Editorial

The Possible Clinical Application of Pharmacogenetics in Rheumatology

Pharmacogenetics is an area of research that has gained much popularity in various fields but most of all in oncology[1], neurology[2], and more recently, in cardiology[3].

Pharmacogenetics has been defined as the study of variability of drug responses due to hereditary factors[4], but more often it has been considered a tool to translate functional genomics into rational therapeutics[5], that is, assessing the role of genetic determinants in the variable response to the same molecule at the same dose. However, pharmacogenetics focused on the variants (or mutants) of single genes was, until recently, considered of little value, even though there was some evidence that drug-metabolizing enzymes played an important role under strict genetic control[6].

There are 3 areas that deserve particular attention: the relationships between (1) genotypes or immunogenotypes and drug metabolism; (2) genotypes or immunogenotypes and adverse drug reactions; and (3) genotypes or immunogenotypes and clinically relevant effects of drugs.

Pharmacogenetics, drug metabolism, and disease susceptibility. Remarkable evidence was obtained when studying CYP2D6 gene function, responsible for the metabolism of nortriptyline. Homozygous carriers of the loss-of-function alleles of CYP2D6 cannot degrade many drugs. It has been clearly shown that 5–10% of Caucasians are poor metabolizers through CYP2D6 and are homozygous for 2 recessive loss-of-function alleles of the gene controlling cytochrome p450 monooxygenase CYP2D6[7,8]. In these subjects, opioid analgesics are activated, thus explaining the variability of response to codeine between individuals. Furthermore, in these subjects the use of tricyclic antidepressants (nortriptyline), amiodarone, and clarithromycin has been associated with toxicity occurrence. On the other hand, carriers of two B1 alleles of cholesterol ester transfer protein were shown to be the best responders to pravastatin used in coronary artery diseases[9]. These were clear indications that a genetic setting could not only influence the pharmacokinetic but also the clinical response to a particular drug. A poor drug metabolism might have some effects also on disease susceptibility. Indeed, examining 383 patients with ankylosing spondylitis (AS) and 269 healthy controls, a significant association was found between homozygous CYP2D6*4 and AS [RR = 2.1, 95% confidence interval (CI): 1.3–3.4, p = 0.002][10]. Since it is well known that xenobiotics produce proinflammatory

effects on T cells[11], an impaired xenobiotic metabolism might be involved in the induction of inflammatory diseases, such as AS, in which major histocompatibility complex (MHC) genes contribute only 20–50% of the genetic risk. At this time, no data are available on any possible relationship existing between cytochrome gene polymorphisms, drug metabolism, and drug response in AS.

Pharmacogenetics and drug transporters. Several transporters of drugs have been detected in the intestine, liver, kidney, and also in the central nervous system and blood–brain barrier. It has been shown that there are 15 allelic variants of the MDR-1 P glycoprotein (P-gp) gene even though their functional basis is at present unknown[1]. It is probable that studies on polymorphic drug transporter genes will be undertaken in the near future.

Pharmacogenetics, immunopharmacogenetics, pharmacometabolism, and adverse drug reactions (ADR). Serious ADR are the sixth leading cause of death in the United States and result in $75 billion in health care costs[12]. In addition, 60% of the drugs listed among those inducing ADR undergo metabolism through one of the type I enzymes, with a variant allele (mutation) possibly impairing their metabolism[13]. Type I enzymes are predominantly oxidative enzymes. Typical phase I enzymes are cytochrome P-450 (CYP) enzymes. Pharmacogenetics might truly improve the personalized approach using molecules that follow such metabolic pathways.

Pharmacogenetics and phase II enzymes. Very few drugs are metabolized by phase II enzymes, which couple the by-products of phase I enzymes. Typical phase II enzymes include methyl-transferases, n-acetyl-transferases, and glutathione-s-transferases. One of the most relevant polymorphisms was found in people carrying a defect in thiopurine-methyl-transferases (TPMT), which metabolize 6-mercaptopurine and azathioprine very slowly. There is one genetic polymorphism of TPMT activity with 90% of the population manifesting a high level of activity, 10% a medium level, and 0.03% a full deficiency. As a consequence, patients homozygous for the wild-type allele need a dose of 100 mg/m^2, while the dose for patients with the homozygous defective allele[14] should be no more than 10 mg/m^2. Of course a poor personalized dosing regimen with

azathioprine could cause an increased toxicity, agranulocytosis, or severe thrombocytopenia due to the accumulation of high concentrations of thioguanine nucleosides. This was clearly shown to be the case by Stolk, et al[15]. Of 33 patients with rheumatoid arthritis (RA) treated with azathioprine, 14 developed ADR. In these patients, TPMT activity was significantly lower than in those without toxicity. In particular, 7 of the 8 patients with intermediate activity developed toxicity, resulting in a significant relationship (p = 0.005) with toxicity and a relative risk of 3.1 (95% CI 1.6–6.2), suggesting that TPMT activity, as determined by the variant alleles, is a strong determinant of azathioprine toxicity. Marra, et al[16] have recently calculated the cost-effectiveness of TPMT polymorphism screening in patients with rheumatic diseases before giving the drug, and were able to conclude that the introduction of polymerase chain reaction (PCR) testing of such functionally relevant polymorphisms may represent good value in certain health care settings. Indeed, the usual dosing strategies were estimated to cost CAN$677, whereas a previously defined genotype with a lower dose cost CAN$665. Therefore, at least concerning azathioprine, pharmacogenetic analysis seems ready to enter the clinical practice.

Pharmacogenetics and drug targets. A possible association of methylene-tetrahydrofolate reductase (MTHFR) gene polymorphisms and drug efficacy or toxicity was recently examined in 106 patients with RA treated with methotrexate (MTX) in Japan. A higher rate of overall toxicity was seen in patients with the 677T allele of MTHFR compared to patients without the allele (RR = 1.25, 95% CI 1.05–1.49, p < 0.05). Patients with the 1298C allele were receiving significantly lower doses compared to patients without the allele (RR = 2.18, 95% CI 1.17–4.06, p < 0.05). Confirmatory data are clearly needed, but this study suggests possible relationships between MTHFR gene polymorphisms and favorable response or toxicity[17]. This is reminiscent of the identification of 3 major variant genes of lipoxygenase A (ALOX 5), inducing the presence of different numbers of binding sites for SP1, a transcriptional factor crucial for the nuclear message. ALOX 5 is the enzyme required for the production of both cysteinyl-leukotrienes (LTC4, LTD4, LTE4) and LTB4. ALOX 5 activity partially determines the level of leukotrienes in the airways. Since pharmacological inhibition of ALOX 5 or antagonism of leukotrienes at their receptor level are associated with improvement of asthma symptoms, it is likely that polymorphic variants of ALOX 5 might determine the response to treatment. More particularly, the mutant genotype (less function leads to lower leukotriene levels) does not respond to antileukotriene treatment because the disease is not mediated by leukotrienes, but by other factors. Indeed, when patients carrying the mutant variant were treated with an ALOX 5 inhibitor (ABT-761), they experienced a decrease of forced expiratory volume (FEV1), while carriers of the wild-type had an improvement of FEV1[18]. The same results were later confirmed with a leukotriene inhibitor, zafirlukast, thus suggesting that genotyping might help define responders and nonresponders. Although less evident, similar results were seen in studies analyzing the differences in the response to ß agonists in asthmatic patients in the presence of variants of ß1 and ß2 adrenergic receptors[19]. In addition, a metaanalysis of 5 studies of patient responders and nonresponders among 5-HT2A receptor variants (T102C and His452Tyr polymorphism) of schizophrenic patients treated with clozapine reported a clear association of the 102-T/C with clozapine response[20]. Immunopharmacogenetics might also help to define the immunogenetic setting driving either the response to drugs or the occurrence of toxicity. Along this line, several attempts have been made to analyze the ADR developing during disease modifying antirheumatic drug therapy in RA and their relationship with the MHC-HLA genotype. An association was seen by several groups between D-penicillamine or aurothioglucose-related ADR and DR3[21-23], or between thiopronine (another drug efficacious in RA) and B35-Cw4 alleles[24]. More recently, a relationship between lack of gingival overgrowth on cyclosporine A (CsA) and the presence of HLA-DR1 has been described[25].

Immunopharmacogenetics and drug efficacy. O'Dell, et al[26] observed that the majority of their patients responding to the triple therapy (MTX, sulfasalazine, and hydroxychloroquine) carried the MHC-HLA shared epitope (DR0401-0404, 0405, 0101, 1001, 1402), while only a minority of responders to MTX alone did so (94 vs 32%). Over the past year our group reported 52% of responders to CsA (vs 6% of nonresponders) carried DR4-DR1, while MTX responders behaved differently, with 80% of responders carrying non DR4-DR1 alleles (vs 28% of nonresponders)[27]. A possible synthesis of these data has been provided by Gonzales-Gay, et al[28], who showed that patients with aggressive RA needing a combination of MTX and CsA were carrying the HLA-DRB1 shared epitope alleles, such as DR4 or DR1, more frequently than those continuing monotherapy with MTX. According to these data, carriers of DR4-DR1 would be expected to require combination therapy.

Immunopharmacogenetics and anti-tumor necrosis factor (TNF)-α therapy. The advent of biological response modifiers (BRM), like TNF-α blockers, offers a unique opportunity to test a genetic setting predisposing either to a favorable response or to ADR. The early identification of patients who will respond positively to these drugs will be of great help in establishing a cost-effectiveness profile of these molecules. So far there are no data in this regard. The few preliminary observations suggest that more studies should prospectively address the issue. Our group has recently provided data showing that genotyping patients for

the TNF receptor type II (RII) gene might be of help in identifying patients who are responsive to anti-TNF. The TNFRII gene shows a polymorphism in exon 6 with 2 alleles, T and G. When the T allele, but not the G allele, is present, the NIa restriction enzyme cleaves the 242 base-pair PCR product, generating 2 smaller fragments of 133 and 109 base pairs. In our study we observed that in RA patients (66 patients all resistant to a combination therapy, therefore receiving TNF-α blockers in addition to MTX), the TG/GG genotype carriers (28 patients), all with disease activity scores > 3.7, had a very low chance of achieving moderate or low disease activity (< 3.7 or < 2.4) after therapy, while the patients with the TT genotype (38 patients) had a 3-fold higher chance of responding (37.8 vs 10.7%; OR 5.1, 95% CI 1.3–19.96, p = 0.03). We believe that patients carrying the G allele are unlikely to benefit from TNF blockers[29].

Recently, Mugier et al[30] observed that carriers of the –308 TNF-α G/G genotype responded better to infliximab. This was also confirmed for etanercept by Padyukov, et al[31], who observed that carriers of the TNF –308 G/G and simultaneously of the IL-10–1097 G/G genotypes had better results with the drug.

This is reminiscent of the clinical pharmacogenetic link observed in breast cancer patients expressing (or not) high levels of HER-2/neu protein (HER-2: surface growth factor overexpressed in 25–30% of metastatic breast tumors). Women overexpressing HER-2 present a particularly aggressive form of the disease with a poor prognosis. Through the Hercep test, a semiquantitative measurement of HER-2 expression, patients with high expression can be given herceptin (a monoclonal antibody against HER-2) with a higher chance of obtaining positive results. Clinical benefits were seen only in patients who were strikingly positive[32]. It is interesting that FDA-approved pharmacogenetic tests detecting HER-2 gene amplification, either directly or indirectly, are already available [www.fda.gov/cdrh/pma/pmasep98.html].

The use of new therapeutics, like BRM, that result in serious ADR in only a minority of patients creates unique opportunities for addressing the crucial question of whether we should treat all patients with RA possessing poor prognostic factors, or only some patients identified with a favorable pharmacogenetic profile in terms of safety and clinical efficacy. Several gene polymorphisms could be prospectively tested for receptors or molecules involved with BRM now used in clinical practice (Table 1). The identification of any possible relationship with ADR or clinical efficacy would be a tremendous step forward. To make this type of testing a reality, we need the support of institutions and companies to perform international studies that will allow us to define the real chance of using this tool at the bedside.

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Table 1. Pharmacogenetics and immunopharmacogenetics at the bedside: examples of genetically determined variations possibly influencing therapeutic responses.

Polymorphic Gene	Target Disease	Drug	Effects
CYP2D6	Depression, arrythmia, infections	Nortriptyline, amiodarone, clarithromycin	Slow metabolizers: increased toxicity Rapid metabolizers: decreased efficacy
CETP (cholesteryl ester transfer protein)	CAD	Pravastatin	Carriers of 2 B1 alleles more benefit
β_2 adrenergic receptor	Asthma	Albuterol	Homozygous Gly16/Gly16 more sensitive than Arg16/Arg16
ALOX5	Asthma	Zileuton	Wild: type (WT): efficacy; mutated-type (MT) worse
TPMT	RA, Crohn's, SLE	Azathioprine	TPMT 1(wild): normal doses; TPMT 3A, B, C: myelotoxicity
TNFRII T/T	RA	Infliximab	Best responders
TNF-α –308 G/G	RA	Infliximab	Best responders
TNF-α –308 G/G + –1087 G/G	RA	Etanercept	Best responders

CYP2D6: cytochrome P 450 enzyme 2D6 isoform (the isoform 2D6 along with the 3A4 and 2C9 are the most important forms and account for 60–70% of all phase I dependent metabolisms); CETP: cholesteryl ester transfer protein (the homozygous B1 genotype associates with a clinically relevant response to the HMG-CoA reductase inhibitor pravastatin); ALOX5: 5 lipoxygenase gene (located on chromosome 10q11.12) with 2 promoter genotypes, the wild and the mutant; TPMT: thiopurine methyltransferase (the most important variants that account for 90% of the defective alleles in Europeans are TPMT 3A, 3B and 3C); DR 1–4 (DRß1): DRß1 rheumatoid epitope; TNFRII-G: allele G of the p75 TNF-α receptor, TNF-α –308 G/G. CAD: coronary artery disease.

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