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Genetics and Genomics of Sjogren's Syndrome: Research provides Clues to Pathogenesis and Novel Therapies

Barbara M. Segal, MD¹, Abu N. M. Nazmul-Hossain, DDS, MSc, PhD², Ketan Patel, BDS, PhD³, Pamela Hughes, DDS³, Kathy L. Moser, PhD⁴, and Nelson L. Rhodus, DMD, MPH⁵

¹Department of Medicine, University of Minnesota, Minneapolis, MN

²Dental Research Institute, University of California, Los Angeles, CA

³Department of Developmental and Surgical Sciences, University of Minnesota, Minneapolis, MN

⁴Arthritis and Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK

⁵Department of Diagnostic and Biological Sciences, University of Minnesota, Minneapolis, MN

Abstract

Purpose—While the key inciting events that drive the progression from autoantibodies to clinical disease remain to be clarified, new light has been shed on the factors contributing to disease susceptibility and the role of genetic factors in determining Sjogren's syndrome (SS) disease phenotypes. The purpose of this review is to provide an update on the role of genetic markers in the susceptibility to and pathogenesis of Sjogren's syndrome. This paper also discusses how genomic and proteomic technology can help in the design of specific therapeutics.

Key Findings—Recent evidence suggests that inflammatory genes associated with interferon pathways, and specific regulatory genes that control the maturation and proliferation of B cells, contribute to the pathogenesis of Sjogren's syndrome. Both gene expression profiling technology and gene association studies have been used to identify these key biologic pathways. Molecularly defined subsets of pSS patients are also being revealed by these studies. Previously identified gene loci which predispose to multiple autoimmune disorders have been confirmed supporting the paradigm of “general” autoimmune disease genes. Association of SS with many additional susceptibility loci are likely to be established through ongoing genome-wide association scans (GWAS). Clues from genetic studies suggest that targeting B cells will prove to be an effective way of reducing the systemic manifestations of pSS and are supported by early clinical trials.

Summary—Genome-wide technologies are likely to identify new genes and molecular pathways in the pathogenesis of SS that will be useful not only to identify patients at risk for SS, but also to identify subsets of patients at risk for variable levels of disease severity. In the future, these studies could identify novel biomarkers that will lead to significant advances in management by providing the means to tailor therapeutic strategies to individual patients.

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Corresponding Author and Address: Dr. Nelson L. Rhodus, DMD, MPH, FICD Department of Diagnostic and Biological Sciences School of Dentistry, University of Minnesota 7-536 Moos Tower, 515 Delaware Street S.E. Minneapolis, MN 55455 Phone: (612) 625-0693 Fax: (612) 624-0477 rhodu001@umn.edu.

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Keywords

Sjogren's; genetics; genomics; autoimmune; inflammatory; Sjögren's Syndrome; Gene Expression Profiling; Interferon; Genetics

Introduction

Sjögren's syndrome (SS) is a common, chronic, heterogeneous systemic autoimmune disorder characterized by lymphocytic infiltration into exocrine glands. Clinical presentations include dry eyes and dry mouth with a wide range of systemic manifestations involving the skin, neurologic, hematologic, respiratory, gastrointestinal, genitourinary, and musculoskeletal systems.^[1] Patients are typically classified as primary SS (pSS) when another connective tissue disease is not evident, and secondary SS (sSS) when patients with a preexisting connective tissue disease have features of SS. Approximately half of all patients with SS have an accompanying autoimmune rheumatic disease, most commonly systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA).^[2] Prevalence of pSS, based on recent population studies, is estimated to be 0.2–0.7% with a strong female predominance of 9:1.^{[3], [4] and [5]} The estimated frequency of autoimmune disorders in family members of SS patients is 30–35% suggesting that genetic factors play a role in the etiology of the disorder.^[6]

Although SS is a relatively common autoimmune disease, it remains one of the most understudied and poorly understood rheumatic disorders. SS patients are often misdiagnosed (particularly with SLE) or under-diagnosed as clinicians fail to recognize SS or are not familiar with the variety of clinical presentations. Multiple classification criteria have been proposed since 1965. Although the 1992 American European Consensus Group criteria^[7] are now widely accepted, diagnosis of SS remains a complex process involving multiple subspecialists and often an invasive procedure, the minor salivary gland biopsy, to fully characterize patients. The classification criteria can be difficult to apply and clear cut differentiation between primary SS and secondary SS is not always possible. In contrast to recent successes in SLE and RA, the genetic contribution to disease in SS is largely unexplored. Nevertheless, new insights into potential disease mechanisms have been suggested by genetic, genomic and proteomic studies completed to date in SS. Furthermore, studies in SLE, RA and other related phenotypes are proving to be informative for SS.

Pathogenesis of pSS

The cause of SS is unknown however; extensive data implicates both the innate and acquired immune systems which are mutually co-stimulatory in the pathogenesis. Lymphoproliferative sialoadenitis in SS is associated with lymphocyte infiltration, epithelial cell proliferation, and apoptosis.^[8] A hallmark of pSS is B-cell hyperactivity as manifested by the production of autoantibodies, hypergammaglobulinemia, formation of ectopic lymphoid structures within the inflamed tissues, and enhanced risk of B-cell lymphoma. The link between autoimmune disease and increased risk of lymphoproliferative disease is not well understood. An increased risk of lymphoma appears to be associated with unopposed chronic inflammation in multiple autoimmune diseases including rheumatoid arthritis (RA), lupus (SLE) and Sjogren's. The development of overt lymphoma is likely to be due to sustained immune stimulation which promotes the expansion of scarce B cell clones and results in the outgrowth of monoclonal aggregates of B cells.^[9] Increased association with lymphoma has been consistently demonstrated in population based studies of primary Sjogren's. In comparison to RA and SLE, pSS is associated with the strongest risk of non-Hodgkin's lymphoma (Odds Ratio 11.7).^[10]

As is the case in other systemic autoimmune disorders, a complex interplay of multiple genes influenced by environmental factors is thought to contribute to the etiology of SS. A genetic contribution to Sjogren's pathogenesis was first demonstrated in the late 1970's and 1980s when it was found that disease susceptibility was associated with MHC Class II HLA alleles.^{[11], [12] and [13]} Convincing data demonstrated that the anti-Ro (SSS-A) and anti-La (SS-B) autoantibody responses in SLE and in SS were most strongly associated with HLA-DQ alleles.^[14] Recent studies have confirmed the association of HLA –DRB1*0301, as well as DQ A1*0501 and DQB1 *0201. A role for non-HLA genes was suspected as well, but results from multiple early small studies which suggested a role for systemic inflammatory genes, cytokine genes, and genes involved in the regulation of apoptosis, were inconsistent.

The precise nature of environmental factors which might influence the onset of clinical disease in the genetically predisposed host in pSS remains hypothetical. Interaction between genetic factors and viral infection is suggested by animal models in which transgenic mice containing either the human T-cell leukemia virus (HTLV-1) tax gene, or the Hepatitis C (HCV) envelope gene, develop sialoadenitis.^{[15], [16]} Expression of viral antigens is hypothesized to perturb the ductal epithelium, and leads to the dysregulation of apoptosis in ductal epithelial cells, which is a prominent finding in the salivary tissue in pSS. Hormonal factors have also been implicated by findings in a series of studies that suggest that systemic and local androgen deficiency is present in SS patients.^{[17], [18] and [19]} Defective regulation of androgen in SS salivary gland is likely to contribute to acinar atrophy and ductal cell hyperplasia.^[20]

Gene Expression Profiling Reveals Insights into Pathogenesis

Genome-wide gene expression profiling is a powerful technology that allows the simultaneous measurement of thousands of mRNA transcripts in a biologic sample. Since 2005, the development of high-throughput transcriptional profiling using microarray technology has dramatically enhanced the ability to characterize comprehensive patterns of gene expression in isolated cells from normal and diseased tissues. Expression patterns, or “signatures” that distinguish patients from controls have been described that provide new insight into molecular pathways that are dysregulated in disease. For example, microarray technology has been used widely in oncology to define dysregulated pathways and provide prognostic insight.^[21] This approach has also been used in a variety of autoimmune disorders including SLE and RA.^{[22], [23]}

Gene expression profiling in SLE

Gene expression profiling using microarray technology in patients with SLE demonstrated up-regulation of the Type 1 interferon (IFN) pathway.^[24] Numerous subsequent studies have confirmed the IFN signature as one of the most distinctive patterns of dysregulated gene expression in SLE.^{[24], [25] and [26]} Studies using gene expression profiling in rheumatic autoimmune diseases were pioneered by our group at the University of Minnesota. The initial study in SLE was performed using the oligonucleotide microarray platform developed by Affymetrix (www.affymetrix.com). This analysis identified gene expression patterns that differentiated most SLE patients from healthy controls.^[22] One of the most striking results was the predominance of over-expressed IFN-inducible genes. Approximately half of the SLE patients exhibited an elevated IFN score, while the others had scores indistinguishable from controls. The IFN signature correlated with more severe manifestations of SLE. Thus, gene expression profiling has brought innate immune mechanisms to the forefront of research aimed at understanding pathogenic mechanisms in SLE.

Gene expression profiling in SS

Success of the SLE studies provided “proof of concept” and suggested that gene expression profiling studies in SS might also be informative. Over-expression of IFN-inducible genes was first reported in pSS salivary gland tissue by Hjelmervik.^[27] Ten patients with pSS and 10 controls with symptoms of pSS were evaluated. Out of the top 200 most differentially expressed genes, the highest ranked transcripts were from the T cell receptor β locus and genes involved in antigen presentation lymphocyte development and activation as well as interferon induced chemokine genes (e.g. CXCL13 and BCA-1).^[27] Numerous interferon regulatory genes were also highly expressed. In addition, down-regulation of the expression of carbonic anhydrase II, essential in saliva production and secretion, was also found, suggesting direct functional abnormalities in SS.

Several gene expression profiling studies in human SS have been reported subsequently which demonstrated up regulation of type I IFN genes in both salivary gland tissue and in saliva.^{[28], [29], [30] and [31]} Gottenberg et al. evaluated minor salivary gland tissue from 7 pSS patients and 7 controls using microarrays containing over 10,000 probes.^[28] Analysis of these data also indicated IFN-mediated innate immune mechanisms in the pathogenesis of pSS. Specifically, 23 genes known to play a role in IFN signaling were identified, including two Toll-like receptors (TLR 8 and TLR9). This study also demonstrated that plasmacytoid dendritic cells, a major producer of IFN, could be detected by immunohistochemistry in all pSS patients, but none of the controls. IFN-related gene expression patterns were also reported in labial salivary tissue in the study of 3 pSS patients and 3 controls by Wakamatsu.^[29] These studies strongly support the role of innate immunity, in addition to adaptive immune mechanisms, in the pathogenesis of pSS. A proposed model (Figure 1) suggests that stimulation of toll like receptors (TLRs) (e.g. by viral or immune complexes) in salivary glands may be dysregulated, very possibly due to genetic variants that predispose to pSS, and that continual stimulation of the innate immune response system through TLRs contributes to the persistence of the IFN signature.^[32]

Using methodology similar to that employed in the SLE study described above, our group demonstrated over-expression of IFN-inducible genes in peripheral blood of pSS patients.^[33] As shown in Figure 2, hierarchical clustering analysis of transcripts differentially expressed between patients and controls demonstrated that the IFN-inducible gene expression signature was most prominent in those patients with high-titer anti-Ro/SSA and anti-La/SSB.

The clinical relevance of the IFN signature in pSS has been confirmed in multiple studies. Functional and proteomic studies provide additional support for the central role played by the IFN-1 pathway in pSS. DNA microarray analysis and quantitative real-time polymerase chain reaction (PCR) was performed to identify key target genes in peripheral blood from patients with Sjögren's syndrome (SS) by Kimoto's group.^[34] Their results suggest that up-regulation of IFN inducible genes is a systemic phenomenon important to the pathogenesis of Sjogren's syndrome. Dysregulated IFN pathways are detectable in 40% of pSS patients and are associated with increased disease activity, as well as multiple laboratory markers of B cell hyperactivity, including depressed C3, and elevated levels of polyclonal IgG.^[35] Significantly, a key regulatory cytokine, the B-cell activating factor (BAFF), which has been shown to be elevated in pSS sera, was also shown to be associated with the IFN-1 signature.^[35]

BAFF is a TNF receptor ligand involved in B-cell differentiation and activation and B lymphocyte survival.^[36] Ittah et al. showed that apoptotic bodies from epithelial cells, in combination with anti-Ro/SSA and IFN, induce local production of BAFF expression by

epithelial cells.^[37] BAFF in turn promotes persistence of auto reactive B cells which may then provide an amplification loop that result in chronic inflammation.^[38] Interestingly, salivary epithelial cells from SS patients exhibit a heightened sensitivity to BAFF, adding further support to the concept of a central role of the glandular epithelial cells in inducing and perpetuating the activity of auto reactive immune cells. This observation is of key importance given that transgenic expression of the human BAFF gene is sufficient to induce an SS-like disease in normal mice.^[39]

Proteomics and Genomics in Saliva

Like serum, saliva offers a convenient body fluid for analysis as well as an obvious source of information relevant to disease status in pSS. In 1989, our group reported significant alterations in salivary proteins in patients with SS as compared to controls and suggested a differential expression of those proteins between pSS and sSS.^[40] Tremendous advances in salivary diagnostics and laboratory analytical technologies have been developed since 1989.^[30]

A recent study utilized microarray technology coupled with mass spectrometry to identify genomic and proteomic biomarkers in whole saliva collected from pSS patients and healthy controls. Twenty-seven mRNA transcripts were found to be significantly up-regulated in patients compared to controls.^[30] Strikingly, 19 of the 27 genes that were found to be up-regulated in saliva were IFN-inducible, or were related to lymphocyte infiltration and antigen presentation known to be involved in the pathogenesis of pSS. These molecular signatures in saliva support the systemic findings indicating a major role for IFN pathways in the pathogenesis of pSS.

Multiple Autoimmune Diseases Share Common Genes

Interestingly, genetic studies have revealed polymorphisms that are associated with multiple autoimmune diseases. Clinically distinct autoimmune disorders including SLE and SS, as well as RA, Psoriasis, Dermatomyositis and Multiple Sclerosis, have now been shown to be associated with dysregulation of interferon pathways supporting common pathogenic mechanisms across related autoimmune disease phenotypes.^{[24], [41]} Multiple genes which are components of the type I IFN system, as well as several intriguing newly described genes such as *MECP2*, are also common to both SLE and SS.^[42]

Genetic studies in SLE have provided additional insight into potential genetic contributions to pSS. In SLE, over 30 genetic polymorphisms have been convincingly established, including variants that alter function in such genes as IFN regulatory factor 5 (*IRF5*) that play a key role in host defense.^[43] IRF-5 is a transcription factor involved in innate immunity that functions in response to toll receptor signaling. In 2007, Mariette's group described the first analysis of *IRF5* rs2004640, rs2070197, rs10954213, and rs2280714 polymorphisms in SS. In their cohort of 212 pSS patients and 162 healthy blood donors, all of whom were of Caucasian origin, the *IRF5* rs2004640 GT or TT genotype (T allele carriers) was identified in 87% of pSS patients compared with 77% of controls (P = 0.01, odds ratio [OR] 1.93 [95% confidence interval (95% CI) 1.15–3.42]). The *IRF5* rs2004640 T allele was found on 59% of chromosomes from pSS patients compared with 52% of chromosomes from controls (P = 0.04, OR 1.36 [95% CI 1.01–1.83]) This study was the first to demonstrate a significant association between pSS and the *IRF5* rs2004640 T allele.^[44] Additionally, new B cell genes including: *FAM 167-A* *BLK* and *TNFSF4* which appear to play a role in PSS as well as RA, SLE, and Scleroderma, are emerging as “general” autoimmune disease genes.^[45] Indeed, as large scale genetic studies continue to reveal additional susceptibility loci, a substantial number of genes identified to date are associated

with more than one autoimmune disease. Table 1 summarizes the genes for which association has been confirmed in pSS as well as in multiple other autoimmune diseases.

Application of Genome Wide Association Scans to identify Novel Genetic Loci

A major recent addition to the genetic research tool box has been the development of genome-wide association studies. In this approach, microarrays can be utilized to genotype hundreds of thousands of single nucleotide polymorphisms (SNPs) spanning the genome that can then be tested for association with a disease phenotype. GWAS technology has enabled the identification of novel associations with loci that would have been unlikely to be selected as high-priority candidate genes for analysis. Since 2008, GWAS investigations have typically involved comparing cohorts of several thousand samples of cases and controls. Dozens of new risk alleles have been identified in SLE and RA. In addition, valuable confirmatory data that is related to previously identified genes has been obtained, including the signal transducer and activator of transcription 4 (*STAT4*) and the IFN regulatory gene (*IRF5*). In 2008, based on the GWAS findings in both SLE and RA, Korman et al. applied the candidate gene association approach to test the hypothesis that association of the variant haplotype of *STAT4* seen in RA and SLE was also associated with pSS.^[42] Their group genotyped *rs7574865*, the most strongly disease-associated SNP in the variant *STAT4* haplotype, in 124 Caucasian pSS subjects and compared them to 1143 Caucasian controls. The disease-associated T allele was more common in chromosomes of the pSS patients (29.6%) than in controls (22.3%), leading to a P-value for association of 0.01.

STAT4 is an important transcriptional regulator of adaptive immunity, that can be phosphorylated by membrane-bound receptors, dimerized and translocated to the nucleus, where *STAT4* molecules differentially regulate gene expression.^[46] *STAT4* transduces interleukin-12, interleukin-23, and type I interferon cytokine signals in T cells and monocytes, leading to T-helper type 1 and T-helper type 17 differentiation, monocyte activation, and production of interferon- γ . As such, *STAT4* has a central role in directing helper T cells toward the pro-inflammatory T-helper type 1 and T-helper type 17 lineages.^[47] Although the evidence for this association is very strong and well replicated, the exact mechanism by which polymorphisms in this gene lead to disease remains unknown. Recently, Nordmark et al. found evidence of a strong additive effect of the major risk alleles of *IRF5* and *STAT4* with an overall significance between the number of risk alleles and pSS of $P=2.5 \times 10^{-9}$.^[48] The odds ratio for pSS increased in an additive manner, with an average increase in OR of 1.78. For carriers of two risk alleles, the OR for primary SS was 1.43, whereas carriers of five risk alleles have an OR of 6.78. Since *IRF5* and *STAT4* are components of the type I IFN system, this data emphasizes the importance of this system in the pathogenesis of primary SS.

Ongoing efforts to define the genetic associations that contribute to altered gene expression patterns are likely to reveal additional genes that explain both commonalities and differences between SS and related disorders. In another recent candidate gene association study, which again followed a lead suggesting an important role for the *MECP2* gene in the pathogenesis of SLE, Cobb et al. examined the potential role of *MECP2* in pSS. *MECP2* is critical in DNA methylation-induced transcription silencing.^[49] The gene is present on the "X" chromosome. In their association study of 460 PSS patients compared to 1828 controls, the *rs1743* allele, which was previously associated with Lupus susceptibility, was shown to have an OR in pSS equal to 1.33. Homozygosity for *MECP2* was associated with an additive effect (OR 2.17). The discovery of this gene loci in pSS is particularly interesting in that the association is not dependent on the presence of anti-Ro (SSA) or anti-La (SSB) antibody.^[49]

The first genome wide association study to be performed in pSS is currently underway and will undoubtedly provide insight into the genetic architectures of this complex syndrome. In the future, the continuing application of genome-wide technologies is likely to identify new genes and molecular pathways in SS that will be useful not only to identify patients at risk for SS, but also to identify subsets of patients at risk for variable levels of disease severity and different extra-glandular features of the disease such as lymphoma. Despite a similar clinical sicca phenotype at presentation, the two disease subsets(anti-Ro(SSA)/anti-La(SSB) positive and seronegative) have a different longitudinal course and are likely to have partly different molecular pathogenesis, analogous to seropositive and seronegative RA (Figure 3). Much more work needs to be done to clarify whether unique genetic factors and environmental agents contribute to the seropositive and seronegative SS subsets. We believe that these studies will have a high likelihood of providing novel biomarkers that will lead to significant advances in management by not only providing information useful in identifying patients at risk for poor outcomes, but also providing the means to tailor therapeutic strategies to individual patients and reduce the likelihood of drug toxicity.

Novel therapies based upon pathogenesis

pSS has long posed a vexing therapeutic dilemma, as the underlying pathophysiologic mechanisms remain obscure, disease activity is challenging to evaluate, and no specific disease modifying treatments known to be effective are well established. Based upon recent convincing evidence that innate immunity, most notably mediated by INF signaling, plays a role in the initial B-cell activation, interest in B cell targeted therapies has increased worldwide. The B lymphocyte pathogenic axis is targeted by numerous drugs currently in evaluation including Epratuzumab a monoclonal antibody directed at the CD22 B cell surface antigen which may preferentially target auto-reactive B cells. Baminercept, a lymphocytotoxin-beta receptor fusion protein which along with BAFF supports the formation of germinal centers within salivary glands is another molecule that is of interest for SS.^[32] Particularly promising in pSS is Belimumab, a monoclonal antibody that targets the BAFF receptor specifically and could disrupt the cycle of B cell activation and antibody production.^[50] Belimumab appears to be effective for SLE and is undergoing early stage development for pSS. Potential new cytokine therapeutic targets, have been suggested recently by data implicating pro-inflammatory Th17 cells in SS.^{[51], [52]} and ^[53] IL-for primary SS was 1.43, whereas carriers of five risk alleles have an OR of 6.78. Since *IRF5* and *STAT4* are components of the type I IFN system, this data emphasizes the importance of this system in the pathogenesis of primary SS.

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Conclusions and Future Prospectives

It is likely that further investigation will clarify the distinctive genetic contribution to the seropositive and seronegative SS phenotypes. The mechanisms which account for the lack of correlation between the severity of inflammatory sialoadenitis and the degree of hypofunction of the exocrine glands which is characteristic of both types of SS are also likely to be clarified. Investigation of the molecular mechanisms suggested by functional and proteomic studies are likely to reveal the pathways involved in the evolution of late complications of pSS such as the emergence of lymphoproliferative disorder. In the future, patient selection for new targeted therapies could be based on specific genetic risk factor profiles such as the additive effects of *IRF5* and *STAT 4* on the interferon signaling system. Additional work is needed to characterize the genetic factors associated with systemic features of SS such as those patients with distinctive neurologic disorders. Comprehensive, genome-wide studies will provide insights into the pathogenesis and treatment of patient subsets comprising this extremely complex heterogeneous syndrome.

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Figure 1.
Model of Sjogren's syndrome pathogenesis

Figure 2.

Peripheral Blood Gene expression profile of pSS

Gene expression profile of seropositive pSS patients differs from seronegative patients.

Hierarchical clustering analysis of 106 differentially over expressed genes between 36 pSS patients (indicated by blue bars), 11 seronegative patients (indicated by gray bars) and 22 controls (indicated by orange bars). The purple bars on the right side of the CLUSTERTREEVIEW diagram indicate the IFN signature gene cluster. Each row represents an individual gene. Red signifies genes that are upregulated relative to controls. Green indicates genes downregulated compared to controls, black indicates genes that are not significantly different from controls.

Figure 3.
Differences in risk factors, immune events, and longitudinal disease course in seropositive and seronegative Sjogren's syndrome subsets

Table 1

Sjogren's syndrome Genetic Factors outside the HLA loci associated Multiple Autoimmune Diseases

GENE	AUTOIMMUNE DISEASES in addition to pSS	FUNCTION
STAT4	SLE, RA, SSc, IBD **, Type I DM, Psoriasis, APS ***	Transcription factor regulating T cell function
IRF5	SLE, RA, SSc,	Transcription factor regulating IFN production
TNFSF4 (Ox40L)	SLE, SSc, RA	Cytokine expressed on multiple cells, including APC, B cells PDC, T cells and Natural Killer Cells. NF-kB regulation, T cell proliferation
FAM167A-BLK	SLE, SSc, RA, APS	B cell development and proliferation
MECP2	SLE	DNA methylation induced transcription silencing

* SSc=Scleroderma

** IBD= Inflammatory bowel disease

*** APS=Antiphospholipid Syndrome