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REVIEW

Human leukocyte antigen polymorphisms and personalized medicine for rheumatoid arthritis

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Human leukocyte antigen (*HLA*) polymorphisms are the most important genetic risk factors for rheumatoid arthritis (RA), a chronic systemic inflammatory disease of unknown etiology. Certain *HLA-DRB1* alleles, known as shared epitope (SE) alleles because they have the same amino-acid sequence at positions 70–74, are associated with susceptibility to RA. A gene dosage effect is present for RA-predisposing SE alleles, and protective alleles show epistasis. An important role of amino-acid polymorphisms at positions 11 and 13 of the HLA-DRβ chain was also reported recently. Rheumatoid factor and anticitrullinated peptide antibodies are present in many RA patients. Similar to extra-articular manifestations, the presence of these autoantibodies is also associated with certain *DRB1* alleles. Different frequencies of RA risk alleles in different ethnicities explain the varying prevalence of RA in different populations and suggest genetic heterogeneity of RA with regard to phenotype and population subsets. Some drug-induced hypersensitivity reactions due to disease-modifying antirheumatic drugs are also associated with *HLA* alleles. Understanding the role of *HLA* as the most important genetic factor relevant to RA susceptibility may help in determining its pathogenesis and pave the way to personalized medicine.

INTRODUCTION

The human major histocompatibility complex is a region of ~ 3.6 Mbp on chromosome 6p21.31, comprising > 200 genetic loci including the human leukocyte antigens (*HLA*).[1] The main gene products of this region that are relevant to immunity are designated class I molecules (HLA-A, C and B) and class II molecules (HLA-DR, DQ and DP). The former consists of a type I transmembrane protein of 45 kD (the heavy chain) and a soluble protein of 12 kD (β2-microglobulin, the light chain). They bind and present intracellular antigens to the T-cell receptors of CD8$^+$ T cells. In contrast, the latter consists of two type I transmembrane proteins (the 32 kD α chain and 28 kD β chain) and present both intra- and extracellular antigens to the T-cell receptors of CD4$^+$ T cells. The *HLA-A, C* and *B* genes encode HLA-A, C and B heavy chains and the *B2M* gene, located elsewhere (on chromosome 15) encodes the β2-microglobulin light chain. The *DRA1, DRB1* genes (and additionally *DRB3, DRB4* and *DRB5* genes in some haplotypes), and the *DQA1, DQB1, DPA1* and *DPB1* genes encode the α and β chains of HLA-DR, DQ and DP molecules, respectively. *HLA* is hyperpolymorphic and > 12 000 distinct alleles of these genes have been identified (IMGT/HLA data base, http://www.ebi.ac.uk/ipd/imgt/hla/). Studies of the frequencies of HLA alleles have revealed genetic associations with > 100 diseases. Most of the different genes of the major histocompatibility complex are in strong linkage disequilibrium, making it difficult to identify the primary causative gene.

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of unknown etiology. It mainly affects synovial joints, reducing quality of life and life expectancy. The development of extra-articular manifestations, including serositis, Felty's syndrome, rheumatoid vasculitis, lymphoproliferative disease and interstitial lung disease (ILD), is frequent complications. RA is thought to represent a set of clinical syndromes comprising different disease entities with specific features. The pathogenesis of RA is likewise considered to be multi-factorial, with disease susceptibility associated with genetic, environmental and stochastic factors.[2] The prevalence of RA is 0.5–1.0% in most populations. The concordance rate in monozygotic twins is 12–30% and the sibling recurrence rate for RA lies between 5 and 10%. The heritability of RA is estimated to be 65%.[3–5] The association of RA with *HLA* was already reported ~ 40 years ago.[6] To date, more than 100 risk loci for RA have been identified in large-scale studies including genome-wide association studies.[7] All of the known genetic risk factors together were estimated to explain 16% of RA risk, 11% of which was due to *HLA* alone.[4,8] The remaining 49% of genetic predisposition to RA represents missing heritability. Thus, *HLA* is the most important known genetic risk factor for RA.

HLA AND RA

Many studies have reported that polymorphisms at the *HLA-DRB1* locus are associated with several different autoimmune diseases including RA. The following *HLA-DRB1* alleles are associated with

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RA in most ethnic groups: DRB1*04:01, *04:04, *04:05, *01:01 and *10:01. In Europeans DRB1*04:01 and in Eastern Asians *04:05 are most strongly associated with RA. Some other DRB1 alleles are also weakly associated with RA susceptibility. Almost all of these share amino-acid sequences at positions 70–74 (QKRAA, RRRAA or QRRAA) in the third hypervariable region of the HLA-DRβ chain. These are designated shared epitope (SE) alleles.[9] DRB1*09:01 is not a SE allele, possessing RRRAE at position 70–74, but is nonetheless also associated with RA in some populations, especially in East Asians.[10–13] A different classification criterion for DRB1 alleles has been established, also based on amino-acid residues at positions 70–74, as follows: S1 (XA/ERAA), S2 (XKRAA), S3P (Q/RRRAA), S3D (DRRAA) and X (without XXRAA).[14,15] As S2 and S3P confer higher susceptibility for RA, S1, S3D and X can be pooled as 'L alleles'. These amino-acid residues form the peptide position 4 binding pocket of the DR molecule. Thus, polymorphisms of these amino-acid residues may change the repertoire of the presented peptides.[16]

DRB1 is considered to be the primary gene for RA susceptibility. DRB1 and DQB1 are in strong linkage disequilibrium. This makes it difficult to exclude the hypothesis that the DQB1 allele is primarily responsible for the association with RA.[17] Certain combinations of DRB1 alleles confer higher risk, such as heterozygosity for *04:01/ *04:04 or *04:05/*09:01.[13,18] Such a heterozygote disadvantage was also reported in type 1 diabetes and systemic lupus erythematosus.[19,20] These data cannot exclude the involvement of trans-complementing heterodimers formed by DQA1 and DQB1 genes.

A gene dosage effect has been noted for the associations of SE alleles with susceptibility to RA. Thus, having two SE alleles confers a higher risk for RA and for more severe bone destruction. Because the molecular mechanisms responsible for the gene dosage effect are not clear, a cusp hypothesis was developed to explain it, as follows. SE molecules act as allele-specific signal transduction ligands that interact with non-HLA cell surface receptors, including cell surface calreticulin, for production of nitric oxide and reactive oxygen species for cell signaling.[21]

DRB1*13:01 and *13:02 are negatively associated with RA in European and East Asian populations, respectively.[22,23] The resistant effect of these DRB1*13 alleles is dominant over the susceptible effect of SE alleles in DRB1 heterozygotes. The protective DRB1 alleles are thought to be recognized by autoreactive T-cell receptors with higher affinity than predisposing DRB1 alleles. As a result, negative selection and development of DR-driven autoreactive regulatory T cells are promoted.[24] An effect of noninherited maternal antigen was also reported as protective against RA. Thus, the protective DRB1 allele that was not inherited from the mother decreases RA susceptibility risk in her child.[25] This phenomenon could be explained by the micro-chimerism of maternal cells that enter the circulation of the child during gestation or birth.

Recent analyses of the association of polymorphisms of amino-acid residues with RA showed important influences of positions 11 and 13 of the HLA-DRβ chain, followed by positions 71 and 74.[26] The amino-acid residues at positions 11 and 13 of HLA-DRβ form the binding pocket for peptide position 6 of DR molecules.[16] It can be concluded that of the many hypotheses proposed to explain the association of HLA-DRB1 with RA; some seem tentative, others credible, but none unequivocal.

HLA AND SUBSETS OF RA
Rheumatoid factor (RF) is composed of autoantibodies specific for denatured IgG-Fc domains and is present in ~ 80% of RA patients, having some roles in the formation of immune complexes or cryoglobulin, which cause some types of RA symptoms. RF has long been used as a biomarker for RA in daily clinical practice, but is often detected in aged individuals without RA. An association of SE with RF-positive RA has been established; however, only a weak association with RF-negative RA has been established.[27] A recent study showed that DR molecules form complexes with intracellular mis-folded proteins without processing and are transferred to the cell surface. The IgG heavy chain was similarly presented by DR molecules with different allele-specific binding affinity,[28] explaining the mechanism by which RF is generated and the important role of SE alleles.

Anticitrullinated peptide antibodies (ACPA) are present in 70–80% of RA patients. Although RF and ACPA are frequently found in the same patient, the specificity of ACPA for diagnosing RA is higher than RF. The presence of ACPA is considered a predictive marker of bone destruction; however, it is still controversial as to whether ACPA can also predict the response to treatment with biologic or nonbiologic disease-modifying antirheumatic drugs.[29–32] Because of their high specificity, ACPA are believed to have some role in the pathogenesis of RA. SE alleles are associated with ACPA-positive RA, but only relatively weakly with ACPA-negative RA.[4,23,27,33,34] Other than SE alleles, DRB1*03 and DRB1*14:54, are associated with ACPA-negative RA in European and Asian populations, respectively.[23,34,35] The differential contribution of HLA was also confirmed in a genome-wide association study between ACPA-positive and -negative RA susceptibility.[36,37] An analysis of the association of polymorphisms of amino-acid residues with ACPA-positive and -negative RA also showed different specificities,[38] suggesting the distinct disease category of ACPA-positive and -negative RA. Gene–environment interactions were detected between SE alleles and smoking for susceptibility to ACPA-positive RA.[39–41] Citrullinated peptides were found in the lung of individuals with a smoking habit, and are believed to be generated by smoking. The citrullinated peptides are thought to be autoantigens presented by SE alleles in the pathogenesis of RA.[42]

Autoantibodies to Ro/SS-A and La/SS-B, components of ribonucleoprotein complexes, are specifically present in RA patients with secondary Sjögren's syndrome that affects salivary and lacrimal glands, causing dry mouth or dry eye. Anti-Ro/SS-A antibodies are present in ~ 20% of RA patients and anti-La/SS-B antibodies in ~ 5%. Anti-Ro/SS-A antibodies are detected in almost all RA patients with anti-La/SS-B antibodies. The DRB1*08:03-DQB1*06:01-DPB1*05:01 haplotype is strongly associated with anti-Ro/SS-A antibodies in Japanese RA patients, whereas DRB1*15:01-DQB1*06:02-DPB1*05:01 is associated with anti-La/SS-B, suggesting the primary role of DPB1*05:01 for the production of autoantibodies to ribonucleoprotein.[43]

Extra-articular manifestations are frequent complications of RA and influence its prognosis. Associations of extra-articular manifestations with the SE or DRB1*04 alleles have been reported.[44–47] However, protective effects of SE and the predispositional effects of the DR2 serological group (DRB1*15 and *16) for ILD are seen in the Japanese RA population,[48–50] although a weak protective effect of SE for ILD in RA has also been reported.[48,50,51] Finally, DR4 is also associated with airway disease in RA.[52]

It was well known that the development of lymphoproliferative disorders is frequent in RA patients, although the incidence of some other malignancies in RA is relatively lower.[53] A tendency toward a higher frequency of DQB1*06:01 was noted in RA patients with lymphoproliferative disorders (Table 1, Furukawa H et al, unpublished results), although the effect was not statistically significant. When the analysis was restricted to patients treated with methotrexate, the frequency of HLA-B*15:11 was significantly higher.[54] Thus, genetic

Table 1 *HLA-DQB1* allele carrier frequency in RA patients with RA-LPD

	RA-LPD(+)RA (n = 14)	RA-LPD(−)RA (n = 1520)	P	OR	Pc	95% CI
*02:01	0 (0.0)	13 (0.9)	1.0000	3.85	NS	
*03:01	2 (14.3)	315 (20.7)	0.7471	0.64	NS	
*03:02	1 (7.1)	162 (10.7)	1.0000	0.64	NS	
*03:03	4 (28.6)	411 (27.0)	1.0000	1.08	NS	
*03:06	0 (0.0)	4 (0.3)	1.0000	11.62	NS	
*04:01	6 (42.9)	766 (50.4)	0.6034	0.74	NS	
*04:02	1 (7.1)	89 (5.9)	0.5727	1.24	NS	
*05:01	4 (28.6)	244 (16.1)	0.2618	2.09	NS	
*05:02	0 (0.0)	62 (4.1)	1.0000	0.80	NS	
*05:03	0 (0.0)	74 (4.9)	1.0000	0.67	NS	
*06:01	8 (57.1)	354 (23.3)	0.0069	4.39	0.1033	(1.51–12.74)
*06:02	0 (0.0)	187 (12.3)	0.3990	0.25	NS	
*06:03	0 (0.0)	2 (0.1)	1.0000	20.94	NS	
*06:04	1 (7.1)	105 (6.9)	1.0000	1.04	NS	
*06:09	0 (0.0)	7 (0.5)	1.0000	6.96	NS	

Abbreviations: CI, confidence interval; NS, not significant; OR, odds ratio; Pc, corrected P-value; RA, rheumatoid arthritis; RA-LPD, RA-associated lymphoproliferative disorder.
Associations were tested by Fisher's exact test using 2×2 contingency tables under the dominant model.

association studies of RA subsets with *HLA* support the notion that RA is a phenotypically heterogeneous syndrome.

The prevalence of RA is different in different ethnic populations, at ~ 1% in Europeans, ~ 0.5% in Asian or African populations, but >2% in some populations of Amerindians.[55,56] The main SE alleles are also differently associated with RA in the different populations. Thus, *DRB1*04:01* and *04:05* are most strongly associated with RA in European and East Asian populations, respectively. In addition, the effect size of SE alleles on associations with RA is weaker in some populations.[57–59] Because the frequency of *DRB1*09:01* is low in European populations, an association of this allele with RA could not be readily observed. An association of *DRB1*03* and *DRB1*13:01* with ACPA-negative RA is seen in European populations,[35,60] whereas it is *DRB1*04:05* and *14:54* that are associated with ACPA-negative RA in Japanese.[23,34] The differences in prevalence of RA among populations could be explained by the different frequencies of RA risk alleles, although the effects of environmental factors cannot be excluded.

Analyses of HLA associations with RA in Asian populations, mainly Chinese and Korean, revealed amino-acid residues, different from those of Europeans, conferring disease susceptibility.[61] The involvement of amino-acid position 57 is noted in Chinese and Korean RA, instead of position 71. Similar amino-acid residues are associated with RA in the Japanese population (Oka *et al.*, unpublished results). The genetic risk of possessing *DRB1* in African Americans is explained by amino-acid positions 11 and 57 of the DRβ chain.[62] The different frequencies of RA risk alleles in each population reflect these different amino-acid residues conferring risk. These data indicate that distinct disease entities observed in each ethnic population reflect the genetic heterogeneity of RA.

HLA AND DRUG-INDUCED HYPERSENSITIVITY REACTIONS IN RA

A strong association between certain *HLA* alleles and the occurrence of adverse cutaneous reactions has been reported, including Stevens–Johnson syndrome and toxic epidermal necrolysis. These patients suffer from blisters and erosions on the skin and mucous membranes; distinguishing between the two syndromes is based on the affected body surface area. Stevens–Johnson syndrome and toxic epidermal necrolysis are known to be induced by several specific drugs, with striking associations with *HLA* alleles. In this regard, allopurinol effects are associated with *B*58:01*,[63] abacavir with *B*57:01*,[64,65] carbamazepine with *B*15:02* in Chinese but with *A*31:01* in Japanese and Europeans[66–68] and methazolamide with *B*59:01* in Japanese.[69] Associations with *HLA* alleles were also confirmed in other drug-induced hypersensitivity reactions. Agranulocytosis induced by methimazole is associated with *DRB1*08:03*.[70] Cholestatic hepatotoxicity induced by ticlopidine and tiopronin is associated with *A*33:03*.[71,72] Liver injury induced by flucloxacillin is associated with *B*57:01*, and by amoxicillin–clavulanate with *DRB1*15:01*.[73,74] Myopathy induced by statins is associated with *DRB1*11:01*.[75] Some of these associations are useful for predicting and preventing drug-induced hypersensitivity reactions by *HLA* typing before starting the medication. The mechanisms responsible for these drug hypersensitivity reactions so strongly associated with *HLA* remain unclear. Several possibilities can be considered. Acting as haptens, drugs or their metabolites may bind to peptides presented by HLA molecules and activate T-cell receptors. Alternatively, drugs may bridge between HLA molecules and T-cell receptors, independently of the peptide presented on HLA, and thus activate T cells.[76] They may bind to the grooves of specific HLA alleles and alter the repertoire of presented peptides.[77]

Disease-modifying antirheumatic drugs have been used for the treatment of RA for many years. Some nonbiologic disease-modifying antirheumatic drugs often cause hypersensitivity reactions. For example, Stevens–Johnson syndrome, toxic epidermal necrolysis and drug-induced hypersensitivity syndrome are induced by sulfasalazine.[78–81] D-penicillamine, gold salts and bucillamine cause proteinuria due to membranous nephropathy.[82–85] Drug-induced ILD can be triggered by gold salts, methotrexate, leflunomide and tacrolimus.[86–89] The Japanese are believed to be more susceptible to drug-induced ILD than other ethnic groups, suggesting the involvement of genetic factors in its pathogenesis. Indeed, an association of *HLA-A*31:01* with drug-induced ILD in methotrexate-treated Japanese RA patients has been reported.[90] Proteinuria induced by D-penicillamine and gold salts is associated with DR3 in Europeans[91] and bucillamine-induced proteinuria with *DRB1*08:02* in Japanese.[92] Further studies are likely to reveal associations with *HLA* alleles of other drug-induced hypersensitivity reactions caused by disease-modifying antirheumatic drugs.

Secondary Sjögren's syndrome or the presence of anti-Ro/SS-A antibodies are thought to be predictive markers for the development of drug-induced hypersensitivity reactions in RA patients.[93,94] The association of DR3 with RA patients possessing anti-Ro/SS-A antibodies[95] can explain why this specificity is associated with drug-induced proteinuria in Europeans.[91,96] However, an association of *DRB1*08:03* with anti-Ro/SS-A antibodies[43] cannot explain the correlation because it is the *DRB1*08:02* allele that is associated with bucillamine-induced proteinuria in Japanese RA.[92] Thus, genetic association studies on *HLA* could provide surrogate markers for drug-induced hypersensitivity reactions in some cases, but can also provide essential individual genetic markers useful for guiding RA treatment. These observations imply that *HLA* may have a substantial role in drug-induced hypersensitivity reactions in RA patients and provide important information for developing personalized medicine for RA.

CONCLUSION

Remarkable recent technical progress in genotyping methods has yielded major advances in our knowledge of the complex genetics of RA susceptibility. Despite the fact that *HLA* remains the most important genetic factor for the pathogenesis of RA, explanations for a large part of RA heritability are still lacking. Alternative assessments of *HLA* risk alleles may not be universally applicable to RA because of disease heterogeneity and ethnic diversity in its manifestations. These also make it difficult to predict the genetic risk for RA, its severity and response to treatment with disease-modifying antirheumatic drugs with sufficient accuracy. However, predictability of drug-induced hypersensitivity reactions is acceptably specific. To go beyond the status quo of disease association studies, the actual genetic and immunological mechanisms responsible for the heterogeneous manifestations of RA in the context of SE alleles still need to be clarified. To paraphrase Sir Arthur Conan Doyle, the words 'of murder' could be substituted by 'between *HLA* and RA' in the following sentence: 'There's the scarlet thread of murder running through the colourless skein of life, and our duty is to unravel it, and isolate it, and expose every inch of it.'[97]

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