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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.

REVIEW

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.

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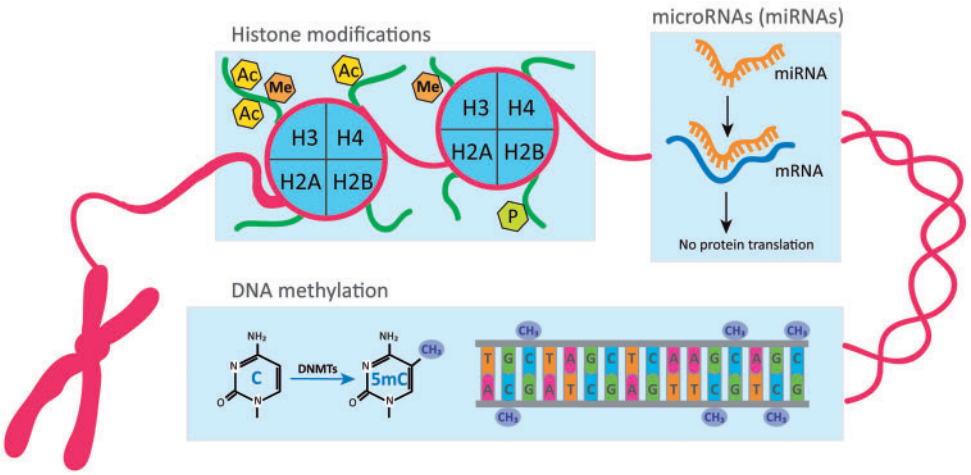
Genetics

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

Fig. 1 Schematic representation of fundamental epigenetic mechanisms involved in the regulation of gene expression



Histone modifications are covalent post-translational modifications within the N-terminal tail of histone proteins. The most studied histone marks include Ac, Me and P of lysine residues at histones H3 and H4. DNA methylation refers to the covalent modification of C residues to 5mC by DNMTs typically in the context of CpG dinucleotides. Histone modifications and DNA methylation modulate chromatin structure and transcriptional accessibility of the DNA, thereby altering gene expression. At the post-transcriptional level, miRNAs, a class of small ncRNA, can bind to mRNA of target genes interfering with their expression. 5mC: 5-methyl-cytosine; Ac: acetylation; C: cytosine; DNMTs: DNA methyltransferases; Me: methylation; P: phosphorylation.

sibling concordance (λs) 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 class II, within *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-DRB9*). 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[a] (95% CI)	*P*-value	Population	References
Risk loci associated at genome-wide significance level ($P < 5.0E-08$)					
HLA-DQB1	rs115575857	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[b]	Taylor *et al*. [12]
	rs9271573	2.29 (2.01, 2.62)	3.00E-34	European	Taylor *et al*. [12]
	rs9271588	0.57 (0.53, 0.63)	8.52E-37	Han Chinese	Li *et al*. [13]
HLA-DQA1, HLA-DQB1	rs3021302	2.24 (1.97, 2.54)	2.00E-35	Multi-ethnic[b]	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	MICA*008	1.90 (1.56, 2.31)	9.37E-09	European	Carapito *et al*. [14]
IRF5-TNPO3	rs3757387	1.44 (1.29, 1.62)	2.73E-19	European	Lessard *et al*. [11]
	rs17339836	1.58 (1.36, 1.84)	2.43E-16	European	Lessard *et al*. [11]
	rs3823536	1.49 (1.34, 1.65)	3.00E-14	Multi-ethnic[b]	Taylor *et al*. [12]
	rs3823536	1.54 (1.36, 1.76)	7.00E-11	European	Taylor *et al*. [12]
	rs59110799	1.72 (1.49, 1.99)	3.00E-13	Multi-ethnic[b]	Taylor *et al*. [12]
	rs3807306	1.50 (1.32, 1.71)	6.00E-10	European	Taylor *et al*. [12]
STAT4	rs10553577	1.43 (1.26, 1.62)	6.80E-15	European	Lessard *et al*. [11]
	rs10168266	1.44 (1.32, 1.57)	1.77E-17	Han Chinese	Li *et al*. [13]
	rs11889341	1.40 (1.26, 1.56)	9.00E-10	Multi-ethnic[b]	Taylor *et al*. [12]
	rs7574865	1.51 (1.31, 1.75)	2.00E-08	European	Taylor *et al*. [12]
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]
CXCR5	rs7119038	0.74 (0.64, 0.86)	1.10E-08	European	Lessard *et al*. [11]
TNIP1	rs6579837	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	rs5029939	1.67 (1.40, 1.99)	7.75E-09	Han Chinese	Li *et al*. [13]
GTF2IRD1-GTF2I-NCF1	rs117026326	2.20 (1.99, 2.43)	1.31E-53	Han Chinese	Li *et al*. [13]
	rs117026326	1.98 (1.67, 2.35)	1.10E-15	Han Chinese females	Song *et al*. [16]
	rs117026326	2.03 (1.06, 2.57)	4.60E-09	Chinese	Zhao *et al*. [17]
IKZF1	rs4917129	0.70 (0.61, 0.79)	4.24E-08	Han Chinese	Qu *et al*. [18]
Suggestive risk loci associated at significance level $P > 5.0E-08$					
HLA-DPB1	rs9277554	1.65 (1.37, 2.00)	3.00E-07	Asian	Taylor *et al*. [12]
HLA-DPB1/COL11A2	rs3117221	1.47 (1.26, 1.71)	9.52E-07	Han Chinese	Li *et al*. [13]
HLA-DQA1	rs9405117	0.54 (0.42, 0.69)	9.83E-07	Han Chinese	Li *et al*. [13]
HLA-DQB1	rs6928482	1.43 (1.23, 1.66)	2.52E-06	Han Chinese	Li *et al*. [13]
HLA-DMB/PSMB9	rs11756897	1.57 (1.32, 1.87)	2.76E-07	Han Chinese	Li *et al*. [13]
LOC105370283-PTMAP5	rs17074492	1.53 (1.31, 1.79)	6.00E-08	European	Taylor *et al*. [12]
TNFAIP3	rs6933404	1.29 (1.13, 1.47)	6.53E-08	European	Lessard *et al*. [11]
PTTG1	rs2431098	0.81 (0.73, 0.91)	2.28E-07	European	Lessard *et al*. [11]
RELN	rs7341475	1.39 (1.23, 1.57)	3.00E-07	Multi-ethnic[b]	Taylor *et al*. [12]
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]
MIS18BP1/LINC00871	rs1957173	0.61 (0.50, 0.74)	7.00E-07	Multi-ethnic[b]	Taylor *et al*. [12]
IRAK1BP1	rs1507153	1.26 (1.11, 1.43)	7.09E-07	European	Lessard *et al*. [11]
HTR2A/LINC00562	rs7999279	1.42 (1.23, 1.63)	1.00E-06	Multi-ethnic[b]	Taylor *et al*. [12]
SHISA9	rs9938751	0.59 (0.48, 0.73)	1.00E-06	European	Taylor *et al*. [12]
ZNF208	rs10416159	1.49 (1.26, 1.75)	1.54E-06	Han Chinese	Li *et al*. [13]
PRCC/SH2D2A	rs16837677	1.54 (1.29, 1.84)	2.00E-06	Multi-ethnic[b]	Taylor *et al*. [12]
NFAT5	rs7192380	1.28 (1.16, 1.42)	2.00E-06	Multi-ethnic[b]	Taylor *et al*. [12]
ITSN2	rs1545257	0.81 (0.71, 0.91)	2.47E-06	European	Lessard *et al*. [11]
LINC00648/RPS29	rs67617551	0.62 (0.50, 0.76)	2.73E-06	Han Chinese	Li *et al*. [13]
EGLN3/SPTSSA	rs712299	1.48 (1.25, 1.74)	2.84E-06	Han Chinese	Li *et al*. [13]
PRDM1	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)

Table 1 Continued

Gene	SNP	OR[a] (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]

[a]The OR for each variant indicates the disease risk conferred by the minor allele. [b]Multi-ethnic cohort: complete SICCA cohort [19].

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 (*IL12A*) and 2′–5′ oligoadenylate synthetase 1 (*OAS1*). All are involved in type I IFN signalling and overexpression of IFN-induced genes, the so-called IFN signature, which is predominanatly seen in pSS patients positive for anti-Ro/SSA and/or anti-La/SSB [11, 41, 42].

IRF5 is a transcription factor that acts downstream of the toll-like receptors (TLRs) and type I IFN receptor to promote the expression of numerous antiviral and pro-inflammatory proteins [43, 44]. *IRF5* is associated with pSS in patients of all investigated ancestries, the most significant association being a CGGGG insertion/deletion (indel) polymorphism in the promoter region [12, 13, 25–27]. An additional risk haplotype that is present only in European ancestry results from variants spanning both *IRF5* and *TNPO3* [28]. A recent model suggests that within this haplotype, the risk allele increases binding of the transcription factor ZBTB3 to the *IRF5* promoter, resulting in upregulated *IRF5* expression as the potential pathogenic mechanism [45].

STAT4 is a transcription factor, activated by type I IFN, IL-12 and IL-23, that is also associated with SLE and RA [26, 29, 30, 46]. Interestingly, genetic models that support additive effects between the major risk alleles in *IRF5* and *STAT4* have been described [26, 46].

IL-12 is an immunomodulatory cytokine primarily secreted by monocytes and dendritic cells that acts upstream of STAT4 and plays a critical role in the differentiation of Th1 cells and production of IFN-γ by T cells and NK cells [47]. The *IL12A* gene encodes the p35 subunit that forms the IL-12 heterodimer together with the p40 subunit encoded by *IL12B* [48]. Variants in the 3′ end of *IL12A* have been reported for primary biliary cirrhosis, 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 depletion [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

Table 2 Primary Sjögren's syndrome risk loci derived from candidate gene studies

Gene	SNP	OR[a] (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]
	CGGGGindel	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*. [28]
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		Palomino-Morales *et al*. [30]
	rs7582694	1.41 (1.14, 1.73)	1.45E-03		Nordmark *et al*. [26]
		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*. [28]
BAFF/TNFSF13B	5′ haplotype[b]	2.60 (1.70, 4.10)	4.00E-05	B cell maturation, proliferation and survival, epithelial cell survival	Nossent *et al*. [31]
	5′ haplotype[c] (TATT, GTTC)	–	<0.05		Nezos *et al*. [32]
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]
	rs2736191	0.56	0.0019		Rusakiewicz *et al*. [33]
PTPN22	rs2476601	2.42 (1.24, 4.75)	0.01	T cell activation	Gomez *et al*. [34]
TNIP1	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, TLR4 signalling, cytokine production (e.g. IL-1B)	Nocturne *et al*. [36]
BAFF-R	His159Tyr	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]
	rs909253	1.59 (1.34, 1.89)	1.25E-07		
MECP2	rs17435	1.33 (1.12, 1.59)	0.0016	Methylation-mediated transcriptional silencing	Cobb *et al*. [39]
CHRM3	rs7548522	1.93 (1.24, 3.01)	0.0033	Muscarinic receptor	Appel *et al*. [40]

[a]The OR for each variant indicates the disease risk conferred by the minor allele. [b]The 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. [c]Nezos *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 ~16 kb 5′ of the coding region of *CXCR5*. *CXCR5* associations have been

reported in multiple sclerosis and primary biliary cirrhosis [59, 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 $CXCR5^+$ B 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-κB 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-κB [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 families 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 dinulceotide (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 [68–74]. 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 lymphotoxin (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

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**
Naïve 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	Imgenberg-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]
		Downregulation of miR200-5p predictive of pSS lymphoma	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	Upregulation of miR-181a	Peng *et al*. [90]
	CD14$^+$ monocytes	Dysregulation of miRNAs in TGF-β pathway	Williams *et al*. [91]
	CD4$^+$ T cells, CD19$^+$ B cells	Inverse correlation of miR-30b-5p and *BAFF* expression in B cells	Wang-Renault *et al*. [92]
	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.

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 naïve 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

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 naïve 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 tissues types from patients with pSS are still lacking.

Histone modifications

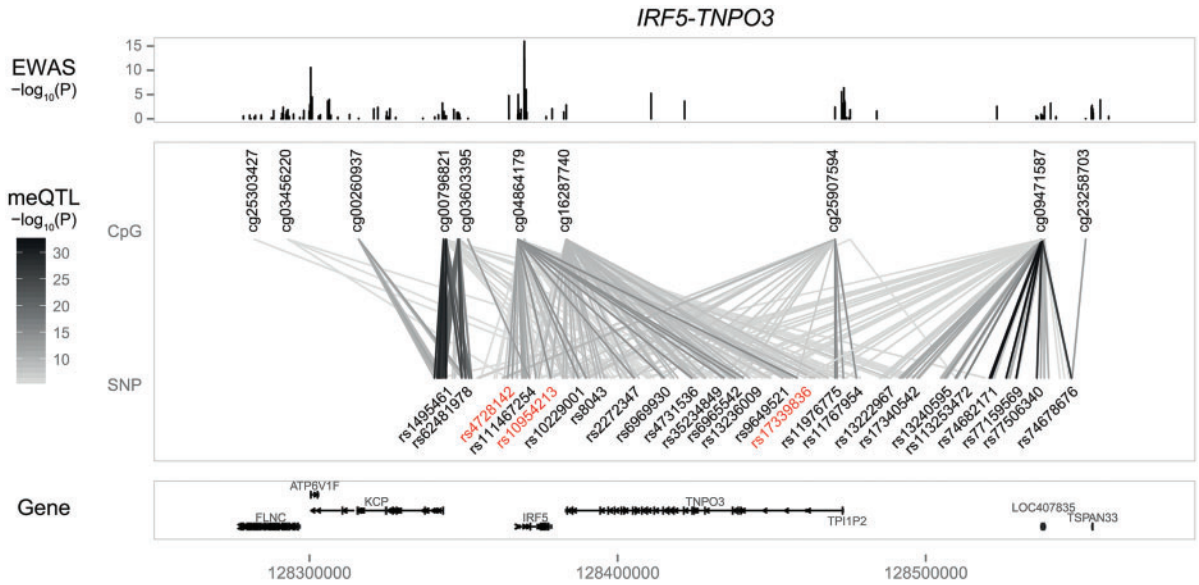
The N-terminal tails of histones protrude out of the nucleosome and are subject to a variety of covalent post-translational modifications with acetylation and methylation of lysine (K) residues at histones H3 and H4 being the most studied modifications (Fig. 1) [102]. Briefly, acetylation leads to a 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.

FIG. 2 Illustration of genetic regulation of DNA methylation at the *IRF5-TNPO3* pSS susceptibility locus



Data presented here are derived from meQTL analysis by Imgenberg-Kreuz *et al.* [73]. Top panel: Results (as −log10 *P*-values) of the pSS case–control EWAS of 100 pSS patients and 400 controls, with cg04864179 being the top differentially methylated CpG site within this locus. Middle panel: Significant meQTLs analysed in 382 controls, genotyped on the ImmunoChip, with the lines connecting the CpG site and corresponding SNPs in darker grey representing a stronger significance of the meQTL. GWAS index SNPs are indicated in red. Bottom panel: The RefSeq genes in the region. meQTL: methylation quantitative trait loci.

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 Kapsogeorgou *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.

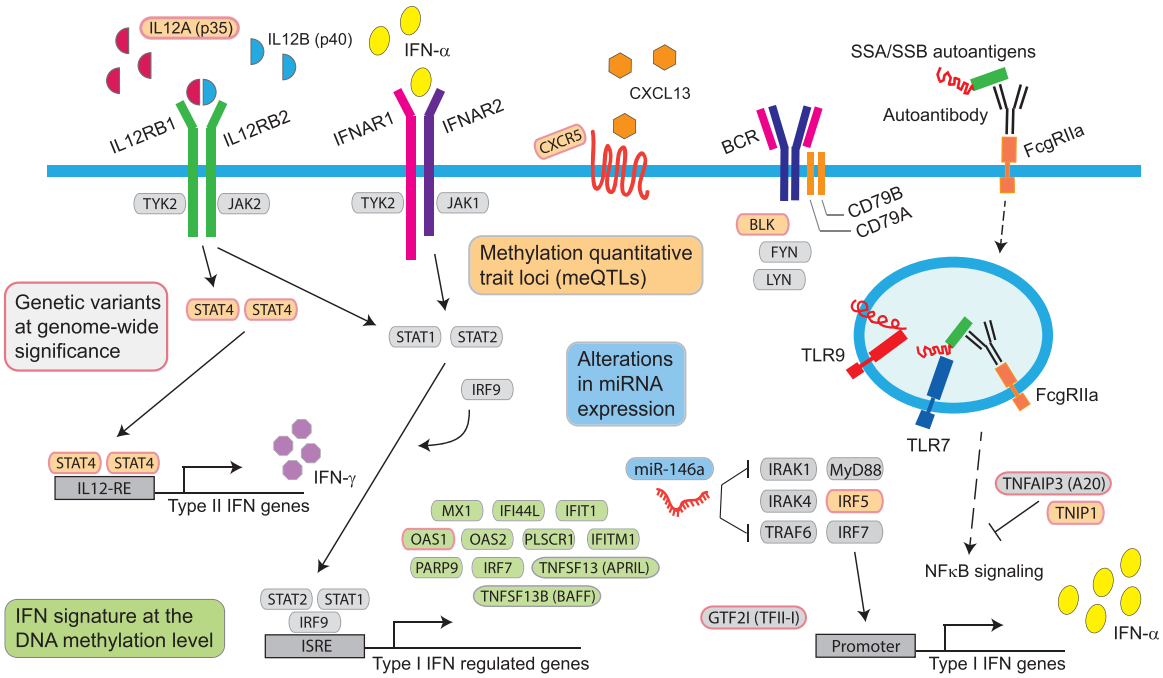
In summary, dysregulated miRNAs are likely to promote excess inflammation and autoimmune responses in pSS. The studies point to aberrant miRNA expression preferentially affecting B cell activation in antibody-positive pSS, a pattern also observed for pSS-related differential DNA methylation.

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

Fig. 3 Schematic summary of genetic and epigenetic mechanisms associated with pSS susceptibility



Boxes with pink borders represent genes with SNPs associated with pSS susceptibility at genome-wide significance outside of the HLA region and include *BLK*, *CXCR5*, *GTF2I*, *IL12A*, *IRF5*, *OAS1*, *STAT4*, *TNFAIP3* and *TNIP1*. Green boxes: IFN-induced genes such as *MX1*, *IFI44L*, *OAS1*, *OAS2*, *TNFSF13B* and *IRF7,* with hypomethylated CpG sites in pSS. Orange boxes: meQTLs, referring to association between a genetic variant (SNP) and the methylation level at a nearby CpG site demonstrated at *BLK*, *CXCR5*, *IL12A*, *IRF5-TNPO3*, *STAT4* and *TNIP1*. Blue boxes: Alterations in miRNA expression, including miRNA-146a, have been identified in pSS. meQTL: methylation quantitative trait loci.

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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