Reumatol Clin. 2009;**5(6)**:268–279

Reumatología Clínica

www.reumatologiaclinica.org

Review article

Gene polymorphisms and pharmacogenetics in rheumatoid arthritis

Ignacio Rego-Pérez, Mercedes Fernández-Moreno, Vanessa Carreira-García, and Francisco J. Blanco *

Unidad de Investigación del Envejecimiento Osteoarticular, Laboratorio de Investigación, INIBIC, División de Reumatología, A Coruña, Spain

ARTICLE INFO

Article history:
Received October 6, 2008
Accepted December 4, 2008
Available online March 21, 2009

Keywords:
Pharmacogenetics
Pharmacogenomics
Rheumatoid arthritis
Tumor necrosis factor
Interleukin-1
Cytokines
Gene polymorphisms

ABSTRACT

Rheumatoid arthritis (RA) is a systemic, chronic, and inflammatory disease of unknown aetiology with a genetic predisposition. The advent of new biological agents, as well as the more traditional disease-modifying anti rheumatic drugs, has resulted in highly efficient therapies for reducing the symptoms and signs of RA; however, not all patients show the same level of response regarding disease progression to these therapies. These variations suggest that RA patients may have different genetic regulatory mechanisms. The extensive polymorphisms revealed in non-coding gene-regulatory regions in the immune system, as well as genetic variations in drug-metabolizing enzymes, suggest that this type of variation is of functional and evolutionary importance and may provide clues for developing new therapeutic strategies. Pharmacogenetics is a rapidly advancing area of research that holds the promise that therapies will soon be tailored to an individual patient's genetic profile..
Chronic and severe forms of gout are frequently wrongly evaluated from the clinical standpoint.

Polimorfismos genéticos y farmacogenética en la artritis reumatoide

Palabras clave:
Farmacogenética
Farmacogenómica
Artritis reumatoide
Factor de necrosis tumoral
Interleucina-1
Citocinas
Polimorfismos genéticos

RESUMEN

La artritis reumatoide (AR) es una enfermedad inflamatoria, sistémica y crónica de etiología desconocida y con predisposición genética. La llegada de los nuevos agentes biológicos, así como los ya conocidos fármacos antirreumáticos modificadores de la enfermedad, condujeron a una eficacia elevada en los tratamientos de la AR. Sin embargo, no todos los sujetos muestran el mismo grado de progresión de la enfermedad como respuesta a estos tratamientos. Estas variaciones demuestran que los sujetos con AR deben tener diferentes mecanismos de regulación génica. Los polimorfismos detectados en las regiones reguladoras no codificantes del sistema inmune y las variaciones genéticas de las enzimas que metabolizan los fármacos demuestran que este tipo de variaciones tiene una importancia funcional y evolutiva elevada, lo que proporciona nuevas pistas para el desarrollo de nuevas estrategias terapéuticas. La farmacogenética es un campo que avanza rápidamente y promete el desarrollo de tratamientos adaptados al perfil genético del sujeto en un futuro cercano.

* Corresponding author.
E-mail address: fblagar@canalejo.org (F.J. Blanco).

1699-258X/$ - see front matter

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic, and inflammatory disease that leads to the destruction of cartilage and has a wide variety of joint manifestations. The hyperplasic synovial membrane intervenes in the process by deeply invading the joint cartilage and the rest of the joint. In this process, there is a great many mediators, both inflammatory and non-inflammatory, including pro-inflammatory cytokines (interleukin [IL]-1β, TNF [tumor necrosis factor] α, metalloproteinases, CD4+ cells, B lymphocytes, macrophages, and synovial fibroblasts), which contribute to the pathogenesis of RA.

Although the efficacy of new drugs to treat RA has been proven, this varies. While there are no trustworthy or useful clinical or molecular markers for treatment response, the concentrations of several cytokines and other inflammatory mediators can correlate with the efficacy of treatment. Pharmacogenetics is centered on the study of gene polymorphisms that code for enzymes metabolizing the drugs, although they are also currently centered in the polymorphisms of the drug transporters as well as in their therapeutic targets.[1]

This review reflects, on one hand, the clinical influence of some of the polymorphisms present in RA related genes and, on the other, the pharmacogenetic principles applied to different treatments, such as the classic disease modifying anti-rheumatic drugs (DMARD) and the new biologic agents. In the imminent future, pharmacogenetic studies could help select the adequate medication and dose for each subject.

Clinical influence of gene polymorphisms in rheumatoid arthritis

Many diseases are multifactorial; in them, both the environment and genetic factors contribute to the etiology or the clinical severity. The genetics of many multifactorial diseases is complex because several genes are involved and also because the mendelian inheritance model does not apply. Gene contribution to the susceptibility to RA is reflected both in family groups and, especially, in monocygotic twins.[2] Several authors suggest that at least 10 different gene regions could be related to RA.[3] The variability in the contribution of multiple genetic factors involved in RA could be related to the variability seen in the clinical manifestations, which oscillates between a mild form of the disease and severe disease. On the other hand, the variability in the response to medication is more appreciated in the population than in a single donor or monocygotic twins. Part of this difference is attributed to genetic factors.[4]

Most of the genes involved in the predisposition to the development of RA are localized in the HLA (human leukocyte antigen) loci DR.[5] Other candidate genes are those encoding different cytokines. Cytokines are important mediators of inflammation and develop a role both in the pathophysiology of joint inflammation as well as in the destruction that characterizes RA progression.[6]

The histocompatibility antigen complex

The MHC (major histocompatibility complex) is a gene region that has been constantly associated to RA. The contribution of this region is approximately 30% of the total genetic effect.[7] RA is associated to specific *HLA-DRB1* alleles that encode a conserved aminoacid sequence (residues 70-74 in the *DRβ1* chain) known as the shared epitope.[8] This sequence is found on the floor of the antigen groove (known as peptide-binding groove). Alleles carrying this sequence are *DRB1**0401, *DRB1**0404, *DRB1**0405, *DRB1**0408, *DRB1**0101, *DRB1**0102, and *DRB1**1001.[9] The presence and the number of copies of the *HLA-DRB1* alleles encoding for the shared epitope have been associated to rheumatic nodules, a larger and faster degree of joint degeneration, Felty's syndrome, vasculitis and, in many cases, to the need for surgery.[10] The *DRB1**0401/*DRB1**0404 genotype is apparently associated to early onset disease, as well as to a more severe clinical phenotype.[9]

Microsatellite sequences have also been described in the HLA region; transcript 2, associated to the *HLA-B* (*BAT2*) and *D6S273* alleles are microsatellites of the class III *HLA* region while *D62223* is a microsatellite of the class I *HLA* region.[11] In this review we will describe how some of these microsatellite markers are related to response to treatment.

Cytokine genes in rheumatoid arthritis

If one takes into account the critical role of several cytokines (such as *TNF* and *IL-1*) in the pathogenesis of RA and considers the heterogeneity of their gene regulation, as well as the presence of these molecules in the joint, it is possible that the polymorphisms that regulate the production of these cytokines affect the natural course of disease.[12] A great number of polymorphisms with possible functional phenotypes (Table 1) have recently been identified, mainly in the promoter region of several cytokines, and it is suspected that they are of great importance to maintain the balance between pro-inflammatory and anti-inflammatory cytokines.

Tumor necrosis factor

One of the molecules that carry out an important role in the pathogenesis of RA is the proinflammatory cytokine *TNF*. This molecule belongs to a family of proteins involved in the regulation of the immune system and in the programming of cell death. The concentration of *TNF* in subjects with RA is chronically elevated in the blood and specifically, in the joints.[13] Two receptors intervene in the functions of this molecule: *TNFRSF1A* and *TNFRSF1B*, present as monomers both on the cell surface and in a soluble form.[14] We know that TNF is involved in the stimulation of cytokine production (it increases the expression of adhesion molecules) and neutrophil activation.

TNF is also a co-stimulator of T cell activation and antibody production on the part of B cells.[15] It also contributes to regulation of homeostasis and plays an important role in inflammation. Sixty percent of the variation in the production of TNF is genetically determined, indicating genetic influence on cytokine production.[16] All of these characteristics, in addition to its localization on chromosome 6 in the class III MHC region between the genes for *HLA-B* and *HLA-DR*,[17] allow us to speculate of the existence of functional polymorphisms for this gene. As a consequence of all of the above, the *TNF* gene has been considered as a disease association candidate gene.

Within the *TNF* gene, mainly in the promoter region, the presence of a SNP (single nucleotide polymorphism) (Figure 1) has been described. The first identified polymorphism was a transition between guanine (G) and adenine (A) in position −308. Allele A, uncommon, has a strong association to the *HLA-A1-B8-DR3-DQ2*[18] haplotype and is also associated to autoimmune disease and phenotypes that lead to a greater production of *TNF*.[19] This allele may facilitate the deregulation of the cytokine network and originate RA.[16]

Polymorphism studies of position −238 of the promoter region of *TNF* showed a greater presence of the G allele versus the A allele. Of the 3 possible genotypes, GG and GA are the most common; the first of these is that it seems to be associated to more severe joint erosions, while subjects with the GA genotype present a slower progression.[20] Other studies showed a similar association in position

Table 2
Gene polymorphisms in rheumatoid arthritis

Gene symbol	Position of the polymorphism	Allele	Possible effect of the polymorphism	Reference
TNF-α	+1304	G A	May contribute to RA susceptibility. Possible linkage disequilibrium	22
	+489	G A	More severe joint erosion	16
	−238	G A	More severe joint erosion Less severe joint erosion	20
	−308	G A	Normal *TNF*-α production Positive regulation of *TNF*-α production	18,19
	−857	C T	May contribute to RA susceptibility. High *TNF*-α production	23
	−863	C A	May contribute to RA susceptibility. High *TNF*-α production	21
	−1031	T C	May contribute to RA susceptibility. High *TNF*-α production	22,24
	*TNF*a6; b5; c1; d3; e3		Increase in RA susceptibility	29
TNFRSF1B	196 codon	T G	More effective increase in *IL-6* production	26
IL-1	*IL-1*α −889	C T	Altered *IL-1*α production	42
	*IL-1*α +4845 (exon 5)	G T	Altered *IL-1*α production. Increase in susceptibility to RA	34,38,41
	*IL-1*β −511	C T	Altered *IL-1*α production	40
	*IL-1*β +3953 (exon 5)	C T	Altered IL-1RA production. Severe joint destruction	39,40,41
	IL-1RA +2018 (exon 2)	C T	Possible proinflammatory effect	43
IL-6	−174	G C	Reduces *IL-6* production	49
	−622	G A	Reduces *IL-6* production	48
IL-10	−1082	G A	Positive regulation of *IL-10* production in lymphocytes Low *IL-10* concentrations. Associated to RA in women	12 52,54
	−819	T C	Low *IL-10* concentrations. Autoimmune manifestations	52,53
	−592	A C	Low *IL-10* concentrations. Autoimmune manifestations	53,52
HLA	Specific shared epitope alleles (*HLA-DR*)	May contribute to susceptibility to RA and severity of RA		8–10

A indicates adenine; C, citosine; G, guanine; HLA, human leukocyte antigen; IL-1, interleukin-1; IL-6, interleukin-6; IL-1RA, IL-1 receptor antagonist; RA, rheumatoid arthritis; *TNF*-α, tumor necrosis factor alpha; *TNFRSF1B*, type 2 *TNF*-α receptor

+489: individuals with the GG phenotype in this position reflected more severe erosions in the development of the disease.[16]

The above described polymorphisms, as well as other present in this gene, such as −1031 T/C, −863 C/A, −857 C/T, or +1304 G/A, may contribute to the susceptibility to RA due to an increase in the production of *TNF*-α,[21-24] and can participate in several haplotypes due both to the great number of potentially relevant polymorphisms as well as to the complex patterns of linkage disequilibrium that take place in the MHC region.[25]

A SNP has been described in the 6 exon of the type 2 *TNF*-α (*TNFRSF1B*) receptor, that consists in a single-base substitution in codon 196 (T to G, from ATG to AGG), leading to a non-conserved aminoacid change (methionine to arginine). Allele 196G seems to be more effective in the production of *IL-6* than allele 196T. Allele 196G may also affect cell membrane receptors.[26]

In the *TNF* locus, as in the case above, sequences of microsatellite deoxyribonucleic acid have also been described. Repetitions consist in A and T base sequences and are localized in non-coding regions. These sequences serve as genetic markers when found in linkage disequilibrium with a functional polymorphism in the proximity of the gene.[27] The *TNF* locus has 5 microsatellites (*TNF*a to *TNF*e) based on the number of repeated sequences.[28] In vitro studies indicate that *TNF*d and *TNF*a2 are associated to high concentrations of TNF-α, while *TNF*a6 is associated to low concentrations.[28] Some microsatellite haplotypes, such as *TNF*a6; *TNF*b5; *TNF*c1; *TNF*d3; and *TNF*e3, have been associated to an increased susceptibility for RA.[29]

Interleukin-1

IL-1 is another cytokine that contributes to the chronic destruction seen in RA. It is accepted that arthritis can be induced in mice through the local injection of recombinant cytokines (*TNF* or *IL-1*) in the knee joint.[30] Biologic activity of IL-1 depends of the balance between both proinflammatory cytokines (*IL-1*α and *IL-1*β) and an anti-inflammatory protein (*IL-1* receptor antagonist [*IL-1RA*]). *IL-1RA* blocks the binding of *IL-1*α and *IL-1*β to their receptor and regulates the activation of these 2 cytokines. *IL-1* is important because it induces the suppression of matrix synthesis carried out by chondrocytes and the release of aggrecanase, an enzyme responsible for proteoglycan loss.[30]

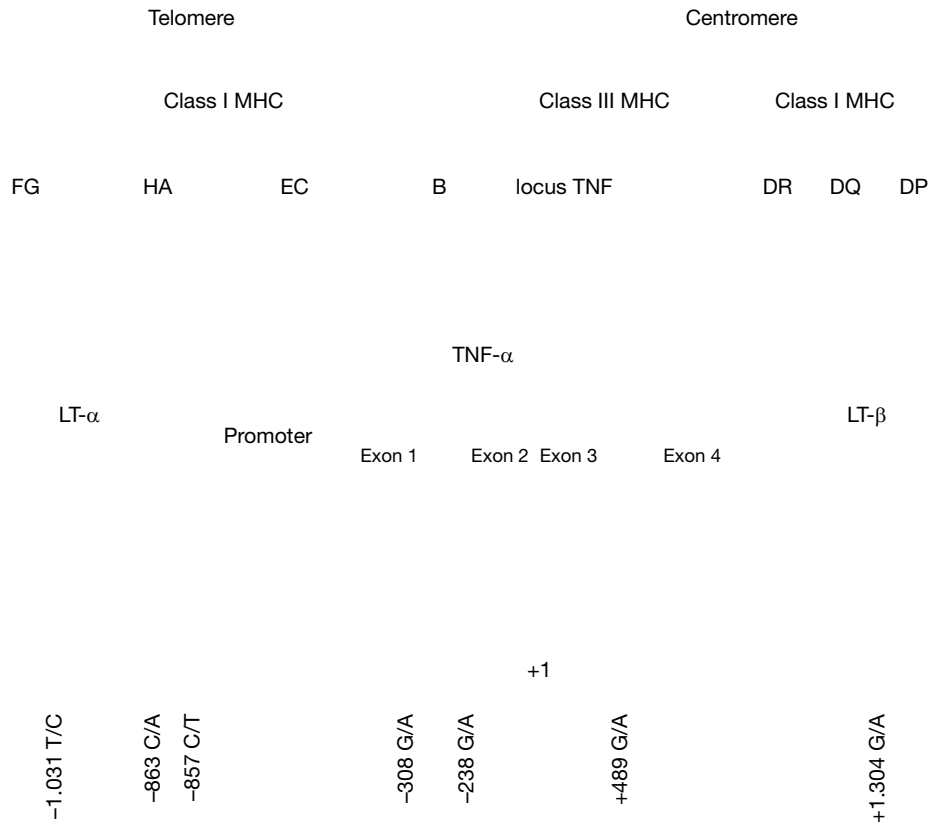

Figure 1. Schematic representation of the gene for the tumor necrosis (TNF) alpha gene, with the most relevant single base polymorphisms reflected. Horizontal arrows indicate the transcriptional orientation of the TNF and lymphotoxin genes. Exon regions 1 and 4, diagonally enhanced, indicate the un-translated region.

Genes encoding these 3 proteins (*IL-1*α, *IL-1*β, and *IL-1RA*) are localized in a 430 kb region in chromosome 2.[31] In each one of these genes there are SNP and other types of alterations that lead to the existence of haplotypes common in the population, given the large number of linkage disequilibrium taking place in this region.[32] Among the most interesting polymorphisms we find: *a)* bi-allelic SNP in the *IL-1* α gene localized in position −889 C/T[33] and in exon 5 in position +4845 G/T[34]; *b)* in the *IL-1*β gene in the position −511 C/T[35] and exon 5 in position +3953 C/T[36]; *c)* in the *IL-1RA* gene in position +2018 C/T in exon 2[37]; and *d)* a penta-allele polymorphic site in intron 2 that contains a VNTR (variable number of tandem repeats) of an 86 bp sequence.

Some studies have reflected the association between the presence of less prevalent alleles in the *IL-1*α (+4845) or *IL-1*β (+3953) genes and an increase both in susceptibility to RA AR[38] and joint destruction.[39] Other studies have related some of the above mentioned polymorphisms, *IL-1*α (−889), *IL-1* α(+4845), *IL-1*β (+3953), or the VNTR of intron 2 of *IL-1RA*, with al altered production of IL-1.[40-42] Genotype in *IL-1*β has also been described as an influence in IL-1RA concentrations.[40] On the other hand, *IL-1RA* +2018 C/T polymorphism seems to have a pro-inflammatory effect.[43]

Interleukin-6

IL-6 is another pleiotropic cytokine with a wide range of biologic activities, including immune response regulation, inflammation, hematopoiesis, and bone metabolism.[44] Overproduction of *IL-6* seems to have a role in the pathogenesis of RA. Serum concentrations of *IL-6* have been described to correlate with disease activity and radiographic joint damage.[45] However, other authors propose that *IL-6* acts as an anti-inflammatory mediator.[46] *IL-6* has been seen to increase *IL-1RA* and peripheral blood soluble *TNF* receptor concentrations and both aspects could lead to an anti-inflammatory effect by suppressing the action of *IL-1* and *TNF*.[47]

Polymorphisms in the *IL-6* promoter region have been described, among which there are a translocation of G/C in position −174 and a transition of G/A in position −622; both in complete linkage disequilibrium.[48] It has been shown that polymorphism of position −174 affects *IL-6* concentrations and has been associated to juvenile idiopathic arthritis.[49] However, this last data seems to rule out the importance of the role of these polymorphisms in RA susceptibility.[48,50]

Interleukin-10

Another cytokine that regulates the inflammatory response is *IL-10*, which acts as a negative regulator of *TNF*-α and other proinflammatory cytokines.[51] There are different polymorphisms in *IL-10* (its gene is located on chromosome 1) that may affect the concentrations of cytokines under production. Point alterations in positions −1082 G/A,

−819 T/C, and −592 A/C may result in an ACC haplotype associated to low expression levels of *IL-10*.[52] These variations also correlate with autoimmune manifestations,[53] in particular the -1082AA genotype, associated to the development of RA in women.[54] On the contrary, genotype -1082GG of this cytokine is associated to a positive regulation in *IL-10* production in lymphocytes.[12]

Pharmacogenomics of antirheumatic drugs

Disease-modifying anti-rheumatic drugs in RA

Theses drugs have the capacity of reducing or preventing damage to the joints and preserving their integrity and function by acting on the immune response. However, the consequences of treatment with these drugs in patients diagnosed with RA are variable and unforeseeable. A possible cause that explains the differences both in efficacy and the appearance of adverse events can be the genetic variations present among individuals when metabolizing these drugs.

DMARDs that have potential use as personalized medications in relation to the genetic profile of the subject with RA are methotrexate (MTX), sulphasalazine (SSZ), and azathioprine (AZT).

Methotrexate

This is the most commonly used drug in the treatment of RA and its main pharmacologic effect seems to be antagonizing folate metabolism. MTX comes into the cell through the RFC (reduced folate carrier) 1 and is intracellularly converted into MTX polyglutamates, leading to intracellular retention of MTX by promoting the inhibition in the synthesis of purines and the formation of adenosine, a potent anti-inflammatory agent.

MTX directly inhibits several enzymes, such as dihydrofolate reductase, 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformilase (ATIC), or timidilate synthase (*TYMS*). MTX directly inhibits other enzymes, such as methylenetetrahydrofolate reductase (*MTHFR*), but its degree of expression can contribute to increasing MTX effects.

An extensive review of the pharmacogenetics of MTX[55] shows the existence of several gene polymorphisms related to MTX transporters though the cell membrane and with enzymes that influence its metabolism (Table 2). Among the polymorphisms that influence MTX cell membrane transport, those of G80A in RFC1 and C3435T in the *ABCB1* gene stand out, which codify a membrane transporter (glucoprotein P) that is really implicated in bioavailability and disposition of different drugs. Gene variations in these transporters can affect the response to MTX in subjects with RA, because both increase the entry of the drug into the cell. Those individuals that have the RFC 80A/A genotype have a better response when compared to subjects with the wild type allele (80G/G).[56] Subjects with the ABCB1 3435C/C and 3435 C/T genotypes have a higher risk of presenting RA, compared to subjects with the 3435T/T genotype, because they respond better to MTX treatment.[57]

Among the polymorphisms influencing the metabolic enzymes involved in the cell route of this drug, 2 SNP located in the gene coding for the enzyme *MTHFR* stand out. This enzyme is very important for the regeneration of reduced folate. The C677T polymorphism in this gene results in a thermosensitive variant with a detriment in enzymatic activity.[58] There is a great range of clinical effects associated to these polymorphisms, such as the increase in gastrointestinal adverse events,[59] an increase in liver toxicity[60] and different adverse events.[61] In addition, recent studies show that *MTHFR* 677TT genotype bearers respond less to MTX in comparison to other genotypes[62]; however, other authors have not found any effect on toxicity or on efficacy.[63,64] A1298C polymorphism confers reduced *MTHFR* activity and also shows discrepancies in its clinical effects. Therefore, some studies propose an increased efficacy of MTX,[60,61] greater susceptibility for presenting RA[63] and an increase in toxicity[62,65,66]; however, another study did not detect any effect on efficacy and toxicity.[64]

Genes encoding *TYMS* and *ATIC* are also related with the MTX cell pathway by being its therapeutic targets. *TYMS* is a key enzyme in the de novo synthesis of thymidilate and converts deoxyuridine monophosphate into deoxythymidine monophosphate. MTX polyglutamates inhibit this enzyme. In the 5′-UTR (un-translated region) of the *TYMS* gene a tandem polymorphic repeat was identified, with a VNTR of 28pb[67]; the larger the number of repeated elements, the larger the expression of messenger ribonucleic acid (mRNA) and the larger the enzymatic,[68] therefore reducing the efficacy of MTX.[64] Another polymorphism has been described in this gene, consisting in a deletion of 6-bp (TTAAAG) in position 1496 of the 3′-UTR[69] region, which may be associated to a reduction in the stability and expression of mRNA[70] of this gene in a way that increases the efficacy of MTX.[64]

ATIC converts aminoimidazole carboxamide ribonucleotide into10-formil AICAR. MTX inhibits *ATIC* directly, originating an accumulation of AICAR and adenosine, an anti-inflammatory purine. A previous

Table 2
Pharmacogenetic data related to the efficacy or toxicity due to methotrexate in rheumatoid arthritis

Gene symbol	Polymorphism	Effect of the polymorphism	Pharmacogenomics	Reference
RFC1	G80A	Increase of cell uptake of MTX	Increase in response to MTX	56
ABCB1	C3435T	Increase of cell uptake of MTX	Increase in response to MTX	57
MTHFR	C677T	Thermosensitive variants of the MTHFR enzyme with a reduction on enzyme activity	Increase in gastrointestinal adverse events and increase of hepatic toxicity	59,60
			Adverse events	61
			No efficacy or toxic effect	63,64
	A1298C	Reduction in MTHFR activity	Increase in MTX activity	60,61
			Increase in susceptibility to RA	63
			No efficacy or toxic effect	62,64
			Increase in the risk of toxicity	62,65,66
TYMS	5′UTR 28 bp repetition	Increase in mRNA expression and enzyme activity	Reduction of MTX activity	64
	3′UTR 6 bp deletion	Reduction in mRNA stability and expression	Increase in MTX efficacy	64
ATIC	C347G	Accumulation of AICAR and increase in adenosine	In combination with SNP *RFC1* G80A correlates with a better response to MTX treatment MTX	71

ABCB1 indicates adenosinetriphosphate-binding cassette B1; *AICAR,* 5-aminoimidazole-4-carboxamide ribonucleotide; RA, rheumatoid arthritis; mRNA, messenger ribonucleic acid; *ATIC*, aminoimidazole carboxamide ribonucleotide transformilase; *MTHFR*, methylenetetrahydrofolate reductase; *MTX,* methotrexate; RFC1, reduced folate transporter; *SNP,* single nucleotide polymorphism; *TYMS,:* thymidilate sinthase; UTR, un-translated region.

study determined that homozigocity for the C347G polymorphism in *ATIC* and the presence of the G80A SNP in *RFC1* could be related to a better response to MTX.[71]

Sulphasalazine

SSZ is another DMARD commonly employed in the treatment of RA. However, its use is limited due to adverse effects.[72] After oral ingestion, intestinal bacteria cleave SSZ into 5-amino salicylic acid and sulphapiridine, and the latter is metabolized in the liver through acetylation. The *NAT2* gene, located on chromosome 8p22 codes the enzyme involved in the acetylation of sulphapiridine and may be ploymorphic. Gene polymorphisms in NAT2 (Table 3) influence acetylation in slow acetylators versus rapid acetylators. Slow acetylations are more liable to toxicity due to SSZ compared to rapid ones.[73]

The wild type allele *NAT2*4* codes for the rapid acetylation mechanism, while its variants (*NAT2*5A, *NAT2*5B, *NAT2*5C, *NAT2*6, and *NAT2*7), which differ in the combinations of several SNP located in exon 2 of the gene, code for the slow acetylation mechanism, which translates into detriment of the enzymatic activity of the *NAT2* enzyme. Because of these slow acetylations, these variants are associated to an increase in the concentration of SSZ intermediaries.[74,75]

The state of acetylation of an individual, which may be influenced by the *NAT2* gene polymorphisms, may be important when determining the risk of SSZ toxicity. Therefore, it would be useful in clinical practice to develop studies to identify the NAT2 genotype in subjects that are about to start SSZ treatment in order to prevent toxicity associated to this drug.[76]

Azathioprine

AZT is a drug employed in the treatment of different types of cancer, in rheumatic disease, and in prevention of organ rejection. In spite of this, AZT is not commonly employed in the treatment of RA due, among other causes, to the development of other DMARDs. Thyopurine methyltransferase (*TPMT*) is one of the enzymes involved in the metabolism of this drug. Different population studies have allowed to establish the activity of *TPMT* in red blood cells as trimodal: approximately 90% of the population has high activity, 10% intermediate activity, and only 0.3% has little or no activity.[77]

There are 3 allelic variants of the *TPMT* gene: *TPMT*2 (G238C), *TPMT*3A (G460A and A719G), and *TPMT*3C (A719G) (Table 4) present in 60% to 95% of the population that presents low or intermediate activity of TPMT.[78] Clinically, these polymorphisms are associated to hematologic and, in some cases, gastrointestinal toxicity.[79] The *TPMT* gene phenotype may be useful when predicting AZT toxicity.

Biologic agents in rheumatoid arthritis

The introduction of biologic gents has notably altered the treatment of RA; these agents not only reduce symptoms and signs of the disease, but also delay its radiologic progression.[80] However, these treatments are substantially more expensive than traditional DMARDs and, moreover, are not effective for everyone.[81] Some studies point out that between 25% and 30% of subjects with RA do not respond to these treatments.[82] Early identification of subjects who respond positively to these drugs may be of help when establishing an effective treatment with these molecules.[83]

Several studies on *TNF* and *IL-1* mediated inflammatory processes have led to the development of agents that block cytokines for the treatment of RA. Three *TNF* blockers are currently approved by the FDA for the treatment of RA: etanercept, infliximab and adalimumab. These blockers derive from a recombinant *TNF* receptor (*TNFRSF1B*) in the case of etanercept or a monoclonal anti-*TNF*-α antibody in the case of infliximab and adalimumab. The molecular mechanism of the different *TNF* blockers is based on the same principle, which consists in impeding *TNF* binding to *TNF* cell surface receptors, in this way inhibiting signal transduction induced or regulated by *TNF*. Although neutralizing treatment can be very effective in reducing symptoms and indications of RA, not all subjects have the same degree of response in disease progression terms.[84] It has been proposed that variability in the promoter and coding regions of TNF-α may modulate the magnitude of the secretion response of this cytokine.[85]

The fourth biologic agent approved by the FDA for the treatment of RA is anakinra, a recombinant form of *IL-1RA*; its molecular mechanism has already been explained.

All of the drugs that have the potential to turn into "personalized treatments" directed to subjects with RA, share problems related to effectiveness and toxicity. In response to the toxicity of these drugs, the risk of presenting lymphoma has been described, but it must be remembered that by themselves, RA patients have a twofold increase in the risk for lymphoma. Only some of these lymphomas are related to the presence of the Epstein-Barr virus. This may, in turn, be related to the elevated prevalence of this type of virus in subjects with RA, reflecting a mild compromise of antiviral immunity in these subjects.[86]

Etanercept

Etanercept is a dimeric fusion protein that includes the p75 receptor of human *TNF* bound to a c fragment of immunoglobulin

Table 3
Pharmacogenetic data related to the efficacy or toxicity of sulphasalazine in rheumatoid arthritis

Gene symbol	Polymorphism	Effect of polymorphism	Pharmacogenomics	Reference
NAT2	NAT2*4	Increased activity of NAT2 enzme	Reduced concentrations of SSZ intermediaries	73
	Wild allele	Rapid acetylation	Less predisposition to SSZ intoxication	
	*NAT2*5A*	Reduced activity of NAT2 enzyme	Increased concentrations of SSZ intermediaries	74,75
	T341C, C481T	Slow acetylation	Larger predisposition to SSZ toxicity	
	*NAT2*5B*	Reduced activity of NAT2 enzyme	Increased concentration of SSZ intermediaries	74,75
	T341C, C481T, A803G	Slow acetylation	Larger predisposition to SSZ toxicity	
	*NAT2*5C*	Reduced activity of NAT2 enzyme	Increased concentrations of SSZ intermediaries	74,75
	T341C, A803G	Slow acetylation	Larger predisposition to SSZ toxicity	
	*NAT2*6*	Reduced activity of NAT2 enzyme	Increased concentrations of SSZ intermediaries	74,75
	C282T, G590A	Slow acetylation	Larger predisposition to SSZ toxicity	
	*NAT2*7*	Reduced activity of NAT2 enzyme	Increased concentrations of SSZ intermediaries	74,75
	C282T, G857A	Slow acetylation	Larger predisposition to SSZ toxicity	

NAT2 indicates N-acetyltransferase 2; SSZ, sulphasalazine.

Table 4
Pharmacogenetic data related with azathiprine toxicity in rheumatoid arthritis

Gene symbol	Polymorphism	Effect of the polymorphism	Pharmacogenomics	References
TPMT	*TPMT*2*	Low to intermediate TPMT activity	Hematologic and gastrointestinal toxicity	[79]
	G238C	Reduction in AZT methylation		
	*TPMT*3A*	Low to intermediate TPMT activity	Hematologic and gastrointestinal toxicity	[79]
	G460A, A719G	Reduction in AZT methylation		
	*TPMT*3C*	Low to intermediate TPMT activity	Hematologic and gastrointestinal toxicity	[79]
	A719G	Reduction in AZT methylation		

AZT indicates azathioprine; TPMT. thiopurine methyltransferase.

G1 (IgG1) (Figure 2a). This medication is produced exclusively with human aminoacid sequences and is 934 residues long.

The efficacy of etanercept for the treatment of RA has been shown as effective both as monotherapy as well as combined with MTX. The progression of joint damage is significantly reduced when this drug is administered for more than 24 moths. In addition, treatment with etanercept is significantly better than MTX monotherapy.

However, opportunistic infections have been described, as well as tuberculosis, heart failure, and lymphoma in subjects receiving this drug. The risk of presenting some of these is increased if, at the same time of treatment, the subject uses steroids or other immunosuppresants. Increased risk for demyelinating diseases has also been described.[87]

Infliximab

Infliximab is a monoclonal antibody that binds *TNF*-α and neutralizes its activity. It is a chimeric mouse-human antibody in which the mouse antibody variable regions are bound with the constant region of human IgG1 (Figure 2b). This drug is the first *TNF* blocker employed in the treatment of RA and shows the important role that TNF-α plays in this disease.

Treatment with infliximab has provided subjects with improvement in their quality of life, the prevention of structural damage to the joint and possibly bone repair. This drug has also been successfully employed, alone or in combination with MTX, for the treatment of other disease, such as Crohn's disease, ankylosing spondylitis, or psoriatic arthritis.

Infliximab has the same problems than etanercept: local reactions at the site of injection, opportunistic infections, tuberculosis and a higher risk of developing lymphoma.[88] In the same way, demyelinating processes, heart failure, and autoimmune diseases have been associated to it.

Adalimumab

Adalimumab is a human monoclonal IgG1 antibody. Its molecular mechanism is similar to that of infliximab: it binds both circulating *TNF* as cell surface bound *TNF* blocking the interaction of *TNF*-α with its receptors p55 and p75 localized on the cell surface (Figure 2c). Adalimumab modulates the biologic response induced by TNF and reduces the concentrations of *IL-6* and matrix metalloproteinases (MMP) 1 and MMP-3.[89]

Adalimumab is used in monotherapy or combined with MTX. A benefit of this drug is the inhibition on the progression of long-term joint structural damage in subjects with RA who have not satisfactorily responded to other DMARD.

Toxic effects associated to the use of adalimumab include the same as those described for the 2 previous drugs: opportunistic infections, tuberculosis, demyelinization processes, autoimmunity, and heart failure.

Anakinra

Anakinra is a recombinant form of human *IL-1RA* that acts as an antagonist of *IL-1* biologic activity through competitive inhibition and binds to the cell surface receptor of *IL-1* blocking cell signaling (Figure 2d). Its efficacy in subjects with RA has been seen when used in monotherapy as well as combined with MTX, etanercept, and other DMARDs.[90-92] In addition, because *IL-1* plays an important role in the development of Still's disease, this drug has been successfully employed for the treatment of said disease.[93,94] Treatment in combination with etanercept has not been shown to be clinically advantageous and is currently contraindicated because it increases the risk of developing opportunistic infections.

The inhibition of structural damage is an important benefit of this drug, while injection site reactions are its major disadvantage; in addition to this, pneumonia and skin infections have been described.[92]

Pharmacogenomics of biologic agents

Several studies have correlated the response to biologic therapy with some of the gene polymorphisms described in this review (Table 5). Different haplotypes that include the *HLA-DRB1* region as well as the *TNF* gene corresponding region influence the response to etanercept in caucasians.[95] In this same study, those subjects with 2 copies of the *HLA-DRB1* shared epitope allele have been shown to have a better response to etanercept when compared to subjects who had one or no copies of that allele. Because of the great number of genes localized in the HLA region that influence both function as well as immune system regulation and the existence of linkage disequilibrium occurring in this region, there are many genes that may influence response to treatment.[96]

In a large study, 78 subjects treated with infliximab were genotyped for alleles *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1*, for a repetitive trinucleotide polymorphism in the MICA gene, as well as for microsatellites o the *TNF* gene from *a* to *e*, and for microsatellites present in other regions of the *HLA* complex, such as *D6S273*, *BAT2* (HLA class III) and *D6S2223* (HLA class I). When analyzing all of the haplotypes, the authors concluded that the *D6S273_4* and *BAT2_2* pair is the most significant in subjects responding to treatment. In the same way, the frequency of *TNFa11*; b4 haplotype, a marker normally present in *D6S273_4* and *BAT2_2*, was also increased, while that of allele *D6S273_3* was reduced in subjects who responded to treatment.*11* These results allowed the authors to speculate that these markers could be localized in the same haplotype that includes the until now unknown "response gene."

Other studies have shown a correlation between *TNF*-α -308G[12] and *TNF*-α -857T[97] polymorphisms and a good response to etanercept. Other authors have analyzed the response to etanercept or infliximab in subjects with severe RA, characterized by a negative response

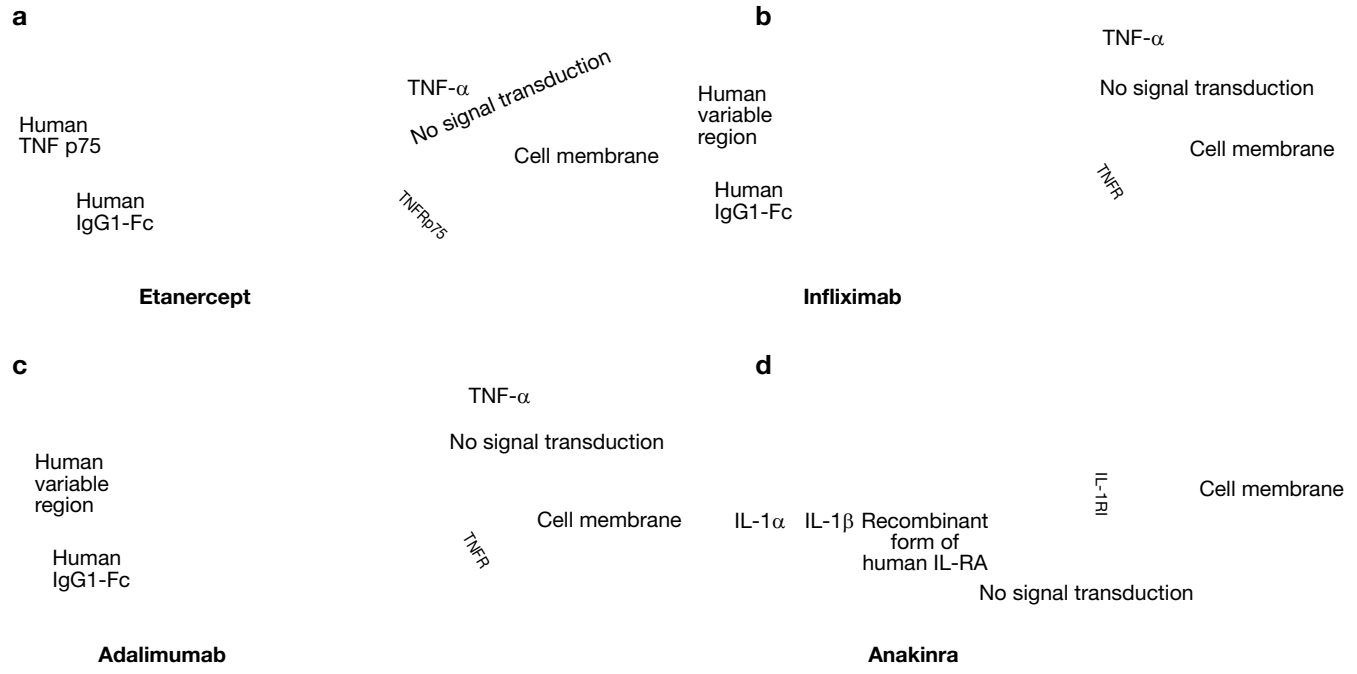

Figure 2. Molecular mechanisms. a) Tumor necrosis factor alpha (TNF-α) blocker, etanercept. b) TNF-α blocker, infliximab. c) TNF-α blocker, adalimumab. d) treatment with interleukin-1 (IL-1) blocker. Anakinra, a recombinant form of human IL-1 receptor antagonist (IL-1RA).

to MTX in combination with other DMARDs. Subjects with the *TNFRSFB* 196TT genotype treated with anti-*TNF* had a larger degree of response during 24 weeks when compared to subjects with the TG/TG genotype. On the basis of these results, the 196TT genotype would correlate with a greater degree of anti-*TNF* treatment response in RA, while the G allele would be associated to a worse response.[98]

The combination of the *TNF* -308 G/G and *IL-10* -1082G/G genotypes (subjects with mild inflammatory responses) also shows a better response to etanercept. Therefore, etanercept seems to be more effective in subjects who have a phenotype that encodes a mild inflammatory response.[12] Another study shows microsatellite polymorphisms in the *IL-10* promoter region, associated to a better response to long term etanercept treatment.[99]

Different pharmacogenetic studies have been developed on the efficacy of infliximab. The presence of the -308 G/A SNP in the *TNF* promoter region influences the response to infliximab[100-102]; subjects that have the G/G phenotype respond better to treatment. Some authors speculate on the possibility that the *TNF* -308 polymorphism could influence the response to infliximab due to the effects that it has on the circulating concentrations of *TNF*[101]; the presence of allele A (high *TNF* producers) may be related to a worse response to infliximab. However, other studies show that there is no relationship with the response to infliximab.[11,98] In the same way, a study carried out by this group in 113 subjects with RA showed that -308G/A and -238 G/A polymorphisms as well as the presence of the shared epitope or the *DR3* allele are not correlated with a better response to infliximab after 30 weeks of treatment, according to the DAS28 score.[103]

Until today there is only one pharmacogenetic study that analyzes the degree of response to anakinra. This shows a relationship between the infrequent *IL-1*α +4845 G/T polymorphism and a significant response to treatment.[104]

Conclusions

In summary, response to treatment is partially determined by an individual's genetics. As has been previously indicated, RA is a well-defined disease with widely accepted criteria,[105] while its clinical aspects and the molecular pathways involved in the process are heterogeneous.[106] Therefore, responses to different treatments vary among individuals. Because of the development of a great variety of new drugs, their price and the lack of detailed information on its adverse events as well as an increased susceptibility to infection make it necessary to develop prognostic genetic markers for response to treatment.[107] These markers can be found in the previously described genes or in those coding for protein involved in the therapeutic targets, their metabolism or the disease pathogenesis.[108] In this sense, the article by Lequerré et al,[109] stands out, in which they detail a profile of 41 transcripts of mRNA susceptible of predicting response to a combined therapy of infliximab and MTX from peripheral blood cells. In this way, the understanding of the genetic contribution in the treatment of RA will turn into an ever more relevant fact, at a moment in which the therapeutic targets of the treatments developed are the same mechanisms that contribute to develop the disease.[107]

There are numerous polymorphisms that have been described until today, such as *TNF, TNFRSF1B,* MHC alleles and other cytokine genes, but their function is controversial and their study yields contradicting results; there are several reasons to explain this, among which the population stratification and linkage disequilibrium stand out.[110] Additionally, the analysis of haplotypes present in candidate gene regions seems to be a more adequate method than the description of individual SNP. However, because pharmacogenetics is a relatively new field in which

Table 5
Pharmacogenetic data related to the efficacy or toxicity of biologic agents in rheumatoid arthritis

Gene symbol	Position of the polymorphism	Allele	Possible effect of the polymorphism	Pharmacogenetics	References
TNF-α	+489	G A	More severe joint erosions	No effect on response to etanercept	93
	−238	G A	More severe joint erosions Less severe joint erosions	No effect on response to etanercept	93
	−308	G A	Normal *TNF-α* production Positive regulation of *TNF-α*	Better response to infliximab	98,99
	−857	C T	Susceptibility to RA. High *TNF-α* production	Better response to infliximab	95
	TNFa11; b4	Haplotype	Influence on *TNF-α* production. Found with *D6S273_4* and *BAT2_2*	Better response to infliximab	11
TNFRSF1B	Codon 196	T G	More effective in the positive regulation of *IL-6* production	Better response to anti-*TNF* treatment	96
IL-1	*IL-1α* +4845 (exon 5)	G T	Positive regulation of lymphocyte *IL-1αRA* production	Better response to anakinra	101
IL-10	−1082	G A	Positive regulation in lymphocyte *IL-10* production Low concentrations of *IL-10*. Associated to RA in women	Better response to etanercept in combination with *TNF-α* -308G/G	12
	−819	T C	Low *IL-10* concentrations. Autoimmune manifestations	Subjects with a low inflammation haplotype respond better to etanercept	
	−592	A C	Low *IL-10* concentrations. Autoimmune manifestations		
HLA	Specific alleles of the shared epitope (*HLA-DR*)	May contribute to susceptibility and severity of RA	Specific *HLA-DRB1* alleles. Certain haplotypes respond better to etanercept		93
	HLA microsatellites *BAT2, D6S273, D6S2223*	Haplotype that may carry the treatment "response gene"	Haplotype *D6S273_4* and *BAT2_2* correlates with a better response to infliximab		11

A indicates adenine; C, citosine; G, guanine; *HLA,* human leukocyte antigen; IL-1, interleukin-1, IL-10, interleukin-10; RA, rheumatoid arthritis; *TNF-α,* tumor necrosis factor alpha; *TNFRSF1B*, type 2 *TNF-α* receptor.

different studies are just being published, it may be speculated that, in a not so distant future, a personalized treatment may be applied in relation to an individual's genotype.[30] To achieve this requires large studies that involve multiple institutions with the objective of obtaining an adequate number of subjects in order to determine if the genetic variants described for cytokine genes, as well as other specific molecules, directly contribute to both the pathophysiology as well as the response to different treatments for RA.

Acknowledgments

The authors of this article wish to thank Ms P. Cal Purriños for her expert assistance as secretary.

Conflict of interest

None.

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