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REVIEW

Pharmacogenetics of rheumatoid arthritis: Potential targets from susceptibility genes and present therapies

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Abstract: Rheumatoid arthritis (RA) is a chronic heterogeneous autoimmune disorder of unknown etiology resulting in inflammation in the synovium, cartilage, and bone. Genetic factors play an important role in susceptibility to RA as the heritability of RA is between 50% and 60%, with the human leukocyte antigen (HLA) locus accounting for at least 30% of overall genetic risk. Outside the major histocompatibility complex (MHC) region, six additional risk loci have been identified and validated including *PTPN22*, *STAT4*, *PADI4*, *CTLA4*, *TNFAIP3-OLIG3*, and *TRAF1/C5*. Genetic factors are also important in RA pharmacotherapy due to the gene-dependent activity of enzymes involved in the pharmacokinetics and/or pharmacodynamics of RA medications. Indeed, there is great variability in drug efficacy as well as adverse events associated with any anti-rheumatic therapy and genetics is thought to contribute significantly to this inter-individual variability in response. This review will summarize the genetic factors that have been implicated in the pathogenesis of RA, and how these determinants may factor into the potential pharmacogenetics of this disease. We will also review the therapeutic agents that are currently being utilized or presently being evaluated in the treatment of RA, along with potential pharmacogenetic markers that have been proposed for such medications.

Keywords: rheumatoid arthritis, susceptibility genes, pharmacogenetics

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease that results in inflammation in the synovium, cartilage and bone.[1] If RA is not adequately treated in a timely manner, there is significant negative impact to patient health accompanied by a large societal burden. RA typically presents in the sixth decade of life with symmetrical small joint pain and swelling in the hands, feet, and wrists. With disease progression, medium to large size joints such as the shoulder, elbow, knees, and hip become involved. Extra-articular manifestations are common in RA and include features related to vasculitis (eg, rheumatoid nodule, episcleritis, peripheral neuropathy or palpable purpura) and/or lymphocytic infiltrate (eg, sicca symptoms, hypothyroidism, interstitial lung disease, splenomegaly, or lymphadenopathy).

The current therapeutic management of RA includes symptomatic management and early disease modification. Nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids are first line therapies used for symptomatic relief. However, if oral corticosteroids are instituted, a conscious effort must be made to minimize dosage. In conjunction with the use of NSAIDs, disease-modifying antirheumatic drugs (DMARDs) are promptly initiated in the management of RA. DMARDs attempt to slow down the progression of RA and include: methotrexate (MTX), azathioprine

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(AZA), sulfasalazine (SSZ), hydroxychloroquine (HCQ), and leflunomide (LFA). These agents are either used as monotherapy in a sequential fashion, or more commonly as part of a cocktail with multiple DMARDs. Unless there is a contraindication to MTX, this agent is the DMARD of choice among most rheumatologists. If significant disease activity persists despite an adequate trial of DMARDs, then more targeted biologic therapies such as inhibitors of tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, co-stimulatory molecule, or B-cells are employed.[2] The aforementioned roles of various DMARDs and biologic agents serve only as a antecedent to a more detailed discussion of their potential pharmacogenetic targets, presented in upcoming sections.

The etiology of RA is still not fully elucidated. Theories regarding the pathogenesis of RA must address three key elements of the disease: onset of autoimmunity, chronic inflammation, and subsequent joint destruction.[3] Autoimmunity is manifested by the presence of immunoglobulin G (IgG) antibodies, clinically detectable prior to the onset of RA, which represents the lymphoid phase of the disease. The lymphoid phase is followed by the articular phase which is triggered by poorly understood environmental and biomechanical events in a genetically susceptible host. The environmental factors most frequently associated with RA pathogenesis include smoking and various infectious agents (eg, parvovirus). The persistent inflammation subsequently initiates articular destruction, a process mediated by osteoclasts. The importance of cytokines in the pathogenesis of RA is highlighted by their involvement in every phase of pathogenesis.

In this review, we will summarize the genetic factors and the cytokines that have been implicated in the pathogenesis of RA, and how these determinants may factor into the potential pharmacogenetics of this disease. Moreover, we will review therapeutic agents currently being utilized or presently being evaluated in the treatment of RA, along with potential pharmacogenetic markers that have been proposed for such medications.

Genetics of RA susceptibility

There is substantive evidence for a genetic basis of RA. There is increased occurrence of disease among first degree relatives (lambdas 2 to 15) and increased concordance of identical twins. The prevalence of RA in the general population varies, but increases among siblings of RA probands.[4] Based on evidence from studies of monozygotic and dizygotic twins, it is estimated that the heritability of RA is between 50% and 60%,[5] which strongly suggests that genetic factors are indeed critical in RA pathogenesis.

Multiple loci are known to contribute to the risk of developing RA.[6–8] The most compelling evidence to support a genetic basis is the consistent and reproducible association of RA within the major histocompatibility complex (MHC), particularly the human leukocyte antigen (HLA) alleles. This region accounts for at least 30% of overall genetic susceptibility to RA.[4,6] Within the HLA locus, the strongest association is with alleles of *HLA-DRB1*, but recent evidence indicates that other HLA genes also contribute to genetic risk. Increasingly genes outside the MHC region on different chromosomes (Figure 1) are being indentified regarding the genetics of RA susceptibility (see also Table 1). To date, the most consistent association outside the MHC region appears to be with a polymorphism in the protein tyrosine phosphatase nonreceptor 22 (*PTPN22*) gene.[6–8,10] Other candidate genes have been identified, but the scope of this review will be limited only to the best studied non-MHC variants.

MHC locus is associated with RA pathogenesis

Association with variations in the MHC or HLA locus on chromosome 6 (6p21.3) was identified over three decades ago, and is the primary locus that has been consistently and reproducibly associated with RA susceptibility across all studied populations.[6] The class II molecules of the HLA locus are recognized as the most powerful genetic determinants for RA contributing at least 30% of the total genetic effect.[11] Numerous classes of *HLA-DRB1* alleles known to be associated with RA susceptibility comprise the shared epitope (SE) region (Table 2).[7,12,13] The *HLA-DRB1* alleles of the class II *HLA-DRB1* gene encode a conserved sequence of amino acids (ie, QKRAA, QRRAA, or RRRAA) at positions 70 to 74 in the third hypervariable region (HVR3) of the class II DRB1 chain.[14] The aforementioned *HLA-DRB1* alleles and their conserved amino acid sequence are collectively referred to as the shared epitope (SE), which distinguishes disease-associated alleles from those that do not confer risk for RA.[14]

As previously mentioned, extensive evidence exists indicating that the *HLA-DRB1* SE is strongly associated with susceptibility to RA.[14–18] While *HLA-DRB1* SE alleles confer risk to RA, not all alleles display the same magnitude of association with RA.[7,14,19] For example, certain alleles (eg, *HLA-DRB1*0401*) confer a much stronger degree of risk compared with other alleles (eg, *HLA-DRB1*0101*).[6–8] Moreover, certain allelic combinations (eg, *HLA-DRB1*0401/*0404*) appear to confer a very high risk to RA susceptibility, and even severity of the disease.[20] The strength of association is influenced

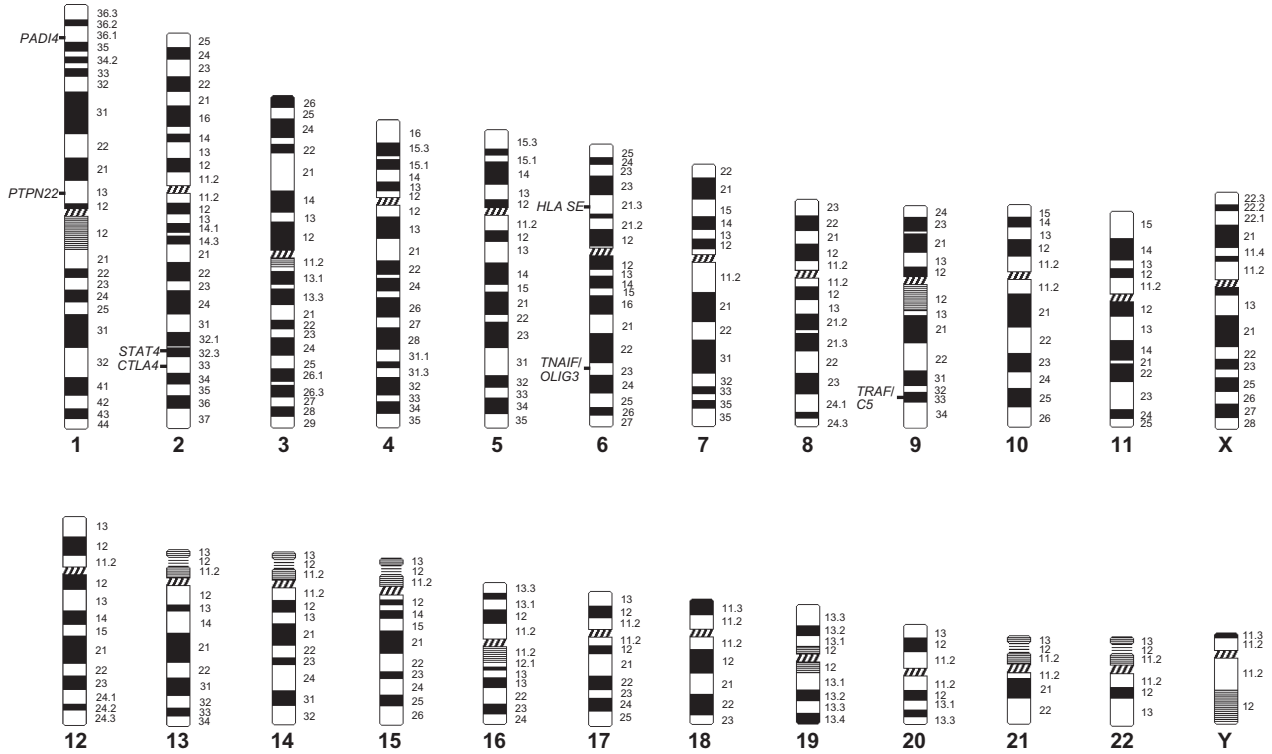

Figure 1 Location of validated risk genes for rheumatoid arthritis.

by factors including, but not limited to the: 1) frequency and number of SE alleles; 2) amino acid at position 70 to 74 in the HVR3 region of the class II DRB1 chain; and 3) anti-citrulline-peptide antibody (ACPA) status. The frequency of the SE alleles varies considerably depending on ethnicity. For example, the *HLA-DRB1*0401* and **0404* alleles are predominately associated with RA in Caucasian populations, whereas the **0405* and **0101* alleles are associated with RA in Asian and Jewish populations, respectively.[6,21,22] In a recent study of French Caucasians, the authors proposed that not only is RA susceptibility conferred by amino acid residues at positions 72 to 74 (ie, RAA), but that the association strength is modulated by amino acid residues at positions 70 and 71 (Table 2).[23] This hypothesis and proposed classification scheme has been subsequently supported by findings in other populations.[13,24,25] However, only a single study has investigated the relevance of this new *HLA-DRB1* classification in terms of RA susceptibility on both Caucasian and non-Caucasian cohorts.[26]

Table 1 Genetic variants demonstrating strong susceptibility to rheumatoid arthritis

Gene/Locus	Number of RA patients	OR	95% CI	*P* value	Ethnicity	Ref.
HLA-DRB1 SE	689	3.0	2.2–4.2	3.0×10^{-9}	Caucasian	31
	2,204	1.9	1.4–2.8	<0.05	Asian	9
PTPN22	2370	1.6	1.4–1.8	4.8×10^{12}	Caucasian	37
STAT4	16,088	1.3	1.2–1.4	<0.001	Caucasian	49
	16,088	1.2	1.1–1.3	<0.001	Asian	49
PADI4	5,591	1.1	1.0–1.1	0.72	Caucasian	71
	3,713	1.3	1.2–1.4	<0.0001	Asian	64
CTLA4	2370	1.1	1.0–1.2	0.004	Caucasian	37
TNFAIP3-OLIG3	3962	1.2	1.1–1.3	2.6×10^{-6}	Caucasian	91
TRAF1/C5	1522	1.4	1.2–1.5	2.8×10^{-8}	Caucasian	95

Abbreviations: HLA-DRBI SE, major histocompatibility complex, class II, DR beta 1; PTPN22, protein tyrosine phosphatase, non-receptor type 22 (lymphoid); STAT4, signal transducer and activator of transcription 4; PADI4, peptidyl arginine deiminase, type IV; CTLA4, cytotoxic T-lymphocyte-associated protein 4; TNFAIP3-OLIG3, tumor necrosis factor, alpha-induced protein 3/oligodendrocyte transcription factor 3; TRAFI/C5, TNF receptor associated factor/complement component 5.

Table 2 A new scheme remodeling the HLA-DRB1 shared epitope classification in rheumatoid arthritis

Allele classification	HLA-DRB1 susceptibility allele	Amino acid sequence	Genetic risk to RA
S_2	*0401	Q-K-RAA	High
	*1303	D-K-RAA	
S_{3P}	*0101, *0102, *0404, *0405, *0408	Q-R-RAA	Intermediate
	*1001, *1402, *1406	R-R-RAA	
S_1	*1501, *1502, *1503	Q-A-RAA	Low
	*0103, *0402, *1102, *1103, *1301, *1302	D-E-RAA	
S_{3D}	*1202, *16	D-R-RAA	

Abbreviations: HLA-DRB1, major histocompatibility complex, class II, DR beta1; RA, rheumatoid arthritis.

In that study ($n_{cases/controls}$ = 759/789), a positive association with RA susceptibility was demonstrated for S_2 allele carriers (odds ratio [OR] = 2.1, 95% confidence interval [CI]: 1.5–3.0; $P < 0.0001$) and S_3P allele carriers (OR = 2.74, 95% CI: 2.0–3.7; $P < 0.0001$).[26]

The RA susceptibility associated with the *HLA-DRB1* SE appears to be limited to a subset of patients who have ACPA-positive and not ACPA-negative RA.[12,27–31] For example, in the largest cohort studied to date, the presence of any *HLA-DRB1* SE allele was strongly associated with an ACPA-positive phenotype (OR = 3.0, 95% CI: 2.2–4.2; $P = 3.0 \times 10^{-9}$).[31] That *HLA-DRB1* SE confers risk specifically to ACPA and that these antibodies are present in approximately 70% of RA patients[32] explains, at least in part, an association between these alleles and susceptibility to RA and perhaps more importantly, suggests a difference in the pathology between ACPA-positive and ACPA-negative RA.

In addition to affecting disease susceptibility, the *HLA* SE appears to be important in onset, progression, and severity of RA. In a large European cohort, the presence of any *HLA* SE allele was associated with an average 3.6 years earlier diagnosis compared with absence of *HLA* SE;[31] a finding replicated in an American cohort.[33] Moreover, large differences were identified in rates of erosion progression between ACPA-positive and ACPA-negative RA with respect to the *HLA* SE.[12] The strength of this association appears to be dependent on the number of copies of the SE allele (two copies: OR = 11.8, $P < 0.0001$; one copy: OR = 4.4, $P < 0.0001$). This finding combined with the report that ACPA-positive has a more aggressive clinical course than ACPA-negative RA,[34] strongly implies that this locus not only plays a significant role in RA susceptibility, but also is important in the severity of RA. Thus, in the clinical setting, these determinants (ie, SE alleles or ACPA status) will likely translate into poor therapeutic impacts by virtue of the fact that these subset of patients have a greater burden of inflammatory disease.

PTPN22 is associated with RA pathogenesis

The tyrosine phosphatase nonreceptor 22 (*PTPN22*) gene, located on chromosome 1 (1p13), was the first non-*HLA* gene associated with RA.[35] Specifically, the minor allele of a non-synonymous 1858C>T single nucleotide polymorphism (SNP; rs2476601), results in an amino acid change from R620W in the *PTPN22* gene.[35] Outside of the *HLA* region, this variant exhibits the strongest and most robust association with RA (Table 1). The well studied associations with *HLA-DRB1* and *PTPN22* explain about 50% of the genetic contribution to RA disease susceptibility.[36] A meta-analysis of 13 RA studies ($n_{cases/controls}$ = 2370/1757) revealed a strong association of the T-allele genotype with the development of RA (OR = 1.6, 95% CI: 1.4–1.8; $P = 4.8 \times 10^{12}$), and an earlier age at disease onset.[37] Similar to *HLA-DRB1*, *PTPN22* is strongly associated with ACPA-positive (OR = 1.43, 95% CI: 1.2–1.7; $P = 0.0001$), but not ACPA-negative (OR = 1.0, 95% CI: 0.8–1.3; $P = 0.73$) RA;[37] a finding that strengthens the theory that ACPA-positive and ACPA-negative RA have different pathologies. The greater risk allele frequency present in Caucasian RA populations (OR = 1.7, 95% CI: 1.2–2.2; $P < 0.0001$)[35] is consistent with the extensive evidence confirming that the *PTPN22* variant is associated with RA in all populations of European or North American descent.[35,37–44] A lack of association of *PTPN22* in Asian and African populations is reflected by the absence or very low frequency of this risk allele in that demographic.[35]

Similar to the SE, the *PTPN22* appears to be important in onset, progression, and severity of RA. In a large European cohort, the presence of any *PTPN22* variant was associated with an average 4.2 years earlier diagnosis compared with absence of a variant;[31] a finding subsequently replicated in another large Caucasian cohort.[37] In contrast to the *HLA* SE, *PTPN22* does not appear to be associated with an erosive phenotype.[31] This finding combined with the report that ACPA-positive has a more aggressive clinical course than ACPA-negative RA,[34] suggests that *PTPN22* not only plays a significant role in RA susceptibility,

but may also be important in severity of RA; an effect similarly reported for the *HLA* SE. Likewise, the presence of the *PTPN22* variant may influence the outcome of various therapeutic agents in RA. Of course, such speculation requires further confirmation.

PTPN22 which encodes the intracellular protein lymphoid tyrosine phosphatase (LYP), a powerful inhibitor of T-lymphocyte activation, plays a critical role in T-lymphocyte antigen receptor (TCR) signaling pathway.[45] The *PNPT22* polymorphism is a gain-of-function variant resulting in greater phosphatase activity which elevates the activation of T-lymphocytes and confers enhanced inhibition of activation.[46,47] Interestingly, T-lymphocytes expressing the *PTPN22* polymorphism produce less IL-2 in response to T-lymphocyte signaling compared to wildtype controls.[46,47] Such a variant which enhances inhibition of T-lymphocyte activation may result in weaker signaling and failure to effectively remove auto-reactive T-lymphocytes, which could explain, at least in part, its relevance to RA.[7]

STAT4 is associated with RA pathogenesis

The signal transducer and activator of transcription 4 (*STAT4*) gene, located on chromosome 2 (2q32.2–q32.3) is another non-*MHC* gene associated with RA pathogenesis.[48] Specifically, a SNP was identified at position 274 (–23582G>A; rs7574865) within the third intron of the *STAT4* gene.[48] Four polymorphisms in tight linkage disequilibrium (ie, rs11889341, rs7574865, rs8179673, and rs10181656) form a susceptibility haplotype which is tagged by the T allele (rs7574865), have the strongest reported association with RA.[48] A meta-analysis conducted on 15 studies ($n_{cases/controls}$ = 16,088/16,509) revealed an significant association between RA and the *STAT4* variant (rs7574865) in both Caucasians (OR = 1.3, 95% CI: 1.2–1.4; $P < 0.001$) and Asians (OR = 1.2, 95% CI: 1.1–1.3; $P < 0.001$).[49] The association of *STAT4* variant (rs7574865) with RA was validated in patients from European, North American, and Asian descent.[49–54] Europeans appear to have the lowest (21.4%) and Asians the highest (32.0%) prevalence of the rs7574865 variant among the populations studied.[49] Stratification of RA patients according to the presence of ACPA antibody revealed a statistically significant association between the rs7574865 variant and RA in both ACPA-positive and ACPA-negative RA patients versus controls.[54,55] That *STAT4* confers risk specifically to ACPA, that these antibodies are present in approximately 70% of RA patients,[32] and that a similar finding has been demonstrated with SE and *PTPN22*, explains, at least in part, an association between *STAT4* and susceptibility to RA and again strongly indicate a difference in the pathology between ACPA-positive and ACPA-negative RA.

The JAK/STAT pathway is the signaling target of a multitude of cytokines that are thought to play biologically significant roles in rheumatoid synovial inflammation.[56] Specifically, *STAT4*, which encodes STAT4, transmits signals induced by several key cytokines, including IL-12, IL-23, and type I interferons (IFNs).[57] Activated STAT4 transcribes specific genes including interferon-γ, and plays a critical role in the development of type 1 helper T (Th1) lymphocyte response thought to drive the chronic autoimmune response.[58–60] STAT4 has also been implicated in the differentiation of Th17 cells; an effect dependent in part on the activity of IL-23.[61] Thus, the JAK/STAT pathway with its many interactions, including various inflammatory cytokines[61,62] may explain, at least in part, the association of the *STAT4* gene with chronic inflammatory disorders including RA.

PADI4 is associated with RA pathogenesis

The peptidylarginine deiminase 4 (*PADI4*) gene, located on chromosome 1 (1p36), is known to be associated with RA, particularly in Asian populations.[63,64] The strongest association has been demonstrated for a SNP located in intron 3 (341-15A>T) of PADI4, called *PADI4_94* (rs2240340) and a recent meta-analysis revealed a significant association between RA and the PADI4_94 SNP in Asian populations (OR = 1.3, 95% CI: 1.2–1.4; $P < 0.0001$).[64] In contrast, findings in cohorts of European ancestry have been inconsistent.[37,63–66,69,70] Whereas *PADI4_94* was found to be associated with RA in North American and German populations, studies in Spanish, Swedish, and UK populations reported no evidence for association of *PADI4* with RA.[37,65] Analysis of data collected in the largest study performed to date ($n_{cases/controls}$ = 5,591/13,638) gave an overall OR of 1.01 (95% CI: 1.0–1.1; $P = 0.72$), indicating that *PADI4_94* genotype is not associated with RA in European Caucasian descent. This result is supported by a recent meta-analysis which confirmed a lack of association between *PADI4_94* genotype and RA in people of European descent (OR = 1.1, 95% CI: 1.0–1.1; $P = 0.12$).[71] This meta-analysis contrasted two earlier meta-analyses which suggested that the *PADI4_94* polymorphism confers susceptibility to RA in those of European descent (OR = 1.1; 95% CI: 1.0–1.2; $P = 0.0096$), albeit to a lesser degree than in Asian subjects.[72,73] The discrepancy between studies of Caucasian descent can be explained, at least in part, by previous studies being underpowered to detect an OR of this level, thus producing false-positive results.[71] Therefore, *PADI4_94* may be in LD with the true disease associated allele in Asian but

not Caucasian populations.[71] A possible explanation for the difference between RA patients from Asian and European descent may be that *PADI4* is associated only with a subgroup of RA patients. Specifically, the association between *PADI4* genotype and RA may be restricted to patients with more severe disease.[74] Unlike the *HLA-DRB1*, *PTPN22*, and *STAT4* genes, there is growing evidence that *PADI4* polymorphisms play a role in the development RA in Asian populations independent of ACPA status. This observation suggests that the aforementioned genes may affect RA susceptibility via different but possibility convergent mechanisms. This does not preclude the possibility that *PADI4* interacts with and modulates the activity of ACPA-dependent genetic factors such as the *HLA* SE.

The *PADI4* gene encodes the type 4 peptidylarginine deiminase enzyme, which catalyses the post-translational modification of arginine to citrulline, generating citrullinated proteins.[75] The mechanism by which *PADI4* genotype may influence RA susceptibility has not yet been elucidated. While the link between synovial intracellular citrullinated proteins and ACPA status emphasizes the role of deimination of synovial proteins in RA, there is no compelling evidence supporting *PADI4* genotypes correlating with ACPA levels or ACPA-positive disease in particular. Antibodies to these citrillinated peptides are highly specific for RA and often precede the development of disease, suggesting a critical role in RA pathogenesis.[63] In addition, PADI4 mRNA was detected in hematological cells and pathological synovial tissues, and is significantly overexpressed in the blood of RA patients.[76,77] Moreover, anti-PAD-4 auto-antibodies were associated with the *PADI4* susceptibility haplotype.[71] Collectively, these reports suggest that critical link between the *PADI4* gene and the pathogenesis of RA in Asian populations.

CTLA4 is associated with RA pathogenesis

The cytotoxic T lymphocyte antigen 4 (*CTLA4*) gene, which is located on chromosome 2 (2q33), has been investigated frequently in relation to RA. While other polymorphisms within the *CTLA4* gene appear to be associated with RA,[78–80] an G>A SNP in the 3′ untranslated region (CT60; rs3087243) has received a more thorough investigation, especially in European populations.[37,52,80–82] Although previous case-control studies in various populations have suggested a possible association of *CTLA4* alleles with RA, the results of these studies are often inconclusive and are sometimes contradictory. A large cohort ($n_{cases/controls}$ = 2,370/1,757) from the North American Rheumatoid Arthritis Consortium (NARAC) and the Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA) collections, provided support for an association of *CTLA4* (CT60 allele) with the development of RA, but only in the NARAC cohort (OR = 1.1, 95% CI: 1.0–1.2; $P = 0.004$).[37] When those results were combined with previously published data for *CTLA4*, it demonstrated continued evidence of association with RA (OR = 1.1, 95% CI: 1.0–1.2; $P = 0.01$).[37] These earlier results correlated well with a recent meta-analysis which confirmed an association of *CTLA4* gene polymorphism with RA in Caucasians (OR = 0.9, $P = 1.8 \times 10^{-3}$) which also revealed that *CTLA4* enhanced the development of ACPA-positive as compared with ACPA-negative RA.[52] Similar to *HLA-DBR1* SE and *PTPN22*, these reports clearly indicate that *CTLA4* influences the development of RA only in ACCP-positive patients and is further evidence pointing to a divergence in pathology dependent on ACCP status.

The *CTLA4* gene, which encodes the CTLA4 protein, plays an important role in downregulation of T-cell activation.[83] Full activation of T-lymphocytes requires both the recognition of an antigen bound to HLA and a co-stimulatory signal between CD80 or CD86 on the antigen-presenting cell and CD28 on the T-lymphocyte.[84–86] CTLA4 protein expressed on T-lymphocytes as CTLA4 binds to CD80/CD86, may influence susceptibility to RA by inhibiting this co-stimulatory signal. However, the exact mechanism by which *CTLA4* genotype may influence RA susceptibility still remains to be determined.

The importance of CLTA4 to RA pathogenesis is indicated by the development of a soluble fusion protein (ie, abatacept) that consists of the extracellular domain of CTLA-4 linked to the modified Fc (hinge, CH2, and CH3 domains) portion of human immunoglobulin G1 (IgG1).[84] Abatacept inhibits T-lymphocyte activation by binding to CD80 and CD86, thereby blocking interaction with CD28. This interaction provides a co-stimulatory signal necessary for full activation of T-lymphocytes.[84–86] Abatacept has been shown to be effective in treating RA and is indicated in the treatment of moderate to severe RA and active polyarticular juvenile idiopathic arthritis.[84–86]

TNFAIP3-OLIG3 is associated with RA pathogenesis

This association locus was first identified in a GWAS[36] and has been subsequently confirmed in multiple Caucasian and Asian cohorts.[87–92] Specifically, a study of 3962 RA patients and 3531 healthy controls demonstrated strong association (OR = 1.2; 95% CI: 1.1–1.3; $P = 2.6 \times 10^{-6}$) with a variant (rs6920220) in an intergenic region of chromosome 6q23 region between oligodendrocyte linear transcription factor 3

(*OLIG3*) and TNF-α-induced protein 3 (*TNFAIP3*).[91] The validity of this study has been confirmed by a recent meta-analysis of seven studies investigating the association of the *TNFAIP3-OLIG3* region with RA, which indicated a strong association of the variant rs6920220 (OR = 1.2, 95% CI: 1.2–1.3; $P = 7.9 \times 10^{-17}$).[92] Since the *TNFAIP3* gene acts as a negative regulator of the transcription factor nuclear factor-κB (NFκB) in response to TNF- and toll-like receptor activation,[93,94] it is an attractive RA susceptibility candidate gene. Unfortunately, currently there is no therapeutic agent specifically targeting this molecule.

TRAF1/C5 is associated with susceptibility to RA

This association locus was first identified in the same GWAS that identified the *TNFAIP3-OLIG3* locus,[36] and has been subsequently confirmed in a large Caucasian cohort.[95] There is strong evidence of association with a variant (rs3761847) in a region of chromosome 9q33–34 (OR = 1.4, 95% CI: 1.2–1.5; $P = 2.8 \times 10^{-8}$); a region spanning the genes for *TRAF1* and *C5*.[95] Both *TRAF1* and *C5* are biologically plausible contributors of RA susceptibility. The product of *TRAF1* appears to act as a negative regulator of signals mediated through TNF receptors and T-lymphocyte receptors, whereas C5 is an important component of the complement pathways, thought to play a role in articular inflammation in RA.[95]

Other RA susceptibility genes

In addition to the already described associations, other genes previously identified by GWAS are highly suggestive for an association with RA susceptibility. Recently, relatively large cohort studies have strengthened the association of multiple highly suggestive genes with RA susceptibility, especially with genes involved in pathways important to RA pathogenesis. In particular, there is growing evidence that the NFκB signaling pathway may be a critical transcription factor implicated in a variety of cellular responses to stimuli, including inflammation.[96] To date, multiple genes known to interact with NFkB signaling have been proposed to be associated with RA susceptibility (eg, *CD40*, *CD244*, *CDK6*, *CCL21*, *PRKCQ*, *TNFRSF14*, *PIP4K2C*, *IL-1B*, *IL-2RB*, and *IL-2RA*). For these genes, replication in large population cohorts is required before validation as true RA risk alleles.

Gene expression studies in RA

The cDNA microarray technology enables simultaneous expression of thousands of RNA's transcribed from both known and unknown genes. This technology is helpful for large-scale gene discovery, as it provides diagnostic fingerprints by comparing gene expression patterns in normal and pathological cells in a single experiment. Expression profiling studies in RA cohorts can be categorized in two broad categories: those involved in identifying susceptibility genes and those looking at disease expression in RA. A recent systemic review summarized gene expression studies that was differentially expressed in at least two studies or genes differentially

Table 3 Genes associated with a differential expression pattern in patients with rheumatoid arthritis

Differential expression of genes in more than one RA study*	
Gene	**Location**
Cleavage stimulation factor, 3 pre-RNA, subunit 2 (CSTF2)	Xq22.1
Solute carrier family genes (*SLC7A7, SLC25A4*)	14q11.2, 4q35
Colony-stimulating factor 3 receptor (CSF3R)	1p35–p34.3
Troponin genes (*TNNI1,TNNT2,TNNI2,TNNT3*)	1q31.3–32, 11p15.5
Argninosuccinate lyase (ASL)	7cen–q11.2
Tumor protein p53 (TP53)	17p13.1
Tyrosine kinase (TXK)	4p12
Ribosomal protein SA (LAMR1)	3p22.2
Bone morphogenetic protein 8 (BMP8)	1p35–p32
Cytokine P450 (CYP3A4)	7q21.1
Kininogen 1 (KNG1)	3q27
S100 calcium-binding protein (S100)	1q21
Protein phosphatase 2 (PPP2R3)	3q22.1
Matrix metalloproteinase (MMP3)	11q22.3
Differential expression of genes in only one RA study but falling within susceptibility region from previous linkage or association studies*	
Gene	**Location**
Kininogen 1 (KNG1)	3q27
Colony-stimulating factor 3 receptor (CSF3R)	1p35–p34.3
Troponin T type 2 (TNNT2)	1q32
Protease subunit beta-type 9 (PSMB9)	6p21.3
EGF receptor pathway substrate 15 (EPS15)	1p32
Membrane cofactor protein (MCP)	1q32
Stomatin (EPB72)	9q34.1
Interferon regulator factor 4 (IRF-4)	6p25–p23
Neutrophil cytosolic factor 4 (NCF4)	22q13.1
Interleukin 8 (GM-CSF)	4q13–q21
Stathmin 1 (STMN1)	1p36.1
Protein tyrosine phosphatase (PTPRK)	6p22.2–23.1
AA598840	1p34.3
AA487590	13q12–q13
Acyl-coenzyme A thioesterase 7 (HBACH)	1p36.31–p36.11

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expressed in at least one study but located within a genetic linkage or association region (Table 3).[97]

With respect to disease expression, microarray studies of synovial biopsies compared expression of early and long standing rheumatoid arthritis.[98] In that study, the authors identified several gene clusters and distinct molecular signatures specifically expressed during early or late RA, suggesting that different mechanisms are in play at various stages of RA.

Pharmacogenetics of RA

There is great variability in drug efficacy as well as adverse events with almost any antirheumatic therapy. Profiling for individual genetic variability holds great promise in treating rheumatic conditions. The identification of such variants has the potential to improve management of patient care by identifying which patients should avoid a specific drug and which patients should be administered a modified dose. A suitable approach in implementing such a strategy could potentially reduce medical costs and improve the overall process and success of drug therapy.[99] This section of the paper will serve to highlight the current landscape of genetic targets for RA pharmacotherapy.

NSAIDs

Nonsteroidal anti-inflammatory drugs (NSAIDs) continue to be the mainstay of therapy for symptomatic relief. NSAIDs are primarily metabolized by the polymorphic CYP2C9 enzyme with a minor contribution from the CYP2C8 enzyme. Functionally important SNPs associated with decreased catalytic activities have been identified in both enzymes, especially for CYP2C9, which vary in frequency according to ethnicity.[100] The metabolic capacities of the *CYP2C9*2* and **3* alleles are approximately 70% and 3% to 11% compared to the wildtype allele, respectively.[100] While NSAIDs are usually well tolerated for short periods of time, chronic use can lead to gastrointestinal complications (eg, ulcer formation, perforations, and bleeding), and renal toxicity.[101,102] Gene-based dosing for NSAIDs aimed at reducing the occurrence of ADRs is hindered by differing metabolic rates among various NSAIDs and the involvement of additional metabolizing enzymes (eg, CYP2C8). Individuals carrying the gene variants *CYP2C8*3* (rs11572080; rs10509681), *CYP2C9*2* (rs1799853) or *CYP2C9*3* (rs1057910) show increased risk of developing acute gastrointestinal bleeding with NSAID therapy.[103] Specifically, significantly higher frequencies of *CYP2C9*1/*3* (34.6% vs 5.8%, OR = 12.9, 95% CI: 2.9–57.9; $P < 0.001$) and *CYP2C9*1/*2* (26.9% vs 15.4%, OR = 3.8, 95% CI: 1.1–13.2; $P = 0.036$) were identified in bleeding versus control patients.[103] However, it is currently unknown whether parent drugs or products of alternative metabolic pathways are responsible for bleeding.

Glucocorticoids

Glucocorticoids, such as prednisone, have been widely used in the treatment of RA by providing rapid symptomatic relief of pain and swelling.[104,105] Although glucocorticoids inhibit inflammation, they also induce the expression of macrophage migration inhibitory factor (MIF), which in turn, possesses the unique ability to override the inhibitory effect of glucocorticoids on immune and inflammatory responses.[106] The *MIF* (–173G>C) variant has recently been linked to clinical response in numerous inflammatory conditions.[107–111] For example, in juvenile RA patients carrying a *MIF* (–173C) allele, the number of DMARDs required for the treatment was increased, the duration of corticosteroid treatment was significantly longer, and the number of joints with active arthritis was significantly higher.[110] Such a strong predictor of poor treatment outcome in juvenile RA patients would suggest a similar effect in RA patients. A study which investigated this possibility failed to demonstrate a strong association of *MIF* (–173G>C) with clinical response to glucocorticoids in RA.[112] However, due to the small sample size and thus, lack of power, this finding requires replication in larger cohorts. In the future, therapeutic MIF antagonism may therefore provide a specific means of 'steroid sparing'.

Methotrexate

Methotrexate (MTX) is a very effective therapy in treating RA and is the initial disease modifying agent for the majority of rheumatologists. Thus, the majority of patients with RA would have been evaluated on methotrexate at some point in their course.[113] The ADRs associated with MTX range from mild and self-limiting (eg, mucositis, gastrointestinal intolerance) to more severe (eg, hematopoietic suppression, hepatotoxicity, pulmonary toxicity). Both the efficacy and toxicity of MTX is governed by transport (eg, RFC-1, MDR1) and metabolizing enzymes (eg, MTHFR, TYMS, DHFR, ATIC, etc). Variants exist within the genes encoding these enzymes which affect efficacy and/or toxicity of MTX and explains at least in part, the inter-individual variability in response to MTX. Several polymorphisms of genes encoding the MTX signaling pathway have been investigated in relation to efficacy and toxicity (Table 4); however, only the most studied variants will be discussed here in detail. RFC-1 transports folate and MTX into cells and some stud-

Table 4 Candidate gene polymorphisms associated with either efficacy and/or toxicity of MTX pharmacotherapy in rheumatoid arthritis patients

Gene	Variant	Function	Effect on MTX therapy	Ref.
Variants affecting transport of MTX				
RFC-1	80G>A	Minimal effect on transport of folate and MTX into cells	AA genotype associated with 3.7-fold greater response (95% CI: 1.7–9.1, $P < 0.01$)	114
			GA/AA genotype associated with increased risk for overall MTX toxicity (OR = 3.574, 95% CI: 1.1–12.0; $P = 0.039$)	118
MDR1	3435C>T	?	TT genotype associated with a higher remission probability (OR = 4.65, 95% CI: 1.7–13.0; $P = 0.003$)	118–120
Variants affecting intracellular metabolism of MTX				
MTHFR	677C>T	Decreased reduction of 5,10-CH_2-THF to 5-CH_3-THF	CT/TT genotype associated with increased ADRs (RR = 2.0, 95% CI: 1.1–3.7)	126
			CT/TT genotype associated with higher rate of MTX toxicity (RR = 1.2, 95% CI: 1.0–1.5; $P < 0.05$)	128
			No effect on efficacy	126, 127
			CC genotype associated with greater response in early RA	117
	1298A>C	Decreased reduction of 5,10-CH_2-THF to 5-CH_3-THF	AC/CC genotype required lower doses of MTX (RR = 2.2, 95% CI: 1.2–4.1, $P < 0.05$)	128
			CC genotype associated with fewer ADRs	127, 129
			AC/AA genotype associated with higher rate of MTX toxicity (OR = 15.9, 95% CI: 1.5–167.0; $P = 0.021$)	127
			AA genotype associated with greater response in early RA	117
			AC genotype associated with more ADRs	117
TYMS	28bp tandem repeat	Decreased conversion of CH_2-THF to DHF	Alleles with three repeats associated with MTX resistance	114, 132
			Alleles with only two repeats associated with improved response	114, 132
ATIC	347C>G	Decreased *de novo* purine synthesis	GG genotype associated with improved response	130, 114
			GG genotype associated with ADRs	131

Abbreviations: RFC-1, reduced folate carrier – 1; MDR1, multi-drug resistance – 1; MTHFR, 5,10-methylenetetrahydrofolate reductase (NADPH); TYMS, thymidylate synthetase; ATIC, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase.

ies suggest that a variant in the SLC19A1 gene encoding RFC-1 (rs1051266; SLC19A1 80G>A) may influence MTX efficacy and toxicity in RA; [114,115] a finding which has not been replicated in subsequent studies.[116,117]

MTHFR, a prominent regulatory enzyme involved in the folate pathway, catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is a substrate for homocysteine remethylation. While seven polymorphisms have been associated with MTX efficacy or toxicity,[116,121] two nonsynonymous SNPs (rs1801133: 677C>T and rs1801131: 1289A>C) have been extensively studied. The homozygous and heterozygous variants of the *MTHFR* 677C>T polymorphism decreases enzyme activity by approximately 70% and 40%, respectively.[122,123] Similarly, the homozygous variant of the *MTHFR* 1298A>C variant has about 60% of wildtype function.[124,125] Previous studies suggest that the *MTHFR* 677C>T variant is not related to MTX efficacy,[126,127] but rather plays a prominent role in toxicity.[126,128,129] A recent meta-analysis of eight MTX studies, totaling over 1,400 RA patients for the *MTHFR* 677C>T analysis, and over 660 for the *MTHFR* 1298A>C analysis revealed that the *MTHFR* 677C>T variant (ie, CT or TT genotype) was associated with increased toxicity (OR = 1.71, 95% CI: 1.3–2.2; $P < 0.001$).[116] In contrast, the *MTHFR* 1298A>C variant was not associated with increased toxicity (OR = 1.12, 95% CI: 0.8–1.6; $P = 0.626$).[116] Unfortunately, the analysis was limited to these two variants due to a lack of sufficient data for other polymorphisms. Similarly, widely disparate definitions of efficacy among the MTX pharmacogenetic studies combined with insufficient data of MTX polymorphisms prevented meta-analysis of efficacy.[116]

The inter-individual variation in MTX dose required to gain clinical efficacy is highly variable and cannot be currently predicted. Likewise, we are unable to accurately predict which patients will develop ADRs. Given the complexity of the MTX pathway, it is highly probable that multiple genetic variants act in concert to predict MTX efficacy and/or toxicity. Interestingly, the formation of a pharmacogenetic index incorporating the additive effect of multiple genetic variants on MTX efficacy and toxicity may be a possible solution, which may ease the transition of MTX pharmacogenetic testing into the clinic.[105] Indeed, there is evidence to suggest that a low pharmacogenetic index is associated with a good response to MTX, while a higher index score is associated with an increased incidence of ADRs.[130,131]

Leflunomide

Leflunomide is an immunomodulatory drug that inhibits the rate-limiting intracellular enzyme in the *de novo* synthesis of pyrimidines, which is crucial for activating lymphocytes.[133] This DMARD is usually an alternative for MTX-intolerant or resistant RA patients.[134] The main molecular target of leflunomide is dihydroorotate dehydrogenase (DHODH), a key enzyme of *de novo* pyrimidine synthesis. The human *DHODH* gene sequence is highly conserved and contains only one common missense polymorphism (rs3213422) located in the first exon of the *DHODH* gene (19C>A; Q7K).[135] The frequency of remission was increased in *DHODH* 19C allele carriers compared with patients with the *DHODH* A allele indicating a potential role of the *DHODH* gene in leflunomide therapy.[135] Consistent with *in vitro* studies, which demonstrated that *CYP1A2* might be involved in leflunomide activation, RA patients with *CYP1A2*1F CC* genotype had a 9.7-fold higher risk for overall leflunomide-induced toxicity compared to carriers of *CYP1A2*1F A* allele (OR = 9.7; 95% CI: 2.3–41.4; $P = 0.002$).[136] It has also been suggested that therapy with leflunomide causes decreased production of mediators such as IL-1β, IL-6, and TNF-α, which are involved in inflammatory process.[137] However, IL-1 β, IL-6, and TNF-α gene variants appear to be insignificant factors influencing the therapy outcome of RA patients with leflunomide.[137]

Azathioprine

The immunosuppressant agent azathioprine (AZA) is a prodrug which is occasionally used in treating RA.[138] However, treatment success is limited as 10% to 28% of patients terminate treatment due to ADRs, specifically myelosuppression.[139,140] The thiopurine methyltransferase (TPMT) enzyme catalyzes the inactivation of purines, including AZA, and it exhibits genetic polymorphism.[141,142] Genetic polymorphism of *TPMT* is evident in all populations studied to date, including Caucasians, Asians, African-Americans, and Africans. *TPMT*3A*, *TPMT*3C*, and *TPMT*2* are the most prevalent variant alleles comprising approximately 95% of *TPMT* variant alleles in these populations.[143–148] Genotyping for the three most studied SNPs (ie, 238G>C, 460A>G, and 719G>A) provides greater than 95% concordance between *TPMT* genotype and phenotype.[150,151] Briefly, approximately 90% of individuals inherit high activity, 10% have intermediate activity attributed to heterozygosity, and 0.3% have low or no detectable enzyme activity because they inherit two nonfunctional *TPMT* alleles.[149] Multiple studies have demonstrated that *TPMT*-deficient patients and *TPMT* carriers are at high risk and an intermediate risk for severe hematological toxicity, respecitvely.[152–154] Patients with intermediate TPMT activity should receive a reduction of approximately 50% of a normal AZA dose, while AZA should be avoided in patients with low or absent TPMT activity.[155] A genotype-based dosing strategy is less costly, more effective, and is associated with a marked reduction in the number of serious ADRs compared to conventional weight-based dosing strategy.[156] Support amongst rheumatologists for prospective *TPMT* genotyping continues to grow, due in part, to an accumulation of evidence supporting the use of genetic diagnostic testing.

Sulfazalazine

Sulfasalazine, a mildly potent DMARD used in the treatment of RA, inhibits neutrophil function, reduces immunoglobulin levels, and interferes with T-lymphocyte function via the suppression of NFκB.[157] Polymorphisms within the gene encoding the N-acetyl transferase 2 (NAT2) enzyme affect the rate of acetylation which are thought to affect the efficacy and/or toxicity of sulfasalazine.[158] RA patients lacking the wildtype haplotype (ie, *NAT2*4*) at *NAT2* appear to be more likely to suffer from overall ADRs (relative risk [RR] = 3.31, 95% CI: 1.8–6.2; $P = 0.001$) and severe ADRs (RR = 24.6, 95% CI: 2.4–254.5; $P = 0.015$).[158,159] Although additional studies are warranted, it is possible that, in the future, RA patients can be classified into groups according to *NAT2* genotyping: rapid type (homozygote for *NAT2*4*), intermediate type (heterozygote for *NAT2*4* and variant alleles), and slow type (homozygote for variant alleles).[160]

TNF-α inhibitors

Tumor necrosis factor-α (TNF-α) blockade represents a major breakthrough in the treatment of RA. Controlled trials

with all four anti-TNF-α agents, etanercept (Enbrel®), infliximab (Remicade®), golimumab (Simponi™), and adalimumab (Humira®), presently approved for the treatment of RA, have demonstrated impressive improvement in most response measures, including clinical, laboratory and radiographic outcomes.[161] Despite the marked improvement of symptoms and radiographic retardation of RA with the use of anti-TNF-α agents, randomized controlled trials reveal that only 60% to 70% of patients with moderate to severe RA achieve a satisfactory response.[161] A lack of clinical and biochemical predictors of efficacy and toxicity of TNF inhibitors in RA patients has focused much attention to the affect of genetic variations on response. Candidate gene polymorphisms that affect response to etanercept or infliximab therapy are listed in Table 5. Although, multiple polymorphisms have been linked to response, the TNF-α gene promoter −308G>A variant (rs1800629) will be the focus of this review.

The functionality and thus relevance of the −308 variant to TNF-α treatment response is reflected by the ability of the *TNF-α* (−308A) variant to influence the magnitude of the TNF-α secretory response[162] and to affect circulating levels of TNF-α.[163] A recent meta-analysis of nine studies representing a total of 692 RA patients, demonstrated that the probability of successful treatment with anti-TNF-α agents is influenced, at least in part, by the variant in the *TNF-α* gene promoter region.[164] The frequency of the *TNF-α* (-308A) variant was 22% in responders and 37% in nonresponders, and the OR was decreased in responders versus nonresponders (OR = 0.4, 95% CI: 0.4–0.7; $P = 0.000245$), irrespective of the TNF-α inhibitor prescribed.[164] This is strong evidence indicating that the *TNF-α* (-308A) variant predicts poor response to TNF-α inhibitors. The clinical utility of prospectively genotyping for this variant when initiating anti-TNF-α therapy for RA should now be formally assessed. Moreover, it is conceivable that those with the *TNF-α* (-308A) variant could either be treated more aggressively with concomitant DMARDs or implement these biologics earlier in the therapeutic regimen. However, larger cohorts involving multiple centres and

Table 5 Influence of candidate genes on response to rheumatoid arthritis for patients receiving etanercept (ETA), infliximab (INF), or adalimumab (ADA) therapy

Candidate gene(s)	Sample size	Response criteria	Follow-up time	Genotype/phenotype correlation	Ref.
TNF-α (*–238G>A*)	40	DAS28	6 months	GA genotype is associated with a negative response to INF	169
TNF-α (–857)	70	ACR20	3 months	CT/TT genotypes are associated with positive response to ETA	170
	58	DAS28	N/A	CC genotype is associated with positive response to INF	171
TNFRII 676T>G	105	ACR20	12 months	TG genotype is associated with a negative response to ETN, INF or ADA	172
	58	DAS28	N/A	TT genotype is associated with positive response in RA	171
FCGR3A 158F>V	91	ACR20	7.5 months	FF genotype is associated with a positive response to INF	173
TNF-α (–308G>A) & IL-10 (–1087G>A) combination	123	ACR20, DAS28	3 months	GG genotype is associated with positive response to ETA	174
IL-1RN VNTR and *TGFB1* 915G>C Combination	123	ACR20, DAS28	3 months	A2/C allele combination is associated with negative response to ETA	174
HLA-DRB1	255	ACR50	12 months	*0404 & *0101 combination is associated with positive response	175
IL-10	50	DAS28	48 months	IL-10.R3 is associated with positive response	176
	50	DAS28	48 months	IL-10.G13 is associated with moderate or negative response	
	50	DAS28	48 months	IL-10.R2 & G13 combination is associated with moderate or negative response	
	50	DAS28	48 months	IL-10.R3 & G9 combination is associated with positive response	

Abbreviations: ACR, American College of Rheumatology based on perceptual improvement (20, 50, 70, 90%) in disease symptoms; DAS28, disease activity score in 28 joints; IL, interleukin; TNF-α, tumor necrosis factor-α.

ethnicities are a prerequisite before definite conclusions can be drawn regarding the functional role of the *TNF-α* -308A variant and response to TNF-α inhibitors.

With respect to expression profiling studies, Lequerre and colleagues set out to identify predictive genes that would determine responsiveness to infliximab in peripheral blood mononuclear cells (PBMCs) by examining 16 responders and 17 nonresponders to infliximab treatment.[165] Forty-one transcripts discriminated infliximab responders from nonresponders, including CYP3A4, LAMR1, and KNG1. A subset of these transcripts were assessed in two new patients and a sensitivity of 90% and specificity of 70% was achieved for these transcripts.[165]

IL-1 antagonists

Although clinical trials of human recombinant IL-1 receptor antagonists in treating RA have been successful, the magnitude of its effect is inferior to that of anti-TNF-α therapy in RA and varies among patients.[166] The prominent role of IL-1 in the immune response combined with an apparent association between the *IL-1* cluster genes and RA susceptibility suggests that this gene may also be related to the likelihood of response to anti-IL-1 therapy in RA patients. Indeed, Camp and colleagues reported that *IL-1* gene does influence reduction in swollen joints in RA patients treated with an IL-1 receptor antagonist.[167] A highly significant association was found between carriage of the rarer allele at *IL-1A* (+4845) and response to treatment (OR = 4.8; 95% CI: 1.8–12.7; $P = 0.0009$).[167] Moreover, the response rate in patients carrying this allele was 63.4% compared with 26.3% in noncarriers.[167] Due to the paucity of pharmacogenetic data on IL-1 receptor antagonists and other biologics (eg, *IL-12/23* variants) in RA patients, much more research is warranted to better define the relationship between the *IL-1* gene cluster and response to IL-1 receptor antagonists.

Conclusion

Diagnostic testing using genetic markers is superior compared to testing with traditional biomarkers, such as markers of the inflammatory process (eg, C-reactive protein, cytokines) and markers of disease-associated autoantibodies (eg, rheumatoid factor, ACPA status) as they afford greater predictive power, drastically reduce the need for repeated biochemical testing and are less affected by environmental factors like diet. Whereas, traditional biomarkers are important helping to establish a clinical diagnosis, genetic markers can help identify high-risk individuals reliably and in a timely manner so that they can either be treated before onset of the disease or as soon as possible thereafter. For example, patients at high risk or who fail to respond to conservative therapy are candidates for earlier, more aggressive strategies using single or possibly combination antirheumatic therapy.

RA is a complex disease with considerable genetic heterogeneity. With the advent of GWAS, much progress has been made in the genetics of RA. However, the non-MHC RA risk alleles still account for only 5% of the overall genetic burden of RA, indicating that additional non-MHC risk alleles remain to be discovered.[168] Likewise, the modest effect size and power of existing studies to detect these effects at genome-wide levels of significance indicates that many more common alleles of modest effect remain to be discovered.[36] In the future, several steps are paramount in elucidating the genetics of RA in relation to improved patient care. Firstly, as sample sizes increase, coverage across the genome is improved and copy number variations are included, GWAS will reveal these common variants of modest risk, the so-called 'hidden' risk alleles. Secondly, in addition to identifying and confirming risk alleles, functional studies will be fundamental to better understand how these variants contribute to RA pathogenesis. Thirdly, it will be important to determine the value of RA risk alleles in providing clinical prediction among healthy individuals, those with early symptoms consistent with RA, and those with established disease. Finally, it will be important to determine whether combinations of risk alleles are able to subset patients into clinically meaningful categories, which would result in superior patient care.

Similar to pathogenesis, the genetics of RA pharmacotherapy is quite complex, with considerable variability in the cost and response to various medications. Likewise, many pharmacogenetic studies are often too small and the results of which can be inconsistent and misleading. The effect sizes are mild to modest, so there is limited clinical predictive value. However, there are some problems inherent to the field of pharmacogenetics. For this field to evolve further in RA, studies employing large genetically diverse cohorts, a standardized study design, and multiple genetic variants in concert, will be a prerequisite to accurately assess the impact of genetic variants on drug efficacy and/or toxicity. *A priori* knowledge of pharmacogenetic mechanisms associated with RA pharmacotherapy has to potential to identify and therefore stratify patients into clinically important treatment categories (ie, responders vs. nonresponders; tolerant vs intolerant). The incorporation of pharmacogenetics into clinical rheumatology practice (ie, personalized medicine) can certainly be a major

advance in RA pharmacotherapy and a clear enhancement of patient care. However, for this to occur many more well executed and adequately powered studies are required.

It would be remiss to not briefly comment on the influence of epigenetic factors on RA pathogenesis. It has become increasingly evident that the influence of epigenetic processes on the development of rheumatic diseases is probably as strong as the genetic background of a patient. The combinatory nature of these processes forms a complex network of epigenetic modifications with the ability to regulate gene expression through activation or silencing of genes. Indeed, environmental triggers are involved in the development of RA as age, infections, smoking, nutrition, and pollution have been suggested to have an effect on the epigenetic background. Genome-wide analyses of the epigenome (eg, DNA methylation, histone modifications) will enable the detection of additional genes involved in the pathogenesis of rheumatoid arthritis. In the future, knowledge of the epigenetic processes combined with enhanced genetic information will be essential for the understanding of the differences seen in the clinical picture of patients with rheumatic diseases such as RA.

Disclosures

The authors report no conflicts of interest in this work.

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