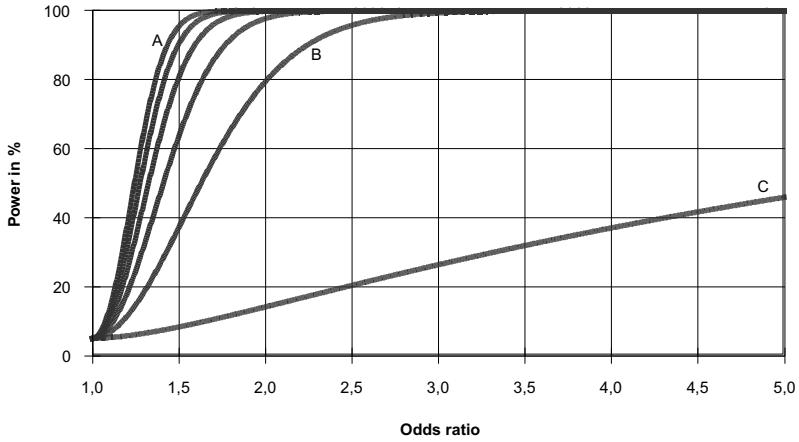

Figure 2. Power calculation analysis to recognize the range of power in which the minimal allele frequencies (MAF) differ to find specific odds ratios[a,b]

a) For a patient population of 325 patients, minimal allele frequencies (MAF) ranging from 5% to 50%, a response of 45% and a chosen type 1 error probability α=0.05, a power of >80% could be achieved to detect an odds ratio ranging from 1.4 (MAF of 50%- **A**) to 2.0 (MAF of 10%- **B**). Line **C** represents a MAF of 5% indicating the lower chance of finding a reasonable odds ratio with the achievement of a power of >80%. Curves between lines A and B represent MAFs of 40% (most left), 30% and 20% (most right).
b) Abbreviation(s): MAF= minimal allele frequency

In many pharmacogenetic studies concerning DMARDs low power is observed due to the use of small sample sizes. Consequently, reported p-values and effect sizes for efficacy and toxicity are difficult to interpret. Also, with the interpretation of underpowered studies a suboptimal reference is provided for future studies (2;3), since replication studies could expect finding smaller effect sizes than originally reported (4;5). For example, if a power calculation is performed to confirm an initial association with an odds ratio of 3.5, hypothetically, a smaller effect size of approximately 2.0 may be expected. Therefore, for the performance of replication studies a larger sample size may be considered to identify the smaller effect size (see also Figure 2 of **chapter 8** in this thesis).
If the calculated power turns out to be small, cooperation with other research groups is attractive. Yet, in practice cooperation is challenging because of regulatory and organizational problems to combine patient cohorts (6).

Ethnicity
Conflicting results may be explained by different frequencies of polymorphisms among ethnic populations, which makes association studies less likely to compare (34). For example, this is highlighted in a study in which an association of *MTHFR* 677C>T and MTX-related alopecia in only African Americans was demonstrated (35). Therefore, to compare results between studies, considering ethnicity of the patient population is appropriate. For *MTHFR* 677C>T the MAF of this SNP in the African American population is 0.098, compared with a MAF of 0.24 in the Caucasian population (NCBI database).
These differences in frequency has also consequences for haplotypes concerning these SNPs. Significant differences in haplotype distribution between Caucasians and African-Americans were observed in the study of Hughes et al (7). Hughes et al (7) reported that the D-prime (D') value, a value

ascribing linkage disequilibrium (LD), for the two SNPs was 0.955, indicating strong LD. However, in African-Americans the D' value is much lower (0.408), indicating less linkage disequilibrium (www.hapmap.org). **Chapter 7** evaluates the role of the number of haplotypes comprising the SNPs *MTHFR* 1298A>C and *MTHFR* 677C>T in treatment outcome to MTX in RA. Analyses were performed in mainly Caucasian patients, which were derived from the BeSt study. 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. No significant associations were seen when differences in number of haplotypes were considered. Alternative values of LD could explain the different results seen in the reports of Urano et al (8) and Taniguchi et al (9), which studied the influence of the haplotype on response in patients with Asian backgrounds. Therefore, if allele frequencies and corresponding haplotypes are substantially differently distributed between ethnic populations, a genuine pharmacogenetic effect is difficult to observe. More important is whether the cohort of patients under study is large enough to limit a random change in genetic variation and to limit a sampling effect.

Nongenetic factors

Besides genetics as factors for drug response, demographic and clinical characteristics of patients are important to include for analysis in pharmacogenetic association studies. For example, several studies linked nongenetic factors to therapy outcome in patients treated with TNF inhibitors. Likewise, concomitant MTX usage and low disability have been demonstrated to predict optimal response to TNF inhibitor therapy (10;11). Moreover, Disease Activity Score (DAS) at baseline determines to a large extend the response of RA patients treated with DMARD therapy as was demonstrated from previous studies (12;13) and **chapters 3-6.** Previously, reciprocal comparison in multivariate regression analyses of 17 polymorphisms and 24 nongenetic factors in the BeSt cohort DAS at baseline was observed as most predictive (13). Scores for prediction of response regarding DAS at baseline were approximately 3 times larger than the SNP *ATIC* 347 C>G (13). This is in correspondence with the results demonstrated in **chapter 5,** which compared the same factors in a different cohort. For DAS at baseline and *ATIC* 347 C>G beta regression coefficient were 0.77 and -0.23, respectively.

Moreover, this was not only the case for MTX therapy, since DAS at baseline was also an important covariate in the multivariate-analyses for response of adalimumab, as demonstrated in **chapter 10**.

In general, the results of a large influence of a nongenetic factor on therapy outcome emphasise the necessity of multivariate-analysis in pharmacogenetic association studies. On order to analyze this predictive effect, studies require longitudinal clinical data regarding the effect of a DMARD on disease activity. Moreover, if a wider focus is applied, interactions between gene and nongenetic factors similar to the observed interactions of genetic studies concerning susceptibility to RA may be concerned (13).

Drug dosage
An important feature in any pharmacogenetic study is drug dosage. In **chapter 5,** it was demonstrated that with the clinical pharmacogenetic model to predict MTX mono-therapy efficacy in patients with established RA smaller predictive values were calculated, compared with the calculated values in the DMARD naïve patients (BeSt cohort). It was found that the model had lower true positive and negative response rates (47% and 81%, respectively) compared with the true positive and negative response rates reported in the BeSt cohort (95% and 86%, respectively). Partly, different control strategy of RA and, consequently, the variance in dosage between the two cohorts leading to different response rates may have been responsible for differences of the model's performance.
Furthermore, drug dosage is necessary for the interpretation and comparison of a functional pharmacogenetic effect on treatment outcome. For example, in theory the cellular amount of Tumor Necrosis Factor-alpha (TNFα) and, thus the amount available for inhibition by TNF inhibiting agents, might depend on the genotype of the *TNF* gene (14;15). However, a higher drug dosage may lead to inhibition of more TNFα and may, therefore, overshadow a genetic effect. In contrast, a genetic effect may be assumed, which may be due to a lower dosage of the TNF inhibitor under study. Future research need to be performed concerning levels of TNFα and TNF inhibiting therapy.

Alternative use of response criteria
Various use of disease activity parameters and/or cutoff levels for the definition of response may contribute to different results observed in pharmacogenetic studies. In order to optimally compare studies or perform meta-analyses, criteria regarding efficacy and toxicity are standardized. Examples are response criteria according to the American College of Rheumatology (ACR) improvement criteria, which are based on a perceptual improvement (20, 50, 70 and 90%) in disease symptoms (termed ACR20, ACR50, ACR70 and ACR90, respectively) and EULAR criteria (defined in **chapter 10**).
Regarding pharmacogenetics and treatment outcome measurements in RA, studying defined groups of patients is challenging. In clinical trials frequencies of response according to disease activity scores after drug therapy are measured. Based on a selected cut off value, several types of frequency distribution curves can be drawn to divide response into two groups. Rarely, the distribution of drug responses is ideal bimodal (16;17). Instead, the frequency distribution curve for measures of response is mostly unimodal distributed (16). A unimodal distribution is consistent with a multifactor configuration caused by effects of many genetic and environmental factors, in which no single factor has a clearly large effect on response. In this way, it is difficult to study a subset of responders and nonresponders by the effects of a single genetic locus (18;19).

Selection of genetic variants for association analysis

Methods of selection
A clear design in selection of genetic variants is relevant for the interpretation of results of pharmacogenetic association studies in RA. Predominantly, the presented candidate genetic factors in these studies are selected based on current knowledge of mechanism of action of the drug (20). With this approach 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. This is demonstrated in **chapter 4**, in which 7 SNPs in genes with proven functional consequences were related to efficacy and toxicity of MTX in the BeSt cohort. Due to the fact that an exact mechanism of action of DMARDs is uncertain, a clear pathway for selection of genetic variation coding for enzymes influencing these agents is challenging. The results from **chapter 3** may indicate that MTX therapy

works via the adenosine pathway, since *AMPD1* 34T allele, *ATIC* 347CC, or *ITPA* 94CC were associated with clinical response, as defined by a DAS of <2,4 (OR [95% confidence interval] 2,1 [1,0–4,5], 2,5 [1,3–4,7], and 2,7 [1,1–8.1], respectively). However, **in chapter 6**, no significant associations of these three SNPs with efficacy were found with a Swedish validation cohort under study (p>0.05). Therefore, it remains unclear, 1] whether the three variants are true markers for MTX response, 2] whether other variants in the three genes are responsible for the effect on treatment outcome, 3] whether other genes are involved. Future research on the mechanism of action of MTX is therefore required.

Since the mechanism of action of DMARDs is considered being polygenetic, selecting SNPs in a single gene will by definition only lead to a limited extent of explained variance of drug response. A solution toward these difficulties is the pathway pharmacogenetic approach, which considers variability in the entire pathway without restricting the analysis to only one gene. This method has advantages over either the candidate gene approach and the genome wide SNP analysis, as highlighted in **chapter 9**. In the same chapter, selection criteria for this approach to effectively explore potential associating SNPs with adalimumab are presented. 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 for analysis in **chapter 10**.

Genome-wide association (GWA) studies may be a promising method for pharmacogenetic studies. GWA concerns a broad approach which rapidly assesses markers across the genome to elucidate genetic variation in patients compared to controls or responders compared to non-responders. As a result, in the last two years GWA studies have presented novel associations with susceptibility to RA taking care of its polygenetic variation (21;22). For example, SNPs within the *TRAF1-C5* gene region have been demonstrated to influence the susceptibility for RA (22). These novel genes form a new source of genetic variation potentially explaining variability in disease activity and treatment response in RA patients (23;24). Ultimately, next to the direct detection of new markers for treatment response, a pharmacogenetic GWA study could give new insights into the mechanism of action of antirheumatic agents.

On the other hand, various remarks could be placed regarding the results from GWA studies. This is due to the overall found small effect sizes (25) and difficult balance between type I errors and type II errors in presenting new associations (26;27).

Concurrent functional SNPs

Genetic variation in metabolic processes may be a confounder for interpretation of pharmacogenetic results. For example, in patients and healthy volunteers with genetic polymorphisms in the cytochrome P450 drug metabolizing enzymes CYP2D6 and CYP2C9 variation in pharmacokinetics of drug therapy have been demonstrated (38-40). CYP2D6 and CYP2C9 are involved in the pharmacokinetics of therapeutics, like anticoagulants, nonsteroidal anti-inflammatory drugs and hypoglycaemic drugs (40). Also, cytochrome P450 enzymes play also a role in metabolism of physiological substrates (41;42). Although the DMARDs in this thesis are not substrates for CYP2D6 and CYP2C9, genetic variation within these enzymes could be relevant for the drug response as outcome. For example, hepatotoxicity could not only be caused by genetic variants encoding enzymes involved in the metabolism of MTX, but also could be enhanced by SNPs encoding the cytochrome P450 enzymes involved in the physiological and pathological processes of the liver.

Confounding genetic variation

Besides SNPs, other types of genetic variation exist which could have an effect on treatment outcome. For example, a factor which can be of influence is copy number variation (CNV). CNV is defined as DNA segments, which are 1 kb or larger and present at variable copy number in comparison

with a reference genome. These segments are collectively termed copy number variants (28). Even though these variants are far less abundant in the genome, CNV account for more nucleotide variation on average than SNPs (29). Subsequently, a SNP effect on treatment outcome could be misinterpreted due to a CNV effect in the same gene region (30). One of the selected SNPs on the custom made array presented in **chapter 10** was the functional SNP *FCGR3A* -158T>G (rs396991). In previous studies, this SNP was associated with treatment outcome to TNF inhibitors (31-33). However, with our analyses presented in **chapter 10** an association with efficacy was absent. Hypothetically, CNV may cause a different interpretation of genotypes resulting in altered findings. From genome wide studies it has been observed that in the FCGR3A gene region CNVs are present (34). In this way, alternative genotyping results of *FCGR3A* -158T>G may be due to CNV: high copy number (more than 2 alleles) may lead to the detection of an inaccurate number of heterozygous genotypes and low copy number (one allele) may lead to more homozygous genotypes (35).
In addition, epigenetics could also be a reasonable confounder in finding (or not finding) genetic associations with treatment outcome. The term epigenetics covers phenotypic changes which are not covered by mutations in DNA sequence. It comprises grossly three different areas in which alteration could lead to changes in gene expression and enzyme activity: methylation of DNA, modification of histones in chromatin and RNA mediated regulation of gene-expression (36).
In summary, it is difficult to assign differences in treatment outcome solely to SNPs. Future research have to be performed to exactly study the weight of SNPs in differences in efficacy and/or toxicity.

Adjustment for multiple testing

Along with the discovery of novel causative loci for treatment response with GWA studies, testing a large number of loci for association creates potential false-positive results and, therefore, the need for adjustment for multiple testing (37). Similarly, but to a lesser extent, adjustment is necessary in studies applying a candidate- or pathway gene approach. The need for multiple testing arises from the assumption that the incidence of false positives is proportional to the number of tests performed and level of significance. For example, If 10,000 genes are tested, 5% or 500 genes might be found significant by chance alone. For this reason correction is important: it adjusts the individual p-value for each gene in order to keep the false positive- rate to less than or equal to the p-value cutoff. **Chapter 10** presented associations between efficacy of adalimumab therapy and SNPs selected by the pharmacogenetic pathway approach (**chapter 9**). It was demonstrated that 19 SNPs, 11 SNPs and 8 SNPs were significantly associated EULAR good response, EULAR remission and relative change in DAS28, respectively ($p<0.05$) Moreover, 4 SNPs, rs1126535 in *CD40LG*, rs6828477 in *KDR*, rs1267067 in *TANK* and rs25648 in *VEGFA* showed consistent associations and, therefore, they appear to be the most predictive for adalimumab efficacy. In this chapter, no adjustment for multiple testing was performed. The most common method for correcting for multiple testing, the Bonferroni correction, involves adjusting the significance level of each test by the total number of performed tests (38). However, this method has a conservative character, since interaction and cooperation between causative genes are circumvented (37;39). Also, other adjustments could be applied, like permutation testing or false discovery rate, but also these methods have specific difficulties (not discussed) (37). Replication of genetic associations in a second comparable cohort of patients is essential, but not always feasible. Therefore, we have decided to present the p-values of this explorative study without adjustments to make the results accessible for clear interpretation. Still, we would underline that multiple independent tests were applied and these results imply suggestive associations with adalimumab efficacy.
All statistical adjustments are focused on a certain level of significance. However, more weight should be on reporting effect size and confidence interval instead of p-values. For example, a mean

decrease in DAS for a specific genetic variant of 1.2 with a 95% confidence interval of 0.8 to 1.6 illustrates a range of values for what the mean decrease might be if the entire population is studied. This range of values highlights the importance of clinical values instead of statistical outcomes. Consequently, effect size and confidence intervals encourage meaningful qualitative decisions about quantitative data. In other words, a rheumatologist becomes more involved in the data and may evaluate its own clinical decision making in e.g. the additive value of genotyping patients in practice.

Future perspectives

Prospective study design for validation of pharmacogenetics in RA

In recent years, pharmacogenetic studies have revealed numerous SNPs that associated with drug response but only a few of these have been introduced as candidates for clinical implementation. Especially, prospective pharmacogenetic studies are scarce. One such prospective study concerns adverse drug reactions to abacavir in HIV-treatment (40). In this large study, the HLA-B*5701 polymorphism was highly linked to hypersensitivity reactions in a cohort of Caucasians and successfully replicated in other but similar cohorts. It was calculated that 14 patients would have to be screened, to prevent one hypersensitivity reaction on abacavir therapy (40). Currently, this polymorphism is increasingly being used as a genetic biomarker in routine clinical practice. Similar studies are needed to demonstrate the value of prospective genotyping for antirheumatic therapy in clinical practice. In figure 3 an ideal study proposal for a prospective study concerning MTX efficacy in RA is presented, in which strategy for therapy is guided by pharmacogenetics.
For this hypothetical study proposal, adult patients with early RA and active disease are enrolled. First a randomization (figure 3) is performed to assign patients to undergo prospective pharmacogenetic screening or to undergo a standard-of-care DMARD treatment without pharmacogenetic screening. Patients assigned to prospective pharmacogenetic testing are divided in predicted responders and predicted nonresponders based on the pharmacogenetic test determining MTX monotherapy efficacy. Predicted responders are allocated to treatment with MTX monotherapy. Predicted nonresponders to MTX are given the alternative traditional DMARD sulphasalazine. Patients assigned to the control group (without pharmacogenetic screening) are given MTX monotherapy as standard-of-care therapy. Therapy is evaluated and adjusted after 6 months. Hereafter, patients with standard-of-care DMARD treatment are screened for the pharmacogenetic test.
Primary analyses are focussed on statistical differences in response percentages between patient groups allocated to pharmacogenetic screening and the patient group allocated to standard-of-care treatment. As secondary analyses, the performance of the pharmacogenetic tests is calculated in the control group with standard-of-care treatment.
Power calculation reveals that enrolment of 300 patients for evaluation of at least 100 patients per group is needed for this prognostic study to have a statistical power of 90% (with a chosen type 1 error probability α=0.05) to detect an improvement in response of 50% in the groups with pharmacogenetic screening compared to the control group without pharmacogenetic screening.
Likewise, a more complex study design could also be applied for a prospective study including the pharmacogenetics of TNF inhibitors.
In conclusion, results of above described study could demonstrate the beneficial value of prospective pharmacogenetic screening compared to current standard therapy. Application of pharmacogenetic tests could reduce inefficacious and unnecessary drug exposure and thus treatment delay and toxicity in clinical practice. However, even with promising results from prospective studies, several challenges appear before a genetic marker can be implemented as a clinical tool (figure 1).

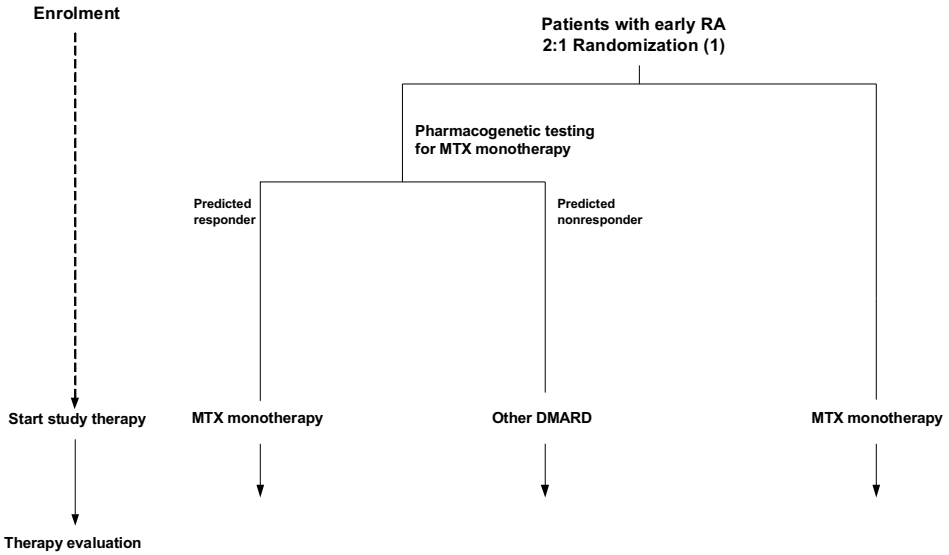

Figure 3. study proposal for a prospective study concerning MTX in RA, in which strategy for therapy is guided by pharmacogenetics[a]

a) Abbreviation(s): MTX= methotrexaat, RA= rheumatoid arthritis, TNF= tumor necrosis factor

Challenging steps towards clinical implication of pharmacogenetics

Social, ethical and legal implications of pharmacogenetics

It is demonstrated from the literature that pharmacogenetics holds the potential to improve therapeutic efficacy, to minimize adverse drug events, to enhance safety and to reduce the overall cost of management of disease, but needs further development for clinical implementation in the near future. Still, this development is not solely a challenge for genetic researchers and clinicians, since several social, ethical and legal implications form large obstacles for authorities, health care organizations, regulatory organizations and individuals.

For (pharmaco)genetic testing, privacy and informed consent may be essential in clinical usage of the genetic information. Personal information could be used adversely to a patient's interests. However, overall the knowledge on genetics is limited in individual patients. As a consequence, the autonomy of patients is reduced and the risk of involuntarily and abusively application of genetic data would be increased (41;42).

Also, it may be considered unethical not to employ pharmacogenetic testing in patients in order to avoid the exposure to the inefficacy and harmful side effects of drugs (43). On the other hand, with the performance of genetic testing the problem of handling 'by-catch' arises. This by-catch is the result of creating a genetic profile by the performance of e.g. whole-genome testing in which not only

searched information is present, but also unsearched information. This was studied by Henrikson and colleagues (44), who demonstrated that 53% of potential pharmacogenetic variants were reported to have a significant association with disease susceptibility. Hereby, genetic profiling could reveal susceptibility to e.g. serious diseases. In the light of self determination, is it mandatory to notify the patient on this by-catch? The psychological impact of this knowledge and concomitant responsibility could be difficult for the patient to handle in the future. This could include a change in health behaviour, quality of life and social surrounding (42;45).
The question remains how the health care insurers would act based on the pharmacogenetic results of their clients. The focus of the health care system on clients is likely to be shifting from a general population view to a more personalized view (46). Likewise, this attitude of the health care system could advance the inclusion of pharmacogenetics in to clinical practice. This could also lead to unwanted situations (46;47). For example, patients that are predicted non responder to conventional medication would be unfavourable to insure, since these patients would require more expensive medication and/or their nonresponse would result in chronic disease.

Economic considerations towards pharmacogenetics
In the short term, implementation of pharmacogenetic testing could result in higher drug related health care costs. Partly, investment in the development and evaluation of pharmacogenetic tests may lead to higher expenses. Also, higher costs could be due to a higher number of individual prescriptions written by confident clinicians, since choice of therapy is scientifically more gratified. In contrast, in the long term, the overall health care costs could be reduced, since e.g. unnecessary and unsuccessful expensive drugs are avoided. Moreover, overall drug related morbidity and mortality could be decreased (47;48).
As in the health care system, a more individualized trend towards the patient may be expected in the pharmaceutical industry. Ideally, application of pharmacogenetic testing can eventually enhance drug discovery and development process leading to market segregation, which is increasing the number of new drugs available on the market for a group of patients. Furthermore, it could result in more effective usage of existing drugs and could assess the efficacy of previously eliminated drugs, which had failed in clinical trials due to e.g. toxicity reasons. However, in practice for a pharmaceutical company this market segregation is difficult to cope, which has invested a substantial time and a significant amount of money in developing a new therapeutic agent for a small part of patients (47;49;50).

Impact of genetics
The impact of the pharmacogenetic test has to be considered. Specifically, in the case of effectively predicting effective drug therapy to antirheumatic agents, the additional value to conventional treatment has to be proven. For example, consulting a rheumatologist in an early phase of RA's disease progress and, hereby, achieving an optimal result on treatment in order to reduce joint damage may overshadow the genetic effects and/or demonstrate over-valuation of a clinical pharmacogenetic test.

Comorbidity, co-medication and adherence to therapy
Individualizing disease and medication of patients remain a problem for clinical usage of pharmacogenetics. If a test is based on a population of patients with RA, mostly the general well being or disease status is assessed. However, regarding the genetic aetiology of RA, previous findings demonstrated that two types of RA, ACPA-positive and ACPA-negative RA, have different genetic origins (51). In this way, estimating a chance of drug response and/or toxicity with a pharmacogenetic test could be limited by individual type of disease(s).

The same limitation arises for a specific test based on one type of drug instead of considering the use of other medication besides antirheumatic agents in RA patients. Indeed, it is demonstrated that statins may have moderate disease modifying effects in RA and these drugs might prevent or slow the development of RA (52). In this way, a clear effect of DMARDs on therapy outcome is difficult to observe. In order to adapt pharmacogenetic tests based on assembled patients, who will have an identical risk of response based on same type of disease, concomitant medication and environmental factors, remain a significant challenge.
Finally, a patients' suboptimal adherence to proposed therapy guidelines could be causative for errors in evaluation of interindividual variability in treatment outcome and hereby interpretation of genotypic effects on treatment outcome. Drug adherence could increase if patients would know they could benefit from their personal pharmacogenetic profile to improve the response to drug therapy.

Education to the clinician less trained in genetics
Along with a growing pharmacogenetic knowledge in rheumatology arises an increasing difficulty to explain to clinicians the use, benefits and pitfalls of pharmacogenetics and how to interpret a pharmacogenetic test. Therefore, additional education for clinicians is required for a successful choice and/or adjustment of drug therapy and, moreover, for an optimal explanation towards the patient.

Conclusion

Results from this thesis have elucidated potential genetic markers, which were associated with treatment outcome to MTX and adalimumab. Furthermore, a model for predicting the efficacy of MTX in patients with RA was validated in two cohorts indicating that predicting efficacy by a pharmacogenetic model is feasible in RA patients treated with MTX. Importantly, definitive conclusions about the role of genetic predictive factors in treatment outcome to DMARDS could not be drawn, since these results have to be further validated and replicated in future pharmacogenetic studies. Large randomized prospective studies should be planned to demonstrate its legitimate predictive and cost-effective value before a genetically individualized approach is applicable in daily clinical practice.

The potential role of pharmacogenetics in the prediction of efficacy and adverse events in RA patients treated with DMARDs is presented in this thesis. Hereby, new knowledge is added to the relatively young research field of pharmacogenetics, which may hopefully lead to a better treatment strategy for RA patients.

References

(1) Bernstein BA. An introduction to sample size and power. J Dev Behav Pediatr 2008 December;29(6):516-22.

(2) Little J, Bradley L, Bray MS, Clyne M, Dorman J, Ellsworth DL et al. Reporting, appraising, and integrating data on genotype prevalence and gene-disease associations. Am J Epidemiol 2002 August 15;156(4):300-10.

(3) Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet 2003 February;33(2):177-82.

(4) Colhoun HM, McKeigue PM, Davey SG. Problems of reporting genetic associations with complex outcomes. Lancet 2003 March 8;361(9360):865-72.

(5) Hattersley AT, McCarthy MI. What makes a good genetic association study? Lancet 2005 October 8;366(9493):1315-23.

(6) Carey TS, Kinsinger L, Keyserling T, Harris R. Research in the community: recruiting and retaining practices. J Community Health 1996 October;21(5):315-27.

(7) Hughes LB, Beasley TM, Patel H, Tiwari HK, Morgan SL, Baggott JE et al. Racial or ethnic differences in allele frequencies of single-nucleotide polymorphisms in the methylenetetrahydrofolate reductase gene and their influence on response to methotrexate in rheumatoid arthritis. Ann Rheum Dis 2006 September;65(9):1213-8.

(8) Urano W, Taniguchi A, Yamanaka H, Tanaka E, Nakajima H, Matsuda Y et al. Polymorphisms in the methylenetetrahydrofolate reductase gene were associated with both the efficacy and the toxicity of methotrexate used for the treatment of rheumatoid arthritis, as evidenced by single locus and haplotype analyses. Pharmacogenetics 2002 April;12(3):183-90.

(9) Taniguchi A, Urano W, Tanaka E, Furihata S, Kamitsuji S, Inoue E et al. Validation of the associations between single nucleotide polymorphisms or haplotypes and responses to disease-modifying antirheumatic drugs in patients with rheumatoid arthritis: a proposal for prospective pharmacogenomic study in clinical practice. Pharmacogenet Genomics 2007 June;17(6):383-90.

(10) Hyrich KL, Watson KD, Silman AJ, Symmons DP. Predictors of response to anti-TNF-alpha therapy among patients with rheumatoid arthritis: results from the British Society for Rheumatology Biologics Register. Rheumatology (Oxford) 2006 December;45(12):1558-65.

(11) Kristensen LE, Kapetanovic MC, Gulfe A, Soderlin M, Saxne T, Geborek P. Predictors of response to anti-TNF therapy according to ACR and EULAR criteria in patients with established RA: results from the South Swedish Arthritis Treatment Group Register. Rheumatology (Oxford) 2008 April;47(4):495-9.

(12) Anderson JJ, Wells G, Verhoeven AC, Felson DT. Factors predicting response to treatment in rheumatoid arthritis: the importance of disease duration. Arthritis Rheum 2000 January;43(1):22-9.

(13) Wessels JA, van der Kooij SM, le Cessie S., Kievit W, Barerra P, Allaart CF et al. A clinical pharmacogenetic model to predict the efficacy of methotrexate monotherapy in recent-onset rheumatoid arthritis. Arthritis Rheum 2007 June;56(6):1765-75.

(14) Marotte H, Arnaud B, Diasparra J, Zrioual S, Miossec P. Association between the level of circulating bioactive tumor necrosis factor alpha and the tumor necrosis factor alpha gene polymorphism at -308 in patients with rheumatoid arthritis treated with a tumor necrosis factor alpha inhibitor. Arthritis Rheum 2008 May;58(5):1258-63.

(15) Louis E, Franchimont D, Piron A, Gevaert Y, Schaaf-Lafontaine N, Roland S et al. Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. Clin Exp Immunol 1998 September;113(3):401-6.

(16) Lindpaintner K. Pharmacogenetics and the future of medical practice. Br J Clin Pharmacol 2002 August;54(2):221-30.

(17) van Vollenhoven RF, Klareskog L. Clinical responses to tumor necrosis factor alpha antagonists do not show a bimodal distribution: data from the Stockholm tumor necrosis factor alpha followup registry. Arthritis Rheum 2003 June;48(6):1500-3.

(18) Johnson JA, Lima JJ. Drug receptor/effector polymorphisms and pharmacogenetics: current status and challenges. Pharmacogenetics 2003 September;13(9):525-34.

(19) Turner ST, Schwartz GL, Chapman AB, Hall WD, Boerwinkle E. Antihypertensive pharmacogenetics: getting the right drug into the right patient. J Hypertens 2001 January;19(1):1-11.

(20) Tabor HK, Risch NJ, Myers RM. Candidate-gene approaches for studying complex genetic traits: practical considerations. Nat Rev Genet 2002 May;3(5):391-7.

(21) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Wellcome Trust Case Control Consortium. Nature 2007 June 7;447(7145):661-78.

(22) Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, Ding B et al. TRAF1-C5 as a risk locus for rheumatoid arthritis--a genomewide study. N Engl J Med 2007 September 20;357(12):1199-209.

(23) Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet. 2008 Aug;40(8):955-62.

(24) Sklar P, Smoller JW, Fan J, Ferreira MA, Perlis RH, Chambert K et al. Whole-genome association study of bipolar disorder. Mol Psychiatry 2008 June;13(6):558-69.

(25) Newton-Cheh C, Hirschhorn JN. Genetic association studies of complex traits: design and analysis issues. Mutat Res 2005 June 3;573(1-2):54-69.

(26) van der Helm-van Mil AH, Padyukov L, Toes RE, Klareskog L, Huizinga TW. Genome-wide single-nucleotide polymorphism studies in rheumatology: Hype or hope? Arthritis Rheum 2008 September;58(9):2591-7.

(27) Yang Q, Cui J, Chazaro I, Cupples LA, Demissie S. Power and type I error rate of false discovery rate approaches in genome-wide association studies. BMC Genet 2005 December 30;6 Suppl 1:S134.

(28) Feuk L, Carson AR, Scherer SW. Structural variation in the human genome. Nat Rev Genet 2006 February;7(2):85-97.

(29) Wong KK, deLeeuw RJ, Dosanjh NS, Kimm LR, Cheng Z, Horsman DE et al. A comprehensive analysis of common copy-number variations in the human genome. Am J Hum Genet 2007 January;80(1):91-104.

(30) Beckmann JS, Estivill X, Antonarakis SE. Copy number variants and genetic traits: closer to the resolution of phenotypic to genotypic variability. Nat Rev Genet 2007 August;8(8):639-46.

(31) Canete JD, Suarez B, Hernandez MV, Sanmarti R, Rego I, Celis R et al. Influence of variants of Fc{gamma}receptors IIA and IIIA on the ACR and EULAR responses to anti-TNF{alpha} therapy in rheumatoid arthritis. Ann Rheum Dis. 2009 Oct;68(10):1547-52

(32) Louis E, El Ghoul Z, Vermeire S, Dall'Ozzo S, Rutgeerts P, Paintaud G et al. Association between polymorphism in IgG Fc receptor IIIa coding gene and biological response to infliximab in Crohn's disease. Aliment Pharmacol Ther 2004 March 1;19(5):511-9.

(33) Tutuncu Z, Kavanaugh A, Zvaifler N, Corr M, Deutsch R, Boyle D. Fcgamma receptor type IIIA polymorphisms influence treatment outcomes in patients with inflammatory arthritis treated with tumor necrosis factor alpha-blocking agents. Arthritis Rheum 2005 September;52(9):2693-6.

(34) Aitman TJ, Dong R, Vyse TJ, Norsworthy PJ, Johnson MD, Smith J et al. Copy number polymorphism in Fcgr3 predisposes to glomerulonephritis in rats and humans. Nature 2006 February 16;439(7078):851-5.

(35) McCarroll SA, Altshuler DM. Copy-number variation and association studies of human disease. Nat Genet 2007 July;39(7 Suppl):S37-S42.

(36) Gomez A, Ingelman-Sundberg M. Pharmacoepigenetics: its role in interindividual differences in drug response. Clin Pharmacol Ther 2009 April;85(4):426-30.

(37) Rice TK, Schork NJ, Rao DC. Methods for handling multiple testing. Adv Genet 2008;60:293-308.

(38) Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ 1995 January 21;310(6973):170.

(39) Perneger TV. What's wrong with Bonferroni adjustments. BMJ 1998 April 18;316(7139):1236-8.

(40) Mallal S, Phillips E, Carosi G, Molina JM, Workman C, Tomazic J et al. HLA-B*5701 screening for hypersensitivity to abacavir. N Engl J Med 2008 February 7;358(6):568-79.

(41) Charo RA. Body of research--ownership and use of human tissue. N Engl J Med 2006 October 12;355(15):1517-9.

(42) Offit K, Groeger E, Turner S, Wadsworth EA, Weiser MA. The "duty to warn" a patient's family members about hereditary disease risks. JAMA 2004 September 22;292(12):1469-73.

(43) Wolf CR, Smith G, Smith RL. Science, medicine, and the future: Pharmacogenetics. BMJ 2000 April 8;320(7240):987-90.

(44) Henrikson NB, Burke W, Veenstra DL. Ancillary risk information and pharmacogenetic tests: social and policy implications. Pharmacogenomics J 2008 April;8(2):85-9.

(45) Clayton EW. Ethical, legal, and social implications of genomic medicine. N Engl J Med 2003 August 7;349(6):562-9.

(46) Collins FS, McKusick VA. Implications of the Human Genome Project for medical science. JAMA 2001 February 7;285(5):540-4.

(47) Robertson JA, Brody B, Buchanan A, Kahn J, McPherson E. Pharmacogenetic challenges for the health care system. Health Aff (Millwood) 2002 July;21(4):155-67.

(48) Lesko LJ, Rowland M, Peck CC, Blaschke TF. Optimizing the science of drug development: opportunities for better candidate selection and accelerated evaluation in humans. Pharm Res 2000 November;17(11):1335-44.

(49) Eisenberg RS. The shifting functional balance of patents and drug regulation. Health Aff (Millwood) 2001 September;20(5):119-35.

(50) Meurer MJ. Pharmacogenomics, genetic tests, and patent-based incentives. Adv Genet 2003;50:399-426.

(51) Plenge RM, Padyukov L, Remmers EF, Purcell S, Lee AT, Karlson EW et al. Replication of putative candidate-gene associations with rheumatoid arthritis in >4,000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4. Am J Hum Genet 2005 December;77(6):1044-60.

(52) Nurmohamed MT, Dijkmans BA. Dyslipidaemia, statins and rheumatoid arthritis. Ann Rheum Dis 2009 April;68(4):453-5.