Genetic variants contribute to differences in response and toxicity to drugs used in autoimmune diseases: Rheumatoid arthritis and systemic lupus erythematosus

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Chapter 9

Summary

Rheumatoid arthritis (RA) is a common autoimmune disease associated with progressive disability and systemic complications. The etiology of this inflammatory disease remains largely unclear due to complexity of interacting factors including genetic and environmental determinants. Disease-modifying antirheumatic drugs (DMARDs) (including methotrexate, MTX), anti-TNF drugs and rituximab in RA have the capacity of reducing progression or preventing damage to the joints and preserving their integrity and function by modulating the immune response.

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease of unknown cause that can affect virtually any organ of the body. Patients present with variable clinical features ranging from mild joint and skin involvement to life-threatening renal, hematologic, or central nervous system involvement. A general approach to treating SLE includes the use of immunosuppressive agents targeting B-cell pathways as belimumab and rituximab.

However, it is widely recognized that interindividual responses both with regard to efficacy and toxicity vary for all drugs used in RA and SLE, including MTX, anti-TNF acting drugs and other agents targeting B-cell pathways as rituximab. A possible explanation is that the genetic profile of an individual influences drug efficacy or drug toxicity. Indeed, genes encoding drug metabolizing enzymes and drug transporters but also pharmacodynamic proteins are found highly variable between individuals. Early identification of subjects who respond or do not respond to these drugs may be of help when establishing the most efficacious and safe treatment with these drugs.

RHEUMATOID ARTHRITIS

In the first part of the thesis studies were presented concerning genetic variability contributing to differences in response to anti-TNF and toxicity to MTX in RA patients. In **chapter 2**, we reviewed the scientific literature for evidence for markers for MTX-induced hepatic injury in RA treatment. These genetic and nongenetic determinants may be useful to predict the individual patients´ risk for MTX-induced hepatotoxicity and could help to reduce the incidence and morbidity of MTX-induced liver injury. The possible nongenetic risk factors include the cumulative MTX dose and duration of treatment, the use of other hepatotoxic drugs or chemicals such as alcohol, impaired renal function and the concomitant use of drugs that decrease the elimination of MTX and history of liver disease. In addition, genetic susceptibility plays an important role in the occurrence of hepatotoxicity and increased risk of developing drug-induced liver injury (DILI). In general, from the published studies, *MTHFR* C677T appears to be the most promising genetic marker

predicting low-dose MTX-induced hepatotoxicity, although because the limited power of studies to identify genetic biomarkers for hepatotoxicity, conflicting results exist limiting its clinical application.

Recently, researchers in pharmacogenetic studies have reported several genetic variants associated with clinical response to anti-TNF treatment. In **chapter 3**, the association of four polymorphisms (rs1532269 and rs17301249, intronic polymorphisms mapped within *PDZD2* and *EYA4*, respectively, and rs12081765 and rs7305646 located at intergenic regions on chromosomes 1 and 12, respectively) previously identified in a genome-wide association study (GWAS) as being associated with anti-TNF treatment response in patients with RA was performed in our study.

These 4 polymorphisms were genotyped in a total of 634 Spanish RA patients treated with anti-TNF drugs. Four results were evaluated: changes in the Disease Activity Score in 28 joints (DAS28) after 6 and 12 months of treatments and classification according to the European League Against Rheumatism (EULAR) response criteria at the same time points. In addition, we combined our data with those of previously reported studies in a meta-analysis including 2,998 RA patients. None of the four genetic variants showed an association with response to anti-TNF drugs in any of the four outcomes analyzed in our Spanish patients. However, rs1532269, mapped within *PDZD2* gene, yielded a suggestive association with the response to anti-TNF when available data from previous studies were combined in the meta-analysis.

This heterogeneity between studies was also seen in the results of **chapter 5**, where we present the results of the first large study on the influence of *FcGR2A* and *FcGR3A* genes on treatment response in a cohort of 302 Dutch RA patients using adalimumab as the anti-TNF therapy. Similarly to chapter 2, treatment outcome was evaluated with the use of the DAS28 criteria and responses were classified according to EULAR criteria. The presence of *FcGR2A*-H allele was associated with EULAR good response at 14 weeks. No significant association was found for *FcGR3A* with good response or remission. The combined effect of both SNPs showed no association with EULAR good response.

In **chapter 4** it was shown that the response to anti-TNF therapy is also influenced by a polymorphism affecting the disease activity, suggesting that increased expression of IL-6 in patients carrying the *-174*C* allele would result in a poorer response to anti-TNF treatment. The original effect on anti-TNF treatment response caused by the change in *IL-6 -174G/C* was replicated in an independent population of 199 Spanish RA patients receiving anti-TNF therapy. Patients were classified according to EULAR criteria as responders and non-responders at 6, 12, 18 and 24 months after the first infusion. The *-174*G* allele was

significantly associated with good or moderate EULAR response at 12, 18 and 24 months. The combined analysis of our data and those previously published showed an association between this genetic variant and the clinical response to anti-TNF.

SLE AND OTHER AUTOIMMUNE DISEASES

The second part of the thesis was focused on studies concerning genetic variability contributing to differences in response to rituximab in several autoimmune diseases, mainly SLE.

Recent studies have provided evidence that antagonizing the action of proinflammatory cytokines, including IL-6 and IL-2, may exert a therapeutic effect in autoimmune disease patients nonresponsive to other therapies. The *-174G/C* genetic variant (rs1800795), located in the *IL-6* gene promoter region, has been found to be associated with autoimmune diseases and involved in increased levels of IL-6 protein in serum in diverse inflammatory diseases; the GG homozygotes have circulating IL-6 concentrations approximately twice higher than those homozygous for the C allele. In **chapter 6** we analyzed the association of the *-174 IL-6* promoter variation with the response to rituximab in a group of 144 Spanish patients that presented diverse systemic autoimmune diseases, including SLE. Six months after the first infusion with rituximab, we evaluated the response to the drug. The CC genotype was more frequent in non-responders as compared to those carrying GC or GG genotypes. A similar trend but not statistically significant was observed when SLE patients were analyzed separately.

In healthy subjects, stratification according to the *IL2–IL21* region polymorphism (rs6822844) revealed significant differences in circulating interleukin-2 with the lowest levels in GG genotype carriers (12). This is in agreement with our results in **chapter 8** where the role of this genetic variant on the rituximab's response was studied in 144 Spanish patients with different systemic autoimmune diseases. The response was evaluated according to EULAR criteria at six months after the first infusion. In the group of SLE patients, both GG genotype and G allele frequency were increased in responders compared with non-responders. No association with response was evident in non-SLE patients. Interestingly, these findings show conflicting results with the results obtained in **chapter 6** where the allele associated previously with lower levels of IL6 were associated with worse response to rituximab.

Rituximab is recognized and bound to the surface of NK cells and macrophages through the FcGR, triggering ADCC immune system mechanism, essential for the activity of rituximab

to deplete B cells. The importance of FcGR3A in the response to rituximab has been shown in studies where mice lacking FcGR3 presented a decrease in the response to this drug (13). In **chapter 7**, a genetic variant in *FcGR3A* gene in the response to rituximab was studied in 132 Spanish patients with different systemic autoimmune diseases. Rituximab was more effective in V allele carriers than in homozygous FF in patients with different autoimmune diseases. We analyzed separately SLE patients and we found a similar trend to those observed in the global analysis but it was not statistically significant.