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Genetics, environment, and gene-environment interactions in the development of systemic rheumatic diseases

Jeffrey A. Sparks, MD, MMSc¹ and Karen H. Costenbader, MD, MPH¹

¹Department of Medicine, Division of Rheumatology, Immunology, and Allergy, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

Abstract

Understanding disease susceptibility factors and gene-environment interactions may offer valuable insights into the biological mechanisms for the etiology of rheumatic diseases. Defining the contributions of genetic and environmental factors to the pathogenesis of rheumatic diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and ankylosing spondylitis (AS), may have important implications for understanding risk prediction, pathogenic mechanisms, cellular pathways, drug discovery, and prevention strategies. However, rheumatic diseases offer distinct challenges to researchers due to heterogeneity in disease phenotypes, low disease incidence, and geographic variation in both genetic and environmental factors. Emerging research areas, including epigenetics, metabolomics, and the microbiome, may provide additional links between genetic and environmental risk factors in rheumatic disease pathogenesis. This article reviews the methods used to establish genetic and environmental risk factors and to study gene-environment interactions in rheumatic diseases and provides specific examples of successes and challenges for identifying gene-environment interactions in RA, SLE, and AS. Finally, we describe how emerging research strategies may build upon previous discoveries as well as future challenges.

Keywords

rheumatoid arthritis; systemic lupus erythematosus; ankylosing spondylitis; RA; SLE; AS; environment; genetics; interaction; smoking

INTRODUCTION

The current paradigm for autoimmune rheumatic disease etiology is that several pre-clinical stages precede the onset of clinically apparent disease. When individuals at increased genetic risk are exposed to environmental or lifestyle factors, early alterations in the immune

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Correspondence: Jeffrey A. Sparks, MD, 75 Francis St., Boston, MA 02114, jasparks@partners.org, Phone: (617) 732-5325, Fax: (617) 732-5766.

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system and the breakdown of self-tolerance ensue, eventually leading to the presentation of overt disease (Figure 1).^{1, 2} Indeed, several genetic and environmental risk factors have been strongly associated with the risk of incident rheumatic diseases, and many more are weakly associated or hypothesized to be related.^{3, 4} However, the pathogenesis and biological mechanisms for the development of autoimmune rheumatic diseases remain poorly understood.

Interactions between genetic and environmental factors may elucidate biological mechanisms for rheumatic disease susceptibility and bridge findings in several fields of research. Greater understanding of rheumatic disease etiology may provide important insights into prevention, screening, and treatment options. Therefore, rheumatic disease researchers are motivated to explore the intersection of genetic and environmental risk factors. However, rheumatic diseases present distinct challenges for the identification of gene-environment interactions (GEIs). These challenges include heterogeneous phenotypes, low disease incidence and prevalence, geographic variation in epidemiology, and the difficulty in identifying individuals at elevated disease risk prior to clinical diagnosis.

This article will serve as an overview to contextualize genetic and environmental risk factors in the development of rheumatic diseases and to highlight future research directions according to study designs and molecular approaches. We will address specific successes and challenges concerning rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and ankylosing spondylitis (AS).

Current research strategies for environmental and genetic risk factors

As in other areas of research, bias, confounding, chance, and generalizability are major threats to validity that must be considered in study designs and analyses investigating genetic and environmental risk factors (Table 1). Investigators studying environmental and lifestyle risk factors for rheumatic disease risk have utilized primarily traditional epidemiologic techniques: the case-control and prospective cohort study designs.

Case-control studies—identify incident disease cases and match them to controls. Advantages of case-control studies are relative efficiency, especially when cases are rare and difficult to identify prospectively. Disadvantages are inherent biases based on the retrospective nature of these studies—selection and recall bias. In particular, the selection of inappropriate controls may introduce biases that lead to erroneous conclusions. Pre-clinical disease changes might also truly influence factors, such as dietary intake, physical activity, or weight changes, introducing the potential for reverse causation. Important environmental exposures may occur early in life and may be difficult to assess. In addition, when genes are associated with established disease, it is unclear whether the effect of these genes is in disease etiology or in disease propagation. Given these nuances, multiple case-control studies in diverse populations are usually warranted to establish an association between an environmental factor and disease susceptibility. Population-based case-control studies, where inclusion is compulsory as in national registries, may overcome some of these challenges.

Cohort studies—offer solutions to some of the biases inherent in case-control studies. This study design follows populations prospectively based on a common exposure or demographic factor before individuals might develop incident disease. The major advantage of cohort studies is that data are collected without bias for all individuals prior to disease onset. Design issues such as selection or recall bias are therefore less problematic, though subtle subclinical manifestations might still introduce potential for reverse causation.⁵ Inclusion criteria, however, might affect generalizability. Nested case-control studies on stored samples performed within cohort studies, can evaluate biomarkers in the pre-clinical period. Confirmation of incident cases is often a limiting factor in cohort studies, since most rheumatic diseases have low incidence and require either extremely large cohorts, long follow-up, or specific populations at increased risk, such as first-degree relatives or those with pre-clinical symptoms (e.g., arthralgias). Meta-analyses of multiple large cohorts may overcome some of these limitations, however heterogeneity in study designs and populations may also be limiting. These different cohort designs (general population, unaffected relative, and symptomatic but non-classifiable individuals) may correspond to particular phases in the development of autoimmune diseases (Figure 1).^{1, 6}

Early studies seeking to identify genetic risk factors often focused on heritability, usually in the context of familial disease. Heritability was estimated evaluating rates of disease within homozygote and heterozygote twins. Linkage analyses utilized entire families, with affected and unaffected family members. These methods were able to establish the familial heritability of a disease, but shared environmental factors might still contribute to this heritability. Linkage analyses were most useful in identifying highly penetrant genes with large effect sizes. In addition, candidate gene studies genotyped a few loci at a time, a slow process focusing on known cellular pathway mechanisms.⁷

Genome Wide Association Studies (GWAS)—The advent of high-throughput genotyping brought about the era of GWAS, which evaluates initially thousands (and more recently hundreds of thousands) of different genetic loci at once for association with disease.⁸ This hypothesis-free method is capable of evaluating the entire genome for potential disease susceptibility genes.⁹ However, GWAS are dependent upon the performance and composition of specific platforms of single nucleotide polymorphisms (SNPs) across the genome, which might vary widely based on race, ethnicity, and geography. Potentially associated loci may not necessarily be included on each platform and therefore would not be tested. The ability of GWAS to detect true associations is dependent upon a homogeneous disease phenotype with similar disease pathogenesis. Appropriate control selection, with similar race and genetic structure is therefore important. For example, most early GWAS in RA were performed for seropositive RA cases among whites with European heritage.^{10–12} Findings from these studies may not be generalizable to seronegative RA or non-white European populations. This concept presents major challenges for diseases such as SLE with heterogeneous subtypes and variation by race, ethnicity, and geography. The genetic components of rheumatic diseases might also be more pronounced in early-onset compared to older-onset disease, in which environmental or age-related factors may be more important.^{13, 14}

GWAS studies usually offer only statistical associations with probabilities of disease development. Disease susceptibility loci detected through this method are not necessarily causal for rheumatic diseases, but may be proxies for causal loci due to linkage disequilibrium. GWAS is most successful at discovering common variants, as opposed to rare variants. The latter, however, often have high effect sizes, and therefore may provide important pathogenic information compared to common variants with lower effect sizes. For example, single mutations of genes involved in the complement cascade (C1q, C2, and C4) occur rarely but are very strongly associated with increased risk of SLE.¹⁵ Methodological issues such as genomic inflation, population stratification, false discovery rates, linkage disequilibria, and multiple comparisons are major design and computational obstacles for GWAS, requiring advanced statistical expertise.¹⁶ Despite these caveats, GWAS have rapidly and efficiently detected numerous rheumatic disease susceptibility loci.¹⁷ To date, however, findings from GWAS for rheumatic diseases have had little clinical impact as significant SNPs are may not be causal and have low effect size estimates, and thus offer little ability to classify individuals according to risk for disease development.

Genetic risk scores (GRS)—have been developed to combine many validated genetic loci into a single summary variable, improving statistical power to detect potential associations.¹⁸ GRS can be calculated simply by counts of risk alleles. However, if effect sizes of individual genetic components vary appreciably, weighted genetic risk scores (wGRS) should be utilized.¹⁹ Typically, wGRS are weighted using the natural logarithm of odds ratios found in large GWAS or meta-analyses.¹⁹ Since *HLA* loci are often more strongly associated with rheumatic disease risk compared to non-*HLA* SNPs, wGRS have been used in rheumatic disease research.^{14, 19–22}

Gene-environment interactions

The rapid emergence of genetic susceptibility loci and the identification and replication of specific environmental risk factors provide the opportunity to evaluate specific GEIs that may offer new biological mechanisms in disease pathogenesis as well as potentially provide personalized medicine approaches where an individual's risk for disease can be calculated using a combination of genetic and environmental factors.²³ However, study design, statistical power and expertise are major challenges in the identification of potential GEIs.²⁴ Since GWAS require very large sample sizes to detect associations of genetic factors with disease, often with modest effect sizes, studies with statistical power to detect GEIs may not be feasible using current techniques. While GEIs may suggest biological mechanisms for disease development, it is not clear that they will be able to provide robust predictive abilities for rheumatic diseases, at least in the foreseeable future.^{25, 26}

GEIs can be identified statistically through additive or multiplicative interactions (Table 2). An additive interaction is an effect beyond the sum of the risks associated with individual factors and can be measured by the relative excess risk due to interaction (RERI), attributable proportion due to interaction (AP), or synergy index (S).²⁷ A multiplicative interaction is an effect greater than the multiplied effects of the individual factors and is measured by the ratio of odds ratios (ROR).²⁷ The interpretation and suitability of interaction terms depend on the scale of the statistical model utilized. Logistic regression

models are on a multiplicative scale, while linear regression models are on an additive scale. Therefore, in a logistic regression model (e.g., with an outcome of development of disease or not), a statistically significant interaction term implies a multiplicative interaction between the two factors. Additive interactions have been thought to represent biologic interaction of two factors within the same pathway.²⁴ However, a statistical interaction does not necessarily imply a biological interaction. In studies of disease pathogenesis, a statistically significant additive GEI should be ideally replicated in independent studies and animal models in order to validate biological plausibility. GEIs usually have statistically significant main effects for both genetic and environmental factors in predicting disease onset, though this may not be a requirement if factors work exclusively in synergy.

Recent advancements in fine mapping of genetic regions of interest and high throughput next-generation sequencing will result in yet more genetic loci associated with the risk of rheumatic diseases, so these issues are timely. We now provide specific examples of genetic factors, environment factors, and GEIs in RA, SLE, and AS susceptibility.

Rheumatoid arthritis

The study of genetic and environmental risk factors for RA is the most developed among the rheumatic diseases, owing to a relatively homogeneous disease phenotype and high disease prevalence.²⁸ Despite these advantages, differences in the risk factor assessment for RA are appreciated based on RA serologic status.²⁹ Early studies of RA risk factors were stratified by the presence or absence of rheumatoid factor (RF). However, RF positivity may be seen in other diseases or as a consequence of aging.³⁰ Recent studies have utilized anti-citrullinated peptide antibody (ACPA) to classify RA, which is more specific for RA so has less potential for misclassification.³¹ Despite validated classification criteria for RA, atypical presentations of other systemic rheumatic diseases have the potential to be misclassified as seronegative RA.^{32, 33}

RA genetic risk factors: The shared epitope and beyond—Genetic variants in the major histocompatibility complex (MHC) region on chromosome 6 were identified to be potentially associated with RA susceptibility and were deemed the “shared epitope.”³⁴ Polymorphisms in three human leukocyte antigen (HLA) genes (*HLA-DRB1*, *HLA-DPB1*, *HLA-B*) in the MHC region are highly associated with RA.³⁵ Polymorphisms in *HLA-DRB1*, in particular the classical shared epitope genotypes of *0401 and *0404, are strongly associated with RA (odds ratios of approximately 4 for risk variants).³⁶ Specific amino acid positions (11, 71 and 74) that correspond to the *HLA-DRB1* shared epitope classical genotypes are located in the peptide-binding groove of the protein HLA-DRβ1, offering a potential biological mechanism for this potent RA genetic risk factor.³⁵

Large genetic consortia have identified many non-*HLA* SNPs associated with RA using GWAS.^{4, 11, 37, 38} Most recently, a large trans-ethnic GWAS associated 101 genetic loci with RA across European and Asian populations.⁴ However, most of the non-*HLA* SNPs are only modestly associated with RA.³⁷ An exception is a SNP near the gene *PTPN22* (encoding a tyrosine-phosphatase protein expressed in lymphoid tissue), which is strongly

associated with RA (odds ratio 1.78).³⁷ SNPs near the genes *TNFAIP3* and *TYK2* also have relatively large effect sizes for RA risk.¹⁷

Most early genetic studies focused on seropositive RA among Caucasian or Japanese populations.⁴ In a recent large genetic consortium study, Han and colleagues made special efforts to correctly classify ACPA-positive and ACPA-negative RA cases by using a highly specific ACPA assay.³⁹ This study identified new genetic risk factors associated with ACPA-negative RA: serine or leucine at amino acid position 11 in *HLA-DRB1* and aspartate in position 9 in *HLA-B*.³⁹ These findings provide further evidence that ACPA-positive and ACPA-negative RA are genetically distinct and thus may have separate pathogeneses.^{4, 17, 39, 40}

Despite the significance of the *HLA* shared epitope to RA risk and the growing number of non-*HLA* SNPs associated with RA, most genetic heritability remains unexplained. The *HLA* shared epitope explains only 12% of the genetic variance for RA, while only about 4% is explained by all other non-*HLA* SNPs.³⁷ Gene-gene interactions for RA may also be present, in particular an association between the *HLA* shared epitope and *PTPN22*, specifically based on presence of the R620W allele, for the development of seropositive RA.^{41, 42} Studies among familial RA and twins suggest that environmental factors have an important role in the development of RA and RA-related antibodies, so GEIs may be important to link these discoveries.^{20, 43}

RA environmental risk factors: Cigarette smoking and more—Strong epidemiologic evidence supports cigarette smoking as an environmental risk factor for RA development. Multiple studies have shown an increased RA risk with smoking among men and women, in varied populations.^{44–53} Furthermore, there is a dose-dependent effect between smoking pack-years and RA development.^{46, 54} After twenty years of smoking cessation, RA risk of former smokers returns to the RA risk of the general population.⁴⁶ Murine models evaluating cigarette smoke exposure have induced inflammatory arthritis, providing further biological evidence.⁵⁵ This association is particularly strong for the development of seropositive RA.⁴⁴ Smoking may contribute up to 25% of RA risk, and up to 35% of risk for ACPA-positive RA.⁵⁶ However, studies of smoking and seronegative RA have revealed less powerful associations.^{44, 47}

Other environmental factors have been associated with the development of RA. It is beyond the scope of this article to review these factors extensively, but they include occupational exposures such as silica, reproductive factors in women, and excess body mass.^{57–59} Dietary factors that might be protective for RA include intake of alcohol and fish.^{60–65}

Given the knowledge of a period of preclinical RA autoimmunity where autoantibodies are present in the absence of clinically apparent joint inflammation, there is growing interest that the induction of RA may occur at extra-articular sites. The induction of autoimmunity in RA may specifically occur at mucosal surfaces in the respiratory tract, mouth, or gut.⁶⁶ Mucosal involvement in the lung has been posited as a possible site for RA initiation due to the increased risk seen in both smoking and particulate silica exposure.^{44, 62} Citrullination of peptides occurs in the lung due to inflammation, and this is most pronounced in

smokers.^{66, 67} Interstitial lung disease is a well known non-articular manifestation of RA and may occur even without articular involvement. Periodontitis has also been associated with both prevalent and incident RA, although smoking and secondary Sjögren's syndrome may confound these associations.^{68, 69} *Porphyromonas gingivalis*, a causative bacterium for periodontitis, expresses the enzyme peptidylarginine deaminase, which can citrullinate both enolase and fibrinogen, self-antigens thought to be involved in the joint-specificity of RA.⁷⁰ A study composed of RA first-degree relatives reported higher concentrations of *P. gingivalis* antibodies in RA relatives who had developed RA-related antibodies, arguing that pre-clinical infection, or its immune response, may be important in the development of altered immunity in RA.⁷¹

RA gene-environment interaction: *HLA-DRB1*-cigarette smoking interaction—

The strong association of smoking with RA risk and evidence for induction of autoimmunity and citrullination in mucosa led to a hypothesized biological mechanism for the role of smoking in the development of RA. Studies initially performed by Klareskog and colleagues demonstrated a strongly significant multiplicative GEI between cigarette smoking and *HLA-DRB1* in determining RA risk.^{42, 72–76} This association was strongest for seropositive RA and has been replicated in several populations, including US women, and Swedish, and Malaysian populations.^{72–74} The presence of heavy smoking and two *HLA-DRB1* genes increased the odds for RA by 23-fold compared to those with neither risk factor.^{42, 73}

Transgenic mouse models have demonstrated a strong immune response between *HLA-DRβ1* and citrullinated self-antigens implicated in smoking, offering more evidence of biologic plausibility for this GEI.⁷⁷ Smoking in particular, but other inflammatory exposures as well, are now thought to cause citrullination of a large array of peptides. The specific responses to these citrullinated peptides are likely genetically determined, generating a diversity of ACPAs to different citrullinated antigens, which arise prior to clinical disease onset and have varying pathogenicity. The *HLA*-smoking interaction may contribute to citrullination processes and autoimmunity and might explain the seroconversion noted in pre-clinical RA (see Figure 2).^{78, 79} This GEI seems to apply only to seropositive RA, and possibly in particular to those with antibodies to citrullinated α-enolase and vimentin.⁸⁰ Other genes (*GSTT1* and *HMOX1*) in which polymorphisms might interfere with the metabolism of cigarette smoke are hypothesized to also enhance RA susceptibility.⁸¹

Although *HLA*-smoking GEIs have led to robust findings about the pathogenesis of ACPA-positive RA among those with both risk factors, they confer little ability to discriminate between RA cases and controls in risk modeling, emphasizing the need for continued research of other biological pathways involved in RA pathogenesis.^{20, 21}

Systemic lupus erythematosus

Due to its elevated morbidity and mortality, there is interest in identifying risk factors for the development of SLE.⁸² SLE has a heterogeneous clinical phenotype, ranging from mild mucocutaneous or musculoskeletal involvement to severe, life-threatening neurologic or renal manifestations.⁸³ The American College of Rheumatology (ACR) classification criteria for SLE considers these varied manifestations as a single disease entity.^{84, 85} This

presents methodological challenges as different SLE subtypes may have separate pathogeneses and therefore different genetic and environmental risk factors. This further partitions an already rare disease, decreasing the statistical power of even large studies with long follow-up periods. Despite these challenges, specific autoantibody and biomarker profiles offer insight into defining distinct SLE subtypes.

SLE genetic risk factors—Like RA and other autoimmune diseases, *HLA* genes are thought to have a central role in SLE susceptibility. The haplotypes *HLA-DRB1**03:01 and *15:01 are strong genetic risk factors for SLE in European populations.³⁶ GWAS have associated over 40 genetic loci with SLE susceptibility.⁸⁶ Several genes, such as *PTPN22*, are implicated in SLE and other autoimmune diseases, RA in particular.⁸⁷ However, many other genes are specifically associated with SLE susceptibility.⁸⁸

SLE-associated genetic loci have been categorized according to putative functionality.⁸⁶ These categories include DNA degradation and cellular debris, immune complex clearance, toll-like receptors, interferon regulation, NF- κ B, and regulation of B-cells, T-cells, monocytes and neutrophils.⁸⁶ The role of DNA repair genes, in particular *ATG5*, *TREX1* and *DNASE1*, may be of particular interest given the nearly ubiquitous presence of antinuclear antibodies in SLE.⁸⁶ A wGRS for SLE, composed of 22 SNPs including *HLA-DRB1*, has been developed and used to calculate GRS of particular SLE subtypes based on validated SNPs for SLE and ACR criteria (such as renal involvement and presence of anti-dsDNA antibodies).²² This wGRS is associated with earlier-onset SLE, consistent with the notion that increased genetic burden may correspond to earlier disease onset.²² Gene-gene interactions may also be present in SLE, specifically between *CTLA4*, *IRF5*, and *ITGAM* with *HLA-DRB1* as well as between *PDCD1* and *IL21*, among others.⁸⁹

SLE environmental risk factors—Smoking and exposure to ultraviolet light have been implicated as possible environmental factors for SLE susceptibility.^{90–93} Ultraviolet-B light is well known to cause SLE exacerbations and its potential pathogenic mechanism in aberrant apoptosis and the removal of cellular debris make this a key candidate in the etiological role for SLE, particularly for cutaneous manifestations.⁹⁴ Epstein-Barr (EBV) virus has been suggested to be involved in the pathogenesis of SLE based on high EBV viral loads in pediatric SLE patients compared to controls, with posited molecular mimicry of EBV antigens and autoantigens targeted by lupus autoantibodies.^{95, 96} However, the association of EBV and risk of SLE remains controversial. Finally, the female predominance in SLE argues strongly for hormonal and reproductive influences. Oral contraceptives and postmenopausal hormones have both been related to increased risk of SLE, in particular among women taking higher doses of ethinyl estradiol.^{97–100}

SLE gene-environment interactions—Unlike RA, there is not yet strong evidence associating particular genetic and environmental factors to SLE susceptibility, which makes GEIs for SLE challenging to identify. However, GEIs for both smoking and ultraviolet-B exposure have been reported.^{101, 102} In the Carolina Lupus Study, women with null homozygous genotypes for *GSTM1* and more than two years of occupational sun exposure had a three-fold increased odds for SLE, with a trend towards statistical significance.¹⁰² Other genes involved in DNA-repair (such as *ATG5*, *TREX1*, and *DNASE1*) have not been

specifically studied for a GEI with ultraviolet-B light for SLE development. A Japanese case-control study found that women who were smokers and had the slow acetylator N-acetyl transferase-2 (*NAT2*) genotype had six-fold increased odds of SLE, compared to never smokers with the rapid acetylator form of *NAT2*. This interaction had a significant additive interaction, an attributable proportion due to interaction of 50%, suggesting that metabolism of oxidants from cigarette smoke may play a role in SLE pathogenesis.¹⁰¹ In addition, genes for toll-like receptors (such as *IRF5* and *TLR7*) may engage microbes inappropriately resulting in SLE autoimmunity. Infections with a number of potential organisms may trigger immune responses that go awry in genetically predisposed individuals leading to SLE autoimmunity, although particular pathogens and interactions have not yet been identified.

Ankylosing spondylitis

The incidence and prevalence of AS varies markedly by geography, perhaps due to the prevalence of *HLA-B27* in these populations.¹⁰³ The identification of at-risk individuals therefore may be comparably easier for AS than other rheumatic diseases based on geography, *HLA-B27*-positivity, or AS relatives. However, AS classification criteria are still evolving, which makes consistent phenotyping challenging.^{104, 105} There is considerable clinical overlap between AS and other *HLA-B27* associated diseases, such as reactive arthritis, psoriatic arthritis, and inflammatory bowel disease. Inconsistent phenotyping could hinder the identification genetic and environmental associations in AS. Finally, since AS patients often present in adolescence or go undiagnosed for many years, identifying exposure windows prior to disease onset is challenging. Cohort studies used in rheumatic diseases are typically not large enough to detect AS in exposed compared to unexposed and most do not follow children or adolescents, who might go on to develop AS in early adulthood.

AS genetic risk factors: *HLA-B27* dominates—Unlike other rheumatic diseases, AS has long been associated with a gene with very large effect size.¹⁰⁶ *HLA-B27* positivity confers an odds ratio of approximately 90 for developing AS compared to *HLA-B27* negative individuals.¹⁰⁷ *HLA-B27* is present in about 90% of patients with AS, but only about 5% of *HLA-B27*-positive individuals develop AS or another form of spondyloarthritis.¹⁰⁷ This illustrates the difficulty in the clinical implementation of genetic testing for rheumatic disease susceptibility. A recent large GWAS associated 31 genetic loci with AS.¹⁰⁸ However, these non-*HLA* loci are overwhelmed by the influence of *HLA-B27*. The overall contribution of *HLA-B27* to AS heritability is estimated to be 20% and about 4% is due to other loci.¹⁰⁸ Other genes implicated in AS risk include *IL23R* and *IL1R2* as well as the intergenic region at 2p15.¹⁰⁷ These associations offer insight into the role of cytokines IL-17/IL-23 and IL-1 in AS pathogenesis.

Several possible explanations might explain the striking association of *HLA-B27* with AS development. The arthritogenic peptide hypothesis states that similarity between microbial peptides and *HLA-B27*-specific CD8-positive lymphocytes could induce autoimmunity.¹⁰⁹ The heavy chain homodimer hypothesis states that *HLA-B27* dimers are resistant to normal degradation and engage natural killer receptors inappropriately and result in

autoimmunity.¹¹⁰ Finally, the protein misfolding hypothesis states that unfolded HLA-B27 accumulates in the endoplasmic reticulum and stimulates the release of proinflammatory cytokines is most widely accepted.^{111, 112}

AS environmental risk factors: Microbial and gut influences?—Microbes have been postulated to trigger altered immunity in AS through molecular mimicry. Some suggest this might specifically occur in the gut through the microbiome. The etiologic role of chlamydiae species and enterobacteria in reactive arthritis has been posited to also apply to AS pathogenesis.^{113, 114} Transgenic HLA-B27 murine models have also demonstrated that the introduction of bacteria is necessary for the development of spondyloarthritis.¹¹⁵ Inflammation in the gut has been consistently observed in spondyloarthritis.¹¹⁶ Cigarette smoking has also been implicated in AS susceptibility, underscoring its role in multiple inflammatory and autoimmune diseases.¹¹⁷ Unlike most other rheumatic diseases, males are more likely than females to develop AS, so male-specific factors such as testosterone may also be involved in the pathogenesis of AS.¹¹⁸

AS gene-environment interactions: Engaging *HLA-B27*—Given the large influence of *HLA-B27* on AS susceptibility, any GEI will need to have biological plausibility with *HLA-B27* functionality.¹¹² In this sense, finding significant GEIs may be more straightforward in AS than in RA and SLE (Table 3). A proteomic analysis of *Chlamydia trachomatis* identified peptides that interact with *HLA-B27* in mouse models and also stimulate T cells from patients with spondyloarthritis.¹¹⁹ The role of environmental factors in AS pathogenesis and why many *HLA-B27*-positive individuals never develop AS or other forms of spondyloarthritis are yet unsolved.^{120, 121}

Emerging research strategies

Epigenetics—Epigenetic regulation is thought to be largely responsible for the cell-specific expression of genes. Epigenetic changes are heritable from one cell cycle to the next, potentially reversible, and do not alter nucleotide sequences.¹²² Biochemical modifications involved in epigenetics include acetylation, histone modifications, methylation, phosphorylation, sumoylation, and microRNA (miRNA).¹²³

Epigenetic modifications provide an important potential link between genes and the environment, since environmental factors influence epigenetic processes, such as methylation and acetylation, while other epigenetic factors are inherited.¹²⁴ It also adds many layers of complexity to our notions of inheritance of complex disease phenotypes. Epigenetic responses to environmental exposures are likely in part genetically determined themselves and can be inherited as stable traits and act as *bona fide* epigenetic quantitative trait loci, perhaps responsible for a great deal of unexplained heritability. This leads to added complexity in understanding the interplay between genetic factors and environmental exposures, and epigenetic control and modification and subsequent genetic expression, in determining disease susceptibility.¹²⁵

Studies performed in rheumatic diseases have mostly focused on inflammatory pathways in distinct immune cell types.¹²⁶ Demethylation has been linked to drug-induced lupus in humans and murine models.^{127, 128} Sophisticated techniques are required to systematically

identify and isolate cells of the same immunophenotype. The differential expression of epigenetic markers between cell types and innumerable immunophenotypes make population-based epigenetic studies difficult to implement. At present, specific hypotheses, often based on known genetic findings and immune function, are often needed for epigenetic investigations. As the technological aspects of high-throughput epigenetic screening evolve, population-based studies utilizing epigenetics are likely to become increasingly feasible. For example, a study examined epigenetic patterns in over 200 neonates to investigate the roles of genetic and maternal factors.¹²⁹ Another study performed a genome-wide methylation analysis of peripheral blood mononuclear cells in a cohort of African American women to find and identified over 900 statistically significant loci potentially related to cigarette smoking.¹³⁰ Methylation patterns have also been implicated as an intermediary of genetic risk seen in ACPA-positive RA.¹³¹ Methods for epigenome-wide association studies are currently being refined and may be ready for large-scale utilization in the near future.¹³²

Microbiome—The microbiome refers to the symbiosis and interaction of individuals and their bacterial flora. It has long been recognized that bacteria occupy the mucosal surfaces in the gut, mouth, respiratory system, and skin. Recent findings implicating autoimmunity at these sites have heightened the search for imbalances of the microbiome and rheumatic disease susceptibility. The relationship of microbiota to diseases such as acute rheumatic fever and reactive arthritis provide a precedent for the potential importance of microbes to other rheumatic diseases.

Scher and colleagues examined the stool from RA cases and healthy controls and found a potential role for the bacteria *Prevotella copri* in RA disease susceptibility.¹³³ While provocative, this study requires replication as factors related to having RA (such as socioeconomic status, healthcare utilization and medications) might explain this association. Studies are currently exploring mechanisms to link specific bacteria in the microbiome and disease susceptibility. Comparisons between high-risk, asymptomatic individuals, the general population, and patients with established disease could elucidate the microbiome's role in rheumatic disease etiology. Exploration of the microbiome of other mucosal surfaces, such as the mouth and lung, are ongoing in RA and other diseases. High-throughput methods to assess microbiota need to be developed so that large population-based studies can be performed in diverse populations and geographic areas, incorporating knowledge of other environmental factors and host genetics.

Metabolomics—Metabolomics is the study of metabolites and their relationship to disease. Importantly, metabolomics may serve as the bridge between host genetics and environmental exposures. Unlike epigenetics and the microbiome, high-throughput methods involving mass spectrometry already exist for metabolomics.¹³⁴ However, metabolites levels may be depend on many factors such as sleep, fasting status, comorbidities, and medication usage making study design challenging and sensitive to many variables. Metabolomic studies require sophisticated statistical support to allow for multiple comparisons, mutual adjustments, and principal components analysis.

Despite these qualifications, studies have been performed to investigate metabolomic profiles in rheumatic diseases. Serum metabolite profiles related to lipids and lactate were

related to the inflammatory burden in RA patients.¹³⁵ Urine metabolite profiles in RA patients could distinguish responders from non-responders to tumor necrosis factor inhibitors.¹³⁶ While provocative, it is unclear whether these methods provide clinically meaningful data beyond currently available biomarkers or whether metabolite dysregulation occurs in the pre-clinical disease period. Identification of novel metabolite biomarkers in the pre-clinical phase may prove to be helpful in identifying individuals at risk for autoimmune rheumatic disease and contribute to the understanding of pathophysiology. Thus, metabolomics may be a powerful tool in identifying biomarker patterns associated with drug response and could influence treatment decisions.

Future considerations and summary

In conclusion, genetic and environmental factors, and interactions between them, some of which may be mediated by epigenetic modification followed by post-translational effects, are associated with the development of rheumatic diseases. The discovery and validation of the *HLA-DRB1*-smoking interaction in RA is a model among rheumatic diseases for GEI studies. For SLE, genetics have elucidated pathways that may lead to the differentiation of disease subtypes and form hypotheses that integrate environmental risk factors. In AS, potential etiologic mechanisms must consider *HLA-B27*. Despite these advances, rheumatic disease etiology remains enigmatic. GEIs have and could continue to provide insights into biological mechanisms that link genetic and environmental risk factors. However, much of the risk for rheumatic disease susceptibility remains unexplained. Future research will investigate whether this unexplained risk can be explained by rare genetic variants, epigenetic controls and modifications, novel environmental factors, gene-gene interactions, or GEIs. In addition, understanding which genes may be involved in the development of autoimmunity, and those that lead to progression of disease will be important to evaluate in longitudinal studies. Emerging research strategies including epigenetics, metabolomics, and the microbiome will likely further elucidate rheumatic disease susceptibility.

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Key points

- Genetic and environmental risk factors have been identified for rheumatic diseases using case-control, cohort, and genome-wide association studies.
- The identification of gene-environment interactions (GEIs) may elucidate biological mechanisms for rheumatic diseases, by causally linking established genetic and environmental risk factors.
- The most well studied example of GEIs in rheumatic disease susceptibility is for cigarette smoking and the *HLA-DRB1* for seropositive RA; the presence of both risk factors greatly increases the risk for RA development.
- Due to the relative rarity of systemic lupus erythematosus (SLE), comprehensive studies of GEIs have not yet been performed for (SLE). However, there is some evidence that genes may interact with smoking and ultraviolet-B light exposure in increasing SLE risk.
- *HLA-B27* is the most potent genetic risk factor for ankylosing spondylitis, and there are suggestions that molecular mimicry by gut microbes might stimulate autoimmunity through GEIs with *HLA-B27*.
- Emerging research frontiers such as epigenetics, metabolomics, and the study of the oral, respiratory, and gastrointestinal microbiome may provide new biologic mechanisms to link genetic and environmental risk factors in rheumatic disease pathogenesis.

Figure 1.

Schematic showing the pre-clinical phases of RA. Other rheumatic diseases likely follow similar phases of progression from genetic susceptibility, immune dysregulation, and sub-clinical disease to classifiable disease. Environmental factors and gene-environment interactions likely occur throughout this process of disease pathogenesis.

From Karlson EW, Deane K. Environmental and gene-environment interactions and risk of rheumatoid arthritis. *Rheum Dis Clin North Am* 2012;38(2):405–26; with permission.

Figure 2.

Schematic of *HLA-DRB1*-cigarette smoking interaction in the development of seropositive RA. Individuals with one or more *HLA-DRB1* shared epitope alleles smoke cigarettes, which induces inflammation in the airways and alveolar mucosa. Peptidylarginine deiminase (PAD) is activated, which citrullinates self-antigens. Antigen-presenting cells stimulate T cells through aberrant HLA-DRβ1 interactions and stimulate B cells to produce anti-citrullinated peptide antibodies (ACPA). Immune tolerance is lost specifically in joints, which manifests clinically as synovitis with signs and symptoms compatible with RA. From Klareskog L, Ronnelid J, Lundberg K, Padyukov L, Alfredsson L. Immunity to citrullinated proteins in rheumatoid arthritis. *Annu Rev Immunol* 2008;26:651–75; with permissions.

Table 1

Threats to validity in study designs.

	Case-control study	Cohort study	Genome-wide association study
Bias	Selection bias Recall bias Inappropriate matching Reverse causation	Selection bias Misclassification Length-time bias Reverse causation Loss to follow-up	Phenotype misclassification Inappropriate controls Genomic inflation
Confounding	Unmeasured confounders	Unmeasured confounders	Population stratification
Chance	Multiple testing Power	Multiple testing Power	Multiple testing False discovery rate Rare variants Power
Generalizability	Study-specific	Cohort-specific	Race/region-specific Phenotype-specific

Table 2

Statistical measures of interaction.

Type of interaction	Statistical measure	Interpretation	Null hypothesis
Additive	RERI	Relative excess risk due to interaction	Additional risk compared to the expected risk from adding the risks for each exposure RERI=0
	AP	Attributable proportion due to interaction	Proportion due to interaction of overall risk among those with both exposures AP=0
	S	Synergy index	Excess risk from combined exposures relative to the risks from each exposure S=1
Multiplicative	ROR	Ratio of odds ratios	Additional risk compared to the expected risk from multiplying risks of each exposure ROR=1

Table 3

Selected genetic, environment, and gene-environment interactions in RA, SLE, and AS.

Phenotype	MHC gene (variance explained)	Number of non-HLA SNPs associated ^{Ref}	Selected non-HLA genes/loci	Key environmental factors	Proposed gene-environment interactions
RA	<i>HLA-DRB1</i> <i>HLA-DPB1</i> <i>HLA-B</i> (12%)	101 ⁴	<i>PTPN22</i> <i>TNFAIF3</i> <i>TYK2</i>	Cigarette smoking Reproductive factors and hormonal exposures Silica Periodontitis Excess body mass Alcohol intake Fish intake	<i>HLA-DRB1</i> -smoking <i>PTPN22</i> - smoking
SLE	<i>HLA-DRB1</i> <i>HLA-DPB1</i> <i>HLA-G</i>	>40 ⁸⁶	<i>ATG5</i> <i>TREX1</i> <i>DNAI1</i> <i>IRF5</i> <i>TLR7</i> <i>FCGR2A</i>	Ultraviolet-B light Current cigarette smoking Silica Reproductive factors and hormonal exposures Alcohol intake Epstein-Barr virus	DNA repair genes-UV-B light and smoking TLR genes-infections
AS	<i>HLA-B27</i> (20%)	30 ¹⁰⁸	<i>IL23R</i> <i>IL1R2</i> 2p15	Gut microbiome Cigarette smoking Testosterone	<i>HLA-B27</i> -infections

Abbreviations: AS, ankylosing spondylitis; HLA, human leukocyte antigen; MHC, major histocompatibility complex; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SNPs, single nucleotide polymorphisms; TLR, toll-like receptor; UV-B, ultraviolet-B.