

Insights into the genetic basis of systemic sclerosis: immunity in human disease and in mouse models

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Abstract: Systemic sclerosis (SSc; scleroderma) is a chronic, multisystem autoimmune disease characterized by vasculopathy, fibrosis, and autoantibodies. In the past decade, great efforts have been made to investigate genetic susceptibility for SSc. To date, over 20 gene loci have been identified as risk factors for SSc in large genome-wide association studies and confirmed by independent replication studies. However, the biological relevance of these genetic associations is still largely unknown. Exploring the mechanism behind these risk loci is essential to better understand disease pathogenesis and to identify novel therapeutic targets. Mouse model studies including knockout, knockin and knockdown of these genes can advance our understanding of pathogenic cellular and molecular mechanisms in human disease. Although such mouse model systems do not exactly correspond to human disease, they can provide insight into pathological mechanisms that influence disease pathways. In this review, we discuss recent findings regarding the genetic basis of SSc in the setting of genetic manipulation of these pathways in murine models.

Keywords: GWAS, ImmunoChip study, type I interferon pathway, genetic mutation animal models

Introduction

Systemic sclerosis (scleroderma, SSc) is a chronic, multisystem autoimmune disease characterized by vasculopathy, fibrosis, and autoimmunity.¹ At present, the etiology of SSc is unknown and disease-related mortality remains high. Reported prevalence and incidence estimates of SSc vary widely (prevalence estimates range from 31–659/million population and incidence rates range from 4–23/million/year) according to geographic location, ethnicity, and methods of case ascertainment.² The interplay among genetic background, environmental factors, and autoimmunity as they relate to SSc pathogenesis is unclear. A comprehensive understanding of the genetic and molecular basis of SSc is crucial in order to develop appropriately targeted therapies.

In this review, we summarize our current knowledge about the genetic basis of SSc susceptibility identified in genome-wide association studies (GWASs) and related studies, and we present recent evidence from mouse models of SSc. We also discuss the consistency and discrepancy between findings derived from human and animal studies.

Heritability of SSc

In spite of recent advances in identifying genetic risk loci for SSc, only a small proportion of heritability has been explained in this complex autoimmune disease. Family studies have demonstrated that the risk of SSc is increased among first-degree

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relatives of patients, compared to the general population. In a study of 703 families in the United States, including 11 multiplex SSc families, familial SSc occurred significantly more frequently in siblings and first-degree relatives of SSc cases compared to in the population as a whole, with a relative risk (RR) of 15.³ A family history of SSc is the strongest risk factor identified to date, although the absolute risk is low. The largest SSc-twin study published thus far, with 42 twin pairs including 24 monozygotic pairs, failed to demonstrate a higher disease concordance in the monozygotic versus the dizygotic twins. However, concordance for the presence of antinuclear antibodies was significantly higher in the monozygotic twin pairs (90%) than in the dizygotic pairs (40%),⁴ suggesting that there was a shared genetic susceptibility to autoimmunity even among the unaffected co-twins. Further study in the same cohort showed a higher level of concordance among monozygotic twins regarding the gene expression profile of cultured dermal fibroblasts,⁴ demonstrating that a stronger genetic predisposition to SSc is apparent at the molecular level in skin fibroblasts in the monozygotic twins. A population-based study utilizing the Utah Population Database identified a significantly increased relative risk for Raynaud's phenomenon (RR =6.38, 95% confidence interval [CI] =3.44–11.83) among first-degree relatives of SSc subjects compared to non-SSc families, as well as increased risk of other autoimmune diseases including systemic lupus erythematosus (SLE), Sjögren's syndrome, dermatomyositis, rheumatoid arthritis, and interstitial lung disease.⁵ Taken together, these studies demonstrate the presence of a genetic predisposition to autoimmunity in general and SSc in particular.

Candidate gene studies, GWAS, and ImmunoChip analysis in SSc

The contribution of different genetic factors to SSc susceptibility in general and to its clinical and autoantibody subsets is now widely accepted. Multiple candidate gene studies in relatively small SSc cohorts^{6,7} have reported susceptibility loci for SSc. Unlike a candidate gene approach in which single nucleotide polymorphisms (SNPs) in a small number of genes are tested for association between cases and controls, a GWAS provides an unbiased view of SNP variants across the human genome. The ImmunoChip array is a custom SNP genotyping array with high density mapping of autoimmune disease (AID)-associated loci. The first large GWAS report of Radstake et al in 2010, followed by the GWAS report of Allanore et al in 2011, both with independent discovery and validation cohorts, identified

the strongest association with SSc to reside in the major histocompatibility complex (MHC) class II region.^{8–10} In the human leukocyte antigen (HLA) region, the *HLA-DQB1* locus was highly associated with the anticentromere antibody (ACA)-positive subgroup of SSc, and the *HLA-DPB1* locus was associated with antitopoisomerase antibody (ATA)-positive SSc. In the MHC class III region, the *NOTCH4* gene was highly associated with both ACA-positive and ATA-positive subgroups.⁹ Susceptibility alleles of the MHC region were well established by GWASs and the ImmunoChip study and are summarized in Table 1. Additional associated genes in the MHC region include the non-HLA genes *TAP2*, *MICA*, and *MICB*, which encode membrane proteins involved in antigen presentation and immune cell activation. These studies also emphasized the contribution of differential genetic components in the development of subphenotypes of SSc.

The GWAS and the subsequent ImmunoChip study also identified and firmly established the role of multiple SSc-susceptibility genes outside the MHC region, including *CD247*, interferon regulatory factors (IRFs) 5 and 8 (intergenic region *TNPO3-IRF5* and *IRF8*), and signal transducer and activator of transcription 4 (*STAT4*). In addition, these and subsequent studies confirmed the association of previously reported genes, including *TNIP1*, *TNFAIP3*, *IL12RB2*, *CSK*, *TNFSF4*, *BANK1*, *BLK*, *PTPN22*, and *PPARG*, as SSc susceptibility loci (Table 2).^{6,10–13}

Table 1 Established susceptibility alleles in MHC region from SSc GWASs and/or ImmunoChip study

Gene	Phenotype	GWAS (P-value**)	ImmunoChip (P-value**)	References
<i>HLA-DQB1</i>	ACA+	2.61×10 ⁻⁵⁴	1.94×10 ⁻¹⁷	8–10,14
<i>HLA-DQA1</i> *	SSc	1.47×10 ⁻¹¹	1.12×10 ⁻²⁵	8–10,14
	ATA+		4.46×10 ⁻²³	
	ACA+			
<i>HLA-DPB1</i> *	SSc	4.64×10 ⁻⁸	3.21×10 ⁻²³	8–10,14
	ATA+	2.42×10 ⁻²⁰		
<i>HLA-DRB1</i> *	ACA+	NA	1.79×10 ⁻³⁹	9,14
	ATA+	1.47×10 ⁻¹¹	3.55×10 ⁻²²	
<i>NOTCH4</i>	SSc	6.82×10 ⁻¹²	NA	8–10
	ACA+	8.84×10 ⁻²¹		
	ATA+	1.14×10 ⁻⁸		
<i>TAP2</i>	SSc	NA	5.87×10 ⁻⁶²	14
	ACA+		2.74×10 ⁻¹¹	
<i>MICA</i>	SSc	7.18×10 ⁻⁹	NA	8
<i>MICB</i>	SSc	5.68×10 ⁻⁹	NA	8

Notes: *Variant at amino acid part of the binding pocket of the molecule; **P-value of the most highly associated SNP for the SSc phenotype.

Abbreviations: ACA, anti-centromere antibody; ATA, anti-topoisomerase antibody; GWAS, genome-wide association study; MHC, major histocompatibility complex; NA, not associated; SSc, systemic sclerosis; SNP, single-nucleotide polymorphism.

Table 2 Selected relevant susceptibility alleles in the non-MHC region found with GWAS, Immunochip, and meta-analysis in SSc

Gene	Phenotype	GWAS (P-value*)	Immunochip (P-value*)	Meta-analysis (P-value*)	References
<i>IRF5</i>	SSc	3.84×10^{-14}	1.01×10^{-10}	7.53×10^{-20}	8–10,14,34
	IcSSc	1.64×10^{-10}		9.63×10^{-14}	
	DcSSc			4.38×10^{-15}	
	ACA+			4.06×10^{-11}	
	ATA+			6.05×10^{-17}	
<i>IRF8</i>	IcSSc	2.32×10^{-12}	3.32×10^{-3}	NA	9,14
<i>CD247</i>	SSc	3.39×10^{-9}	NA	4.26×10^{-1}	8–10
	IcSSc	2.66×10^{-6}			
<i>STAT4</i>	SSc	1.90×10^{-10}	2.94×10^{-12}	2.26×10^{-13}	8–10,25
	IcSSc	7.70×10^{-8}	NA		
<i>TNIP1</i>	SSc	4.68×10^{-9}		3.26×10^{-5}	10,14,17
	IcSSc			1.23×10^{-3}	
	dcSSc			5.57×10^{-4}	
	ATA+		NA	1.31×10^{-5}	
	ACA+		2.33×10^{-3}	2.27×10^{-3}	
<i>DNASE1L3</i>	SSc	NA	5.95×10^{-10}	3.36×10^{-16}	14
	IcSSc		1.36×10^{-11}	1.28×10^{-20}	
	ACA+		7.50×10^{-18}	4.25×10^{-31}	
<i>SCHIP1-IL12A</i>	SSc	NA	9.74×10^{-8}	1.22×10^{-11}	14
	IcSSc		1.05×10^{-6}	1.53×10^{-11}	
	ACA+		1.53×10^{-5}	2.40×10^{-8}	
<i>IL12RB2</i>	SSc	4.74×10^{-6}		2.82×10^{-9}	9,11,14
	ACA+		3.72×10^{-5}		
<i>TNFSF4</i>	SSc		NA	1×10^{-5}	9,69
	IcSSc	7.70×10^{-4}		7.70×10^{-4}	
	ACA+			1.62×10^{-2}	
	ATA+			9.05×10^{-3}	
<i>BANK1</i>	DcSSc	1.03×10^{-2}	2.47×10^{-3}	1.03×10^{-2}	9
<i>BLK</i>	DcSSc		2.60×10^{-5}	1.39×10^{-3}	9,14
	IcSSc			1.54×10^{-4}	
<i>TNFAIP3</i>	ACA+	1.45×10^{-4}		1.45×10^{-4}	14,70
	SSc	NA		1.16×10^{-7}	
	ACA+		8.78×10^{-4}		
	DcSSc			5.2×10^{-9}	
	Fibrosing alveolitis			2.5×10^{-6}	
	Pulmonary hypertension			1.3×10^{-5}	
<i>PTPN22</i>	ATA+	NA	7.10×10^{-3}	2.20×10^{-16}	14,53
<i>CSK</i>	SSc	7.19×10^{-7}	NA	5.04×10^{-12}	69
<i>PPARG</i>	SSc	1.64×10^{-6}	NA	5.0×10^{-7}	10,13
<i>MECP2</i>	DcSSc	NA	NA	5.26×10^{-4}	16
<i>IRAK1</i>	DcSSc	NA	NA	1.56×10^{-4}	15
	ATA+			1.07×10^{-6}	
	SSc-related fibrosing alveolitis			9.05×10^{-4}	
	Pulmonary fibrosis			3.90×10^{-2}	

Note: *P-value of the most highly associated SNP for SSc phenotype.

Abbreviations: ACA, anti-centromere antibody; ATA, anti-topoisomerase antibody; DcSSc, diffuse cutaneous systemic sclerosis; GWAS, genome-wide association study; IcSSc, limited cutaneous systemic sclerosis; MHC, major histocompatibility complex; NA, not associated; SSc, systemic sclerosis; SNP, single-nucleotide polymorphism.

Utilizing the Immunochip array, three new SSc risk loci were identified in a large multiple cohort study, including *DNASE1L3* at 3p14, the *SCHIP1-IL12A* locus at 3q25, and *ATG5* at 6q21, as well as a suggested association of the *TREHDDX6* locus at 11q23.¹⁴ The associations of SSc risk loci *STAT4*, *IRF5*, and *PXK* genes, which had been previously reported in GWASs, were again confirmed in the Immunochip analysis.

In addition, two genes on the X chromosome have been associated with SSc. These include a polymorphism in the interleukin (IL)-1 receptor-associated kinase 1 gene (*IRAK1*) associated with SSc-related pulmonary fibrosis, and the methyl-CpG-binding protein 2 gene (*MECP2*) associated with diffuse SSc.^{15,16} However, there were discrepant results for *PSORS1C1* and *RHOB* polymorphisms that were not confirmed in an independent cohort.^{10,17}

Together, these studies have clearly established that SSc is a complex autoimmune disease with multiple genetic variants in different loci. Many of these genes are also known to be risk factors for other autoimmune diseases, including rheumatoid arthritis and SLE.^{18–20} SSc and SLE are two archetypal systemic autoimmune diseases sharing some features, like antinuclear antibodies, but exhibiting very different clinical phenotypes. In order to gain additional insight into the genetic basis of these two diseases, a pan-meta-analysis of two GWASs in SSc and SLE cohorts was performed. The sample size included a total of 21,109 subjects (6,835 cases and 14,274 controls). This analysis determined that the SLE susceptibility loci *PXK* (PX domain containing serine/threonine kinase) and *JAZF1* (juxtaposed with another zinc finger gene 1) shared susceptibility loci with SSc. This study also validated a new KIAA0319-like gene (*KIAA0319L*) as a susceptibility locus for SSc and SLE. In addition, the gene product of *KIAA0319L* was found to be over expressed in peripheral blood cells from both SSc and SLE patients, suggesting that this polymorphism was related to immune function.²¹

These findings confirmed and added new evidence for the strong autoimmune genetic component underlying SSc pathogenesis and further reinforced the concept that there are shared genetic and pathogenic mechanisms among autoimmune diseases.

Type I interferon pathway gene variants associated with SSc and relevant animal studies

It is widely known and accepted that type I interferons (IFNs) are important key regulators of host defense in the innate immune system. IFN regulatory factors are transcriptional regulators of type I IFNs, and IFN-inducible genes and play a pivotal role in regulation of many facets of innate and adaptive immune responses.²² Both SSc GWAS reports and the Immunochip study confirmed genetic variants of *IRF* genes as susceptibility loci for SSc (Tables 2 and 3). Based on its unique contributory role in type I IFN pathways in the immune system, *IRF* genes and their protein products may represent valuable targets for therapeutic interventions in autoimmune diseases, including SSc.

Animal models are very useful to test a specific hypothesis about disease pathogenesis in vivo. Although animal models that exhibit all aspects of SSc are not currently available, several general SSc mouse models are well characterized and widely used, as noted in Table 4. More specific mouse models, developed by forward genetic studies of experimentally

Table 3 Interferon pathway genes associated with human SSc

Gene	Expression cells	Function
<i>STAT4</i>	Macrophages, DCs, Th1 cells, NK cells	<ul style="list-style-type: none"> • Required for most IL-12 biological responses including IFN-γ production • Th1 differentiation (Kaplan et al²⁷)
<i>IRF5</i>	B-cells, splenic cells, DCs	<ul style="list-style-type: none"> • Regulate TLR signaling pathway proinflammatory cytokine genes-Type I IFNs, IL-6, TNF-α, IL-12 et al (Tamura et al²²)
<i>IRF8</i>	B-cells, T-cells, macrophages, CD11b-negative DCs	<ul style="list-style-type: none"> • Required for TLR9 signaling • Promote Type I IFN production in DCs • Stimulate IFN-γ and PAMP-inducible genes-IL-12, iNOS, FeγRI (Tamura et al²²)

Abbreviations: DCs, dendritic cells; IFN, interferon; IL, interleukin; NK, natural killer; PAMP, pathogen-associated microbial pattern; SSc, systemic sclerosis; Th1, T helper 1; TLR, toll-like receptor; TNF, tumor necrosis factor.

induced mutations, can selectively test for the role of genes and proteins that are relevant to the corresponding human disease. Some of these mouse models with specific targeted deletions in type I interferon pathway genes are described in Table 5.

STAT4

STAT4 is one of the seven members of the signal transducer and activator of transcription (STAT) family, which are important transcription factors for T-cell differentiation and T-cell receptor signaling pathways.²³ It is expressed in macrophages, dendritic cells (DCs), T helper (Th)1 cells, and natural killer (NK) cells. STAT4 plays a key role in type I IFN receptor signaling by being activated and trans located to the nucleus after type I IFN receptor ligation.²⁴ It is also required for IFN- γ production and Th1 cell differentiation (see Table 3). Candidate gene studies in European and Japanese cohorts and both large SSc GWASs have identified and validated the association between SSc with variants in the *STAT4* gene.^{8–10,25} In addition, the *STAT4* SNP rs7574865 and the *IRF5* SNP rs2004640 were observed to have additive effects in terms of susceptibility to both SSc and SSc-related fibrosing alveolitis/interstitial lung disease.²⁶ In an animal study, STAT4-deficient (*stat4*^{-/-}) mice showed reduced IFN- γ production and reduced IL-12 response in lymphocytes, along with an increased propensity toward the development of Th2 cells.²⁷ The role of STAT4 deficiency has been studied in two murine models of SSc: the bleomycin model and the tight skin 1 (TSK1) model.²⁸ STAT4-deficient (*stat4*^{-/-}) mice showed protection against the inflammatory and fibrotic processes in the bleomycin-induced SSc animal model. However, when the STAT4-deficient mice were crossed with the non inflammatory TSK1 model to generate TSK1 mice

Table 4 Overview of general mouse models in SSc

Model	Features	Innate immunity	Additive immunity	Autoantibodies	IFN signature	References
Bleomycin-induced mouse model	Skin inflammation; fibrosis	Yes	Yes	No	Yes	71
TSK1 mouse	Skin subcutaneous fibrosis; lung emphysema	Yes	Yes	Yes	No	72
TSK2 mouse	Skin inflammation; fibrosis	Yes	Yes	Yes	Not evaluated	73
GVHD mouse	Skin, lung and kidney inflammation; fibrosis	Yes	Yes	Yes	Yes	74

Abbreviations: GVHD, graft-versus-host disease; IFN, interferon; SSc, systemic sclerosis; TSK, tight skin.

deficient for STAT4 (*stat4^{-/-}/tsk1*), collagen deposition was not noted to be decreased. These findings suggested that the transcription factor STAT4 exerts its potent profibrotic effects by controlling T-cell activation and proliferation with subsequent inflammatory cytokine release. These findings also underscore the important role of STAT4 implied by the *STAT4* genetic association with SSc.²⁸

Further fine-mapping and functional studies are crucial for elucidating the role of genetic variants in the IFN signaling pathway of the immune system in the pathogenesis of SSc.

IRF5

As noted above, the *IRF5* gene codes for IFN regulatory factor 5 (IRF5), which is a transcription factor that induces the transcription of genes involved in the immune response, including type I IFN, macrophage inflammatory protein-1 α , and - β , 2,5-oligoadenylate synthetase, protein kinase R, pre-B-cell colony enhancing factors, and IFN-induced hepatitis C-associated micro tubular aggregate proteins.^{29,30} IRF5 negatively regulates cell growth and proapoptotic genes, including *p21*, *Bak*, *DAP kinase 2*, and *Bax*.³⁰ It is mostly expressed in B-cells, splenic cells and DCs.²² Genetic variants

of *IRF5* were found to be associated with SSc susceptibility in multiple cohorts.^{8,26,31–34} This may have functional significance, as the minor allele of the *IRF5* SNP rs4728142 was shown to be predictive of longer survival and milder pulmonary involvement in two independent SSc cohorts.³⁵ This minor allele was also associated with lower IRF5 transcript expression in monocytes from SSc patients and controls, suggesting functional relevance of SNP rs4728142, or its association with other SNPs, for IRF5 expression. A recent study by Carmona et al³⁴ revealed that *IRF5* SNP haplotype blocks containing three haplotypic risk alleles (including rs4728142, rs2004640, rs10488631) showed strong association with overall SSc and was not associated with any specific sub-phenotype.

Animal studies have shown that mutation at exons 2–9 of the *IRF5* gene in mice (resulting in *irf5^{-/-}* null mice) caused increased sensitivity to viral infection and lower expression levels of type I IFN in the serum. IFN production was impaired in the toll-like receptor ligand-stimulated macrophages from these *IRF5* mutant mice.³⁶ These studies demonstrate the important role of IRF5 in type I IFN function leading to immune-system abnormalities, which could be relevant to SSc pathogenesis.³⁴ Further studies are needed

Table 5 Interferon pathway gene knockout in mouse model

Model	Deletion location	Features	References
STAT4 deficient	Exon of the <i>STAT4</i> gene	<ul style="list-style-type: none"> Reduced IFN-γ production; reduced IL-12 response; impaired Th1 cell development and propensity towards to Th2 cells; abrogated NK cells cytotoxicity; mice are viable, fertile Protected against bleomycin-induced skin fibrosis; do not abrogate skin fibrosis in TSK1 model by cross-breeding mice 	27,28
IRF5 deficient	Exons 2–9 of the <i>IRF5</i> gene	<ul style="list-style-type: none"> Normal IFN-α induction; reduced IL-2, IL-6, TNF-α induction in DCs; deficiency in Th1 response; mice developed normally; normal hematopoietic cell population Not yet confirmed in SSc animal models 	36
IRF8 deficient	Exon 2 of the <i>IRF8</i> gene; BXH2 mutation in <i>IRF8</i> gene	<ul style="list-style-type: none"> Abnormal populations of B-cells, T-cells, granulocytes and macrophages; mice are viable and fertile as young animals Not yet confirmed in SSc animal models 	41

Abbreviations: DCs, dendritic cells; IFN, interferon; IL, interleukin; NK, natural killer; SSc, systemic sclerosis; Th1, T helper 1; Th2, T helper 2; TLR, toll-like receptor; TNF, tumor necrosis factor; TSK1, tight skin 1.

to explore the functional role of *IRF5* in SSc animal models such as the bleomycin model or the TSK1 mouse.

IRF8

The *IRF8* gene product is an immune cell-specific IRF family member that is mostly expressed in macrophages, DCs, and B-cells.²² The *IRF8* SNP rs11642873 was identified and confirmed as a risk factor for limited cutaneous systemic sclerosis and ACA-positive SSc in a large GWAS and meta-analysis conducted in European and North American cohorts.⁹ The subsequent Immunochip study identified SNP rs16941172 in the *IRF8* gene as being highly associated with the ACA-positive sub phenotype of SSc.¹⁴ Another independent study in a Japanese population further added evidence that the *IRF8* gene is a susceptibility locus for SSc.³⁷ These studies support involvement of IRF8-expressing innate immune cells including B-cells, DCs, and macrophages in disease development.

Animal studies further support a contributory role for IRF8 in the pathogenesis of autoimmune disease (Table 4). BXH2 mice carry a point mutation in the *IRF8/Icsbp* gene that changes arginine to cysteine in position 294 (*IRF8* R294C).³⁸ The mutation is within the IRF association domain important for the interaction of IRF8 with partner proteins.^{39,40} The *IRF8* R294C mutation abolished the development of CD8 DCs without impairing plasmacytoid DC development, and it eliminated production of some inflammatory cytokines, while retaining that of type I IFNs.⁴¹ These studies demonstrated the complex role of IRF8 in DC and monocyte differentiation and immune responses. Further research efforts utilizing SSc animal models are needed to investigate a functional role of IRF8 in human SSc.

Other selected gene variants in human SSc and related animal models

NOTCH4

The *NOTCH4* gene codes for the neurogenic locus notch homolog 4 (Notch4) protein, which is a transmembrane receptor that plays a key role in a variety of developmental processes by controlling cell fate decisions. Notch4 is mostly expressed in blood vessels and the notch signaling pathway controls key functions in vascular smooth muscle and endothelial cells, which may be relevant to the microvascular damage in SSc. Although the gene resides in the MHC region of chromosome 6, the *NOTCH4* polymorphism is associated with SSc independently from HLA genes or alleles. A recent study has identified a consistent association of the *NOTCH4* gene with both ACA-positive and ATA-positive subgroups in

four GWAS SSc cohorts (German, Spanish, Dutch, and US) independent of the HLA associations.⁹ In addition, *NOTCH4* polymorphisms have been associated with other autoimmune disorders, including type I diabetes,⁴² rheumatoid arthritis,⁴³ and SLE.^{44,45}

In an animal study by Villa and colleagues in 2001,⁴⁶ Notch4 was the only receptor expressed in the mouse endothelium among all of the four Notch family receptors. Furthermore, mouse gain-of-function and loss-of-function studies revealed that *NOTCH1* plus *NOTCH4* knockouts resulted in embryonic lethality with vascular remodeling defects.⁴⁷ Although mice deficient in Notch4 alone were viable and fertile,⁴⁷ angiogenesis was disrupted in these mice, suggesting that Notch signaling plays a more important role in angiogenesis. Endothelial cell-specific expression of an activated form of Notch4 driven by the *flk1*-promoter led to embryonic lethality with abnormal vessel structure and patterning in mice,⁴⁸ whose phenotype is similar to that seen in Notch1/Notch4-deficient mice.⁴⁷ The similar vascular phenotypes observed in both the transgenic (gain-of-function) and the knockout mice (loss-of-function) suggest that there may be an appropriate balance needed in Notch expression levels. To date, how the Notch4 signaling pathway is involved in the pathogenesis of SSc is unclear. Further investigation including *NOTCH4* gene manipulation in different SSc mouse models studies may provide better understanding of this pathway.

The search for causal variants

For the most part, the multiple SNPs that have been associated with SSc (and several other autoimmune diseases) are not in coding regions or in known regulatory regions, and therefore it is not clear how these variants affect downstream pathways resulting in disease. However, there are two exceptions to this situation, which are functional variants believed responsible for the *PTPN22* and *DNASE1L3* associations.

PTPN22

The *PTPN22* gene encodes protein tyrosine phosphatase non receptor 22 (PTPN22) which is an intracellular protein tyrosine phosphatase. Polymorphisms in this gene are strongly associated with susceptibility to several autoimmune diseases, including type 1 diabetes, rheumatoid arthritis, and SLE.^{49–51} The risk allele of *PTPN22* results in the exchange of arginine residue at position 620 to tryptophan (R620W). The *PTPN22* R620W SNP was also significantly associated with the ATA-positive subset of SSc in large cohort case-control

studies.^{52,53} The Arg620Trp substitution increases the intrinsic lymphoid-specific phosphatase activity of PTPN22, which reduces the threshold for T-cell receptor signaling and thus promotes autoimmunity.⁵⁴

The corresponding homologue in mice is the PEST (proline–glutamic acid–serine–threonine) domain-enriched tyrosine phosphatase (PEP).^{55–58} Recently, several animal models of the PEP gene have been extensively investigated. PEP knockout mouse,⁵⁹ PEP 619W knockin mouse^{60,61} and PEP knockdown mouse⁶² studies were established.^{59,63} In particular, PTPN22 619W mouse mutation is the homologue for the human Arg620-Trp substitution and results in spontaneous autoimmunity in susceptible mouse strains, essentially replicating the risk effect of the PTPN22 620W in human autoimmune diseases. Furthermore, findings from PTPN22 genetic mouse models shed new light on both cellular as well as molecular mechanisms of the effect of PTPN22 on adaptive and innate immunity.⁶³

DNASE1L3

The DNASE1L3 gene codes for deoxyribonuclease 1-like 3, a member of the human DNase1 family. It is expressed primarily in liver and spleen and is secreted by macrophages.⁶⁴ As one of three human homologs of DNase1, DNASE1L3 functions as an endonuclease capable of cleaving both single- and double-stranded DNA. DNase1 is the major nuclease present in serum, urine, and secretions and is believed to be responsible for the removal of DNA from nuclear antigens at sites of high cell turnover. The non synonymous rs35677470 SNP in exon 8 of this gene results in a substitution of Arg/Cys at the amino acid position 206 of the protein (R206C). An in vitro study indicated that this substitution results in absent DNase activity.⁶⁵ Recent GWASs and Immunochip analysis identified and confirmed DNASE1L3 genelocus as a risk factor for autoimmune disease, including rheumatoid arthritis, SLE, and SSc.^{14,66,67} Furthermore, animal studies revealed that DNASE1L3 deficiency in the Murphy Roths Large (MRL) and New Zealand black x New Zealand white (NZB/W) F1 murine models of SLE resulted in increased susceptibility to and severity of SLE.⁶⁵ DNASE1 knockout mice showed the presence of antinuclear antibodies, deposition of immune complexes in glomeruli and full-blown glomerulonephritis in a DNase1 dose-dependent manner.⁶⁸ Altogether, these data suggest a link between defective apoptotic DNA breakdown and the production of autoantibodies and autoimmunity. Further studies to explore regulatory mechanism of DNASE1L3 utilizing animal models in SSc may lead to a better understanding of the pathogenesis, new diagnostic methods, and new therapeutic approaches to SSc and other autoimmune diseases.

Conclusion

To date, only a small proportion of the observed heritability in SSc has been explained. Multiple SSc genetic approaches, including candidate gene studies, the two large GWASs, and the Immunochip studies, have firmly established and confirmed the association of SSc with multiple non-MHC loci including STAT4, IRF5, IRF8, CD247, BANK1, TNFAIP3, TNIP1, IL12RB2, CSK, TNFSF4, BLK, DNASE1L3, SCHIP1-IL12A, and ATG5, as well as HLA-DQA1/B1, HLA-DOA, HLA-DPB1, HLA-DRB1, NOTCH4, TAP2, and MICA/B genes within the MHC region. With few exceptions, such as PTPN22 and DNASE1L3, the causal variants responsible for these disease associations are not known. In addition, the functional relevance of the identified polymorphisms is not clear. Animal studies manipulating the SSc susceptibility gene homologues are needed to identify and characterize the new gene variants and for further confirmatory studies of the “missing heritability” in the larger scale of GWAS and family studies. Interaction between multiple susceptibility alleles of genes and gene-environmental interaction may have greater effects in pathogenesis of SSc and other autoimmune diseases. Animal model studies not only significantly extend our understanding on the mechanism of disease progress, but also may provide new ideas and tools for identifying novel therapeutic target genes and molecules for human diseases.

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Disclosure

The authors report no conflicts of interest in this work.

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