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

Kooloos, W.M.

Chapter 9

Criteria for the selection of single nucleotide polymorphisms in pathway pharmacogenetics: TNF inhibitors as a case study

Wouter M. Kooloos[1], Judith A.M Wessels[1], Tahar van der Straaten[1], Tom W.J. Huizinga[2] and Henk-Jan Guchelaar[1]

[1] *Clinical Pharmacy & Toxicology, Leiden University Medical Center, Leiden, The Netherlands.*
[2] *Rheumatology, Leiden University Medical Center, Leiden, The Netherlands.*

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Abstract

Pharmacogenetics aims to identify genetic variation to predict drug response or to establish an individual optimal dose. Classically, explorative pharmacogenetic studies are performed concerning a limited number of SNPs in genes encoding enzymes involved in the drug's metabolic route. Alternatively, potential markers across the genome are elucidated by the performance of the hypothesis-free genome-wide method. Besides their successful use, both methods provide substantial disadvantages. 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. In this article, we present selection criteria for this approach to effectively explore potential associating SNPs. As an illustration, the method is applied to the biological adalimumab as a case study.

Introduction

The concept of pharmacogenetics is that germline genetic variability causes variable drug response among individual patients. Knowledge about related genetic variants, mostly single nucleotide polymorphisms (SNPs), may help to predict drug response or optimal dose in the individual patient (1). Classically, explorative pharmacogenetic association studies are aimed at finding potential predictive SNPs. These concern a limited number of SNPs in genes encoding enzymes or proteins representing the drug's major metabolic route or target. For example, to explain variable drug response of the anticoagulant warfarin, association studies showed that bleeding time (INR) was associated with cytochrome P450 2C9 (the major metabolic route of warfarin) genotype and VKORC1 genotype (the pharmacodynamic target of warfarin) (2,3). Obviously, the selection of SNPs within the candidate gene is essential, because only some of them may be related to drug response whereas others are not.
This approach has its limitations, however, because of an incomplete knowledge of the pharmacology of a substantial number of drugs and the wide variety of SNPs in the human genome. Thus it may not be surprising that the candidate gene approach has led to poor reproducibility with regard to potential predictors of drug response. Therefore, systematic selection remains a challenge to scientists in obtaining
a potentially successful set of SNPs for predicting drug response.
In this article, SNP selection for pharmacogenetic association studies is discussed. Additionally, a pharmacogenetic pathway approach is presented, together with proposed criteria for systematic selection of SNPs. We have applied this method for the selection of potential interesting SNPs within genes related to the mechanism of action of TNF inhibiting drug adalimumab. This drug has been effective in the treatment of progressive rheumatoid arthritis (RA) by reducing inflammation and joint destruction (4). Approximately 40–60% of individuals with RA, however, do not respond adequately to this drug (5,6). Moreover, the use of TNF inhibitors is accompanied by adverse events and unintentional immune suppression. Pharmacogenetics has the potential to increase efficacy and ameliorate adverse events and its application can translate into clinical benefit for patients with RA (7)

Candidate gene method

Selection of SNPs in hypothesis driven pharmacogenetic association studies is based on their functionality, in which the genetic variant leads (or is predicted to lead) to alteration in protein
function and hence differences in drug response. This approach has led to the discovery of a substantial number of relevant SNPs in pharmacogenetics [3,8,9]. This approach, however, also demonstrated associations that could not be replicated by other investigators [10,11] and thus could result in possibly false-positive findings. Moreover, in a substantial number of studies, SNP selection is not systematically performed but seems to be arbitrary or extensions of previous findings. Also, because complex traits are mostly considered not to be monogenetic, selecting SNPs according to this hypothetical approach will repeatedly lead to a limited explanation of variance in drug response.

Genome-wide method

A more comprehensive, and more expensive, approach is the genome-wide method using SNP arrays (WGA). A clear advantage of this method is that it is hypothesis-free and that this may reveal unexpected SNPs related to drug response. Hence this method does not rely on current knowledge with regard to the metabolism and mechanism of action of the drug. Indeed, in the past two years genome-wide association studies have presented novel associations of SNPs with drug response

[12–14]. Moreover, novel information about the pathogenesis and progression of complex diseases, like RA and Crohn's disease, could be revealed using the genome-wide SNP approach [15–17]. An advantage of this approach is that complex traits can be explored, accommodating polygenetic variation. Yet, various remarks can be placed regarding clinical overvaluation of the results from this approach because of the overall limited effect sizes found [18]. Additional problems arise regarding the discrepancy between type I errors (false-positive results) and subsequently adjusted type II errors (false negative results) in detecting an associated SNP [19,20]. Specifically, the appliance of rigorous criteria for significance (owing to multiple testing) to oppose type I errors can eventually lead to type II errors (missing a real effect).

Pathway gene method

A third method is the pathway gene approach that combines the advantages of the candidate gene approach and the genomewide approach. Moreover, with this method fewer disadvantages are experienced. Namely, by applying the pathway gene approach fewer false-positive results will be found than with the genome-wide method owing to the limitation of multiple testing. A characteristic of the pathway gene method is that a set of SNPs is selected based on a description of pathways regarding the mechanism of action and pharmacokinetics of the drug under study. In this systems pharmacology approach, one considers variability in the entire pathway without restricting the analysis to a single gene, of which the impact on the drug's mechanism of action is unknown. With the candidate gene method, SNPs that are responsible for the rate limiting or extending step in mechanism of action are easily missed. For example, if SNPs in the signal transduction routes of the beta-adrenergic receptor are explored, a complex quandary of proteins come across which are involved in the signal transduction route. Assumably, for most drugs pharmacogenetics has the greatest potential to be clinically useful if information on multiple genes is used. In this context, the pharmacogenetics of most drugs is likely to be comparable to the genetics of complex diseases. In both cases numerous proteins are involved, and genetic variability in each might contribute to the overall variability observed clinically [21].

Before SNP selection in pathway pharmacogenetics

Exploration of the pathway and gene selection

Before SNP selection, an extensive literature search regarding the hypothetical mechanism of action of TNF inhibitors was performed to select candidate genes coding for involved proteins. Pubmed/National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) was searched for original research concerning in vivo and in vitro studies, published in the past five years, regarding this subject. This search was performed using the Mesh terms RA, TNF-alpha, pharmacology, monoclonal antibodies, etanercept, adalimumab and infliximab.

The anti-TNF drug adalimumab is a complete humanized IgG1 monoclonal antibody which binds to and neutralizes both soluble and transmembrane forms of TNF-alpha. Generally, a summary of seven groups can be created (table 1): neutralization and blockage [22,23]; interaction with Fc receptor (crosslinkage) [24]; initiation of reverse signaling, leading to blockage, increased apoptosis or growth arrest [25,26]; reduction of inflammatory cytokine production and angiogenic factor expression [23,27–29]; restoration of immune regulation (Treg cell) [30]; mediation of complement-dependent cytotoxicity (CDC) and antibodydependent cytotoxicity (ADCC) [22,26]; downregulation or discontinuation of bone and cartilage destruction [31,32]. These mechanisms of action have mainly been demonstrated in vitro and, to a lesser extent, in vivo [33,34]. In the defined pathway 124 genes related to the mechanism of action of TNF inhibitors were explored.

SNP sources

After candidate genes had been selected, a SNP search within these genes was performed. SNPs were assessed using the database of the NCBI (http://www.ncbi.nlm.nih.gov/SNP). The NCBI has compiled a dataset of over 10 million SNPs throughout the entire human genome resulting from publicly and privately funded genome sequencing projects in the dbSNP [35,36]. Other databases of SNPper/CHIP-Bioinformatics (http://snpper.chip.org/bio) and Snap/SNP Annotation platform (http://snap.humgen.au.dk/views/index.cgi), mainly related to the NCBI, were consulted. A total of 51,793 SNPs in 124 genes were available for the SNP selection procedure.

Criteria for the selection of SNPs

Primary selection aims to obtain SNPs, with a high probability to detect, as a result of reported heterozygosity frequencies and the number of previous genotyping techniques applied. When these two primary criteria are applied, fewer SNPs are included for the secondary selection with more extensive parameters. Because of this time-effective aspect, namely not analyzing each SNP in each gene, this two-step design was chosen for the selection process.

Primary selection

The primary selection of SNPs is based on the criteria:

- Genetic region and heterozygosity: introns with a heterozygosity between 0.400 and 0.480 and all exons with a heterozygosity of more than 0.095.
- Validation status: only SNPs with a validation status of 2 or more measurements as reported in the NCBI.

When these criteria were applied, 2629 SNPs out of the total of 51,793 SNPs in 124 genes were selected (Fig. 1).

Mechanism of action	Selected genes	N of genes	Refer-ences
Neutralization and blockage	TNF, LTA, TNFRSF1A, TNFRSF1B, ADAM17, IL1A IL1B, IL1R1, IL1R2, IL1RAP, IL1RN	11	(22,23)
Interaction with Fc receptor (cross-linkage)	FCGR2A, FCGR2B, FCGR3A, FCGR3B	4	(24)
Initiation of reverse signaling, leading to blockage or increased apoptosis or growth arrest	TRADD, FADD, RIPK1, TRAF2, TANK, TNFAIP3, MAP3K7IP1, MAP3K7IP2, MAP3K7, IKBKG, CHUK, IKBKB, NFKB1, NFKB2, NFKB3, MAPK8, TP53, BAX, BAK1, CASP3, CASP7, CASP8, MAPK14, BCL2L1, BIRC2, BIRC3, XIAP, CFLAR	28	(25,26)
Reduction of inflammatory cytokine production and angiogenic factor expression	IL6, IL6R, CSF2, CSF2RA, CSF2RB, CSF1, CSF1R, CSF3, CSF3R, LIF, LIFR, OSM, OSMR, IL2, IL2RA, IL3, IL3R, IL7, IL7R, IL8, IL8RA, IL8RB, IL9, IL9R, IL12A, IL12B, IL12RB1, IL12RB2, IL18, IL18R1, IFNA1, IFNB, IFNG, IFNGR1, IFNGR2, IL15, IL15RA, CD11, CD28, CD40, CD40L, CD69, APOA1, IL4, IL4R, IL10, IL10RA, IL10RB, IL11, IL11RA, IL13, IL13RA1, IL13RA2, TGFB1, VEGFA, VEGFB, VEGFC, FIGF, KDR, FLT1, FLT4, SELE, ICAM1, VCAM1, vWF, PECAM1	66	(23,27-29)
Restoration of immune regulation (Treg cell)	FOXP3	1	(30)
Mediation of complement-dependent cytotoxicity (CDC) and antibody-dependent cytotoxicity (ADCC)	C1QA, C1QB, C1QC, CR1, C2, C3, C4A, C4B, C5, C5AR1	10	(22,26)
Down-regulation or discontinuation of bone and cartilage destruction	TNFRSF11A, TNFSF11, TNFRSF11B, TRAF6	4	(31,32)
Total number of genes		**124**	

Table 1. Candidate gene selection

Abbreviations and accessory full names of formal genes can be relocated in the NCBI gene database *(http://www.ncbi.nlm.nih.gov)*

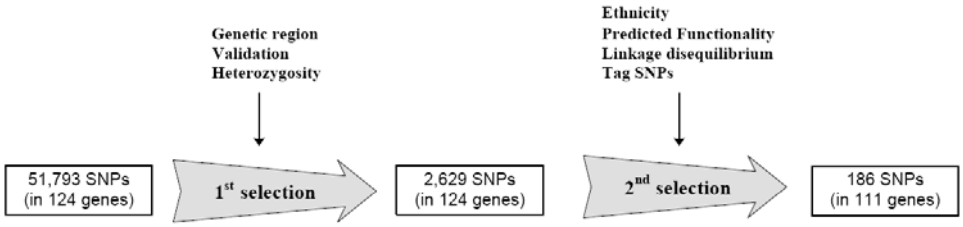

Figure 1. Design stepwise SNP selection
Abbreviation(s): SNP= single nucleotide polymorphism

Genetic region

In the NCBI SNP database, a subdivision is made between different regions of genes: 3' and 5' near a gene, introns and exons. Additionally, functional characteristics in these regions are: noncoding, nonsynonymous, frameshift, synonymous, promoter or untranslated. Important for SNP selection, on the basis of alteration of a gene product and in this way protein function, is the presence of SNPs in exons. Still, noncoding SNPs, like introns, which maybe, for example transcribed to noncoding RNA, could have functions in transcriptional interference and promoter inactivation, as well as indirect effects on transcription regulatory proteins and in genomic imprinting [37].

In the NCBI database, specific regions within each gene were examined. A subdivision was made into different regions: unknown, 3' and 5' near gene, introns and exons. Additionally, exons were subdivided into synonymous, nonsynonymous, 3'UTR and 5'UTR subgroups.

Heterozygosity

True associations in case–control studies depend on the precise definition of response criterion, power and sample size of the study. For the detection of small differences in allele frequencies, a study has to be sufficiently powered. Additionally, selecting SNPs with a low minor allele frequency (MAF) will require very large sample size cohorts to achieve an association which is statistically sufficiently powered [38]. Figure 2 presents examples of number of cases needed to detect significant differences in variable allele frequencies in a case–control (1:2) study design. Paired lines represent number of cases required to detect differences with significance level of 1.10-4 and 1.10-6 with 80% power depending on the MAF in controls and hypothetical odds ratios for obtaining good response in cases relative to controls. For example, to detect a significant difference with a MAF in controls of 0.3 with a hypothetical odds ratio of 2.0 for obtaining good response in cases relative to controls, at least 147 cases and 294 controls are needed.

A constructive tool in selection based on frequency is the usage of a SNP's heterozygosity, which is the frequency of the occurrence of heterozygous individuals for a particular SNP. To use a specific range of heterozygosity as a criterion, the heterozygosity can be calculated from a preferred MAF within a sample size regarding the power for an association study.

In this case study, SNPs were included on the basis of a total sample size of 400–500. In this way, for all SNPs, except exons, cutoff values regarding heterozygosity were chosen between 0.400 and 0.480. If heterozygosity was lower than 0.400 and higher than 0.480, SNPs were excluded, except for SNPs with a significant predicted functional change of protein (defined below). Because SNPs in exons are less abundant, cut-off values regarding heterozygosity were lowered. In this way, SNPs in exons with a heterozygosity of more than 0.095 were included.

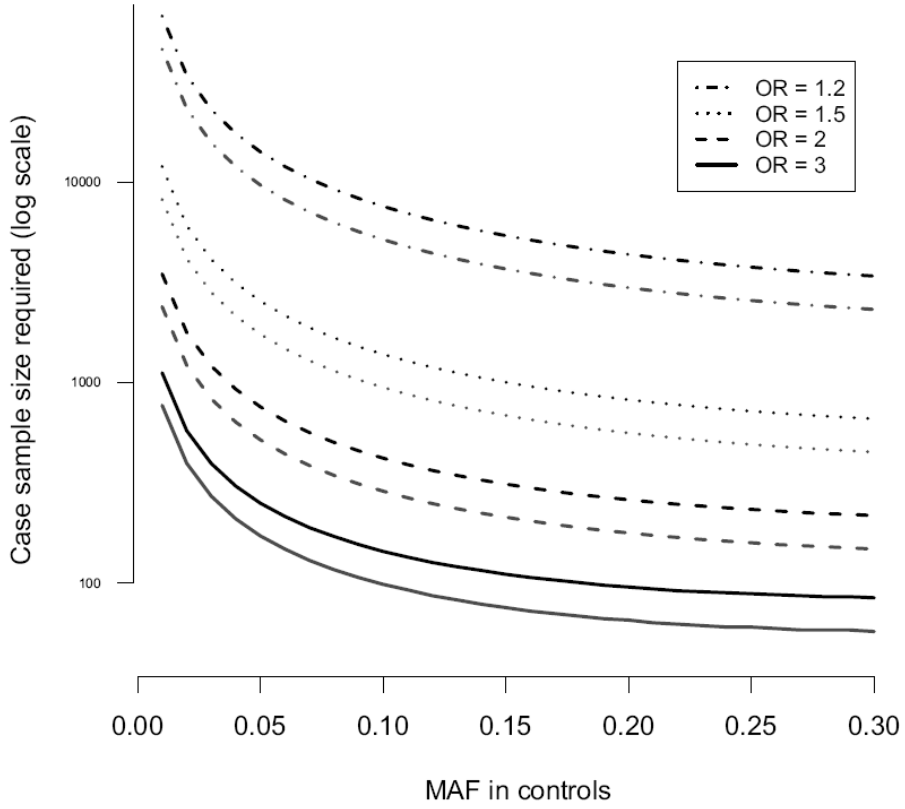

Figure 2. Schematic representation of the number of cases needed to detect significant differences in a case-control (1:2) study design[a,b]

a) Lines represent number of cases required to detect differences with significance level of 1.10^{-4} (lower light line of pair of hypothetical odds ratio- OR) and 1.10^{-6} (upper dark line of pair of hypothetical OR) with 80% power depending on the MAF in controls and hypothetical odds ratios for obtaining good response in cases relative to controls.
b) Abbreviation(s): MAF= minimal allele frequency, OR= odds ratio

Validation

The NCBI has created several descriptions of validation status for SNPs, which have been observed in individual experiments and accepted in this database without validation evidence. These descriptions are important in distinguishing high-quality validated data from unconfirmed data. Subsequently, this will lead to an increase in certainty of selecting a genuine polymorphic SNP. Validation status was assembled in six groups depending on the number of validation measurements:
- by multiple, independent submissions to the refSNP cluster,
- by frequency or genotype data: minor alleles observed in at least two chromosomes,
- by submitter confirmation regarding the SNP,
- all alleles have been observed in at least two chromosomes a piece,
- the SNP was genotyped by the HapMap project.

In this way, a validation score system (number of measurements) was created to distinguish high-quality validated data from unconfirmed data.

Secondary selection

The secondary selection of SNPs is based on three criteria:

- Predicted functionality
- Tag SNPs and linkage disequilibrium (LD)
- Ethnicity

When these criteria were applied, 223 SNPs out of 111 genes of the total remaining 2629 SNPs in 124 genes were selected.

Functionality

SNPs that affect gene expression occur in all regions of the genome. SNPs causing amino acid alterations (nonsynonymous SNPs) have been extensively studied. Less examined are variants located within the noncoding regions of the genome because mechanistic roles of noncoding genome sequences remain poorly defined. Moreover, the analysis of their functional consequences is complex [39]. While mostly regarded as nonfunctional, these variants can impact gene regulatory sequences, like promotors, to change gene expression and enzyme activity.

Another important feature is the exploration of possible functionally important regions in candidate genes within different species, which are identified within evolutionarily conserved sequences [40]. Several web software tools have been developed to assess these regions but this aspect is not further discussed in this article [41].

Functional change of a SNP was qualified and estimated using the Internet tools SNPs3D (http://www.snps3d.org/) [42] and/or PMut (http://mmb2.pcb.ub.es:8080/PMut) [43]. These resources provide a method of identifying those nonsynonymous SNPs that are likely to have a deleterious impact on molecular function in vivo.

For each SNP in the second step of the selection, predicted functionality according to the above resources was examined. If a predicted significant effect of a SNP was demonstrated, according to SNPs3D, this SNP was favorable to include in comparison with other SNPs with the same validation score, same heterozygosity for Caucasians and location within a gene region.

Tag SNPs and linkage disequilibrium

Tag SNPs usually occur in haploblocks or subregions. SNPs in different haploblocks or from different genes may, however, also be in LD. It is useful to search for both in association studies [44]. The degree of LD between alleles at two loci can be described with the correlation coefficient (r^2). This coefficient is informative in association analyses because it is inversely proportional to the sample size that is required for detecting a pharmacogenetic association given a fixed genetic risk [45]. An r^2 of 1 indicates full linkage, which means that there is no loss of power when using a marker Tag SNP instead of directly genotyping the disease causal variant. LD blocks (including tagged SNPs) can be relocated using the metric D0, which is closely related to r^2, and provides information about the recombination breakpoints of chromosomes. These parameters are required for the search of Tag SNPs in the HapMap database (http://www.hapmap.org). To limit the effort and costs of association studies, taking account of Tag SNPs is important [45].

Tag SNPs were explored in the database provided by the International HapMap Project [46]. Additional criteria were ethnicity (Caucasian, discussed below), $r^2 > 0.8$, MAF > 0.20 and maximum segment size of 250 basepairs.

Additionally, available software for the exploration of LD, are the HapMap database and WGAviewer (http://www.genome.duke.edu/centers/pg2/downloads/wgaviewer.php) [47]. This last tool provides an interface to automatically annotate, visualize and interpret the set of P-values emerging

from a whole genome association study [17]. HapMap data are used to identify nongenotyped polymorphisms that associate with the phenotype of interest through LD with genotyped variants. Regarding LD, SNPs with $r^2 > 0.7$ and $D0 = 1$ were regarded as SNPs in LD. Within a demonstrated LD, the most favorable SNP based on validation status and heterozygosity was selected. This was also the case for Tag SNPs: regarding SNPs in LD only the most favorable SNPs, based on validation status and heterozygosity, were included in the final selection.

Ethnicity
During a first exploration of frequency in SNPs the mean heterozygosity was assessed. Yet, it is also important to be aware of the differences in frequency mutation among ethnic populations [48,49]. In the NCBI, the MAF for each ethnic group is presented. Hereby, the consistency of the patient population under study should be examined, before accomplishing a SNP selection. For our case study we used the heterozygosity of each SNP for Caucasian population.

Characteristics of the selected SNPs

After applying these criteria, 186 SNPs were finally selected to analyze in RA patients treated with the TNF inhibitor adalimumab. Percentages of SNP selection according to mechanism of action are displayed in figure 3. The largest group of genes, 58% of all SNPs (N = 107), are located in genes involved in the reduction of inflammatory cytokine production and angiogenic factor expression. None of the SNPs within the gene coding for proteins related to immune regulation (Table 1) was finally selected.

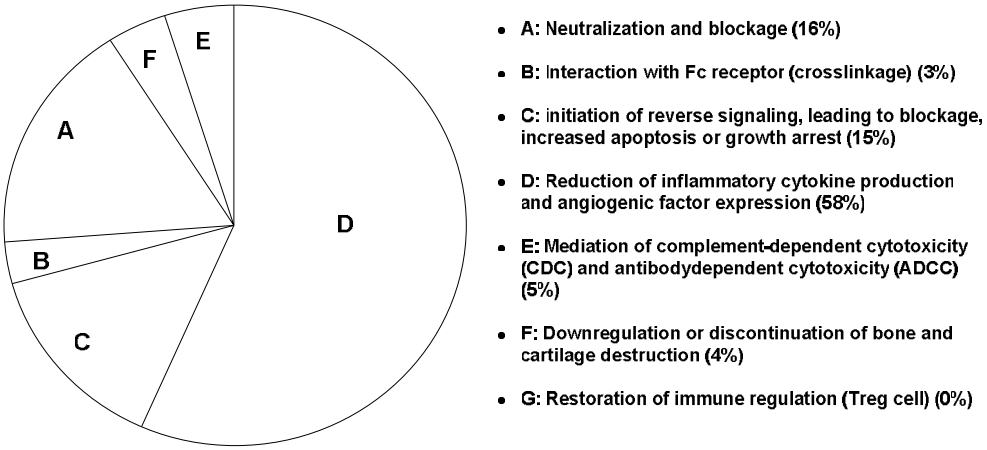

Figure 3. Percentages of selected SNPs according to mechanism of action.

In table 2, characteristics of the finally selected SNPs are presented. The majority of the selected SNPs are located in an intron or exon region (N = 170; 91.4%). In the exon region, 32 SNPs are thought to influence amino acid replacement, while 20 SNPs are substitutions that are synonymous.

More than half of the SNPs have a heterozygosity between 0.400 and 0.480. Of all available criteria scores, derived from the NCBI validation criteria, SNPs with several four criteria were abundant. Less effective criterion was the functionality of the finally selected SNPs using SNP3D. In 11 SNPs, a subdivision could be made based on deleterious (N = 3) and nondeleterious (N = 8). Additionally, 93 Tag SNPs were included for SNPs in a region of the genome with high LD, which facilitate a reduction of genotyping 467 SNPs, earlier selected in our primary selection. Finally, the number of SNPs selected with the capacity of representing a LD block was 20. Within our selection, these SNPs represent a total of 55 SNPs. During this selection, a large percentage within the criterion functionality remains unknown (94%).

Criteria	N of SNPs selected (%)
Gene region:	
- Unknown/N.A.	2 (2.2)
- 3' near gene	2 (2.2)
- 5' near gene	12 (6.5)
- Intron	84 (45.2)
- Exon:	86 (45.7)
o Synonymous	20 (10.8)
o Nonsynonymous	32 (17.2)
o 5'UTR	7 (3.8)
o 3'UTR	27 (14.5)
Heterozygosity:	
- Unknown/N.A.	1 (0.5)
- <0.400	37 (19.9)
- ≥0.400 or ≤0.480	118 (63.4)
- >0.480	30 (16.1)
Number of NCBI validation criteria:	
- 2	17 (9.1)
- 3	57 (30.6)
- 4	86 (46.3)
- 5	26 (14.0)
Functionality:	
- Unknown/N.A.	175 (94.1)
- Deleterious	3 (1.6)
- Non-deleterious	8 (4.3)
Tag SNPs	93 (50.0)
SNPs representative for LD	20 (10.8)

Table 2. Characteristics of finally selected SNPs according to defined criteria

Abbreviation(s): LD= linkage disequilibrium, N.A. = not available, UTR= untranslated region, SNP= single nucleotide polymorphism

Discussion

We present a rational approach for the selection of SNPs for pathway pharmacogenetic association studies. This method was applied in the presented case study by describing the pathways regarding the mechanism of action and pharmacokinetics of the TNF inhibitor adalimumab. 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. Second, this approach has an important statistical advantage: the chance of false-positive results is lower compared to the genomewide method, because of decreased multiple testing.

The next step would be to bring this pathway pharmacogenetic approach into practice. Namely, a pharmacogenetic study may be considered to validate the functionality of the selected SNPs in the pathway with respect to the therapeutic outcome to TNF inhibitors. Interestingly, an association study is projected by our group in the near future. Hereby, the efficacy of treatment with adalimumab in RA patients is linked with genetic variants systematically selected by this approach. Gene ontology analysis software may be useful in identifying novel pathways associated with mechanism of action of TNF inhibitors. One free-available software program is the Gene Ontology project, which is a large bioinformatics initiative to unify genomic databases and to increase convenient usage for biological scientists [50]. This software tool, however, was not used in our exploration of genes involved in the mechanism of action of adalimumab.

With the application of proposed criteria, objective selection of SNPs can be achieved. Defined steps were made to include 186 SNPs in 111 genes out of 51,793 SNPs in 124 genes in our case study. However, several crucial remarks can be placed.

Because the SNP selection is performed based on in vivo and in vitro studies concerning assumed pathways and targets in the mechanism of action of TNF inhibitors, there could be issues owing to limited understanding or changing opinion about the mechanism of action of the drug. For example, scientists thought that the drug imatinib was an inhibitor of several tyrosine kinases (TKs), like the BCR-Abl and platelet-derived growth factor (PDGF) receptor. Reports of inhibition of the c-kit signal transduction pathway by imatinib mesylate gave new insights into the mechanism of action of this drug [51,52]. Irrespective of whether or not the mechanism of action of the group of TNF inhibitors, such as infliximab, etanercept and adalimumab, is similar, clinical trials have demonstrated that the patient response differs within and between RA patients, as seen in results of several studies in which anti-TNF treatment has been switched [53,54]. Hypothetically, the
variation in clinical results can be explained by differences in the mechanism of action. This makes a class-effect and a complete similar mechanical pathway less probable.

Although many SNPs have been reported in the past decade, only a very small minority of the genetic variants published have proven functional consequences. Generally, functionality remains an important SNP selection criterion if compared with other used criteria in our presented method. During our stepwise selection, however, a predicted functionality could be assessed in only 6% of the SNPs. Future research has to be performed to explore the functional ability of a SNP. Subsequently, more predictive tools for functionality may be available for scientists to use.

A third crucial remark is related to prognostic versus predictive nature of the biomarker. A substantial number of published SNPs have been described to potentially associate with drug therapy outcome and with disease susceptibility under study [55,56]. If a high qualitative association is demonstrated between a SNP and the susceptibility to RA, as is seen in genome-wide studies [16,57], these results may be of interest for pharmacogenetic studies. Moreover, next to a significant association of

a SNP with susceptibility to RA, a more than random chance of this SNP being related to treatment outcome could be intelligible. Likewise, a pharmacogenetic condition can have implications for understanding susceptibility of disease [58,59]. Still, despite the necessity of prospective validation of our approach compared with the other methods, so-called 'literature-SNPs' were not taken into account during our selection. Namely, significant results based on literature may influence the objectivity aiming at a systematical pathway gene method to obtain optimal, original and detectable SNPs. Interestingly, this is the case for the SNP TNFa -308A > G, which is extensively studied in association studies with responsiveness to TNF-alpha-blockers in RA. Because the heterozygosity of this SNP is 0.163 (according to NCBI) and its position is not within an exon region, this SNP would not be selected according to our objective criteria.

Finally, costs are an important limiting factor in the SNP selection process. Costs of assays are indirectly correlated with the number of SNPs that could be examined and leads to an unwanted constraint to objectively select SNPs [60].

In this paper we have presented a feasible pathway gene approach with defined selection criteria to effectively explore potential SNPs with adalimumab as a case study. The comparison of this approach with the candidate gene- and whole genome methods requires further investigation.

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