Review

Genetics and epigenetics in primary Sjögren’s syndrome

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Abstract

Primary Sjögren’s syndrome (pSS) is considered to be a multifactorial disease, where underlying genetic predisposition, epigenetic mechanisms and environmental factors contribute to disease development. In the last 5 years, the first genome-wide association studies in pSS have been completed. The strongest signal of association lies within the HLA genes, whereas the non-HLA genes IRF5 and STAT4 show consistent associations in multiple ethnicities but with a smaller effect size. The majority of the genetic risk variants are found at intergenic regions and their functional impact has in most cases not been elucidated. Epigenetic mechanisms such as DNA methylation, histone modifications and non-coding RNAs play a role in the pathogenesis of pSS by their modulating effects on gene expression and may constitute a dynamic link between the genome and phenotypic manifestations. This article reviews the hitherto published genetic studies and our current understanding of epigenetic mechanisms in pSS.

Key words: primary Sjögren’s syndrome, genetics, GWAS, HLA, IRF5, STAT4, epigenetics, DNA methylation, histone modification, non-coding RNA

Rheumatology key messages

- The strongest signal of genetic association with primary Sjögren’s syndrome is within the HLA genes.
- Genes relevant to the innate and adaptive immune systems are associated with modest effect sizes.
- DNA hypomethylation at interferon-induced genes is an epigenetic modification in different cells and tissues.

Introduction

Primary Sjögren’s syndrome (pSS) is a complex disease where genetic, epigenetic and environmental factors are thought to contribute to disease pathogenesis. The overwhelmingly strongest genetic predisposition to pSS is attributed to the HLA genes, where early studies reported different HLA class II gene haplotypes associated with pSS in different ethnic groups [1]. Initial attempts to identify non-HLA susceptibility genes employed a candidate gene approach, later followed by genome-wide association studies (GWASs) as the technology for large-scale genotyping advanced. Genetic risk variants in pSS are predominantly found at intergenic regions and deciphering the functional impact of identified variants is a challenge. Epigenetic modifications have emerged as important mechanisms for understanding how the interaction of genetic predisposition and environmental factors may give rise to chronic autoimmunity, including pSS (Fig. 1) [2]. In this review, current studies of genetic variants and epigenetic alterations in pSS and their implications for disease pathogenesis will be discussed.

Genetics

The genetics of pSS are largely understudied compared with other systemic autoimmune diseases. Initial evidence supporting an important role of inherited factors in pSS came from reports of familial aggregation, increased concordance rates among monozygotic twins and increased prevalence of other autoimmune diseases amongst relatives of pSS patients [3–5]. The estimated odds of female
Sibling concordance (s) for pSS ranges from 8 to 30 and dizygotic twin concordance rates from 2 to 4% [6]. The genetic contribution to pSS has been investigated by studies of X chromosome aneuploidy, candidate genes and GWASs in populations of European and Asian ancestry.

**X chromosome**

The single most important risk factor for pSS is being female. Given the remarkable gender disparity (14 female:1 male), the X chromosome has been a particularly promising candidate for genetic studies [7]. Harris et al. [8] showed that men with Klinefelter’s syndrome (47,XXY) had a similar risk of pSS as 46,XX women. Furthermore, the coexistence of Turner syndrome (45,X) with pSS is very rare, whereas the estimated prevalence of pSS in women with 47,XXX is 2.9 times higher than that in women with 46,XX [9]. The identification of structural chromosomal aberrations resulting in partial triplications of the X chromosome (Xp11.4::pter) in three patients with pSS (a mother–daughter pair and an unrelated female) suggests that dosage-sensitive risk genes may be located within this chromosomal interval [10]. The gene(s) that mediates this effect remains undefined.

**MHC**

Like in many autoimmune diseases, the strongest pSS genetic associations are within the MHC, and risk variants vary by serological status and among different ethnic groups (Table 1). The most consistent associations to date have been with DR2 and DR3 alleles at the DRB1 locus in Caucasian populations [20, 21]. Very strong associations with anti-Ro/SSA and/or anti-La/SSB were identified in patients that carry DRB1*03 and DQB1*02 alleles or were heterozygous for DQw1 and DQw2 [22, 23]. A meta-analysis of 1166 cases and 6470 controls of diverse ethnic backgrounds derived from 23 studies confirmed that significant risk was associated with pSS and HLA class II alleles DRB1*03:01, DQA1*05:01 and DQB1*02:01 [24].

A large-scale study by Lessard et al. [11] replicated previously identified associations in the MHC, which represent the strongest genetic risk factors in pSS [odds ratio (OR) ~3.5]. Two highly significant associations were found. The first was with an extended 5 Mb haplotype peaking at HLA-DQB1 that carries multiple loci with expression levels that are altered by risk genotypes. A second independent effect was comprised of variants within a narrow region of association peaking at HLA-DQA1. These loci were also associated with pSS in a study of Europeans by Taylor et al. [12]. Li et al., in their GWAS of Han Chinese pSS patients, obtained the strongest associations with HLA-DRB1/HLA-DQA1 and HLA-DPB1 [13], while Taylor et al. [12] observed much weaker effects in their Asian subpopulation (HLA-DPB1 and HLA-DRB1). A non-canonical MHC-linked but HLA-independent risk locus for pSS is MHC class I polypeptide-related sequence A (MICA). The MICA gene is highly polymorphic and a strong association of pSS with the MICA*008 allele has been shown in two European cohorts [14].
## Table 1

Established and suggestive primary Sjögren’s syndrome risk loci based on GWASs

| Gene                          | SNP            | OR* (95% CI) | P-value     | Population          | References       |
|-------------------------------|----------------|--------------|-------------|---------------------|------------------|
| Risk loci associated at genome-wide significance level (P < 5.0E-08)                      |               |              |             |                     |                  |
| HLA-DQ81                      | rs11557587     | 3.53 (3.03, 4.11) | 7.65E-114  | European            | Lessard et al. [11] |
| HLA-DQA1                      | rs116232857    | 2.53 (2.24, 2.86) | 1.33E-96   | European            | Lessard et al. [11] |
| HLA-DRB1, HLA-DQA1            | rs9271573      | 2.02 (1.82, 2.23) | 3.00E-42   | Multi-ethnic        | Taylor et al. [12] |
| HLA-DQA1, HLA-DQB1            | rs3021302      | 2.24 (1.97, 2.54) | 2.00E-35   | Multi-ethnic        | Taylor et al. [12] |
| HLA-DQB1, HLA-DQA2            | rs9275572      | 2.28 (1.99, 2.61) | 7.00E-33   | European            | Taylor et al. [12] |
| HLA-DPB1, COL11A2             | rs4282438      | 1.58 (1.45, 1.72) | 8.77E-25   | Han Chinese         | Li et al. [13]   |
| MICA                          | MICA008        | 1.90 (1.56, 2.31) | 9.37E-09   | European            | Carapito et al. [14] |
| IFR5-TNPO3                    | rs3757387      | 1.44 (1.29, 1.62) | 2.73E-19   | European            | Lessard et al. [11] |
| IL12A                         | rs13739836     | 1.58 (1.36, 1.84) | 2.43E-16   | European            | Lessard et al. [11] |
| CXC5R5                        | rs7119038      | 0.74 (0.64, 0.86) | 1.10E-08   | European            | Lessard et al. [11] |
| TNIP1                         | rs8579837      | 1.43 (1.20, 1.71) | 3.30E-08   | European            | Lessard et al. [11] |
| OAS1                          | rs10774671     | 0.75 (0.66, 0.86) | 2.59E-09   | European            | Li et al. [15]   |
| TNFAIP3                       | rs5029393      | 1.67 (1.40, 1.99) | 7.75E-09   | Han Chinese         | Li et al. [13]   |
| GTF2IR1-GTF2I-NCF1            | rs117026326    | 2.20 (1.99, 2.43) | 1.31E-53   | Han Chinese         | Li et al. [13]   |
| STAT4                         | rs10553577     | 1.43 (1.26, 1.62) | 6.80E-15   | European            | Lessard et al. [11] |
| VR1A                           | rs10168266     | 1.44 (1.32, 1.57) | 1.17E-10   | European            | Lessard et al. [11] |
| IL12A                         | rs485497       | 1.30 (1.16, 1.46) | 1.17E-10   | European            | Lessard et al. [11] |
| BLK                            | rs2736345      | 1.30 (1.16, 1.47) | 4.97E-10   | European            | Lessard et al. [11] |
| RELN                          | rs7341475      | 1.39 (1.23, 1.57) | 3.00E-07   | European            | Lessard et al. [11] |
| PDRM1/ATG5                    | rs548234       | 1.52 (1.29, 1.78) | 3.61E-07   | Han Chinese         | Li et al. [13]   |
| STAT4                         | rs3821236      | 1.47 (1.27, 1.71) | 2.92E-07   | Han Chinese         | Li et al. [13]   |
| KLRG1                         | rs1805673      | 0.62 (0.51, 0.74) | 6.00E-07   | Asian               | Taylor et al. [12] |
| RPS29                         | rs6717551      | 0.62 (0.50, 0.76) | 2.73E-06   | Han Chinese         | Li et al. [13]   |
| CD222                         | rs1545257      | 0.81 (0.71, 0.91) | 2.47E-06   | European            | Lessard et al. [11] |
| LINCO0648/RR5S29              | rs67617551     | 0.62 (0.50, 0.76) | 2.73E-06   | Han Chinese         | Li et al. [13]   |
| ECLN3/SPSSI                  | rs712999       | 1.48 (1.25, 1.74) | 2.84E-06   | Han Chinese         | Li et al. [13]   |
| PRMD1                         | rs526531       | 1.22 (1.09, 1.38) | 2.93E-06   | European            | Lessard et al. [11] |
| PDE8B                         | rs181851       | 0.67 (0.56, 0.79) | 3.00E-06   | European            | Taylor et al. [12] |
| NACC2                         | rs4842091      | 1.39 (1.21, 1.61) | 5.00E-06   | European            | Taylor et al. [12] |
| GRIP2/CCDC174                 | rs79407237     | 0.61 (0.49, 0.75) | 5.00E-06   | European            | Taylor et al. [12] |
| ZNF43/ZNF208                  | rs2522092      | 1.45 (1.24, 1.70) | 5.86E-06   | Han Chinese         | Li et al. [13]   |

(continued)
**Non-MHC genes**

Beyond the associations with the MHC and X chromosome, the majority of the early studies of pSS genetics focused on candidate genes previously described as implicated in immune function or other autoimmune diseases. Larger genetic studies of pSS using unbiased, genome-wide approaches were first completed in 2013, one in patients of European ancestry and the other in a Han Chinese population [11, 13]. Genetic associations have now been established for pSS at levels reaching a stringent genome-wide significance threshold ($P < 5.0E-8$) and most of these findings have been replicated in independent studies [11-13, 15-18]. Additional candidate genes with suggestive evidence of association are summarized in Table 2. In general, the locations of peak association lie outside the protein coding regions and the functional consequences are not precisely known, but they are thought to involve regulation of gene expression and function. Candidate genes for these associated regions involve both innate and adaptive immune functions and offer interesting biological clues to the potential pathogenesis of pSS.

Genetic susceptibility to dysregulation of innate immunity is exemplified by associations of pSS with IFN regulatory factor 5 (IRF5), signal transducer and activator of transcription 4 (STAT4), IL-12A and FAM167A and C7orf72, IKZF1. While 5' effects have been described for celiac disease [49-51]; however, the effect in the region of IL12A in pSS appears to be distinct from those previously reported and is stronger in Europeans than in Asians [12].

OAS1 is a type I IFN-induced gene often seen among the overexpressed genes in autoimmune disease. The pSS risk allele gives rise to a splicing shift of the p46 isoform to alternative transcripts that lack translational response to type I IFN stimulation. At the protein level, the risk variant of OAS1 is associated with decreased OAS1 enzymatic activity and viral clearance [15].

NK cells are a critical cytotoxic lymphocyte type that plays a major role in innate immune responses. Based on the potential role of NK cells in animal models of sialadenitis, a candidate gene case–control study of NCR3/Nkp30 showed that two promoter single nucleotide polymorphisms (SNPs) are protective from pSS, suggesting that NK cells may promote an Nkp30-dependent inflammatory state in salivary glands [33].

The pathogenesis of pSS also involves dysregulation of adaptive immune responses. Several genes associated with pSS are primarily involved in B cell function. Variants in a region comprising two genes, FAM167A and the BLK (B lymphocyte kinase) locus, were first found to be associated with pSS in a candidate gene study of Swedish and Norwegian patients, and later replicated in other European and Asian cohorts [11, 28, 52]. FAM167A and BLK are transcribed in opposite directions, possibly from common promoter elements, and expression levels are inversely correlated. FAM167A encodes a novel protein, DIORA-1, which is highly expressed in the lung, suggesting a potential role in pulmonary involvement [53]. BLK is involved in B cell signalling that results in activation of multiple nuclear transcription factors. Reduced expression of BLK is hypothesized to lead to a breakdown in tolerance by allowing autoreactive cells to escape deletion [28].

Significantly increased levels of B cell activating factor (BAFF/BLyS), a member of the TNF family, have been demonstrated in serum of pSS patients compared with

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### Table 1 Continued

| Gene         | SNP       | OR* (95% CI) | P-value | Population      | References |
|--------------|-----------|--------------|---------|-----------------|------------|
| PHIP         | rs10943608| 1.23 (1.08, 1.40) | 6.22E-06 | European        | Lessard et al. [11] |
| C7orf72, IKZF1 | rs4917014 | 0.68 (0.57, 0.81) | 9.86E-06 | Han Chinese     | Li et al. [13] |
| RBMS3        | rs13079920| 1.35 (1.17, 1.55) | 2.90E-05 | Han Chinese     | Song et al. [16] |
| GTF2IRD1-GTF2I-NCF1 | rs117026326 | 3.12 (1.36, 7.17) | 7.30E-03 | European American | Zhao et al. [17] |

*The OR for each variant indicates the disease risk conferred by the minor allele. *Multi-ethnic cohort: complete SICCA cohort [19].
TABLE 2 Primary Sjögren’s syndrome risk loci derived from candidate gene studies

| Gene            | SNP                        | OR* (95% CI) | P-value | Function                                                                 | References                                |
|-----------------|----------------------------|--------------|---------|--------------------------------------------------------------------------|-------------------------------------------|
| IRF5-TNPO3      | rs2004640                  | 1.93 (1.15, 3.42) | 0.01    | Type I IFN and TLR signalling, cytokine production (e.g. IL-6, IL-12), apoptosis | Miceli-Richard et al. [25]               |
|                 | rs10488631                 | 1.57 (1.23, 1.99) | 3.23E-04|                                                                         | Nordmark et al. [26]                     |
|                 | CCGGGIndel                 | 2.00 (1.50, 2.70) | 6.60E-06|                                                                         | Miceli-Richard et al. [27]               |
|                 |                            | 1.49 (1.24, 1.79) | 2.41E-05|                                                                         | Nordmark et al. [26]                     |
|                 | rs13246321                 | 1.70 (1.35, 2.13) | 5.50E-06|                                                                         | Nordmark et al. [26]                     |
| STAT4           | rs7574865                  | 1.46 (1.09, 1.97) | 0.01    | Type I and II IFN pathways, NOD2 signalling                               | Korman et al. [29]                      |
|                 |                            | 1.40 (1.21, 1.62) | 4.70E-06|                                                                         | Palomin-Morales et al. [30]              |
|                 | rs7582694                  | 1.41 (1.14, 1.73) | 1.45E-03|                                                                         | Nordmark et al. [28]                     |
|                 |                            | 1.40 (1.15, 1.70) | 7.00E-04|                                                                         | Nordmark et al. [28]                     |
| BLK-FAM167A     | rs12549796                 | 1.37 (1.15, 1.63) | 4.70E-04| B cell signalling and development, thymopoiesis, insulin secretion and synthesis, NF-κB signalling | Nordmark et al. [28]                     |
| EBF1            | rs3843489                  | 1.68 (1.29, 2.18) | 9.90E-05| Enhancer of transcriptional activity during B cell development            | Nordmark et al. [28]                     |
| TNSF4/OX40L     | rs1234315                  | 1.34 (1.14, 1.64) | 7.40E-04| Co-stimulation, T cell proliferation, cytokine production                | Nordmark et al. [26]                     |
| BAFF/TNFSF13B   | 5’ haplotype               | 2.60 (1.70, 4.10) | 4.00E-05| B cell maturation, proliferation and survival, epithelial cell survival   | Nezos et al. [31]                       |
|                 | 5’ haplotype               | -             | 0.05    |                                                                         | Nezos et al. [32]                       |
|                 | (TATT, GTTC)               |               |         |                                                                         |                                           |
| NCR3/NKp30      | rs11575837                 | 0.48          | 0.0039  | Regulation of dendritic and NK cell communication, regulation of Th1 (IL-12 and IFN-γ) cytokines | Rusakiewicz et al. [33]                 |
| PTPN22          | rs2736191                  | 0.56          | 0.0019  |                                                                         | Rusakiewicz et al. [33]                 |
| TNIP1           | rs2476601                  | 2.42 (1.24, 4.75) | 0.01    | T cell activation                                                        | Gomez et al. [34]                       |
|                 | rs3792783                  | 1.33 (1.16, 1.52) | 3.40E-05| NF-κB signalling, EGF/ERK signalling, TNF-induced apoptosis              | Nordmark et al. [35]                     |
|                 | rs7708392                  | 1.21 (1.08, 1.36) | 1.30E-03|                                                                         | Nordmark et al. [35]                     |
| TNFAIP3         | rs2230926                  | 3.26 (1.31, 8.12) | 0.011   | NF-κB signalling (repressor), TNF-induced apoptosis, cytokine production (e.g. IL-1B) | Nocturne et al. [36]                    |
| BAFF-R          | His159Tyrr                 | 4.13 (1.19, 14.3) | 0.01    | NF-κB2 signalling                                                        | Papageorgiou et al. [37]                 |
| LTA/LTB/TNF     | rs1800629                  | 2.00 (1.61, 2.49) | 2.48E-10| Lymphoid organogenesis, tertiary lymphoid tissue maintenance             | Bolstad et al. [38]                     |
| MECP2           | rs909253                   | 1.59 (1.34, 1.89) | 1.25E-07| Methylation-mediated transcriptional silencing                           | Cobb et al. [39]                        |
| CHRM3           | rs7548522                  | 1.93 (1.24, 3.01) | 0.0033  | Muscarinic receptor                                                      | Appel et al. [40]                       |

*The OR for each variant indicates the disease risk conferred by the minor allele. bThe haplotype in the 5’ regulatory region of the BAFF gene is constituted by the SNPs rs9514827:-2841T>C, rs3759467:-2704T>C, rs1041569:-2701T>A and rs9514828:871C>T. cNezos et al. [32] analysed the 5’ regulatory region BAFF haplotype and also identified risk haplotypes for lymphoma. EGF/ERK: epidermal growth factor/extracellular signal-regulated kinase; NOD2: nitric oxide synthetase 2.

healthy controls and directly correlate with the degree of clinical activity and titre of circulating autoantibodies [31, 54–58]. Disease susceptibility for anti-Ro(SSA- and anti-La(SSB-positive pSS has been associated with a haplotype of four SNPs located in the 5’ regulatory region of the BAFF gene [31], where Nezos et al. [32] further characterized a different haplotype associated with pSS-lymphoma. A novel association in Lessard et al. [11] was attributed to a SNP located ~16kb 5’ of the coding region of CXCR5. CXCR5 associations have been
reported in multiple sclerosis and primary biliary cirrhosis [58, 60], and studies in pSS have found dysregulated CXCR5 gene expression in B cells in both the periphery and in salivary gland tissues [61, 62]. Carrying the CXCR5 pSS risk allele correlates with downregulated CXCR5 gene expression in peripheral blood B cells with concomitant low numbers of CXCR5+ peripheral blood B cells and homing of CXCR5+ cells in the minor salivary glands [63]. Targeting the CXCR5–CXCL13 axis might be a novel therapeutic approach in pSS.

Associations of pSS with risk variants in TNFAIP3 and TNIP1 that are important in NF-kB signalling may affect multiple cell types through a variety of mechanisms, including regulation of TNF-induced apoptosis, TLR4 activation and cytokine production [36]. The variants in TNIP1 identified by Lessard et al. [11] have so far not been replicated in Asian GWASs. The exact function of TNIP1 has not yet been defined, but it binds to TNFAIP3, which in turn suppresses TLR-induced apoptosis by negatively regulating NF-kB [64, 65].

Several additional associations with pSS that appear to be exclusive to Asian ancestry have been reported. The GWAS of Han Chinese pSS patients by Li et al. [13] identified an association with the novel GTF2I; the strongest associations were with SNPs extending from GTF2I to GTF2IRD1–GTF2I. A GWAS of Taiwanese Han Chinese pSS females replicated the association, which is also strongly associated with SLE in Asians, and further identified a suggestive variant in RBMS3, a modulator of acinar apoptosis and TGF-β signalling in the exocrine system [16, 17]. Taylor et al. [12] found an Asian-only association with KLRG1 and IKZF1; the latter was replicated in Han Chinese pSS patients [18]. IKZF1 is a transcription factor implicated in chromatin remodelling that regulates lymphocyte differentiation and shares a common interaction with members of the histone deacetylase family with GTF2I [18]. Zhao et al. [17] fine-mapped the region spanning GTF2IRD1–GTF2I and determined that the pSS risk allele tags the p.Arg90His variant in the neighbouring NCF1 gene, which is associated with pSS in Chinese.

In summary, apart from the HLA genes, the effect sizes of identified genetic associations are generally small and the functional impact of identified genetic variants has in most cases not been elucidated. Studying the interaction of the genetic risk loci with epigenetic alterations may increase our understanding of pSS genetic risk.

**Epigenetics**

Epigenetic modifications comprise mitotically heritable changes that can potentially influence the phenotype without involving any alteration to the DNA sequence itself. Epigenetic marks are relatively stable over time, facilitating the maintenance of cell identity, yet dynamic enough to change in response to various external and internal stimuli [66]. Epigenetic regulation is primarily accomplished by DNA methylation, histone modifications and non-coding RNAs (ncRNAs).

**Role of DNA methylation in pSS**

The most widely studied epigenetic mark is DNA methylation, depicted in Fig. 1. DNA methylation can modulate gene expression through chromatin rearrangements, leading to altered accessibility of gene regulatory regions for binding of transcription factor complexes [67]. In addition, a considerable number of Cytosine-phosphate-Guanine dinucleotide (CpG) sites are located in intergenic regions, indicating their contribution to long-distance regulatory processes.

Early investigations in pSS have assessed methylation at specific CpG sites in candidate genes or determined global methylation without single CpG site resolution in different cell and tissue types [68–71]. More recently, epigenome-wide association studies (EWASs) have applied the Infinium HumanMethylation450 (HM450k) BeadChip array [96], summarized in Table 3.

**EWASs in peripheral blood cells**

The first EWAS in pSS was published by Altorok et al. [75], where differentially methylated CpG sites in naïve CD4+ T cells were investigated. A main finding was hypomethylation at multiple sites of lymphotxin (LT)-α. Signalling of LT-α/β expressed on immune cells through the LT-β receptor (LTβR) expressed on epithelial cells is essential for the formation of ectopic lymphoid tissue, and upregulated expression of LT-β mRNA in pSS minor salivary glands has been described previously [38, 62]. Further results included hypomethylation at IFN-induced genes such as STAT1, IFI44L and IFITM1 and genes encoding members of solute carrier proteins. This study benefits from the analysis of a single cell type of naïve CD4+ T cells, which allows assessment of DNA methylation changes preceding immune activation.

In a later EWAS, Miceli-Richard et al. [76] interrogated methylation patterns in peripheral CD19+ B cells and CD4+ T cells. A more pronounced differential methylation was seen in B cells compared with T cells, which may indicate aberrant gene regulation in pSS B cells as an important disease mechanism. Hypomethylated CpG sites in pSS B cells were found at IFN-induced genes, more prominently when only patients positive for anti-Ro/SSA and/or La/SSB antibodies were included. Patients with high disease activity also presented with more abundant differential methylation [97]. However, the relatively low number of study subjects precludes any firm conclusion regarding the impact of methylation changes on disease activity.

A larger EWAS was performed by Imgenberg-Kreuz et al. [77]. In whole blood, the most significantly hypomethylated sites were found at type I IFN-induced genes such as MX1, IFI44L, PARP9 and IFITM1, where the difference in methylation levels was more pronounced when only anti-Ro/SSA and/or anti-La/SSB antibody-positive patients were included, confirming results by Miceli-Richard et al. [76]. A larger difference in methylation levels at IFN-regulated genes was detected when analysing peripheral blood B cells rather than whole blood, suggesting that single cell type analyses may be advantageous. This study also investigated gene expression patterns in B cells, which showed
increased mRNA expression of IFN-induced genes in pSS, corroborating the IFN signature both at the methylation and gene expression level.

Epigenetic signatures are cell type specific and whole blood cell composition differs between patients with pSS and controls [77]. This can be partly overcome by reference-based methods for statistical deconvolution of major blood cell types, although the results may still be confounded by cellular subtypes. In order to fully dissect epigenetic changes in different immune cell types in pSS, analyses of fractionated cell populations are warranted. Given the central role for B cells in pSS pathogenesis, longitudinal studies of DNA methylation patterns of peripheral naive B cells and activated B cell subtypes would be of great interest to decipher epigenetic changes during immune activation. Further, studying plasmacytoid dendritic cells (pDCs), the main IFN-producing cell type, would be of importance, but these cells are infrequent in the circulation and epigenetic analysis may prove difficult. To summarize the results from the three EWASs, hypomethylation at IFN-induced genes is observed in different cell types and is more pronounced in patients with anti-Ro/SSA and/or anti-La/SSB antibodies. This is in concordance with previous findings of a gene expression

| **TABLE 3** Epigenetic studies in primary Sjögren’s syndrome |
|---------------------------------------------------------------|
| **Genome-wide studies of DNA methylation using HM450k array technology** |
| **Cells/tissues** | **Number of cases/controls** | **Main outcome** | **References** |
| Naive CD4+ T cells | 11/11 | Hypomethylation at LTA and IFN-induced genes | Altorok et al. [75] |
| CD4+ T cells, CD19+ B cells | 26/22 | Larger methylation differences in B cells than in T cells | Miceli-Richard et al. [76] |
| Whole blood, CD19+ B cells, minor salivary glands | 100/400, 24/47, 15/13 | Hypomethylation at IFN-induced genes; meQTL effects at pSS GWAS risk loci | Imingenberg-Kreuz et al. [77] |
| Whole blood | 24/24 (case–case) | Hypomethylation at a ncRNA in high fatigue | Braekke-Norheim et al. [78] |
| Minor salivary glands | 13/13 | Enrichment for differential methylation in promoters | Cole et al. [79] |
| SGECs | 8/4 | Hypomethylation at IFN-induced genes | Charras et al. [80] |

| **Studies of differential miRNA expression** |
| **Method** | **Cells/tissues** | **Main outcome** | **References** |
| RT-qPCR | PBMCs | Dysregulation of miR-146a/b, miR-155, miR-223, miR-483-5p | Pauley et al. [81], Kapsogeorgou et al. [82], Zilahi et al. [83], Shi et al. [84], Gourzi et al. [85], Chen et al. [86] |
| | SGECs | Upregulation of miR-200b-5p, in AB-positive pSS downregulation of let-7b | Kapsogeorgou et al. [82], Gourzi et al. [85], Kapsogeorgou et al. [87] |
| | Minor salivary glands | Dysregulation of miR-16 and miR-181a | Gourzi et al. [85], Wang et al. [88] |
| RT-qPCR OpenArray | Serum | Correlation of miRNA expression with clinical parameters | Lopes et al. [89] |
| Microarray | PBMCs | Correlation of miRNA expression with clinical parameters | Peng et al. [90], Williams et al. [91] |
| | CD14+ monocytes | Uregulation of miR-181a | Wang-Renault et al. [92] |
| | CD4+ T cells, CD19+ B cells | Inverse correlation of miR-30b-5p and BAFF expression in B cells | Alevizos et al. [93] |
| | Minor salivary glands | Inverse correlation of miR-768-3p and miR-574 with focus score | Alevizos et al. [93] |
| Next-generation sequencing | PBMCs | Downregulation of miR-105-5p | Chen et al. [94] |
| | Minor salivary glands | Upregulation of miR-5100 | Tandon et al. [95] |

AB: autoantibody; meQTL: methylation quantitative trait loci; RT-qPCR: reverse transcriptase quantitative PCR; SGECs: salivary gland epithelial cells.
IFN signature in whole blood, different peripheral blood mononuclear cells (PBMCs) and salivary gland tissue from patients with pSS, preferentially seen in antibody-positive patients with pSS [41, 62, 98–100].

EWASs in salivary glands and salivary gland epithelial cells

In the study by Imgenberg-Kreuz et al. [77], analysis of minor salivary gland biopsies identified the most significantly hypomethylated CpG site at the IFN-induced gene OAS2. Cole et al. [79] also performed an EWAS on minor salivary glands; among genes enriched for promoter differential methylation were transcription factors, long ncRNAs, miRNAs and the cell surface marker genes CXCR5 and TNFRSF13B (TACI). A limitation of both studies is the fact that minor salivary gland tissue consists of multiple cell types, where inflammatory cells are only sparsely present in control glands, and therefore the results must be interpreted with caution.

To overcome the effects of cellular heterogeneity, Charras et al. [80] studied long-term cultured salivary gland epithelial cells derived from minor salivary glands of patients with pSS and controls. IFN-induced genes were among differentially methylated genes, corroborating the results obtained with whole minor salivary glands. These findings emphasize that immunologically active epithelial cells display similar signalling pathways as blood cells, supporting the concept of pSS as autoimmune epithelitis [101]. Analyses of immune cells dissected from patient salivary glands may be another possibility. Comparing DNA methylation levels in, for example, peripheral naive and infiltrating activated B cells from the same patient could yield important insights into gene regulation during B cell homing and activation, providing additional information to the results of epigenetic analyses of peripheral blood cells only.

DNA methylation patterns of clinical subphenotypes

DNA methylation reflects the epigenetic status at a certain time point that might correlate with disease activity or specific disease manifestations. In a case–case EWAS, Braekke Norheim et al. [78] investigated differential DNA methylation in whole blood samples from pSS patients with either high or low fatigue, assessed by the fatigue visual analogue scale. Hypomethylation of a ncRNA associated with high fatigue was the main finding. Limitations of this study are the relatively small sample size and the study design analysing fatigue as a categorical variable.

Genetic regulation of DNA methylation

Genetic risk variants in pSS may potentially have functional consequences by influencing DNA methylation patterns of target genes, thereby modulating transcriptional accessibility. In the study by Imgenberg-Kreuz et al. [77], genetic variants at seven pSS GWAS loci were tested for association with methylation levels in whole blood from control individuals in a methylation quantitative trait loci analysis [11]. The results indicate that all tested pSS GWAS alleles have the potential to affect DNA methylation levels at nearby genes, most notably within the HLA region and at the IRF5-TNPO3 locus (Fig. 2), pointing to a connection between pSS susceptibility loci and epigenetic regulation. However, studies investigating methylation quantitative trait loci effects in fractionated cells and additional tissue types from patients with pSS are still lacking.

Histone modifications

The N-terminal tails of histones protrude out of the nucleosome and are subject to a variety of covalent post-translational modifications with acetylation and methylation of lysine (K) residues at histones H3 and H4 being the most studied modifications (Fig. 1) [102]. Briefly, acetylation leads to a relaxed chromatin conformation permissive to transcription, while deacetylation results in transcriptional repression by inducing chromatin compaction [103]. Konsta et al. [104] found an association of genetic risk variants with marks for promoters and enhancers in reference B cells and with enhancers in reference monocytes. In the study by Imgenberg-Kreuz et al. [77], hypomethylated sites in pSS were enriched for enhancer regions in reference B and T cells, whereas hypermethylated sites predominantly overlapped with a histone mark that indicates actively transcribed genes. For now, there are no studies available analysing histone marks directly in primary cells from patients with pSS. However, attempts at integration of multiple layers of genome-wide data such as SNP genotyping, DNA methylation and histone modifications hold great promise to provide further insights into the functional roles of pSS-associated variants.

ncRNAs

ncRNAs comprise a large family of different RNAs, with miRNAs being the most extensively studied. miRNAs are a class of evolutionary highly conserved small single-stranded RNA molecules with a length of 19–25 nucleotides that can bind to target mRNA transcripts and interfere with translation (Fig. 1). Aberrant expression of miRNAs has been demonstrated in numerous autoimmune diseases [105].

In pSS, studies in peripheral blood cells, minor salivary gland tissue and salivary gland epithelial cells have identified a number of differentially expressed miRNAs, summarized in [81–95]. However, the target genes and the functional role of these miRNAs remain elusive in many cases. General limitations of these studies are small sample sizes, heterogeneous clinical parameters and potential confounding of the results by variation in cell type distribution. A consistent finding of several studies is upregulated expression of miR-146a/b in PBMCs from patients with pSS [81, 83, 84, 86]. A proposed mechanism for miR-146-dependent regulation of immune responses is via negative feedback mechanisms targeting TLR signalling [106, 107]; dysregulated miR-146 expression may promote excess inflammation, leading to autoimmune responses.
The development of expression microarray and next-generation sequencing technologies has facilitated simultaneous analysis of expression patterns of a larger number of miRNAs. Peng et al. [90] identified overexpression of miR-181a in PBMCs in a Chinese pSS cohort compared with controls. In contrast, a study by Wang et al. [88] found miR-181a, along with miR-16 expression, to be downregulated in labial salivary gland tissue in pSS. Additional studies investigating miR-181a and miR-16 expression in different tissue types in larger, clinically well-characterized cohorts are needed to ascertain the role of these miRNAs in pSS pathogenesis. In a recent study, Wang-Renault et al. [92] analysed miRNA expression patterns in peripheral T cells and B cells from patients with pSS and healthy controls. When only anti-Ro/SSA-positive patients were included in the analysis increased differential miRNA expression was seen in B cells, but not in T cells.

A few studies have correlated clinical manifestations with miRNA expression. Lopes et al. [89] performed unsupervised hierarchical clustering based on serum expression levels of nine small ncRNAs and found correlations with a number of laboratory parameters. A recent study by Kapsokefalou et al. [87] reported that downregulated miR200b-5p expression in minor salivary gland biopsies preceded lymphoma development, suggesting miR200-5p levels as a predictive biomarker for lymphoma. Additional studies comprising larger sample sizes are required to replicate these intriguing findings and to further explore the role of miRNA expression for lymphomagenesis in pSS.

In summary, dysregulated miRNAs are likely to promote excess inflammation and autoimmune responses in pSS. The studies point to aberrant miRNA expression preferentially affecting B cell activation in antibody-positive pSS, a pattern also observed for pSS-related differential DNA methylation.

**Conclusion**

As of today, genetic studies in pSS on a genome-wide scale comprising large cohorts have been successful in establishing convincing associations, albeit restricted to individuals of European and Asian ancestry. The most robust association has been determined for HLA class II genes, reflecting that antigen presentation to CD4+ T cells and subsequent immune activation is an important disease mechanism. The consistent genetic associations with IRF5 and STAT4 in the type I IFN system are in agreement with DNA hypomethylation and overexpression of IFN-induced genes present in different pSS cell types [41, 42, 61, 98, 99, 108]. A schematic summary of genetic and epigenetic mechanisms associated with pSS susceptibility is depicted in Fig. 3.

Genetic and epigenetic studies continue to reveal the complexity of pSS. To advance the field further, next-generation whole-genome sequencing of large cohorts of clinically well-characterized pSS patients are warranted, intersected with genome-wide transcriptional profiling and
epigenetic data. Application of advanced analytical approaches, such as imputing genotypes across the genome to increase density, and hence information content of SNP data, will further aid in genetic studies of pSS. Continuing to increase sample sizes will be critical to enhancing statistical power for discovery of additional novel genes and identifying patient subsets based on molecular classifications. Analysis of patient subphenotypes will advance our understanding of the disease mechanisms underlying the diverse clinical manifestations and add novel insights into biomarkers and potential therapeutic targets.

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