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Systemic Sclerosis is a Complex Disease Associated Mainly with Immune Regulatory and Inflammatory Genes

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Abstract: Systemic sclerosis (SSc) is a fibrotic and autoimmune disease characterized clinically by skin and internal organ fibrosis and vascular damage, and serologically by the presence of circulating autoantibodies. Although etiopathogenesis is not yet well understood, the results of numerous genetic association studies support genetic contributions as an important factor to SSc. In this paper, the major genes of SSc are reviewed. The most recent genome-wide association studies (GWAS) are taken into account along with robust candidate gene studies. The literature search was performed on genetic association studies of SSc in PubMed between January 2000 and March 2014 while eligible studies generally had over 600 total participants with replication. A few genetic association studies with related functional changes in SSc patients were also included. A total of forty seven genes or specific genetic regions were reported to be associated with SSc, although some are controversial. These genes include HLA genes, *STAT4*, *CD247*, *TBX21*, *PTPN22*, *TNFSF4*, *IL23R*, *IL2RA*, *IL-21*, *SCHIP1/IL12A*, *CD226*, *BANK1*, *C8orf13-BLK*, *PLD4*, *TLR-2*, *NLRP1*, *ATG5*, *IRF5*, *IRF8*, *TNFAIP3*, *IRAK1*, *NFKB1*, *TNIP1*, *FAS*, *MIF*, *HGF*, *OPN*, *IL-6*, *CXCL8*, *CCR6*, *CTGF*, *ITGAM*, *CAV1*, *MECP2*, *SOX5*, *JAZF1*, *DNASEIL3*, *XRCC1*, *XRCC4*, *PXK*, *CSK*, *GRB10*, *NOTCH4*, *RHOB*, *KIAA0319*, *PSD3* and *PSOR1C1*. These genes encode proteins mainly involved in immune regulation and inflammation, and some of them function in transcription, kinase activity, DNA cleavage and repair. The discovery of various SSc-associated genes is important in understanding the genetics of SSc and potential pathogenesis that contribute to the development of this disease.

Keywords: CD247, genetics, genome-wide association studies, HLA class genes, IRF5, scleroderma, STAT4, Systemic sclerosis.

INTRODUCTION

Systemic sclerosis (SSc) is a heterogeneous disorder of unknown etiology characterized by extensive skin fibrosis, microvascular changes, and autoimmunity. Based on the extend of skin fibrosis, SSc can be classified into two clinical subsets: limited cutaneous (lcSSc) and diffuse cutaneous (dcSSc) SSc. The latter one is more severe with rapid progression of skin and visceral involvement, as well as poorer prognosis [1, 2]. SSc is also marked by the mutually exclusive anti-nuclear and -nucleolar autoantibodies, primarily anti-topoisomerase I (ATA), -centromere (ACA), -RNA polymerases (ARA), -fibrillarin, and -U3 RNP, placing it as an autoimmune disease [3, 4].

Although the causes of SSc are not well understood, accumulating evidence suggests that genetic factors play import roles. Compared to in the general population, prevalence of SSc is increased when there is a positive family history of disease (0.026% *vs* 1.6%) [5]. Concordance rates of anti-nuclear autoantibodies (ANA) in SSc were significantly higher in monozygotic twins [6]. SSc also has unusually high incidence in certain, relatively genetically-isolated populations, such as Choctaw Indians [7]. The earliest genetic studies indicated that specific alleles in the human leukocyte antigen (HLA) region were associated with SSc [8-10].

In addition to HLA genes, many other candidate genes also have been examined, and some were confirmed for association with SSc and its subsets. The identification of these genes often comes from genes found to be associated with other related autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome (SS). Recently, emergence of the genome-wide association study (GWAS) has made possible the identification of new loci that would not have been discovered with the traditional hypothesis-driven approach [11, 12]. This review summarizes the major and most well-confirmed genes that have been associated with SSc in the past 14 years.

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CANDIDATE GENE STUDIES – IMMUNE REGULATORY GENES

HLA Region

The HLA region contains many genes that are important for immune function. HLA class II genes have been extensively examined for their association with SSc. Strong correlations have been discovered between specific SSc autoantibodies and HLA class II, but due to small sample sizes and other factors, results have been mixed [13-17]. Recently, a large study with a multi-ethnic US cohort of 1300 SSc patients and 1000 healthy controls analyzed HLA class II (*DRB1*, *DQB1*, *DQA1*, and *DPB1*) alleles, haplotypes and shared epitopes for association with SSc and subtypes [18]. In white and Hispanic SSc patients, the alleles *HLA-DRB1**1104, *DQA1**0501, *DQB1**0301, and *DQB1**26 epitope (absence of leucine in position 26) were significantly increased. The *HLA-DRB1**0701, *DQA1**0201, *DQB1**0202 haplotype and *HLA-DRB1**1501, *DQA1**0102, *DQB1**0602 haplotype were protective for SSc in whites, in a dominant and recessive pattern, respectively. In African Americans, *HLA-DRB1**1104 was not associated with SSc, but *DRB1**0804 was, along with *DQA1**0501 and *DQB1**0301. The strongest associations with the anti-centromere antibody (ACA) subtype were *HLA-DQB1**0501 and *DQB1**26 epitope. The anti-topoisomerase I antibody (ATA) subtype was best explained by *HLA-DPB1**1301 and the *HLA-DRB1**1104, *DQA1**0501, *DQB1**0301 haplotype [14, 15]. The anti-RNA polymerase III antibody (ARA) subset was best explained by *HLA-DRB1**0404, *DRB1**11 and *DQB1**03 in whites and Hispanics and *DRB1**08 in black subjects. Using a genome-wide approach comparing 137 Korean SSc patients to 564 controls, another study found the single nucleotide polymorphisms (SNPs) rs3128930, rs7763822 and rs7764491 on *HLA-DPB1* and *DPB2* to be associated with SSc, especially dcSSc and ATA+ SSc, in Koreans [19], and these results were confirmed in the replication set of North American subjects [19]. Further, association between *DPB1**1301 and ATA positive SSc was consistent with studies in the Korean cohort [19], UK Caucasian [20] and Han Chinese [21]; *DQB1**0501 with ACA positive SSc was consistent with the reports in Spanish [22], Japanese [15] and Han Chinese [23]; *DRB1**0701 with SSc as a protective role was consistent with Spanish cohort [18], and *DRB1**11 with SSc as a risk factor was concordant with Spanish [22], UK [20, 24] and South African cohorts [25]. As sample sizes become larger and methods more advanced, the HLA associations of SSc should become consistent and clear.

STAT4

STAT4, stands for signal transducer and activators of transcription-4, is part of a family of transcription factors that influence T cell differentiation in response to stimulation from cytokines and growth factors. STAT4, in particular, is activated by IL-12 and IL-23 signals and is involved in the differentiation pathways for Th1 and Th17. It was speculated that prolonged STAT4 activity due to genetic differences may cause an imbalance in Th1/Th2 cells [26, 27]. Several polymorphisms in the STAT4 gene have been shown to be associated with SSc [27-29]. The rs7574865 T allele was found to be associated with lcSSc in a Spanish-Caucasian case-control study (332 patients/1296 controls), and replicated in five independent Caucasian cohorts [27]. In contrast, a case-control study with a French-Caucasian population (885 patients/970 controls) found an association between rs7574865 and SSc, with no significant difference in correlation between the diffuse and limited versions of the disease [28]. This study also found an additive effect on SSc susceptibility between the *STAT4* rs7574865 and the interferon regulatory factor 5 (*IRF5*) rs2004640 T allele, especially with regard to the development of fibrosing alveolitis (FA). The association with *IRF5* may be related to the fact that the STAT4 pathway is activated by interferon (IFN)-α in monocytes [30]. Additionally, the *STAT4* rs11889341-A allele was found to increase SSc susceptibility in a dominant model in North American patients (880 patients/507 controls and 522 patients/531 controls), but only in the presence of the *TBX21* rs11650354 major allele [29]. Association of *STAT4* with SSc was confirmed in recent GWAS on the disease [11, 12].

CD247

T-cell surface glycoprotein CD3 zeta chain (CD247), a component of T cell receptor (TCR)/CD3 complex, plays an important role in assembly and transport of the TCR/CD3 complex to the cell surface and in receptor signaling function [31, 32]. Low expression of CD3 zeta has been shown to impair immune function [31, 32]. *CD247* was associated with SSc in a GWAS that was followed by a replication cohort of Caucasian ancestry (2753 patients/4569 controls) [11]. An independent study with a French Caucasian cohort (1031 patients/1014 controls) again confirmed the association between rs2056626 of *CD247* and SSc, and additionally found that the rs2056626G minor allele conferred a protective effect in a dominant model [33]. Further studies are necessary to discover the functional link between *CD247* and SSc.

TBX21

T-bet (TBX21) is a transcription factor downstream of STAT4 that promotes the differentiation of Th1 through enhancement of Th1 cytokines [34]. Like STAT4, it regulates the Th1/Th2 balance [34, 35]. A case-control study with two independent cohorts of a Caucasian American population (880 patients/507 controls and 522 patients/531 controls) revealed that TT genotype of rs11650354 was a risk factor for SSc under a recessive model [27]. Through examination of plasma cytokine levels, the TT genotype was found to be associated with a Th2 cytokine profile, while the CC (wild-type) genotype was associated with a proinflammatory cytokine profile [27].

PTPN22

PTPN22 encodes a non-receptor protein tyrosine phosphatase involved in the suppression of T cell activation. The R620W variant of *PTPN22* is a gain-of-function mutation that leads to the down-regulation of T cell receptor signaling, which may prevent the destruction of self-reactive T cells and reduce the activity of T regulatory cells [36]. The

T allele of R620W (1858T) may also result in reduced BCR signaling ability, which could lead to proliferation of autoreactive B cells. This last function is notable as SSc is a disease marked by the presence of autoantibodies [36]. In addition, IL-10, a cytokine synthesis inhibitory factor, was also found to be lowered in patients with the 1858T allele. A case-control study on a multi-ethnic North American population (1100 patients/740 controls) revealed that the R620W CT/TT genotype was associated with ATA+ and ACA+ in SSc in white patients [37]. A meta-analysis of three independent studies showed only association with SSc, however with ATA+ patients accounting for most of the association [38]. Another meta-analysis of eight European Caucasian cohorts and two other studies with large statistical power revealed an association between the T allele of R620W (1858T) and ACA+ SSc [39].

TNFSF4

The tumor necrosis factor ligand superfamily member 4 (TNFSF4) gene encodes for the OX40L ligand, expressed on dendritic cells and endothelial cells in acute inflammation, which binds to the OX40 receptor to impart a co-stimulatory signal to T lymphocytes, leading to T cell proliferation and cytokine production [40-42]. A case-control study on a Caucasian population (1059 patients/698 controls) found that the minor alleles of rs1234314, rs2205960 and rs844648 of *TNFSF4* were associated with SSc, while the rs844644 minor allele had a protective effect [43]. The rs1234314 SNP was associated with ACA+ and ATA+, while rs2205960 was associated with ATA+ and rs844648 with ARA+. The minor alleles (G) at rs1234314 and rs2205960 were more common in lcSSc. A replication study with 8 European Caucasian cohorts (3014 patients/3125 controls) revealed mixed results [44]. The rs1234314 G allele was found to be associated with SSc, lcSSc, and ACA+, but not ATA+. The other alleles (rs844644, rs844648, and rs12039904) showed similar association signals with ACA+ and lcSSc.

IL23R

Interleukin-23 (IL-23) is an essential cytokine for the proliferation of Th17 cells, which promote inflammation and are implicated in many autoimmune diseases [45]. IL-23 activity is mediated by the IL-23 receptor. Concentrations of IL-23 and IL-17 have been reported to be increased in SSc patients [46, 47]. A study with a Dutch cohort (143 patients/246 controls) and Spanish replication cohort (365 patients/515 controls) did not find association between *IL23R* and SSc [48]. However a larger study on a North American Caucasian population (1402 patients/1038 controls) found that rs11209026 of *IL23R* was associated with ATA+ SSc and rs11465804 of *IL23R* with ATA+ and dcSSc [49]. The major alleles of these two SNPs were found to be protective for pulmonary arterial hypertension (PAH) in SSc [49].

IL-21

Interleukin-21 (IL-21), a cytokine gene located on the 2q27 locus, contributes to T helper 17 (Th17) development and is produced by Th17 cells [50]. IL-21 has been shown to be up-regulated in the epidermis of SSc patients, and may work in conjunction with IL-23 to increase risk for autoimmune diseases in patients [51]. A study with a large Caucasian cohort (4493 patients/5856 controls) revealed a considerable association between the rs6822844 SNPof *IL-21/IL-2* and SSc susceptibility [52]. This SNP is particularly associated with lcSSc and ACA+ patients.

CD226

CD226 encodes DNAX accessory molecule 1, which is involved in T-cell co-stimulation [53]. The T allele of *CD226* rs763361 SNP was recently identified as a risk factor for autoimmune diseases [54]. A study on a European Caucasian population found that rs763361T allele was associated with SSc in both the discovery and replication cohorts (991 patients/1008 controls and 999 patients/634 controls) [55]. The rs763361 TT genotype was also correlated with dcSSc, ATA+, and FA subsets. In this study, the authors did not find any relation between rs763361 and expression of *CD226* in cells.

BANK1

BANK1, the B-cell scaffold protein with ankryn repeats, is a B-cell specific substrate of tyrosine kinases that functions downstream of the B-cell receptor (BCR) and enhances B cell calcium mobilization [56]. A case-control study with French (874 patients/955 controls) and German Caucasian cohorts (421 patients/182 controls) found that 2 polymorphisms, rs3733197 and rs10516487, together called the *BANK1* A-T haplotype, were found to have a protective effect on dcSSc susceptibility [57]. An additive effect between *BANK1*, *STAT4* (rs7574865-T), and *IRF5* (rs2004640-T) was additionally discovered. Another study with six different Caucasian cohorts (2380 patients/3270 controls) confirmed the association with dcSSc and also revealed that three polymorphisms, rs17266594, rs3733197 and rs10516487, were associated with the presence of ATA [58]. The latter finding is unsurprising as ATA is highly specific to dcSSc.

C8orf13-BLK REGION (ALSO KNOWN As FAM167A-BLK)

The B lymphoid kinase (BLK) is a kinase specific to the B lymphocyte that plays an important role in BCR signaling and B cell development. *C8orf13* is a ubiquitous gene with unknown function. Two SNPs at the *C8orf13-BLK* intergenic region, rs13277113 and rs2736340, were examined for association with SSc in a case-control study [59]. Combined analysis of the North American cohort (1050 patients/694 controls) and Spanish cohort (589 patients/722 controls) found an association between both polymorphisms and ACA+ SSc and lcSSC. Whole blood mRNA gene expression

profiling followed by pathway analysis suggested that *C8orf13-BLK* region was associated with the dysregulation of BCR and NF-κB signaling pathways. A case-control study with a small Japanese cohort (309 patients/769 controls) confirmed the association of rs13277113A of *C8orf13-BLK* with SSc [60]. Meta-analysis of the above two studies along with a large French cohort (1031 patients/1014 controls) also found association of rs13277113 with SSc and dcSSc [61]. An additive effect was discovered between *C8orf13-BLK* and *BANK1* in the dcSSc subset. These data suggest that dysregulation of B-cell signaling and development may play an important role in the pathogenesis of SSc.

PLD4

Phospholipase D4 gene (PLD4) is involved in the phagocytosis of microglia and in the immune system, although its exact functions are not known [62]. In an initial study (415 patients/16,891 controls) and replication study (315 patients/21,054 control) of Japanese subjects, eighteen genes associated with RA were analyzed for any association with SSc [63]. The *PLD4* rs2841277 was found to be significantly associated with SSc in Japanese patients [63].

TLR-2

Toll-like Receptors (TLR) are fundamental members of the immune system response because they provide a first-line pathogen recognition system. TLRs are present in macrophage and dendritic cells, and they identify microbial antigens as part of the innate immune response [64]. In a study consisting of a discovery cohort and a replication cohort (452 patients/537 controls and 1,170 patients/925 controls) from European populations, fourteen polymorphisms of TLR genes were analyzed for their role in SSc susceptibility [65]. A rare polymorphism called Pro63His in *TLR-2* was found to be associated with ATA+, dcSSC, and the development of PAH in SSc patients [65]. Of notes, when the TLR-2 was stimulated, increased quantities of inflammatory mediators (such as interleukin-6) were produced by the Pro63His variant [65].

NLRP1

The NLR family pyrin domain containing 1 (NLRP1) gene functions in the innate immune system alongside inflammasomes, which influence pro-interleukin-1β production in SSc patients [66, 67]. This cytoplasmic protein is implicated in the development of inflammatory cytokines IL-1β, IL-33 and IL-18, and genetic variants were associated with autoimmune diseases, such as vitiligo, Addison's disease and type 1 diabetes [68, 69] A study with a discovery set and replication set (870 patients/962 controls and 1059 patients/625 controls) conducted with individuals of European Caucasian origin, revealed an association between the *NLRP1* rs8182352 variant, and ATA+ and SSc-related fibrosing alveolitis (FA) [70].

CANDIDATE GENE STUDIES – INFLAMMATORY GENES

IRF5

IRF5 stands for interferon regulatory factor 5. It is a crucial regulator of type I interferon signaling. IFN is a mediator of innate immunity, responding to infections by stimulating natural killer cells, cytotoxic T cells, monocyte maturation, plasma cell maturation, and more [71]. A recent study found an IFN signature similar to that of SLE in the peripheral blood cells of SSc patients [72]. A case-control study with a French Caucasian population (881 patients/760 controls) found an association between the *IRF5* intron 1 rs2004640 T allele and SSc, especially the dcSSc [73]. A strong association between *IRF5* and FA was additionally discovered. A Japanese study (281 patients/477 controls) confirmed the rs2004640 association, and found that rs10954213 and rs2280714 also associated with SSc, especially in the ATA+ and dcSSc subsets. The strongest association was found with rs2280714, which was correlated with overexpression of IRF5 mRNA [74]. Investigation of phenotype-haplotype correlations of *IRF5* with the SNPs rs3757385, rs2004640, and rs10954213 found the "R" haplotype (C-T-A) was found to be associated with dcSSc, and the "P" haplotype (A-G-G) with both dcSSc and FA [75]. Association of *IRF5* with SSc was also confirmed in the GWAS of SSc [11, 12].

TNFAIP3

Tumor necrosis factor-α–induced protein 3 (*TNFAIP3*) encodes A20, an ubiquitin-modifying enzyme that inhibits NF-κB activity and is a key regulator of inflammatory signaling pathways [76]. A study with a large French Caucasian cohort (1018 patients/1012 controls) revealed an association between the rs5029939G allele of *TNFAIP3* and SSc susceptibility [77]. The study also found the SNP to be associated with the dcSSc, ATA+, FA, and PAH subsets. An independent French study with a discovery set (985 SSc patients and 1,011 controls), and replication analysis (622 SSc patients and 493 controls) indicated that rs117480515 of *TNFAIP3* to be associated solely with the SSc polyautoimmune subset [78]. A study of a Japanese cohort indicated an association of rs6932056 of *TNFAIP3* with SSc [79].

IRAK1

IRAK1 represents the IL-1 receptor–associated kinase 1 that regulates NF-κB through Toll-like receptor activation and T cell receptor signaling, making it a crucial factor in the body's immune system response to infection [80]. A study with a discovery cohort (849 patients/625 controls), and two replication cohorts on the Italian Caucasian (493 patients/509 controls) and German Caucasian (466 patients/1,083 controls) populations revealed that the rs1059702 TT genotype of *IRAK1* was associated with dcSSc, ATA+ and SSc-related fibrosing alveolitis [81]. Another study consisting of five different Caucasian cohorts

(3065 patients/2630 controls) showed that the association between *IRAK1* rs1059702 and dcSSc was explained by a nearby SNP rs17435 of the *MECP2*. However, *IRAK1* rs1059702 was consistently associated with presence of pulmonary fibrosis (PF) [82].

FAS

The FAS (Apo-1/CD95) antigen, part of the tumor necrosis factor family, is a cell-surface receptor molecule implicated in the apoptosis of a wide variety of cells, including immune cells [83, 84]. T lymphocytes isolated from patients with SSc were shown to exhibit a lower apoptotic rate than controls [85]. Dysregulation of FAS-mediated apoptosis might contribute to the pathogenesis of SSc through the suppression of autoreactive immune cell apoptosis [86]. A small study utilizing an Italian cohort (350 patients/232 controls) found that the *FAS*-670G>A polymorphism was associated with susceptibility to both lcSSc and dcSSc, and also discovered increased levels of serum FAS (sFAS) in patients with *FAS*-670AA genotype [87]. Functionally, sFAS antagonizes FAS-mediated apoptosis by down-regulating the number of surface FAS receptors and binding to the FAS ligand. However, a meta-analysis of 9 distinct ethnic cohorts (2900 patients/3186 controls) found an association of *FAS*-670GG genotype with lcSSc [88]. The apparent contradiction between these two conclusions should be researched in further studies.

MIF

The macrophage migration inhibitory factor (MIF) is a cytokine that impedes p53-dependent, activation-induced apoptosis, which has been implicated in sustained pro-inflammatory and immunoregulatory pathways. MIF has anti-apoptotic actions on fibroblasts and its deficiency results in lower amounts of IL-6, IL-2, tumor necrosis factor α (TNFα), and interleukin-1β (IL-1β) in the body [89]. Higher levels of MIF expression have been reported in patients with SSc [90]. In a study on *MIF* (486 patients/254 controls), the pro-inflammatory haplotype represented by 7 CATT repeats (C7), was found to be lower in lcSSc patients [89]. An additional, larger study (3800 patients/4282 controls) on the *MIF*-173*C allele in the European population supported previous findings; higher frequencies of the *MIF*-173 SNP were observed in dcSSc patients when compared to the controls and lcSSc patients [90].

HGF

Hepatocyte growth factor (HGF) has an antifibrotic effect and counteracts many of the profibrotic actions of TGF-β [91]. However, HGF was found in higher levels in SSc patients suggesting that increased HGF levels alone are not sufficient to inhibit tissue fibrosis [92]. A small study on the Japanese population (314 patients/103 controls) revealed that the *HGF*−1652 SNP was associated with end-stage lung disease in SSc patients [93]. This SNP may also regulate transcriptional efficiency in the *HGP* promoter region, influencing the severity of interstitial lung disease (ILD) in SSc patients [93].

OPN

Osteopontin (OPN) is a matricellular protein with profibrotic properties. It enhances the proinflammatory Th1 cell response and also presents profibrotic properties [94-97], which is believed to be important in SSc pathogenesis. In a study of Italian population (357 patients/864 controls), two *OPN* SNPs at the +1239A/C in the 3′UTR region and −156G/GG in the 5′ region were associated with SSc [98]. A further study suggested the role of *OPN* as a mediator in dermal fibrosis and in pro-inflammatory responses [99]. In SSc patients, reported OPN levels were higher than normal and correlated with the ACA+, ATA+ and ARA+ subsets of SSc [99].

CXCL8

CXCL8 (also known as IL-8) is a member of the CXC chemokine family with proinflammatory and immunoregulatory function [100, 101]. A study on the Brazilian population (151 patients/147 controls) revealed that *CXCL8* (-251) A, in association with the *CXCR2* (+1208) CC genotype, conferred an increased risk for SSc, whereas *CXCL8* (-251) A in the presence of the TT and TC genotypes of *CXCR2* (+1208) had a protective roles in SSc [102].

CCR6

CCR6 (CC chemokine receptor 6) is the selective receptor for chemokine CCL20 [103]. In an animal model of rheumatoid arthritis, IL17-produing Th17 cells predominantly express CCR6 and produce its ligand, CCL20 [104]. A recent study genotyped twelve tag SNPs of *CCR6* in 2411 SSc patients and 7084 healthy controls from 3 European populations (France, Italy, and Germay) [105]. The analyses revealed an association between SNP rs10946216 and SSc susceptibility [105]. Moreover, the data showed that rs3093023 A allele and rs10946216 T allele were in high linkage disequilibrium, and demonstrated that these two SNPs were associated with ATA positive SSc patients [105].

CTGF

Connective tissue growth factor (CTGF), a gene linked to fibrosis through proliferation of fibroblasts and production of extracellular matrix, was found to have increased expression in the serum of patients with SSc [106]. An association between the -945G allele in the promoter region of *CTGF* and SSc was reported in a UK cohort (500 patients/500 controls), and the result was confirmed by a Japanese replication study (395 SSc patients/269 controls) [107, 108]. However, both of these studies had relatively small sample sizes in the cohorts. A large multicenter study comprised seven independent case-control sets of European ancestry including Spanish, French, Dutch, German, British, Swedish and North American (a total of 1180 patients/1784 controls) found no associations between -945G and any form of SSc [109]. A report with a North American cohort (1311 patients/1004 controls) also failed to show association of the

same SNPs with SSc [110]. Meta-analysis might help solve this apparent contradiction. Recently, another SNP, rs9399005, which may alter the CTGF mRNA transcript, was associated with SSc in a study with a small French cohort (241 patients/269 controls) [111].

ITGAM

ITGAM has been recently identified as an autoimmune disease risk gene, and it encodes the α subunit of the αMβ2-integrin [112]. It is expressed on the surface of leukocytes, and regulates adhesion of neutrophils and monocytes, cell activation, which is important for innate immunity [113]. A study on seven independent European cohorts found an association between the *ITGAM* rs1143679 *A and SSc [114]. Trends between the rs1143679 allele and lcSSc and ATA+ patients have also been observed [114]. Furthermore, a meta-analysis (4337 patients/5326 controls) of the rs1143679 SNP revealed a significant association between *ITGAM* and SSc [115]. A study consisting of French (1031 patients/1014 controls) and American (1038 patients/691 controls) cohorts analyzed the *ITGAM* rs9937837 polymorphism and found no association with SSc [116].

CAV1

Caveolin-1 (CAV1) is an integral membrane protein. It can regulate CTGF gene expression, and function in intracellular signaling cascade that is associated with fibrosis in SSc [117, 118]. CAV1 up-regulates insulin receptor signaling and low levels of this protein have been linked to overexpression of profibrotic markers, such as collagen [119, 120]. A study assessed 23 SNPs in the French population (564 patients/1776 controls), and the three most prominent *CAV1* SNPs (rs926198, rs959173, rs9920), were then genotyped in an Italian population (791 patients/843 controls) [119]. The *CAV1* rs959173 C minor allele was found to be protective against SSc (especially lcSSc) [119].

CANDIDATE GENE STUDIES – TRANSCRIPTION REGULATION

MECP2

MECP2 encodes for methyl-CpG-binding protein 2, a chromatin-associated protein that can both activate and repress transcription [121, 122]. It participates in epigenetic control of neuronal function [121, 122], and its mutations were implicated in Rett syndrome and autism [122]. A study consisting of five different Caucasian cohorts (3065 patients/2630 controls) showed that the *MECP2* rs17435 was associated with dcSSc [82].

GENOME-WIDE ASSOCIATION STUDIES IDENTIFIED IRF8, GRB10, SOX5, NOTCH4, TNIP1, PSORS1C1 AND RHOB

Unlike traditional candidate gene studies, GWAS can scan the entire genome for SNPs associated with a chosen disease. In 2010, a robust GWAS on SSc scanned over 300,000 SNPs for association with SSc [11]. The association of *CD247* with SSc was confirmed in the studies. Subsequent re-analysis of the data of this GWAS for association to specific SSc subtypes discovered three new non-HLA loci [123]. The rs11642873 of *IRF8* and the rs12540874 of *GRB10* were both associated with lcSSc, while the rs11047102 of *SOX5* was associated with ACA. *IRF8* stands for interferon regulatory factor 8 that is a part of the IRF family and may contribute to the cross-talk between IFN-γ and Toll-like receptor (TLR) signaling [124]. *GRB10* encodes growth factor receptor-bound protein 10 that is an adaptor protein known to interact with several tyrosine kinase receptors [125]. It has been particularly implicated in insulin signaling through the insulin receptor [126] and insulin like growth factor I (IGF I) receptor [127]. *SOX5* stands for sex determining region Y-box 5, a member of SOX family. As a transcription factor, it is involved in the regulation of embryonic development and in the determination of the cell fate. It plays an essential role in chondrocyte differentiation through activation of COL2A1, leading to formation of cartilage [128].

In addition to non-HLA region genes, a gene in the HLA region, *NOTCH4* (neurogenic locus notch homolog 4), was also found to be associated with both ACA and ATA [123]. *NOTCH4* encodes a receptor protein that is involved in multiple cell processes, such as cell differentiation, proliferation and apoptosis [129, 130].

The latest GWAS was conducted in 2011 with 564 patients and 1776 controls and scanned almost 500,000 SNPs [12]. Three novel loci were discovered. TNFAIP3 interacting protein 1 (*TNIP*1) with SNP rs3792783 was strongly associated with SSc. TNIP1interacts with TNFAIP3 to negatively regulate NF-κB activity [131, 132]. Interestingly, analysis of SSc patient dermal fibroblasts showed reduced expression of TNIP1, and adding recombinant TNIP1 to skin cells lowered collagen synthesis levels [12]. These results suggest that NF-κB may play an important role in fibrosis and SSc pathogenesis. Psoriasis susceptibility 1 candidate gene 1 (*PSORS1C1*) is a gene contributing to psoriasis, and is located in the HLA-DQB1 region [133, 134]. SNP rs3130573 of *PSORS1C1* was associated with SSc [12]. A weaker association was also found between the SNPs (rs342070 and rs13021401) of the ras homolog gene family member B (*RHOB*) and SSc [12]. The RHOB gene regulates cell morphogenesis and motility [135, 136].

IMMUNOCHIP STUDIES IDENTIFIED DNASE1L3, SCHIP1-IL12A AND ATG5

In addition to GWAS, immunochip is a custom SNP genotyping array that provides high-density mapping of autoimmune disease (AID)-associated loci for large cohorts at reduced costs [137]. In an immunochip study of SSc, three novel non-HLA loci were found to be associated with SSc in the European and North American discovery and replication cohorts. They are missense SNP (rs35677470) in *DNASE1L3*, a SNP (rs77583790) in the intergenic region between *SCHIP1* and *IL12A*, and a SNP (rs9373839) intronic to *ATG5* [137].

DNASE1L3 encodes for deoxyribonuclease I-like 3, a member of human DNase I family and functions as an endonuclease capable of cleaving both single- and double-stranded DNA [138, 139]. The nonsynonymous rs35677470

SNP in *DNASE1L3* produces a protein that lacks DNase activity [138]. DNASE1L3 has also been demonstrated to be involved in DNA fragmentation and apoptosis [140]. Studies have suggested that microvascular injury, a feature of SSc, may be a result of endothelial cell apoptosis mediated by antibody-dependent cell-mediated cytotoxicity [86, 141]. Moreover, there has been implication that the production of autoantibodies could be a consequence of unrepaired DNA breakages [142]. In the immunochip analysis, *DNASE1L3* (SNP rs35677470) was significantly associated with ACA positive SSC [137].

SCHIP1-IL12A stands for Schwannomin-interacting protein 1/Interleukin 12A. *SCHIP1-IL12A* locus is an intergenic region that contains a genetic variant that has been associated with celiac disease [143, 144]. The function of SCHIP1 is unknown. *IL12A* encodes interleukin 12 (IL12), which is involved in the proliferation and differentiation of activated T and natural killer cells as well as induces IFN-γ production and Th1 polarization of T-cells [145]. An increased IL12 level was observed in SSc [145]. The immunochip analysis revealed that *SCHIP1-IL12A* (SNP rs77583790) has a significant association with lcSSc [137].

ATG5 (autophagy-related gene 5) encodes for ATG5 protein that forms a complex with ATG12, and this conjugation system is required for autophagosomal elongation [146]. Autophagy proteins, including ATG5, are involved in both innate and adaptive immunity [147, 148]. They function in degradation of microorganisms, antigen presentation, and regulating immune signaling [147, 148]. They have also been implicated in the pathogenesis of autoinflammatory diseases, such as Crohn's disease, as well as autoimmune diseases including systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, and diabetes mellitus [147-149]. The immunochip data showed that *ATG5* (SNP rs9373839) was associated with SSc [137].

The association of *DNASE1L3*, *SCHIP1-IL12A*, and *ATG5* with SSc provides evidence that defects in DNA fragmentation in apoptosis, the role of the IL12 pathway, and autophagy possibly play important roles in the pathogenesis of SSc [137]. With these findings, this immunochip analysis contributes to the growing knowledge of the underlying pathogenic mechanism of SSc.

META-ANALYSIS IDENTIFIED IL2RA, IL6, JAZF1, CSK, PSD3 NFKB1, PXK AND KIAA0319L

Meta-analysis is a statistical tool for combining results from multiple studies. It becomes popular as a method for resolving discrepancies in genetic association studies and improving statistic power [150]. A meta-analysis study with Caucasian European cohorts (3023 patients/2735 controls) revealed an association between Interleukin 2 receptor α (*IL2RA*) and SSc [151]. In particular, the rs2104286 of *IL2RA* was associated with ACA+ lcSSc patients [151].

IL2RA has also been associated with several autoimmune diseases, primarily because of its importance as is a regulatory T-cell marker. IL2RA is involved in various pathways that help control the differentiation of effector cells, T-cell proliferation, and immune tolerance [152].

Another meta-analysis on the mixed European populations (2749 patients/3189 controls) found a risk effect of rs2069840*G allele of interleukin-6 (*IL6*) to lcSSc and a trend of association between the *IL6* rs1800795*G allele and dcSSc with a protective effect [153]. Furthermore, the rs2069827-rs1800795-rs2069840 allelic combination of *IL6* demonstrated an association with SSc overall [153]. IL6 is a pleiotropic cytokine that is released in lymphocytes, monocytes, and fibroblasts as part of the pro-inflammatory response to stimuli [154]. It is involved with numerous innate and adaptive immune system responses. In the studies of SSc, IL6 stimulates the production of collagen by dermal fibroblasts [155-157].

A larger meta-analysis study (5270 patients/8326 controls) on people with European ancestry revealed that the *CSK* rs1378942 was associated with SSc susceptibility [158]. CSK stands for c-src tyrosine kinase that causes the inactivation of the src kinases through the phosphorylation of tyrosine at the C-terminus [159, 160]. Overexpression of CSK was reported to induce fibrosis [161], making *CSK* another potential risk factor to SSc. In addition to *CSK*, *PSD1* and *NFKB1* also were found in association with SSc in this meta-analysis [158]. The former encodes a protein with a Sec7 domain and a pleckstrin domain, although its exact function is unknown. The rs10096702 SNP of *PSD1* was associated with SSc in various European populations [158]. The latter stands for the nuclear factor of kappa light polypeptide gene enhancer in B-cell 1 that encodes a 105kDa protein responsible for the regulation of various immune system functions, such as the inflammatory response [162, 163]. The rs1598859 of *NFKB1* was marginally associated with SSc [158]. However, a more recent study with smaller sample size (151 patients/147 controls) found no statistically significant associations between *NFKB1* polymorphisms and SSc [164]. More research must be conducted on this gene to reach a valid conclusion about its association with SSc.

Recently, a pan-meta-analysis study based on two GWASs and various replication cohorts (6835 patients/14,274 controls) from Europe and the USA was performed [165]. This GWAS based meta-analysis found genetic association of *PXK* (SNP rs2176082), *JAZF1* (SNP rs1635852) and KIAA0319L (SNP rs2275247) with SSc [165]. The *PXK* stands for PX domain containing serine/threonine kinase. This gene encodes a kinase binds and modulates both Na,K-ATPase enzymatic and ion pump activities [166]. The polymorphisms of *PXK* were associated with autoantibody production in SLE [165]. Juxtaposed with another zinc finger gene 1 (*JAZF*1) encodes a nuclear protein, and functions as a transcriptional repressor [167]. It is associated with skeletal frame height and size, which relates to collagen deposits and bone morphogenesis in the body [167]. *KIAA0319L*, a gene on chromosome 1p34 with unclear function is highly expressed in immune cells, such as natural killer cells and macrophages, which suggest its potential role to autoimmunity [168].

Table 1. Summary of the reported genes associated with SSc.

Function	Gene	Replication	Largest Size (SSc/Control)	Reference
Immune Regulation				
T cell differentiation proliferation activation	HLA	yes	1300/1000 for HLA alleles	[8-23, 11, 12]]
	STAT4	yes	2753/4569	[11, 12, 25-27]
	CD247	yes	2753/4569	[11, 33]
	TBX21	replicated in a single study	880/507	[27]
	PTPN22	inconsistent in subtype association	1100/740	[37-39]
	TNFSF4	yes	3014/3125	[43, 44]
	IL23R	inconsistent reports	1402/1038	[48, 49]
	IL2RA	no	3023/2735	[151]
	IL-21	no	4493/5856	[52]
	SCHIP1IL12A	replicated in a single study	4017/5935	[137]
	CD226	replicated in a single study	991/1008	[55]
B cell signaling	BANK1	yes	2380/3270	[57, 58]
	C8orf13-BLK	yes	1031/1014	[59-61]
innate immunity	PLD4	no	315/21054	[63]
	TLR-2	replicated in a single study	1170/925	[65]
	NLRP1	replicated in a single study	1059/625	[70]
	ATG5	replicated in a single study	4017/5935	[137]
Inflammation				
interferon regulation	IRF5	yes	2753/4569	[11, 12, 73-75]
	IRF8	replicated in a single study	3175/4971	[123]
NF-κB signaling	TNFAIP3	yes	1018/1012	[77-79]
	IRAK1	inconsistent reports	3065/2630	[81, 82]
	NFKB1	no	5270 /8326	[158]
	TNIP1	replicated in a single study	564/1776	[12]
cytokine/chemokine	FAS	inconsistent genotypes	2900/3186	[87, 88]
	MIF	yes	3800/4282	[89, 90]
	HGF	no	314/103	[93]
	OPN	no	357/864	[98]
	IL-6	no	2749/3189	[153]
	CXCL8	no	151/147	[102]
	CCR6	no	2411/7084	[105]
	CTGF	inconsistent reports	500/500	[107-111]
regulation of monocytes	ITGAM	inconsistent reports	4337/5326	[114-116]
regulation of cytokines	CAV1	replicated in a single study	791/843	[119]
Transcription	MECP2	replicated in a single study	3065/2630	[82]
	SOX5	replicated in a single study	3175/4971	[123]
	JAZF1	no	6835/14274	[165]
DNA cleavage	DNASEIL3	replicated in a single study	4017/5935	[137]
	XRCC1	no	177 case only	[172]
	XRCC4	no	177 case only	[172]
Kinase activity	PXK	no	6835/14274	[165]
	CSK	no	5270/8326	[158]
	GRB10	replicated in a single study	3175/4971	[123]
Cell differentiation	NOTCH4	replicated in a single study	3175/4971	[123]
Cell morphogenesis	RHOB	replicated in a single study	564/1776	[12]
Unknown	KIAA0319L	no	6835/14274	[165]
	PSD3	no	5270/8326	[158]
	PSOR1C1	replicated in a single study	564/1776	[12]

FUNCTIONAL ALLELIC STUDIES ON XRCC1 AND XRCC4

XRCC1 and *XRCC4* are two DNA repair genes that encode protein that forms a complex with other enzymes involved in repairing DNA double strand breaks [169-171]. Patients with SSc were reported to have an increased frequency of unstablized DNA breaks and spontaneous chromosomal damage [172-175]. Moreover, this increased DNA damages were found in even higher rate in patients with anti-centromere antibodies (ACA) [174]. In a study that evaluated DNA damage and polymorphic sites in these two genes in patients with SSc, patients with the *XRCC1* Arg399Gln allele showed increased frequency of ANA and ACA compared to patients with the *XRCC1* Arg399Arg allele [172]. Patients with the *XRCC4* Il3401Thr allele exhibited increased DNA damage compared with those with the Ile401Ile allele [172]. Interestingly, in a group of healthy subjects, *XRCC1* Arg399Gln and *XRCC4* Ile401Thr alleles are associated with a higher degree of DNA damage than the *XRCC1* Arg399Arg and *XRCC4* Ile401Ile alleles [172].

CONCLUSION

The set of genetic factors for SSc grows increasingly larger and more complex. Based on current susceptibility genes, several biological processes that seem important in SSc pathogenesis are immune regulation, inflammation, transcription, kinase activity, DNA cleavage and repair, which is summarized in Table **1**.

In the category of immune regulation, HLA genes are the major ones that are critical to initiate immune response. *STAT4*, *TBX21*, *PTPN22*, *TNFSF4*, *IL23R*, *IL2RA*, *IL21*, *IL12A*, *CD247* and *CD226* are involved in T cell differentiation, proliferation and/or activation. *BANK1* and *C8orf13-BLK* are involved in B cell signaling. *PLD4*, *TLR-2*, *NLRP1* and *ATG5* play important roles in innate immunity.

In the category of inflammation, *IRF5* and *IRF8* are regulators for interferon type I and II, respectively. *TNFAIP3*, *IRAK1*, *NFKB1* and *TNIP1* are involved in NF-κB activation and signaling. *FAS*, *MIF*, *HGF*, *OPN*, *IL-6*, *CXCL8*, *CCR6*, and *CTGF* are cytokines or chemokines that may be associated with tissue fibrosis in SSc. *CAV1* may control CTGF signaling. *ITGAM* regulates adhesion of neutrophils and monocytes.

While the genes functioning in immunity and inflammation appeared to be two major categories to SSc susceptibility, the genes involved in transcription regulation including *MECP2*, *SOX5* and *JAZF*, different kinase activities including *PXK*, *CSK* and *GRB10*, and DNA cleavage or repair including *DNASEIL3*, *XRCC1* and *XRCC4*, as well as *NOTCH4* and *RHOB* that control cell fate and morphogenesis, respectively, are also important to SSc. Together, a variety of SSc associated genes discussed herein indicate a heterogeneous nature of SSc genetics.

A sign of the heterogeneity of SSc genetics also lies in the pattern that has emerged in which some susceptibility genes were typically associated with the dcSSc and ATA+ subsets (i.e. *IRF5*, *BANK1*) or the lcSSc and ACA+ subsets (i.e. *STAT4*). While not all genes fall neatly into one category or the other, it might be useful to in future studies to view the pathogenesis of these two types of SSc as related but distinct. The highly auto-antibody specific HLA associations support this idea. Association of genes with sub-phenotypes is additionally helpful because they may allow physicians to predict the onset of pulmonary arterial hypertension, fibrosing alveolitis, and scleroderma renal crisis, and thus start treatment earlier.

Much research remains to be done on the genetics of SSc. Many newly identified SSc associated genes, such as *IRF8*, *SOX5*, *TNIP1*, *CSK*, *PSD3*, and *NFKB1* should be investigated further in replication studies to firmly establish their role in SSc genetics. Meanwhile, genes that have been verified by many studies, such as *STAT4* and *IRF5*, should be explored for their functional link to SSc.

ABBREVIATIONS

SSc	=	Systemic sclerosis
lcSSc	=	Limited cutaneous SSc
dcSSc	=	Diffuse cutaneous SSc
ATA	=	Anti-topoisomerase I autoantibodies
ACA	=	Anti-centromere autoantibodies
ARA	=	Anti-RNA polymerases autoantibodies
GWAS	=	Genome-wide association studies
HLA	=	Human leukocyte antigen
STAT4	=	Signal transducer and activators of transcription-4
CD247	=	T-cell surface glycoprotein CD3 zeta chain
TBX21	=	T-box 21
PTPN22	=	Protein tyrosine phosphatase nonreceptor type 22
TNFSF4	=	Tumor necrosis factor ligand superfamily member 4
IL23R	=	Interleukin-23 receptor
IL-21	=	Interleukin-21
BANK1	=	B cell scaffold protein with ankyrin repeats 1
BLK	=	B lymphoid kinase
PLD4	=	Phospholipase D4 gene
TLR	=	Toll-like Receptors
NLRP1	=	NLR family pyrin domain containing 1
IRF5	=	Interferon regulatory factor 5
TNFAIP3	=	Tumor necrosis factor-α–induced protein 3
IRAK1	=	IL-1 receptor–associated kinase 1
MIF	=	Macrophage migration inhibitory factor
HGF	=	Hepatocyte growth factor ()

OPN = Osteopontin

CXCL8 = Chemokine CXC motif ligand 8

CCR6 = CC chemokine receptor 6

CTGF = Connective tissue growth factor

ITGAM = Integrin alpha-M

CAV1 = Caveolin-1

MECP2 = Methyl-CpG-binding protein 2

IRF = Interferon regulatory factor

GRB10 = Growth factor receptor-bound protein 10

SOX5 = Sex determining region Y-box 5

NOTCH4 = Neurogenic locus notch homolog 4

TNIP1 = TNFAIP3 interacting protein 1

PSORS1C1 = Psoriasis susceptibility 1 candidate gene 1

RHOB = Ras homolog gene family member B

DNASE1L3 = Deoxyribonuclease I-like 3

SCHIP1-IL12A = Schwannomin-interacting protein 1/ Interleukin 12A.

ATG5 = Autophagy-related gene 5

IL2RA = Interleukin 2 receptor α

IL6 = Interleukin-6

CSK = c-src tyrosine kinase

PSD1 = Pleckstrin domain 1

NFKB1 = Nuclear factor of kappa light polypeptide gene enhancer in B-cell 1

PXK = PX domain containing serine/threonine kinase

JAZF1 = Juxtaposed with another zinc finger gene 1

XRCC = X-ray repair, complementing defective, in Chinese hamster

CONFLICT INTEREST

The authors confirm that this article content has no conflict of interest.

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