

The genetics of scleroderma (systemic sclerosis)

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Purpose of review

To determine the advances made in the genetics of scleroderma in candidate gene association studies.

Recent findings

Over the past 18 months, a number of candidate gene studies using large case–control series in scleroderma have been reported. The studies have identified multiple genes involved in immune regulation including *BANK1*, *C8orf13-BLK*, *IL-23R*, *IRF5*, *STAT4*, *TBX21*, and *TNFSF4* as susceptibility genes for the development of SSc. Furthermore, gene–gene interaction studies suggest that *IRF5*, *STAT4*, and *BANK1* as well as *TBX21* and *STAT4* interact with regard to scleroderma susceptibility. Many of the genetic variants associated with SSc susceptibility are shared among other autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus.

Summary

Candidate gene association studies have substantially advanced our understanding of the pathogenesis of SSc and demonstrate that SSc is a polygenic, autoimmune disease.

Keywords

genetics, polymorphisms, scleroderma, systemic sclerosis

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Introduction

Scleroderma or systemic sclerosis (SSc) is a multisystem, autoimmune disease clinically characterized by progressive fibrosis in the skin and internal organs [1]. Although fibrosis and endothelial dysfunction are clinical hallmarks of SSc, inflammation and autoimmunity are likely the root cause [1]. Autoimmunity and inflammation are currently best exemplified by the presence of multiple but nonoverlapping SSc-associated autoantibodies [e.g., antitopoisomerase I (topo), anticentromere ACA] and anti-RNA polymerase III (POL) subsets in scleroderma patients, which, in turn, tend to identify distinct clinical subsets [2,3]. Skin biopsies of early scleroderma skin demonstrate perivascular infiltrates of mononuclear inflammatory cells [4]. Furthermore, patients with SSc have increased circulating levels of cytokines that have distinct patterns based on the SSc-associated autoantibodies [5]. Recent studies in SSc patients have identified dysregulation of type I interferon (IFN) pathways similar to those seen in systemic lupus erythematosus (SLE) [6–8]. First described using whole-genome gene expression profiling of peripheral blood from SSc patients, this original observation has been confirmed in subsequent studies that demonstrate increased expression of type I IFN-induced genes in CD14⁺ monocytes, CD4⁺ T cells, and skin biopsies from SSc patients compared with healthy controls [8–10]. Together, these data strongly

implicate inflammation and autoimmunity in the pathogenesis of SSc. The origins of these immune alterations are not known, but it is likely that the genetic background of these individuals strongly influences their immune regulation and disease susceptibility.

SSc is a complex disease that occurs in genetically predisposed individuals who have encountered specific environment exposures and/or other stochastic factors [11]. Candidate gene studies have implicated multiple genetic factors that increase the risk of individuals to develop SSc (recently reviewed in [11]). These studies are often limited by small cohorts, the clinical heterogeneity of SSc, and lack of replication. Significant recent efforts have been made to develop large collaborative cohorts of SSc patients to identify and confirm candidate genes that are SSc susceptibility factors. Furthermore, given that the modest magnitude of the risks of individual genetic polymorphisms, these studies are exploring how gene–gene interactions might impart greater risk than the individual gene. In the current review, we will discuss the recent evidence from candidate gene studies that support a strong genetic link to scleroderma.

Human leukocyte antigen associations

Polymorphisms in the human leukocyte antigen (HLA) region have been linked to SSc susceptibility, and these

Table 1 Recent candidate gene associations with susceptibility to scleroderma

Gene	Reference	Cohorts (single/multiple)	Number of subjects		Replication	
			SSc	Controls	Yes	No
<i>BANK1</i>	Rueda <i>et al.</i> [20**]	Multiple	2380	3270	[21*]	
<i>CTGF</i>	Fonseca <i>et al.</i> [16]	Multiple	500	500	[17*]	[18*,19*]
<i>C8orf13-BLK</i>	Gourh <i>et al.</i> [22*]	Multiple	1639	1416	–	–
<i>IL-23R</i>	Agarwal <i>et al.</i> [23*]	Single	1402	1038	–	[24,25]
<i>IRF5</i>	Dieude <i>et al.</i> [26**]	Multiple	881	760	[27*]	–
<i>STAT4</i>	Rueda <i>et al.</i> [28**]	Multiple	1317	3113	[29*,30*,31**]	–
<i>TBX21</i>	Gourh <i>et al.</i> [30*]	Multiple	1402	1038	–	–
<i>TNFSF4</i>	Gourh <i>et al.</i> [32*]	Single	1059	698	–	–

studies been previously reviewed [11–13]. A large case–control study was recently performed on white, black, and Hispanic SSc patients to determine the class II HLA associations with the SSc-specific autoantibodies (topo, ACA, POL) [14]. The strongest positive associations in whites and Hispanics were the *HLA-DRB1*1104*, *DQA1*0501*, *DQB1*0301* haplotype, and *DQB1* alleles encoding a nonleucine residue at position 26 (*DQB126*epi*). In contrast, the *HLA-DRB1*0701*, *DQA1*0201*, *DQB1*0202* haplotype, and *DRB1*1501* haplotype were negatively associated with SSc susceptibility in whites and Hispanics. SSc in blacks was associated with *HLA-DRB1*0804*, *DQA1*0501*, and *DQB1*0301* alleles. With regard to the SSc-associated autoantibodies, *HLA-DPB1*1301* showed the highest odds ratio for ATA-positivity. *HLA-DQB1*0501* and *DQB1*26* epi-alleles showed the highest association with ACA-positivity. Lastly, *HLA-DRB1*0404*, *DRB1*11*, and *DQB1*03* alleles in whites and Hispanics and *DRB1*08* in blacks were associated with POL-positivity. These data indicate unique and multiple HLA class II effects in SSc and that there are distinct associations with the SSc-associated autoantibody subsets.

In a smaller study from Spain, the *HLA-DRB1*11* was also associated with SSc susceptibility, whereas the *HLA-DRB1*0701* had a protective effect [15]. These investigators also found that *HLA-DRB1*1104* was associated with topo-positivity and *HLA-DRB1*01* and *HLA-DRB1*05* alleles were associated with ACA-positivity. These data are similar to those reported in the U.S. cohort above [14].

Candidate gene associations

Many candidate gene studies have demonstrated associations of specific single nucleotide polymorphisms, in genes involved in immune regulation, vascular function, and extracellular matrix (ECM), with SSc susceptibility (reviewed in [11]). In the last year, several reports have sought to confirm one of these associations (*CTGF*) [16,17*–19*]. In addition, a number of reports have investigated novel associations of multiple genes involved in inflammation and autoimmunity with SSc

susceptibility and these that will be discussed in this review (Table 1) [20**,21*–23*,24,25,26**,27*,28**,29*,30*,31**,32*].

Connective tissue growth factor

CTGF induces proliferation, ECM production, and chemotaxis of mesenchymal cells, processes central to fibrosis [33,34]. *CTGF* is upregulated in scleroderma skin biopsies as well as cultured fibroblasts from scleroderma patients [34–36]. The first report of an association of the G-allele at position –945 of *CTGF* was in discovery and replication cohorts totaling 500 scleroderma patients and 500 controls [16]. These data were confirmed in a Japanese cohort [17*]. Interestingly, in this study, 46% of the patients had diffuse disease, 48% had interstitial lung disease, and 31% were topo-positive [17*]. Secondary analyses demonstrated that the association is primarily in patients within these groups.

Two additional studies have attempted to replicate the association of the *CTGF* –945 promoter polymorphism with SSc [18*,19*]. The North American cohort failed to identify an association of this polymorphism using 749 SSc patients (including 257 with diffuse SSc and 124 topo-positive patients) and 429 controls [18*]. In a study with 1180 SSc patients of European descent, Rueda *et al.* [19*] were not able to confirm the association of the *CTGF* –945 polymorphism with SSc. The inability to replicate the association of *CTGF* in two larger cohorts of SSc patients suggests that *CTGF* may not be a strong genetic determinant for SSc susceptibility, but given the importance of *CTGF* in SSc and the intriguing observation of stronger associations in the diffuse and topo-positive patients, additional studies are needed to clarify these discrepancies.

T-box expressed in T cells (T-bet, *TBX21*)

The *TBX21* gene, located on chromosome 17q21.32, encodes for the transcription factor, T-box expressed in T cells (T-bet) [37]. Initially identified in CD4⁺ T cells, where it is the critical transcription factor for the development of Th1 cells, it has also subsequently been shown to regulate dendritic cell and B-cell function [38]. Furthermore, T-bet-deficient mice develop more

severe dermal fibrosis in the bleomycin-induced skin fibrosis model [39,40]. Given these observations, it was of interest to determine whether *TBX21* polymorphisms were associated with SSc. In a recent study involving two large independent cohorts of North American white SSc patients and controls, the rs11650354 variant of *TBX21* was associated with SSc susceptibility with a recessive pattern of inheritance [30]. The association was observed in limited and diffuse SSc patients as well as patients who were topo-positive, ACA-positive, and POL-positive. In addition, plasma from SSc patients with the susceptible 'TT' genotype of the *TBX21* demonstrated elevated levels of Th2 cytokines. In contrast, the 'CC' genotype was associated with the type I IFN pathways by whole-genome expression analysis. These data must be replicated in additional cohorts.

Signal transducer and activators of transcription-4

Cytokines regulate cellular behavior through binding of their receptors, leading to activation of signal transducers and activators of transcription (STATs). STAT4 is involved in signaling through the IL-12 and IL-23 receptors and may also be activated in response to type I IFN receptors [41]. STAT4 promotes Th1 cell development and is a negative regulator for Th2 cell differentiation [41]. *STAT4* polymorphisms have been associated with rheumatoid arthritis (RA) and SLE [42].

Therefore, it was of interest to determine whether *STAT4* polymorphisms are associated with SSc. Using five independent European cohorts totaling 1317 SSc patients and 3113 healthy controls, an association of *STAT4* rs7574865 T allele with limited SSc but not diffuse SSc was reported [28]. The association of *STAT4* 7574865 with limited SSc was subsequently confirmed in a Japanese cohort, which also demonstrated an association with the ACA positivity [31]. Two additional reports have confirmed the association of *STAT4* with SSc susceptibility; however, in both of these large studies, the association was observed in both limited and diffuse SSc [29,30]. Together, these four independent studies identify and confirm that *STAT4* is an important genetic risk factor for the development of SSc.

Interferon regulatory factor 5

Type I IFNs are potent regulators of innate and adaptive immunity, and multiple lines of evidence point to their importance in the pathogenesis of SLE [6,7,43]. Interferon regulatory factor 5 (IRF5) plays a role in Toll-like receptor signaling, is a critical transcription factor in the activation of IFN associated genes, and its polymorphisms have been associated with SLE susceptibility [44,45]. Similar to SLE, the type I IFN signature is observed in peripheral blood and skin of SSc patients [8,10]. Therefore, it was of interest to determine

whether *IRF5* polymorphisms are associated with SSc susceptibility.

The association of *IRF5* polymorphisms with SSc susceptibility was first reported in a recent study using a discovery cohort of 427 SSc patients and a confirmatory cohort of 454 SSc patients [26]. The association as noted with the *IRF5* rs2004640 functional polymorphism with limited and diffuse SSc as well as topo-positive and ACA-positive SSc. Multivariate analyses suggested that the association was strongest in the topo-positive group and in patients with interstitial lung disease. A study using a Japanese cohort of SSc patients has subsequently confirmed these findings [27]. Unpublished data from our group in collaboration with multiple groups in Europe have also confirmed the association of *IRF5* polymorphisms with SSc susceptibility. The consistent associations of *IRF5* with SSc susceptibility places *IRF5* as an important gene for the SSc development.

B-cell scaffold protein with ankryn repeats (*BANK1*)

The importance of B cells in SSc pathogenesis is best supported by the presence of multiple specific autoantibodies [2,3]. *BANK1* is a B-cell adaptor protein that links the B-cell receptor (BCR) to downstream kinases such as Lyn. *BANK1* polymorphisms have been associated with SLE and RA [46,47]. Similar to SLE, these *BANK1* variants have now been associated with SSc susceptibility in a multicenter case-control study of 2380 white SSc patients and 3270 matched controls [20]. An association of the rs10516487 G and rs17266594 T alleles was observed, which was strongest in the diffuse SSc and topo-positive groups. These data have subsequently been confirmed, once again demonstrating the strongest association of *BANK1* polymorphisms with the diffuse SSc subset [21].

C8orf13-BLK region

Two genome-wide association studies have identified the *C8orf13-BLK* region of chromosome 8p23.1 as a susceptibility locus for SLE [48,49]. B lymphoid kinase (Blk) is a Src kinase that is expressed in thymocytes [50]. Blk transduces signals downstream of the BCR [51]. Using two independent case-control series totaling 1416 SSc patients, we recently demonstrated an association of two variants in the *C8orf13-BLK* region with limited and diffuse SSc [22]. No association with topo-positive patients was observed. However, rs2736340 was associated with ACA-positive patients and rs13277113 was associated with both ACA-positive and POL-positive patients. Functional studies using microarray expression profiling of peripheral blood demonstrated alterations in BCR pathways in patients grouped according to their genotype. Although this study had a discovery and a replication cohort as well as functional data, it will be important to replicate these findings in other cohorts.

Tumor necrosis factor superfamily-4

Tumor necrosis factor superfamily-4 (TNFSF4) encodes for the protein OX40 ligand (OX40L). OX40L is expressed on dendritic cells, macrophages, B cells, T cells, and nonimmune cells such as endothelial cells [52]. It is the ligand for the OX40 on T cells, where it provides costimulation to the T-cell [52]. *TNFSF4* has been identified as a susceptibility gene for SLE [53–55]. Using a case–control series of 1059 North American white SSc cases and 698 controls, multiple polymorphisms within the *TNFSF4* gene region were found to be associated with limited and diffuse SSc susceptibility as well as specific SNPs that were associated with topo-positive, ACA-positive, and POL-positive individuals [32]. Although these observations were made in a large cohort, they must be confirmed in other cohorts.

Interleukin-23 receptor

Current paradigms point to Th17 cells involvement in the pathogenesis of multiple autoimmune diseases, such as RA, inflammatory bowel disease (IBD), psoriasis, and ankylosing spondylitis (AS) [56]. IL-23 promotes the expansion of the Th17 population by inducing the proliferation of Th17 cells [57]. Genome-wide association studies have demonstrated that interleukin-23 receptor (*IL-23R*) polymorphisms, which encodes for the IL-23R, confer a significant risk for susceptibility to IBD, psoriasis, and AS [58–60]. It has been hypothesized that Th17 cells might play a role in SSc pathogenesis [61,62]. Two published studies, however, have suggested that *IL-23R* SNPs do not confer risk to SSc susceptibility [25,63]. This has recently been challenged in a larger case–control series of 1402 SSc cases and 1038 controls [23]. No association was observed in SSc patients as a group; however, autoantibody analyses demonstrated that the *IL-23R* rs11209026 (Arg381Gln variant) and rs11465804 were associated with topo-positive SSc. Interestingly, an association of pulmonary hypertension was noted with polymorphisms at rs11209026 and rs11465804. There are many reasons for the differences in the three studies. An important difference may be that the two initial studies did not focus on the topo-positive group and it is possible that the association of *IL-23R* with SSc resides only in this subset. These data will have to be further investigated before firm conclusions can be made.

Gene–gene interactions in scleroderma

The candidate gene studies focus on identifying the risk of common variants in single genes with SSc susceptibility. These associations are of modest magnitude, and if one simply examines the large number of genes, often contained within the same molecular pathways, it becomes evident that interactions between these genes must be elucidated. Gene–gene interaction, or epistasis [64], is in the exploratory phases but will be critical to

advancing our understanding of how multiple common variants lead to the development of polygenic diseases such as SSc.

Using logistic regression modeling of *STAT4* (rs7574865) and *IRF5* (rs2004640), an additive effect of *STAT4* and *IRF5* with regard to SSc susceptibility and the development of interstitial lung disease was demonstrated [29]. The same group has subsequently demonstrated that *BANK1* polymorphisms are also additive to *STAT4* and *IRF5* polymorphisms with regard to diffuse SSc susceptibility [21]. Lastly, Classification and Regression Tree Analysis (CART) has been used to explore the potential interaction between *TBX21* and *STAT4*, which encode for proteins that are both involved in Th1 development [30,65]. This study demonstrated that the *STAT4* polymorphism was mostly associated with SSc susceptibility in SSc patients who carried the ‘CC’ genotype at *TBX21* rs11650354 (the group without the *TBX21* ‘TT’ susceptibility genotype). Clearly, additional studies are needed not only to improve our models and understanding of gene–gene interactions in general but also as it pertains to the complex genetics of SSc.

Shared genetic associations with other autoimmune disease

One of several themes that begin to emerge from the list of SSc candidate genes revolves around the emerging concept of shared genetic risk factors for the development of autoimmune diseases. *PTPN22* has been associated with SSc susceptibility in two large studies and has also been associated with the development of type I diabetes mellitus, RA, and SLE [66–70]. *STAT4* and *IRF5* are associated with SSc susceptibility [26,27,28,29,30,31] and have also been identified as susceptibility genes for the development of SLE and RA [42,45]. Recently, *TNFSF4*, *BANK1*, and *C8orf13-BLK* have also joined the list of shared autoimmune genes with risk association with SSc and SLE [22,28,32,46–49,53–55]. Together, these studies implicate dysregulation of common immune pathways due to polymorphisms in several genes in the development of a variety of autoimmune diseases.

Although the genetic similarities between SSc and SLE are striking, there must also be unique factors within each disease that contribute to the distinct clinical features between SSc and SLE. Clearly, the HLA class II associations between the diseases are distinct [14,71]. In addition, studies in SSc have implicated *TBX21*, *CTGF*, and *AIF1* as SSc susceptibility factors, but they have not (yet) been shown to be associated with SLE. Perhaps our knowledge of the pathogenesis of SSc will point to genes in the ECM or vascular pathways that are distinct. Fortunately large collaborative studies using genome-wide

approaches are nearing completion. These studies will facilitate the identification of completely novel genes involved in SSc susceptibility that would not have otherwise been considered using the traditional candidate gene approach.

Conclusion

From the existing data, it is evident that susceptibility to SSc is polygenic, in part linked to autoantibody subsets of the disease. The candidate gene studies have provided a substantial amount of information about SSc pathogenesis. Although extensive and impressive on their own, these studies are only the beginning. We must increase our understanding of how these polymorphisms lead to the development of SSc. Central to this understanding will be defining complex gene–gene and gene–environment interactions. In addition, biological confirmation of these genetic alterations into functional studies is essential to determine whether these associations are in fact causal. Lastly, efforts should also focus on translating these findings into the care of the SSc patient where these genetic factors may ultimately lead to advances in the diagnosis, prognosis, and treatment of patients with scleroderma.

References and recommended reading

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 230).

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