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Genetics of Systemic Sclerosis: An Update

Jasper C. A. Broen · Marieke J. H. Coenen · Timothy R. D. J. Radstake

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Abstract Systemic sclerosis (SSc) is an autoimmune disease characterized by vasculopathy, immune cell activation, and fibrosis of the skin and internal organs. Over the past few years, a role for genetics in the susceptibility for SSc has been established. This review aims to provide an update on the progress made in the past year or so within the field of SSc genetics research. This year has been of particular interest due to the publication of a large genome-wide association study, further investigations into gene–gene interactions, and the tendency to validate genetic results in functional models.

Keywords Systemic sclerosis · Genetics · Polymorphisms · Family studies · Genome-wide association studies

Introduction

Systemic sclerosis (SSc) is a multifaceted autoimmune disease. Its main manifestations are vasculopathy, immune activation, and ultimately extensive fibrosis of the skin and internal organs [1]. Until recently, the precise pathogenesis had not been elucidated, despite the growing interest and efforts from the scientific community in unraveling the factors involved in SSc susceptibility. Over the years, it has been suggested that environmental as well as genetic risk factors predispose individuals to the onset of SSc [2, 3]. Environmental factors are likely to play a role in triggering disease because genetics does not provide a full justification for the development of SSc. However, reports on predisposing environmental factors are ambiguous, and meta-analyses are severely hindered by differences in the methodologies used for exposure measurement by individual studies [4], which unfortunately leaves a convincing environmental risk factor to be discovered. Despite the difficulties involved with composing large, clinically well-defined cohorts, genetic research was able to discover multiple genetic risk factors that have been replicated or challenged by other studies. Many small and underpowered studies scrutinizing genetic risk factors were published in an earlier period, but thanks to the remarkable evolution of genetic research, SSc genetic cohorts and the genetic analyses performed became more comprehensive. Extensive reviews of these small studies and other findings in the field of genetic research throughout history have been published recently [5]. The aim of this review is to provide an update on the key findings emerging from studies from the past year that have explored the genetic background of SSc.

J. C. A. Broen · T. R. D. J. Radstake (✉)
Department of Rheumatology,
Radboud University Nijmegen Medical Centre,
Geert Grooteplein 8,
6500 HB Nijmegen, The Netherlands
e-mail: t.radstake@reuma.umcn.nl

M. J. H. Coenen
Department of Human Genetics,
Radboud University Medical Centre,
Nijmegen, The Netherlands

Family Studies

Although SSc is not inherited in a mendelian fashion, the highest risk factor for developing SSc today remains having a sibling (15- to 19-fold increase) or other first-degree relative (13- to 15-fold increase) with SSc. It must be noted that the relative risk (RR) of developing SSc remains rather low: 1.6% in families versus 0.026% in the general population [6, 7]. In 2010, these findings were further substantiated by a study investigating the heritability of SSc

in relation to Raynaud's phenomenon (RP), other autoimmune disease (AID), and fibrotic interstitial lung disease [8]. This study utilized a very powerful genealogic resource, the Utah Population Database, which contains both family structure data and medical records from more than 7 million individuals. The genetic structure of Utah residents is most similar to descendants from Northern Europe in that very low inbreeding rates are present. The cohort harbored 1,316 unique SSc patients, 1,037 of whom had a child and/or a parent and hence could be included in the study. For each SSc patient, 10 age-, gender-, and place of birth–matched healthy controls were randomly selected. From these 1,037 individuals, 50 families were identified that had a significantly larger than expected number of SSc cases. In these families, the RR ranged from 3.07 (95% CI, 1.25–7.57) in first-degree relatives to 2.14 (95% CI, 1.16–3.95) in third-degree relatives. In fourth-degree relatives, no significantly increased risk was present. An additional prerequisite of this study was that it included the heritability of RP among relatives of individuals with SSc. The study found a significant increase in RP risk in first- and second-degree relatives (RR, 6.38 [95% CI, 3.44–11.83] and 2.39 [95% CI, 1.21–4.74], respectively). The results concerning fibrotic disease heritability were somewhat less straightforward, as first- to fourth-degree relatives all had a significantly increased risk of developing interstitial lung disease, except the second-degree relatives. The authors did not provide a clear explanation for this observation but stated that the analysis may be limited by the selection of only interstitial lung disease as an outcome for inheritance of fibrosing disease, excluding, for instance, morphea and biliary cirrhosis. The third result presented in this study—the increased prevalence of other AIDs in families with SSc—matches the results of another study published in 2010 [9]. This study described a markedly increased prevalence of systemic lupus erythematosus (SLE) in North American families from 1,071 SSc probands (OR, 16.98 [95% CI, 1.02–227.82]). To a lesser extent, hypothyroidism (OR, 2.51 [95% CI, 1.46–4.62]) and hyperthyroidism (OR, 2.76 [95% CI, 1.16–7.97]) were found to have an increased prevalence in these families. The Achilles heel of this kind of study is the validation of family-derived data. The study conducted in Utah based the identification of cases on billing codes. Unfortunately, the data used were de-identified, so no independent validation could be carried out. In addition, the authors noted that not all SSc patients had a record of RP, which is generally considered to be almost universally present in SSc. Cases in the second study were well-ascertained by chart review or interviews of all first-degree relatives with reported AID by the same rheumatologist. Intriguingly, a study comparing 987 SSc cases with their 3,088 unaffected siblings found that the risk of developing SSc increased with increasing order of birth and number of pregnancies, suggesting that immune development very soon after birth or during pregnancy plays a role in SSc development [10•]. These findings might be the start of the unveiling of an apparent role for (epigenetic) imprinting and may wipe the dust from older studies describing a role for microchimerism in SSc [11].

Candidate Gene Studies

Over the past few years, several single nucleotide polymorphisms (SNPs) have been found to be enriched in patients with SSc or its clinical phenotypes compared with healthy controls. The SNPs were usually selected based on their locus in a gene likely to be involved in SSc pathogenesis, or when the SNP already posed a risk to another AID. Using these strategies, several novel genetic risk factors were identified. Herein we review new insights into the role of established candidate genes and describe novel associations. We focus on studies of sufficient size and power or findings that are, in our opinion, of special interest. In the last paragraph of this section, we briefly highlight some well-designed studies with negative results, which fortunately seem to be accepted for publication more frequently in recent years, counterbalancing positive publication bias. Table 1 displays the effect sizes and summarizes the results of the studies described below.

STAT4

After the discovery that signal transducer and activator of transcription 4 (*STAT4*) polymorphisms play a role in rheumatoid arthritis (RA) and SLE susceptibility [12], a role for this gene in SSc susceptibility was found as well. An initial study in 1,317 SSc patients and 3,113 healthy controls found the *STAT4* rs7574865 T allele to be associated with limited cutaneous SSc (lcSSc), but not with diffuse cutaneous SSc (dcSSC) [13]. The same association was substantiated in a Japanese cohort [14]. Subsequently, two other studies corroborated these results and strongly implicated *STAT4* in SSc susceptibility [15, 16]. Last year, these results prompted a combined European effort to investigate the functional role of the *STAT4* gene in mice. Both *STAT4*-deficient wild-type mice were injected with bleomycin. *STAT4* knockout mice had a decreased T-cell activation, proliferation, and cytokine release, which led to less fibrosis. Of interest, *STAT4*-deficient Tsk1 mice (a well-recognized murine SSc model) did not have a more severe fibrotic phenotype. From these observations, the authors drew the conclusion that *STAT4* is intricately involved in fibrosis and activation of fibroblasts. However, it must be noted that *STAT4* is a T-helper type 1 (Th1) promoter as well; therefore, a more straightforward explanation might be that *STAT4*-deficient mice have fewer Th1 circulating cells and

Table 1 Newly described non-*HLA* susceptibility genes for SSc and its clinical phenotypes

Gene	SNP (associated allele/genotype)	Association	Population	Size (tested phenotype/HC)	OR (95% CI)	Reference
TNFSF4	rs2205960 (T allele)	SSc	US	1,059/698	1.2 (1.1–1.5)	[19]
		ATA$^+$	US	174/698	1.4 (1.1–1.9)	[19]
	rs1234314 (G allele)	SSc	US	1,059/698	1.2 (1.04–1.4)	[19]
		ACA$^+$	US	300/698	1.3 (1.1–1.6)	[19]
		ATA$^+$	US	174/698	1.3 (1.02–1.7)	[19]
		SSc	European	2,856/2,920	1.15 (1.02–1.31)	[20]
		lcSSc	European	1,608/2,920	1.22 (1.07–1.38)	[20]
		ACA$^+$	European	828/2,920	1.23 (1.10–1.37)	[20]
	rs844648 (A allele)	SSc	US	1,059/698	0.8 (0.7–0.97)	[19]
		ATA$^+$	US	193/698	1.4 (1.1–1.8)	[19]
		lcSSc	European	1,673/2,977	1.1 (1.01–1.20)	[20]
		ACA$^+$	European	860/2,977	1.12 (1.01–1.25)	[20]
	rs844644 (A allele)	lcSSc	European	1,653/2,946	0.91 (0.83–0.99)	[20]
		ACA$^+$	European	856/2,912	0.90 (0.80–1.00)	[20]
	rs12039904 (T allele)	SSc	European	2,894/2,991	1.18 (1.08–1.29)	[20]
		lcSSc	European	1,639/2,991	1.20 (1.09–1.33)	[20]
		ACA$^+$	European	840/2,991	1.22 (1.07–1.38)	[20]
BLK	rs2736340 (TT genotype)	SSc	European and US	1,639/1,416	1.71 (1.2–2.4)	[22]
		ACA$^+$	European and US	510/1,416	2.27 (1.5–3.5)	[22]
	rs2736340 (CT genotype)	SSc	European and US	1,639/1,416	1.31 (1.1–1.5)	[22]
		ACA$^+$	European and US	510/1,416	1.6 (1.2–2.0)	[22]
	rs13277113 (AA genotype)	SSc	European and US	1,639/1,416	1.25 (1.1–1.4)	[22]
		ACA$^+$	European and US	510/1,416	1.42 (1.2–1.7)	[22]
	rs13277113 (A allele)	SSc	Japanese	309/769	1.45 (1.17–1.79)	[22]
TNFAIP3	rs5029939 (T allele)	SSc	European	1,667/1,318	2.08 (1.59–2.72)	[25]
		dcSSc	European	523/1,318	2.71 (1.94–3.79)	[25]
		FA	European	532/1,318	2.26 (1.61–3.17)	[25]
		PAH	European	119/1,318	3.11 (1.86–5.17)	[25]
KCNA5	rs10744676 (C allele)	PAH	European	192/1,008	0.36 (0.21–0.63)	[27]
PTPN22	rs2476601 (T allele)	SSc	European and US	3,422/3,638	1.15 (1.03–1.28)	[32]
		ACA$^+$	European and US	1,376/4,126	1.22 (1.05–1.42)	[32]
NLRP1	rs8182352 (C allele)	FA	European	674/1,587	1.19 (1.05–1.36)	[34]
		ATA$^+$	European	536/1,587	1.23 (1.07–1.41)	[34]
HGF	*HGF −1652* (*TT* genotype)	ESLD	Japanese	30/268	8.1 (2.5–26.0)	[36•]
CD226	rs763361 (T allele)	SSc	European	1,990/1,642	1.22 (1.10–1.34)	[39]
	rs763361 (TT genotype)	dcSSc	European	600/1,642	1.86 (1.42–2.43)	[39]
		FA	European	662/1,642	1.82 (1.38–2.40)	[39]
		ATA$^+$	European	553/1,642	1.61 (1.25–2.08)	[39]
CD247	rs2056626 (G allele)	SSc	European and US	5,049/9,740	0.85 (0.81–0.89)	[47•]
		SSc	French	1,031/1,014	0.78 (0.68–0.88)	[49]
		dcSSc	French	308/1,014	0.79 (0.65–0.95)	[49]
		lcSSc	French	643/1,014	0.79 (0.69–0.92)	[49]
		FA	French	346/1,014	0.78 (0.65–0.93)	[49]
IRF8	rs11642873 (A allele)	lcSSc	European and US	3,360/10,143	0.75 (0.69–0.81)	[50•]
GRB10	rs12540874 (A allele)	lcSSc	European and US	3,360/10,143	1.15 (1.09–1.22)	[50•]
SOX5	rs11047102 (C allele)	ACA$^+$	European and US	1,791/10,143	1.36 (1.21–1.52)	[50•]
TNIP1	rs2233287 (A allele)	SSc	European	2,246/4,684	1.31 (1.15–1.43)	[51]
RHOB	rs13021401 (T allele)	SSc	European	2,246/4,684	1.21 (1.12–1.31)	[51]

ACA anticentromere antibody; *ATA* antitopoisomerase antibody; *dcSSc* diffuse cutaneous systemic sclerosis; *ESLD* end-stage lung disease; *FA* fibrosing alveolitis; *HC* healthy controls; *lcSSc* limited cutaneous systemic sclerosis; *PAH* pulmonary arterial hypertension; *SNP* single nucleotide polymorphism *SSc* systemic sclerosis

consequently cannot mount the same response as *STAT4* wild-type mice—something the authors did not address [17•]. Consequently, these results might be coming from an overall impaired immune system rather than specifically less fibrotic potential, as the authors state. In addition, SSc patients carry an SNP in the gene that probably has a less pronounced effect on the gene function than a complete deletion of the gene, complicating translation of findings in mice to human patients. More subtle approaches, such as making use of immune cells from carriers of this particular variant, are welcome to better gauge the precise implication of this polymorphism in SSc.

OX40L

Tumor necrosis factor superfamily-4 (*TNFSF4* or OX40 ligand [*OX40L*]) is the natural ligand of OX40, which is present on T cells and influences T-cell survival and expansion. *OX40L* binding partner OX40 is more highly expressed in the serum of SSc patients than in healthy controls. Moreover, polymorphisms in this gene have been associated with SLE previously [18]. An initial American cohort consisting of 1,059 Caucasian SSc cases and 698 healthy controls found the rare variants of SNPs rs2205960, rs1234314, and rs844648 to be associated with SSc susceptibility. Additional analysis of clinical phenotypes demonstrated significant associations of *OX40L* variants with lcSSc and dcSSC, as well as antitopoisomerase antibody (ATA) and anticentromere antibody (ACA) profiles [19]. These results were recently corroborated by a European effort investigating the influence of *TNFSF4* polymorphisms in SSc susceptibility. This study found the most marked associations in patients with lcSSc and ACAs. In addition, this study identified novel *OX40L* haplotypes involved in SSc susceptibility [20].

The *FAM167A-BLK* Region

The *FAM167A-BLK* region, previously known as *C8orf13-BLK*, has been associated with multiple immune diseases [21]. In 2010, two studies addressing two polymorphisms in this region, rs13277113 and rs2736340, found an association with lcSSc and dcSSc. The T allelle of rs2736340 was associated with SSc in both a US and a Spanish case–control population [22]. Both variants were enriched in patients with ACAs and lcSSc in a combined analysis. A role for the rs13277113 variant in SSc was further substantiated by a Japanese study, which did not investigate rs2736340 [23]. Moreover, RNA expression profiling using peripheral blood elucidated significantly differentially expressed genes in the B-cell receptor–signaling pathways between patients with different genotypes [22].

TNFAIP3

Tumor necrosis factor-α–induced protein 3 (*TNFAIP3*) is a cytoplasmic zinc finger protein that inhibits nuclear factor-κB activity and tumor necrosis factor–mediated programmed cell death. Polymorphisms in this gene have been repeatedly associated with AID susceptibility [24]. A European study investigated 3 SNPs in 1,018 French patients with SSc and 1,012 French Caucasian controls. The *TNFAIP3* rs5029939 was found to be associated with SSc and subsequently replicated in a second set of 649 patients and 306 controls of German and Italian descent (OR, 2.08 [95% CI, 1.59–2.72]). In addition, the strongest association of rs5029939 was observed for dcSSc (OR, 2.71 [95% CI, 1.94–3.79]), fibrosing alveolitis (OR, 2.26 [95% CI, 1.61–3.17]), and pulmonary arterial hypertension (PAH) (OR, 3.11 [95% CI, 1.86–5.17]) [25].

KCNA5

Based on the observations of potassium voltage-gated channel, shaker-related subfamily, member 5 (*KCNA5*) polymorphisms contributing to idiopathic pulmonary hypertension, a study was initiated in 638 SSc patients and 469 controls to determine whether *KCNA5* polymorphisms confer susceptibility to SSc and, more specifically, to PAH [26]. Four *KCNA5* SNPs were genotyped, from which the rs10744676 variant was found to be associated with SSc (OR, 0.62 [95% CI, 0.48–0.79]). In addition, this polymorphism influenced SSc-related PAH (OR, 0.31 [95% CI, 0.13–0.71]). Subsequently, the results were replicated in 938 SSc patients and 564 controls. The above association with PAH in SSc was replicated, but not the association with SSc overall. However, some care must be taken with interpretation of these results because this study did not only include patients diagnosed with PAH on the basis of right heart catheterization, but also included those with PAH based on isolated diffusing capacity of the lung for carbon monoxide less than 50% predicted, and with unexplained dyspnea [27].

PTPN22

Protein tyrosine phosphatase non-receptor 22 (*PTPN22*) has been found to be associated with a broad array of AIDs, including RA and SLE [28]. Although not fully conclusive, a role for the *PTPN22* R620W polymorphism has been evaluated in SSc multiple times [29–31]. This year, a study was published again addressing the role of this variant but also the role of a novel described *PTPN22* variant in SSc susceptibility. In total, 3,422 SSc patients and 3,638 healthy controls from 7 different ethnic cohorts were included in this study. The meta-analysis performed in these cohorts identified the rs2476601 T allele as a risk factor for SSc

susceptibility (OR, 1.15 [95% CI, 1.03–1.28]). Moreover, the rs2476601 T allele was significantly associated with ACAs (OR, 1.22 [95% CI, 1.05–1.42]). The study suggests that the *PTPN22* R620W polymorphism influences SSc genetic susceptibility, whereas the novel R263Q genetic variant does not [32].

NLRP1

Based on the idea that T cells are able to dampen the inflammasome through *NLRP1* responses and the observation that *NLRP1* variants were found to confer susceptibility to AIDs, an endeavor was initiated to investigate this gene in SSc [33]. This study focused on 5 polymorphisms in a discovery set composed of 870 SSc patients and 962 controls and a German and Italian replication set consisting of 1,060 SSc patients and 625 controls. The discovery cohort revealed a significant association for the *NLRP1* rs8182352 variant with both ATAs and fibrosing alveolitis. In the replication cohort, no association was detected in the German population. However, the association with ATAs was replicated in the Italian population. When a meta-analysis was performed considering an additive model for the rs8182352 SNP, the investigators found this variant to confer susceptibility to ATAs and fibrosing alveolitis under an additive model (OR, 1.23 [95% CI, 1.07–1.41] and 1.19 [95% CI, 1.05–1.35], respectively). In addition, logistic regression analysis showed an additive effect of *IRF5* rs2004640, *STAT4* rs7574865, and *NLRP1* rs8182352 risk alleles on SSc-related fibrosing alveolitis [34]. Although the authors state that *NLRP1* is a new genetic susceptibility factor for SSc-related pulmonary fibrosis and ATA-positive SSc, this study would benefit from an independent replication study. As the results coming from the replication cohort are not straightforward, it would be interesting to know the pooled results of the replication cohort.

Hepatocyte Growth Factor

Hepatocyte growth factor (*HGF*) is an antifibrotic factor involved in regenerating damaged tissue by inhibiting fibrosis and promoting angiogenesis [35]. *HGF* seems to be on the other side of the fibrotic mediator spectrum as transforming growth factor-β and therefore might be involved in regulating fibrosis as observed in SSc. A recent Japanese study examined four SNPs within *HGF* and its receptor *c-met* for a role in SSc or SSc clinical subset pathogenesis. The authors did not find a difference in the distribution of the alleles of *HGF* or *c-met* SNPs between SSc patients and controls. Interestingly, they found the *HGF* −1652 TT genotype significantly more frequently in SSc patients with end-stage lung disease compared with patients without that disease (41% vs 8%). This association was confirmed in a relatively small replication study consisting of 155 SSc patients. Compensating for the relatively small replication cohort is a Kaplan-Meyer survival analysis showing that *HGF* −1652 TT carriers had a higher probability of developing end-stage lung disease in time than did carriers of the CT or CC genotype. In addition, the HGF promoter harboring the *HGF* −1652 T allele displayed decreased transcriptional activity compared with the promoter carrying the C allele, which was investigated by a luciferase reporter assay. Electrophoretic mobility shift assays unveiled the presence of a potential negative transcriptional regulator specifically binding to the *HGF* promoter and harboring the *HGF* −1652 T allele [36•]. Despite its small size, this study is of particular interest because it describes a variant of the *HGF* gene distinctively involved in the fibrosis observed in SSc and shapes out its functional relevance. Moreover, the results are analyzed and presented in a Kaplan-Meyer curve that points out clinical relevance more clearly than plain tables.

CD226

CD226, encoding DNAX accessory molecule 1, is important in monocyte extravasation and was recently found to be a genetic risk factor for AID [37, 38]. This observation prompted investigation of a possible association of the *CD226* rs763361 nonsynonymous polymorphism with SSc. To this purpose, a discovery cohort of 991 SSc patients and 1,008 controls and a replication cohort of 999 SSc patients and 634 controls from European Caucasian descent were utilized. The *CD226* rs763361 T allele was found to be associated with SSc in the discovery and the replication samples (OR, 1.22 [95% CI, 1.10–1.34]). In addition, the *CD226* TT risk genotype was associated with dcSSc (OR, 1.86 [95% CI, 1.42–2.43]), ATAs (OR, 1.82 [95% CI, 1.38–2.40]), and fibrosing alveolitis (OR, 1.61 [95% CI, 1.25–2.08]). To investigate the functional relevance of the identified genetic risk factor, expression of *CD226* was assessed on $CD3^+$, $CD4^+$, $CD8^+$, $CD56^+$, and natural killer T^+ cells obtained from 21 healthy donors genotyped for *CD226*, and rs763361 was measured. The polymorphism did not result in a significant change of *CD226* expression among these different sets of T cells [39].

The results described above, together with previously described findings, might imply that the genetic background is exactly similar to that of other AIDs or the idiopathic form of the SSc complication. However, although it is undeniable that there is a large resemblance between the genetic backgrounds of AIDs, some recently published studies also point out genes previously associated with AID that are not associated with SSc. This was true for the SLE-associated genes *CD89, FCGR2A, FCGR3A, ITGAM, ITGAX*, and *CD58* [40–42]. In addition, a

polymorphism previously associated with idiopathic or familiar hypertension and situated in the *SLC6A4* gene was not found to be associated with SSc-related PAH [43]. Finally, some potential genetic defects involved in SSc pathogenic events were discarded, including the *MMP2*, *MMP9*, and *MMP14* genes [44]. These studies, if well-performed and well-powered, are important because they carve out the differences between AIDs more explicitly.

Genome-Wide Association Studies

Before 2010, two genome-wide association studies (GWAS) were published addressing genetic susceptibility to SSc in a non–hypothesis-driven manner. Although very relevant at the time, they were premature considering the current standards for marker resolution and patient and control numbers. The first study utilized 400 markers and targeted 20 Choctaw patients with SSc and 76 matched controls [45]. The second (Korean) GWAS used an efficient number of 500,568 SNP markers but included only 137 SSc patients and 564 controls. Unfortunately, the identified risk factors for SSc were not replicated in a large US cohort. Noteworthy, SNPs in genes previously associated with SSc, including the aforementioned *PTPN22*, showed low *P* values but failed to reach genome-wide significance [46]. In 2010, however, a large and robust GWAS was published. This study included 2,296 SSc patients and 5,171 healthy controls originating from The Netherlands, Germany, Spain, and the United States. Different sets of markers were used that ranged in density from 308,349–488,793 SNPs. This study adjusted for multiple testing by regarding the genome-wide significance threshold ($P \leq 5 \times 10^{-7}$) as the limit for significance. The strongest association was observed at the 6p21 locus in the midst of the major histocompatibility complex region. At this region, rs6457617, situated in *HLA*DQB1*, showed the strongest association. In addition, five non-*HLA* loci showed genome-wide significance: *TNPO3/IRF5* region in 7q32, *STAT4* in 2q32, *CD247* in 1q22-23, *CDH7* in 18q22, and *EXOC2/IRF4* near 6p25. These results further establish *STAT4* and *IRF5* as genetic risk factors for SSc. To verify the novel findings, a case–control set comprising 2,753 SSc patients and 4,569 controls was genotyped for the 3 not previously described variants. Two SNPs identified in the discovery cohort in the *EXOC2/IRF4* and *CDH7* regions were not replicated in the validation cohort. However, the initial association with rs2056626 in the *CD247* gene was replicated [47•]. *CD247* is of particular interest considering it has an important role in the immune system by encoding the T-cell receptor zeta subunit, which is a component of the T-cell receptor complex; this gene has also been associated with susceptibility to SLE previously [48]. The association of *CD247* was recently replicated independently in a French Caucasian population consisting of 1,031 SSc patients and 1,014 healthy controls [49].

A follow-up study exploiting the above-mentioned GWAS data focused on determining genetic components contributing to lcSSc, dcSSc, ACA positivity, and ATA positivity. To this purpose, a meta-analysis was conducted in 4 cohorts comprising 2,296 SSc patients and 5,171 healthy controls. Subsequently, 18 polymorphisms with a *P* value lower than 1×10^{-5}—7 in the lcSSc subtype, 5 in the dcSSc subtype, 2 in ACA positives, and 4 in ATA positives—were further tested in 9 independent cohorts made up of an additional 3,175 SSc patients and 4,971 controls. Overall analysis revealed one variant in the interferon regulatory factor 8 (*IRF8*) gene (rs11642873) to be associated with lcSSc at genome-wide significance. Variants in the growth factor receptor–bound protein 10 (*GRB10*) and sex determining region Y-box 5 (*SOX5*) were just below the genome-wide significance threshold associated with lcSSc and ACA-positive subgroups, respectively (Table 1). Intriguingly, the authors proposed a model of *IRF8* and *SOX5* affecting the formation of extracellular matrix through collagen, type II, α1 in the skin and other organs of SSc patients. Furthermore, this study revealed genome-wide significant results in the *HLA-DQB1* locus with ACA-positive (OR, 2.48), in the *HLA-DPA1/B1* loci with ATA-positive (OR, 8.84), and in *NOTCH4* with ACA-positive (OR, 0.55) and ATA-positive (OR, 0.54). This study is of particular interest because it indicates that the heterogeneity of SSc phenotypes is likely to be the reflection of a different genetic foundation [50•].

Very recently, the fourth GWAS was published that used a high-resolution marker set comparable to that of the previous study (~500,000 markers). The study used a two-step approach; the first step consisted of a GWAS and was conducted in a French cohort consisting of 564 cases and 1,776 controls. Although only 1 SNP showed genome-wide significance, again in the *HLA*DQB1* region, the authors validated 20 SNPs (the top 17 significant SNPs and 3 previously associated SNPs) in a replication cohort of 1,682 SSc cases and 3,926 controls. Follow-up of the top 17 SNPs revealed associations at *PSORS1C1* (*HLA* region), *TNIP1*, and ras homolog gene family, member B (*RHOB*) loci (Table 1). Furthermore, the associations of previously identified candidate loci *STAT4*, *IRF5*, and *CD247* were substantiated. This study also addressed the functional relevance of the findings by investigating the *TNIP1* gene and protein expression. *TNIP1* was expressed at a lower level in both SSc lesional skin tissue and cultured dermal fibroblasts from SSc patients based on genotype. Intriguingly, *TNIP1* showed in vitro inhibitory effects on cytokine-induced collagen production. Although this study presents

novel and interesting candidate loci, it must be noted that only one locus reached genome-wide significance initially. The other loci became highly significant after pooling of the first and second step but are, strictly speaking, not significant yet in the initial French GWAS. These loci not being identified from the first step may be a power problem resulting from the relatively low number of patients included in the genome-wide association step. In addition, the study does not indicate if there is an overlap between cohorts used for previous studies identifying SSc susceptibility genes *STAT4*, *IRF5*, and *CD247* and the ones used in this study for replication, which would be welcome in order to place these results in the correct perspective [51].

Gene–Gene Interaction Studies

As discussed in the previous sections, a large number of genetic variants seem to be involved in SSc susceptibility and pathogenesis. These associated genes all have a very modest but reproducible effect on susceptibility throughout populations. In line with the paradigm of multifactorial diseases, one would expect to be at higher risk of SSc when harboring more susceptibility variants. Not until recently did genetic research in the field of SSc start to combine genetic data to determine whether some variants together formed an additive risk for SSc susceptibility. An additional thought that justifies these attempts is that many SSc candidate genes map to the same biological pathways [5].

The first successful attempt showed that *STAT4* (rs7574865) and *IRF5* (rs2004640) variants form an additive risk for development of SSc and interstitial lung disease [15]. After this first study, this research group repeated the analysis including the *BANK1* polymorphisms and was able to display an additive effect with regard to diffuse SSc susceptibility [52]. In a subsequent analysis, they added an *NLRP1* polymorphism to the list of variants interacting with *STAT4* and *IRF5* [34]. In contrast, the SSc-associated *BLK* region had an additive effect with *BANK1* in the dcSSc subset [53]. An American study showed that the *STAT4* polymorphism was predominantly enriched in SSc patients who carried the CC genotype at *TBX21* rs11650354 [16]. Next to this, an interesting study was published in 2010 describing a three-factor model comprising two SNPs: *IL-6 -174 C> G* and the *IL-2 -330 G> T*, and the *HLA-B*3501* allele that was predictive of the occurrence of digital ulcers in 200 Italian SSc patients [54•].

These initial studies on interactions of susceptibility genes look promising, but they still display only a small proportion of the risk of development of SSc. The most promising findings of gene–gene interactions playing a role likely will come forward from genome-wide interaction analysis. However, this kind of analysis is currently hampered by a lack of computational power and robust statistical methods.

Conclusions

This review aimed to provide an overview of the genetic research that has been conducted in the field of SSc in the past year. When reviewing this research, it becomes apparent that exciting progress has been made in several aspects of genetic research, including family-based studies, candidate studies, and (most noteworthy) GWAS. These observations together further substantiate the role of genetic susceptibility in SSc.

Although association studies of SSc in previous years mainly addressed genetic variants emerging from observations in other AIDs, last year, investigators turned with an increasing frequency toward genes involved in distinct biological processes by function that were possibly involved in SSc pathogenesis. This, however, yielded positive and negative results, with on one hand associations of *NLRP1* and *KCNA5* with PAH, and on the other hand no associations with fibrosis-related *MMP* genes. An attractive future perspective is the increasing number of multicenter initiatives focusing on unraveling the genetic background of SSc. This has the potential to lead to large genetic consortia with more than 10,000 SSc DNA samples, well-powered to discover even smaller genetic effects. The single most important step forward is the large and robust

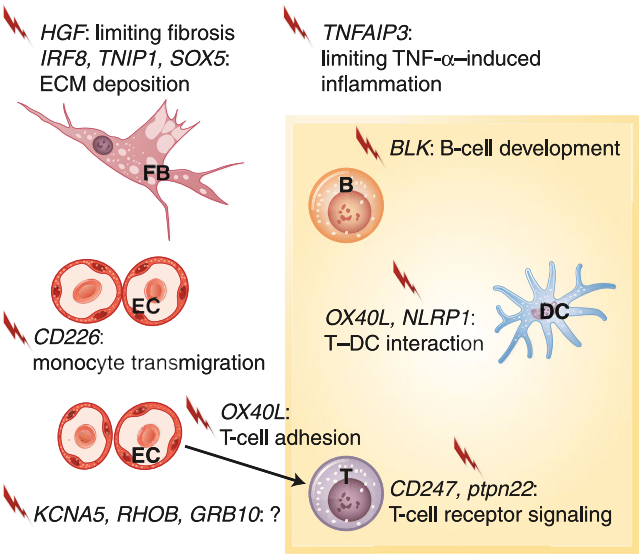

Fig. 1 Graphical representation of the immunologic point of impact of the newly discovered systemic sclerosis susceptibility genes. B—B cell; DC—dendritic cell; EC—vascular endothelial cell; ECM—extracellular matrix; FB—fibroblast; T—T cell; TNF—tumor necrosis factor

GWAS published last year; this study identified susceptibility genes similar to other AIDs, including RA and SLE. The challenge for the following years will be to identify whether certain combinations of risk alleles can drive the development of a particular AID, such as SSc, on a genetically similar background. In parallel with this, epigenetic research should focus on investigating the connections between the genetically susceptible host and the impact of environmental factors on expression of this susceptible genotype. The observation that birth order is a risk factor for SSc, reported last year, strongly hints in this direction.

An important gap in our understanding of the genetics involved in SSc is the lack of functional validation of genetic findings in immune cells or fibroblasts. Fortunately, this is being addressed more and more. Adding this functional validation step to candidate gene studies has become more mainstream in the past year, which enables researchers dedicated to investigating SSc pathogenesis on a cellular level to pick interesting molecules more efficiently from genetics research. This is of particular interest because the newly discovered variants affect multiple immunologic processes (Fig. 1). Another point, well-recognized as problematic in genetic research, is the translation from bench to bedside or, more precisely, the relevant clinical consequences of carrying a genetic variant that increases the risk of developing SSc. This is slowly becoming more apparent to researchers in the realm of SSc genetic research, as complication risk analysis using follow-up data is published at an increased rate. Ultimately, genetic risk factors might be used to better predict the development of scleroderma renal crisis, pulmonary hypertension, and pulmonary fibrosis. This might enable rheumatologists and other physicians to identify patients at risk early and start preventive treatment or check-ups at a higher frequency. A problematic subject in this aspect remains the low OR reported for genetic variants associated with complication development. However, if the investigations into applicable gene–gene interactions continue to expand in the way they did last year, combinations of polymorphisms might be discovered that provide enough risk to become clinical relevant for testing and a prelude to preventive treatments in the upcoming years.

In summary, there has been a steep increase in knowledge regarding the genetic background of SSc in the past year. This knowledge together with previous investigations forms a solid base for the next and most important step: the translation of genetic findings to more efficient patient care and therapeutic interventions.

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