Pharmacogenetics in rheumatology: the prospects and limitations of an emerging field

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Abstract

Objectives. To review the fundamental concepts of pharmacogenetics and analyse how the broad principles of this rapidly emerging field may influence the treatment of rheumatic disease in future.

Methods. The names of common rheumatic drugs and the terms ‘pharmacogenetics’, ‘pharmacogenomics’ and ‘genetic polymorphism’ were used as keywords to search the Medline and Current Contents databases. General review articles on pharmacogenetics were also examined.

Results. Pharmacogenetics is the study of how genetic differences influence the variability in drug toxicity and efficacy. Although the principles of pharmacogenetics have been known for several decades, recent technological advances have hastened the possibility of direct clinical applications. Most studies so far have been phenotypic analyses, but genotyping is now readily available for many polymorphisms. There are several examples pertinent to rheumatology that illustrate the important principles and foretell the usefulness of pharmacogenetics in individualizing therapy. However, further studies are needed.

Conclusions. Because traditional pharmacotherapy in rheumatology has been empirical and because of the slow acting nature of many anti-rheumatic medications, the risk of significant side-effects and the increasing armamentarium of drugs available, pharmacogenetics is particularly relevant to rheumatology. There are many scientific and non-scientific concerns that should be addressed in future studies.

KEY WORDS: Pharmacogenetics, Pharmacogenomics, Polymorphism, Arthritis, Lupus, Rheumatology, Rheumatic drugs, Rheumatic diseases.

Even though drug studies of large populations of patients may generally indicate efficacy and lack of toxicity, rheumatologists are familiar with the inability to predict a priori whether an individual patient will respond to a given medication without adverse effects. While there are myriad other reasons that might explain individual variability in drug response, including age, gender, weight, pharmacokinetics, disease severity, concomitant diseases and environmental factors, genetic differences among individuals in drug metabolism and/or cellular drug targets may explain a significant component of this variability. Importantly, identification of genetic determinants of drug efficacy and toxicity will be valuable because they can be ascertained in the individual patient before initiation of therapy.

Pharmacogenetics focuses on the genetic variations in genes responsible for drug metabolism, drug transport and drug targets to determine how these variations result in inherited alterations in medication outcomes [1]. The advances spawned by the Human Genome Project have hastened the potential applications of this field and the broader, overlapping field of ‘pharmacogenomics’, which also includes identification of drug targets from genetic information [1]. Currently there are only a few examples that have practical relevance to rheumatologists. Nonetheless, the objective of this article is to highlight how the broad principles of this rapidly emerging field may influence rheumatic disease therapy in the future.

General principles

For any given gene, there may be sequence variations between individuals, termed allelic variants. Some alleles
may represent mutations that give rise to non-functional proteins. An example is the mutant allele of the cystic fibrosis transmembrane regulator (CFTR) gene, which is responsible for cystic fibrosis. Here the reference normal allele can be termed the wild-type allele. At the other extreme, because the genetic code is redundant (i.e. more than one codon can encode the same amino acid), some of these variations may be of no consequence. In general, the sequence differences in coding portions are most relevant. However, non-functional segments, such as introns and the UTR (untranslated region) sequences of a given gene, may have consequences, as in the case of CYP3A5, the principal genetic contributor to inter-individual and inter-racial differences in CYP3A-dependent drug clearance [2, 3]. Variation in the CYP3A enzymes, the most important subfamily of the cytochrome P450 enzyme system, influences circulating steroid levels and the oxidative metabolism of half of all drugs. There may also be functional differences between the gene products of the allelic variants of a given gene. For example, an allelic variant may produce a receptor with variable affinity for its ligand and this may have functional consequences. Another type of allelic difference is the relative expression of a gene due to differences in the gene promoter. The significance of most human gene variation, however, is still unknown.

Genetic variation between individuals is very common, with approximately 3 000 000 single-nucleotide polymorphisms (SNPs) throughout the human genome. Until the advances made in the Human Genome Project, ascertaining DNA sequence differences between individuals on a wide scale was difficult. However, it is now possible to identify allelic variants of human genes, utilizing differences in repetitive DNA sequences, such as microsatellite markers [amplified by the polymerase chain reaction (PCR)] and sequence-tagged sites (STSs). Even outside repetitive DNA segments, SNPs have been identified by other methods [4, 5]. SNPs are scattered throughout the human genome, occurring approximately every 1000–2000 base pairs. The availability of these SNPs and STSs provides the means to determine if any of the vast arrays of markers is associated with individual patient characteristics.

While the broad application of knowledge from a genome perspective, termed genomics, to pharmacology may be very extensive, the focus of the area of pharmacogenetics is the study of how genetic differences among individuals influence the variability in drug toxicity and efficacy [1, 6]. Even when no prior relationship is known, it is possible to determine if any given genetic marker is associated with any clinical parameter, such as drug response. To accomplish this goal, high-throughput strategies may be used efficiently to evaluate hundreds if not thousands of markers, representing the entire genome. However, it is still not known if most genetic polymorphisms of obvious candidate genes that encode drug-metabolizing enzymes explain pathogenesis, drug responses or toxicity in certain individual patients with rheumatic diseases.

Pharmacogenetics and pathogenesis of rheumatic diseases

Since the 1960s it has been known that there is wide individual variation in the metabolic pathways of drugs within human populations. Early enthusiasm dwelled on whether these polymorphisms contributed to the pathogenesis of certain rheumatic disorders seen with drug exposure [7, 8]. Indeed, a clear association between acetylation (N-acetyltransferase activity) phenotype and drug-induced lupus was established. Slow acetylators (low N-acetyltransferase activity) were found to be more susceptible to the development of drug-induced systemic lupus erythematosus (SLE) after the initiation of hydralazine therapy [9]. Thus, genetic differences between individuals may result in susceptibility to drug-induced rheumatic illnesses.

The possibility of a genetic basis for drug-induced SLE led to speculation that differences in the metabolism of environmental agents might be responsible for idiopathic disease. However, extensive phenotypic analyses established that there is no association between acetylation polymorphisms and rheumatoid arthritis (RA) or idiopathic SLE [10]. Furthermore, recent detailed genetic mapping studies have indicated that most inflammatory rheumatic diseases, such as idiopathic SLE, are polygenic and that the majority of genetic factors involved in SLE are probably distinct from those influencing drug metabolism [11]. While it remains possible that some of the disease-associated loci contain genes that are also involved in drug metabolism, one current application of pharmacogenetics to rheumatology is in the area of metabolism of drugs that are commonly used for treatment.

Pharmacogenetics and toxicity: the example of azathioprine

Azathioprine, used in SLE and inflammatory myopathies and less commonly in RA, is converted to 6-mercaptopurine in vivo after oral intake. It is then activated to thioguanine nucleotides that are incorporated into DNA, thereby blocking DNA replication (Fig. 1). This is a major cytotoxic mechanism, though not the only one, of thiopurine drugs. For example, methylated thiopurines can inhibit de novo purine biosynthesis [12]. While this anti-proliferative effect explains its use in oncology, its mode of action at the lower doses used in rheumatic diseases is attributed to modulation of several immunological functions. 6-Mercaptopurine can also be inactivated by thiopurine methyltransferase (TPMT). There is genetic polymorphism of TPMT enzymatic activity, approximately 90% of the population having high activity, 10% having intermediate activity and about 0.03% having TPMT deficiency. The molecular basis for altered TPMT activity has been defined for the majority of patient populations. So far, eight TPMT alleles have been identified, including three (TPMT*2, TPMT*3A and
TPMT*3C) that account for 80–95% of cases of intermediate or low enzyme activity [13, 14]. Patients with TPMT deficiency rapidly accumulate high concentrations of thioguanine nucleotides, resulting in potentially fatal bone marrow toxicity. Individuals with intermediate TPMT activity accumulate 50% more thioguanine nucleotides than individuals with high TPMT activity and thus are also at risk of marrow toxicity, especially on larger doses of azathioprine [15, 16].

Ethnic variation in TPMT has been observed, with a median 20% lower activity in African–American subjects compared with Caucasians from the same location [17]. Interestingly, TPMT*3A, the most common variant allele among Caucasians, is not observed in continental Africans (Ghana, Sudan, Kenya) or East Asian populations, such as Japanese, Chinese and Koreans. Instead, among these populations, the variant allele TPMT*3C appears to be most prevalent [18–20].

Variation in TPMT enzymatic activity is relevant to rheumatic disease therapy. Among three RA patients with azathioprine-related bone marrow toxicity, two had TPMT deficiency and one had decreased activity of 5-nucleotidase, another enzyme involved in purine metabolism [21]. In a cohort of 33 azathioprine-treated RA patients, those with intermediate TPMT activity had a three-fold higher risk of developing side-effects, most frequently gastrointestinal intolerance [22]. However, genotyping was not done in these studies and there have been no further reports on the correlation of gastrointestinal intolerance with TPMT activity.

An example of the potential use of contemporary pharmacogenetics in rheumatology is the study of Black et al. [15], who performed a prospective cohort study in which 67 patients with rheumatic diseases (most with RA) who received azathioprine were genotyped for TPMT alleles. The five patients who were heterozygous for mutant TPMT alleles discontinued therapy within 1 month of starting treatment because of low leucocyte counts, and received therapy for a median duration of only 2 weeks. By contrast, patients with wild-type TPMT alleles received therapy for a median of 39 weeks, most patients discontinuing therapy because of gastrointestinal intolerance or loss of efficacy, indicating a strong association between mutant TPMT alleles and drug-induced marrow toxicity.

The circumstances in SLE may be more complex. In a retrospective study of 120 unselected patients with SLE [23], polymorphic TPMT alleles were identified in seven patients (5.8%). One patient with a homozygous

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**Fig. 1. Pharmacogenetics of azathioprine.** The presence of mutations in thiopurine methyltransferase (TPMT) influences the formation of cytotoxic nucleotides and systemic toxicity. (A) Wild-type TPMT results in drug inactivation. (B) Variant TPMT shunts the drug down the activation pathway and increases the production of toxic metabolites by hypoxanthine/guanine phosphoribosyl transferase (HGPRT). Inosine monophosphate dehydrogenase (IMPD) and guanosine monophosphate synthase (GMPS) are two other enzymes involved in the biotransformation of 6-mercaptopurine to thiopurine nucleotides.
TPMT*3A mutation had developed severe marrow aplasia, while three patients with heterozygous mutations seemed to tolerate the medication (duration not specified). An additional five patients had leucopenia even though they possessed the wild-type TPMT allele, and SLE was excluded as the cause of leucopenia. However, no comorbidities, patient characteristics or environmental factors were described for these patients. Even though the TPMT genotype does not account for all cases of myelosuppression in SLE patients treated with azathioprine, detection of mutant TPMT alleles may augmente the prediction of an adverse effect from azathioprine.

A few authors advocate complete avoidance of azathioprine in patients who are homozygous for mutant TPMT alleles, and there are also differing opinions on the safety of azathioprine in heterozygous patients [24], some advocating reduced doses in these patients [25]. While further studies are indicated [26], a priori knowledge of TPMT alleles may permit practitioners to prevent azathioprine-induced myelosuppression in their patients. PCR-based methods for the detection of these alleles in the genomic DNA of patients have been developed and validated [27]. These CLIA (Clinical Laboratory Improvement Amendments)-certified molecular diagnostics are now offered by reference laboratories in the USA (Prometheus, DNA Sciences) and Europe.

Pharmacogenetics and efficacy: the example of opioid analgesics

In addition to its value in predicting toxicity, pharmacogenetics may play a significant role in the efficacy of certain medications. To be an effective analgesic, codeine must be metabolized to morphine by the cytochrome P450 enzyme CYP2D6. Genetic polymorphism of this enzyme results in three separable phenotypes: poor metabolizers, extensive metabolizers and ultra-rapid metabolizers. Poor metabolizers are homozygous for an inactive or deficient CYP2D6 enzyme caused by mutations in the CYP2D6 gene, while ultra-rapid metabolizer subjects have duplication of the gene, resulting in increased enzymatic activity [28]. These molecular alterations can be determined in patients using PCR-based assays. There is a significant degree of ethnic or racial variation in allele frequency. For example, the frequency of the ultra-rapid metabolizer phenotype, with increased capacity to metabolize opioid drugs, is only 1–2% in Northern European populations [29], whereas the frequency is much higher (20% or more) among Ethiopians [30] and Saudi Arabians [31]. The prevalence of the poor metabolizer phenotype in the Caucasian population is 7–10% [32]. Individuals who are poor metabolizers have decreased activation of CYP2D6-dependent analgesic prodrugs such as codeine, hydrocodone, oxycodone and tramadol [33].

In a randomized, placebo-controlled, double-blind trial using an experimental pain model, codeine administration resulted in analgesia (improved pain threshold) in extensive metabolizers but had no effect in poor metabolizer patients [34]. In addition, poor metabolizer individuals suffered an equal incidence of side-effects in comparison with extensive metabolizer subjects, despite not receiving any analgesic benefit. Similar studies have shown that poor metabolizers also obtain weaker analgesia from tramadol [35]. Even though these findings may not have a significant impact on clinical practice, as opioid effects can be monitored readily and quickly by clinical (non-genetic) means, they demonstrate the potential for pharmacogenetics to guide therapy selection and optimization.

Avenues for further exploration

The metabolism of most drugs is complex and involves numerous pathways, so that individual polymorphisms in only one pathway may not be sufficient to predict the clinical phenotype. For example, the phenotypic differences secondary to genetic polymorphisms of N-acetyltransferase (NAT2 gene) alone do not predict the efficacy of sulphasalazine among RA patients [36, 37]. However, the slow acetylator phenotype of the NAT2 polymorphism does seem to correlate with early discontinuation due to significant gastrointestinal side-effects [36, 37], and among chronic discoid lupus erythematosus patients the rapid acetylator phenotype seems to correlate with a better therapeutic response to sulphasalazine treatment [38].

The pharmacogenetic complexity introduced by multiple pathways is also exemplified by methotrexate metabolism, a detailed discussion of which is beyond the scope of this article. The mechanism of action of methotrexate, with respect to both efficacy and toxicity, has not been elucidated definitively but numerous hypotheses have been proposed [39]. Whether there are genetic polymorphisms in the various enzymes involved and, if present, whether they correlate with drug effects need to be explored (Fig. 2). For example, in the methylene tetrahydrofolate reductase (MTHFR) gene, a well-characterized C677T polymorphism, with a prevalence of approximately 8% in the normal population, has been described [40]. In a group of RA patients, the presence of a homozygous (C677TT) or heterozygous (C677CT) mutation was associated with an increased risk of discontinuing methotrexate treatment because of adverse events (relative risk 2.01, 95% confidence interval 1.09, 3.70), mainly due to an increased risk of elevated liver enzyme levels (relative risk 2.38, 95% confidence interval 1.09, 5.34) [41]. In an earlier randomized trial, the same group compared sulphasalazine, methotrexate and a combination of the two drugs in RA and noted that patients homozygous for the mutation in the MTHFR gene had significantly higher baseline homocysteine levels and that the heterozygous MTHFR genotype induced a significantly higher plasma homocysteine at week 52 compared with no mutation [42]. Although patients with gastrointestinal toxicity had a significantly greater increase in
homocysteine levels, the mutation by itself was not predictive of methotrexate-related gastrointestinal toxicity [42]. Further elucidation of such a pharmacogenetic basis for the variable efficacy of methotrexate and its idiosyncratic toxicity will be clinically useful.

Pharmacogenetics: future trends

Current rheumatology practice generally involves the empirical use of medications while monitoring for efficacy and side-effects. Instead of this trial-and-error method, an alternative paradigm can be proposed. In the new model, identification of informative polymorphisms of genes involved in the metabolism, transport or targets of a candidate medication will provide the practitioner with the ability to predict the outcome of therapy. To establish this new model, population studies will be needed on other drugs to identify relevant and informative polymorphisms and establish links between polymorphisms and drug effects in patients [5]. Prospective controlled studies to demonstrate the utility of such predictions will also be necessary.

Translating this information to routine practice will also require that practitioners become aware of the general principles of the technology that will form the basis of the putative new laboratory tests. Currently available gene chip technologies involve the semi-automated use of immobilized oligonucleotides or DNA exposed to labelled patient DNA samples. The gene chip is then scanned for binding of labelled samples. This technology allows researchers to rapidly screen multiple genes simultaneously [43, 44]. In the clinical laboratory, a gene chip (GeneChip®; Affymetrix, Santa Clara, CA, USA) is already on the market for screening alleles of the CYP2D6 and CYP2C19 genes, which are involved in the metabolism of certain drugs. It is not unreasonable to envisage a future in which this technology will become readily available and affordable for routine clinical practice. Rheumatologists may then be able to genotype patients before initiating therapy, thereby individualizing treatment. As more therapeutic options become available, this capability will provide practitioners with important information to guide efficient, effective and safe therapies, even for diseases for which there is incomplete understanding of the pathogenesis.

Limitations and concerns

While pharmacogenetics generates tremendous enthusiasm, caution is warranted [45]. Several concerns, which are universal and not necessarily specific to rheumatology, revolve around our limited knowledge. Pharmacogenetic studies on selected populations may not be applicable to other population groups around the world [28]. Thus far, mostly monogeneic determinants of drug effects have been described, which are the easiest to recognize in population studies [1]. Polymorphisms predictive of minor adverse effects that resolve rapidly and are easy to ascertain clinically may not warrant predictive genotyping and hence may not have practical relevance. Given that so many other factors may play significant roles, the contribution of genetics as a determinant of the treatment response may
need to be assessed for each disease even when the same drug is used. This is applicable to all medical specialties, including rheumatology, in which concomitant disease states often complicate the scenario. For example, modified drug disposition due to liver dysfunction may significantly alter the impact of any genetic difference. There are also major ethical concerns, which are applicable to the genomics era in general and are not necessarily limited to pharmacogenetics. Currently, bioethicists advocate regulations and policies to prevent potential or theoretical abuses of genetic information with respect to individuals. Would health-care organizations insist on knowing a patient’s genetic profile before approval of specific drug therapy? This would be in conflict with the suggestion made by some that the right to privacy of genetic profiles should be made an inviolable civil right. However, the term ‘genetic testing’ is currently used indiscriminately to refer to very different applications of genetics. Advocates of pharmacogenetics urge greater specificity of language and terminologies to avoid misconceptions, among both the lay public and the scientific community [4]. Is designing individualized therapy cost-effective or even feasible from an economic standpoint? For selected situations, testing for individual polymorphisms may already be cost-effective. A pharmacoeconomic study [24] of screening for TPMT mutations in patients receiving azathioprine estimated that there was cost-neutrality, even if the model assumed that only the homozygotes develop severe myelosuppression. As heterozygotes also develop marrow toxicity [15], actual cost savings should be achieved. Similar pharmacoeconomic analyses need to be integrated into clinical pharmacogenetic studies to further define the practical utility of genotype analysis in routine clinical practice.

Nevertheless, the ultimate goal of pharmacogenetics is to individualize therapy by predicting its outcome in any given individual with a much higher degree of certainty than is currently available. As achievement of this goal appears to be on the horizon, new methods for evaluating the cost–benefit of such treatment paradigms for rheumatic diseases will also need to be developed.

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