Review

The genetics of systemic sclerosis: an update

E. Romano[1], M. Manetti[1,2], S. Guiducci[1], C. Ceccarelli[1], Y. Allanore[3], M. Matucci-Cerinic[1]

[1]*Department of Biomedicine, Division of Rheumatology, University of Florence, AOUC, DENOthe Centre, Florence, Italy;*
[2]*Department of Anatomy, Histology and Forensic Medicine, University of Florence, Florence, Italy;*
[3]*Université Paris Descartes, INSERM U1016, and Service de Rhumatologie A, Hôpital Cochin, Paris, France.*

Eloisa Romano, PhD
Mirko Manetti, PhD
Serena Guiducci, MD
Claudia Ceccarelli, MedLabTech
Yannick Allanore, MD, PhD
Marco Matucci-Cerinic, MD, PhD

Please address correspondence and reprint requests to:
Eloisa Romano
Department of Biomedicine,
Division of Rheumatology,
University of Florence,
AOUC, DENOthe Centre,
Villa Monna Tessa,
Viale Pieraccini 18,
50139 Florence, Italy.
E-mail: eloisaromano@libero.it

Received on February 17, 2011; accepted in revised form on April 21, 2011.

Clin Exp Rheumatol 2011; 29 (Suppl. 65): S75-S86.

Key words: systemic sclerosis, scleroderma, gene polymorphism, genetics

Competing interests: none declared.

ABSTRACT

Systemic sclerosis (SSc) is a complex systemic disease characterised by fibrosis of the skin and internal organs, vasculopathy, and activation of the immune system. The complex pathophysiology of SSc implies the potential involvement of 'culprit' genes, either individually or, more likely, together, in driving the disease process. Most of the studies that have provided evidence for the contribution of various genes/loci in SSc pathogenesis are based on a candidate gene approach, on the basis of a shared autoimmune genetic background with other autoimmune diseases, such as systemic lupus erythematosus. In fact, autoimmune genes seem to play a pivotal role in SSc pathogenesis, while less is known about the genetic involvement in vasculopathy and fibrosis. Recently, the availability of genome-wide association studies, which make it possible to screen single-nucleotide polymorphisms across the entire genome without previous knowledge of candidate regions or genes, has yielded a wealth of new genetic susceptibility loci leading to the identification of new pathogenetic mechanisms of complex genetic disorders. In this article, we aim to provide a comprehensive review of recent studies on the genetics of SSc, including genes associated with autoimmunity, fibrosis, and vascular disease. We also discuss the most relevant data obtained in genetic association studies of large populations that included a replication strategy, or studies for which independent replication was available.

Introduction

Systemic sclerosis (SSc, scleroderma) is a chronic, multisystem connective tissue disorder affecting the skin and internal organs characterised by widespread microangiopathy, fibrosis, and autoimmunity. The precise etiology of SSc has not been established, but epidemiologic and genetic studies reflect a complex genetic component, along with a largely unknown and presumably variable environmental contribution (1). A study of familial clustering of SSc in three North American cohorts showed a significant increase in the prevalence of SSc compared to the general population (2.6% *vs*. 0.026%, respectively) (2). In twin studies, concordance rate for the presence of antinuclear antibodies was found to be significantly higher in the monozygotic twins (90%) than in the dizygotic twins (40%), suggesting that the genetic component may selectively contribute to susceptibility to the SSc-related autoimmune processes (3). In the last few years, many progresses have been made in the field of SSc genetics. The complex pathophysiology of SSc implies the potential involvement of 'culprit' genes, either singly or, more likely, together, in driving the disease process. Many of these genes have been found to be associated with other autoimmune diseases, such as systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA), which suggests a shared genetic pathway for autoimmunity. Increasing evidence indicates that autoimmunity associated genes may play a pivotal role in the pathogenesis of SSc, while less is known about the contribution of genes involved in the vasculopathy and fibrotic processes. This review summarises recent advances on the complex genetic component of SSc, including studies on genes implicated in the three major areas of disease expression: autoimmune response, vascular disease and fibrosis.

SSc susceptibility genes

Immune response

Major histocompatibility complex (MHC)-Human leucocyte antigen (HLA) region: The association be-

tween *HLA class II* genotype and autoantibody profile in SSc has been reported in many studies (4-8). *HLA-DRB1*01–DQB1*0501* haplotypes are more common in anticentromere antibody (ACA)-positive SSc patients, while *HLA-DRB1*11–DQB1*0301* haplotypes have been associated with anti-topoisomerase I (anti-topo I) antibody-positivity (9). In the first reported genome-wide association study (GWAS) (10), 137 SSc Korean patients were compared with 564 healthy controls. The study was then replicated in North Americans. Five single-nucleotide polymorphisms (SNPs) located at the *HLA-DPB1* and *HLA-DPB2* locus formed a distinctive peak with log *p*-values for association with SSc susceptibility (p=8.16x10^{-13}). Fine mapping analysis of this genetic region confirmed that rs3128930, rs7763822 and rs7764491 were the SNPs accounting for the observed association, which was particularly strong in anti-topo I-positive SSc patients. In the replication step (1,107 SSc patients and 2,300 controls of North American Caucasian origin), the genotyping of these five SNPs revealed that rs7763822 and rs7764491 were significantly associated with anti-topo I-positive SSc patients (10). Furthermore, a recent large case-control association study analysed *HLA-class II* (*DRB1, DQB1, DQA1*, and *DPB1*) alleles, haplotypes and shared epitopes in a large multi-ethnic American cohort (9). The strongest positive class II associations with SSc observed in white and Hispanic populations were the *DRB1*1104*,*DQA1*0501*,*DQB1*0301* haplotype, and *DQB1* alleles encoding a non-leucine residue at position 26 (*DQB126**epi). Instead, *DRB1*0701*, *DQA1*0201, DQB1*0202* haplotype and *DRB1*1501* haplotype were negatively correlated and possibly protective in dominant and recessive models, respectively (9). In the very recent, much larger GWAS (11), a strong association with SSc susceptibility was found for a cluster of SNPs in an extended region of the 6p21 locus within the MHC region. In particular, the rs6457617 SNP located in the *HLA*DQB1* gene region gave the highest *p*-value (p=2.31x 10^{-18}) (11). No details about the subtypes and autoantibody status of SSc patients have yet been reported from this study. Further large GWAS studies will be necessary to investigate whether SNPs located in the MHC region may be associated with specific SSc phenotypes, such as the autoantibody pattern, disease subset and organ involvement.

STAT4: The protein encoded by *STAT4* gene is a member of the STAT family of transcription factors. In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate into the cell nucleus where they act as transcription activators (12). STAT4 protein is essential for mediating responses to interleukin-12 (IL-12) in lymphocytes, and regulates the differentiation of T helper cells. In particular, STAT4 appears to critically regulate the Th1/Th2 balance, which is known to be dysregulated in SSc (12). Independent studies have analysed the possible role of *STAT4* gene in the genetic predisposition to SSc. In the first study, the intronic rs7574865 polymorphism has been analysed in a case-control set of Spanish Caucasian ancestry with replication in five independent cohorts of European Caucasian ancestry (12). The rs7574865-T allele was significantly associated with susceptibility to limited cutaneous SSc (lcSSc) in the Spanish population (p=1.9x10^{-5}; odds ratio [OR] 1.61; 95% confidence interval [CI] [1.29–1.99]) and the association was validated in all the replication cohorts with different effect sizes (OR ranging between 1.15 and 1.86). In all the cohorts, no association was found between *STAT4* and diffuse cutaneous SSc (dcSSc) subset. A meta-analysis to test the overall effect of the rs7574865 polymorphism further showed a strong risk effect of the T allele for lcSSc susceptibility (pooled OR 1.54; 95% CI [1.36–1.74]; p<0.0001) (12). The second study, performed in a Japanese population, confirmed the association between *STAT4* rs7574865 polymorphism and lcSSc (13). Another study, performed in 1,855 individuals of French Caucasian origin, showed a significant association between the rs7574865 polymorphism and SSc, without restriction to a particular phenotype (14). This study also revealed an additive effect between *STAT4* and *IRF5* genes, in particular in terms of susceptibility to SSc-related fibrosing alveolitis (14). Another *STAT4* gene SNP, the rs11889341 variant, was recently investigated in 1,039 SSc patients and 3,322 controls of North American Caucasian background. These investigators found that the rs11889341-A allele increased the susceptibility (p=2.4x10^{-5}) to SSc in a dominant pattern (15). The role of *STAT4* as SSc genetic risk locus has been confirmed in the recently published GWAS, which identified an association with rs3821236 (11). Finally, the role of *STAT4* gene in tissue fibrosis has been demonstrated in a recent translational study using different animal models of SSc (16).

TBX21: TBX21 is another critical transcription factor that regulates the Th1/Th2 balance. Multiple SNPs in *TBX21* gene were found to be associated with SSc in a Caucasian American population of 902 SSc patients and 4,745 controls (15). The rs11650354-T allele showed a recessive pattern for disease susceptibility (p=1.4x10^{-15}; OR 3.37; 95% CI [2.4–4.6]). Furthermore, the authors identified gene-gene interaction among the *TBX21* rs11650354 and *STAT4* rs11889341 variants. In particular, the *STAT4* genotype increased the risk of SSc only in the *TBX21* rs11650354-CC genotype group. SSc patients carrying the *TBX21* CC genotype had higher IL-6 and tumour necrosis factor alpha (Th1 cytokines) levels, while those with the TT genotype had elevated IL-2, IL-5, IL-4, and IL-13 (Th2 cytokines) levels, compared with controls. Moreover, whole blood expression profiles revealed dysregulation of type I interferon pathways in the CC group and T-cell pathways in the TT group of the *TBX21* SNP, respectively. Collectively, these data indicate that *TBX21* and *STAT4* genes contribute interactively to SSc susceptibility, and may have a role in the altered Th1/Th2 cytokine balance and immune dysregulation (15).

Interferon regulatory factor 5 (IRF5): Type I interferon (IFN) is a central mediator of innate immunity. IFN regulatory factors coordinate the expression

of type I IFNs, which are believed to play a pivotal role in the pathophysiology of connective tissue disorders such as SLE, Sjögren's syndrome (SS), and SSc. Recently, the *IRF5* gene, a member of the IFN regulatory factors, was identified as a susceptibility gene for both SLE and SS (17, 18). A first case-control study showed that there was a significant association between the IRF5 rs2004640 G/T functional polymorphism and SSc (19). The *IRF5* rs2004640-T allele creates a donor splice site in intron 1 of the gene resulting in transcription of the alternative exon 1B (19). This polymorphism has been genotyped in 1,641 subjects of French European Caucasian origin split into discovery and replication cohorts. In both the discovery set and the replication set, the TT genotype was significantly more common in SSc patients than in controls (OR 1.58; 95% CI [1.18–2.11] in combined cohorts). Analyses of the whole SSc population showed a significant association between homozygosity for the T allele and the presence of antinuclear antibodies and fibrosing alveolitis (OR 2.07; 95% CI [1.38–3.11]) (19). The association of *IRF5* rs2004640 polymorphism and SSc has also been replicated in a Japanese population (20). In particular, an association was preferentially observed with dcSSc and anti-topo I antibody-positive SSc (20). Recently, additional functional *IRF5* variants have been identified as autoimmune susceptibility factors. *IRF5* rs377385, rs2004640, and rs10954213 were genotyped in 1,623 individuals of French European Caucasian origin (21). In this report, the autors found that a risk haplotype defined by the rs377385-C, rs2004640-T, and rs10954213-A SNPs (C-T-A) confers susceptibility to SSc, and defines subsets of patients with dcSSc and lung fibrosis (21). The role of *IRF5* gene as SSc genetic risk factor has also been confirmed in the recently published large GWAS of SSc (11).

Protein tyrosine phosphatase, non-receptor type 22 (lymphoid) (PTPN22): The *PTPN22* gene encodes a protein tyrosine phosphatase which is expressed primarily in lymphoid tissues. It is involved in the suppression of T cell activation, and thereby in T cell-dependent antibody production, potentially contributing to the pathogenesis of autoimmune diseases. The minor allele of the R620W missense SNP (C1858T, rs2476601) in the *PTPN22* gene was firstly analysed in a French (22) and in a Spanish cohort (23) in two independent studies. No association was found between rs2476601 and SSc in both studies. Subsequently, the first evidence of association was found in a large cohort of 1,120 patients belonging to 4 ethnic groups in the US (white, black, Hispanic, and Choctaw Indian individuals). In particular, the *PTPN22* R620W polymorphism was associated with both ACA-positive and anti-topo I antibody-positive subsets of SSc (24). A first meta-analysis of studies on this polymorphism in SSc provided evidence that the *PTPN22* 1858T allele is involved in the genetic susceptibility to SSc in European Caucasian (p=8.39x10^{-3}; OR 1.08; 95% CI [1.02–1.15]) and mixed (p=3.11x10^{-3}; OR 1.09; 95% CI [1.04–1.16]) populations, particularly in the anti-topo I-positive subset (25). Recently, an additional meta-analysis of seven Caucasian cohorts from Spain, Belgium, England, Germany, Italy, the Netherlands, and Sweden, and including the findings from two previous studies on US and French Caucasian populations (24, 25), showed evidence for an association of the 1858T allele restricted to the ACA-positive SSc subset (p=0.02 pooled, OR=1.22, 95% CI [1.05–1.42]) (26). Instead, no association was found between SSc and the rs33996649 (R263Q) *PTPN22* polymorphism (26). Collectively, these findings highlight the importance of meta-analyses to solve contradictory results from previously published independent case-control studies.

B cell scaffold protein with ankyrin repeats 1 (BANK1): BANK1 is a B cell-specific scaffold protein that functions in B cell receptor-induced calcium mobilisation from intracellular stores. Two *BANK1* gene missense variants (rs10516487 and rs3733197) were studied in 874 SSc patients and 955 controls of a French cohort and 421 SSc patients and 182 controls of a German cohort (27). The rs3733197-A rare allele and the rs10516487-T rare allele were found to be associated with dcSSc in both cohorts. The G-C haplotype was shown to be a risk factor (p=0.008; OR 1.25; 95% CI [1.06–1.47]), while the A-T haplotype was reduced among dcSSc patients, indicating a protective effect (p=3.39x10^{-4}, OR 0.70; 95% CI [0.57–0.86]) (27). The association between rs10516487-G (C) and rs3733197-G polymorphic alleles with dcSSc was then confirmed in a large multicentre case-control study from six independent cohorts of Caucasian ancestry (28). The association was not restricted to dcSSc, but was also found to be linked to the anti-topo I antibody-positive subset. In particular, a significant association of the rs10516487-G and rs17266594-T alleles with SSc susceptibility was observed (pooled OR=1.12; 95% CI [1.03–1.22]; p=0.01 and pooled OR=1.14, 95% CI [1.05–1.25]; p=0.003, respectively) (28). Taken together, the association of *BANK1* gene with the SSc phenotype found in independent studies suggests an important role of B cells in the diffuse cutaneous subset of SSc patients. In this context, recent preclinical and clinical studies lend support to the notion that B cell depletion may be a promising therapeutic target in patients with dcSSc (29).

Tyrosine-protein kinase or B lymphocyte kinase (BLK): BLK is a Src kinase that is expressed in thymocytes. It transduces signals downstream of the B cell receptor and plays an important role in B cell development. GWASs in SLE have implicated the *C8orf13-BLK* gene region of chromosome 8p23.1 as a susceptibility locus for SLE. Two variants in the *C8orf13-BLK* region (rs13277113 and rs2736340) were tested for association with SSc in a population consisting of 1,050 SSc cases and 694 controls of North Americans of European Caucasian descent with replication in a second series of 589 SSc cases and 722 controls from Spain (30). Both variants were associated with ACA-positive SSc (p=2.2x10^{-6} and p=5.5x10^{-4}, respectively) and lc-SSc (p=3.3x10^{-5} and p=2.9x10^{-3}, respectively) in the combined analysis

(30). The rs13277113 variant was also found to be associated with SSc in the Japanese population, without restriction to specific autoantibody and cutaneous phenotypes (31). Recently, the association of *C8orf13/BLK* locus with SSc has been investigated in a large French Caucasian cohort (1,031 SSc patients and 1,014 controls), and a meta-analysis of all the available data (for a total of 6,149 individuals) was also performed (32). Minor allele frequencies for rs13277113 revealed an association restricted to the dcSSc subtype (p=0.012, OR 1.29) in the French sample. Meta-analysis of combined Caucasian populations showed association with both SSc (p=0.0013, OR 1.16 [1.06–0.26]) and dcSSc (p=0.0012, OR 1.23 [1.08–1.39]). Inclusion of the Japanese population (31) confirmed the overall association with the disease, with the strongest association for dcSSc (p=3.27x10^{-5}, OR 1.27). An additional analysis in the French sample revealed additive effects between *C8orf13/BLK* and *BANK1*, mainly for dcSSc susceptibility (32). Taken together, these findings extend the evidence for a genetic association of SSc with B cell-specific genes (*BANK1* and *BLK*).

T cell surface glycoprotein CD3 zeta chain or T cell receptor T3 zeta chain (CD247): *CD247* gene encodes the T cell receptor zeta (CD3ζ) subunit, a component of the T cell receptor (TCR)-CD3 complex. The CD3ζ chain plays an important role in the assembly of the TCR-CD3 complex and its transport to the cell surface, and is crucial to receptor signalling function. The expression of the CD3ζ chain is altered in chronic autoimmune and inflammatory disorders and its low expression results in impaired immune response. The first large GWAS, including a total of 2,296 individuals with SSc and 5,171 controls of European Caucasian ancestry, identified a new susceptibility locus, previously found in SLE, at *CD247* (1q22–23, rs2056626, p=3.39×10^{-9}) (11). Recently, the rs2056626 SNP has been genotyped in a French Caucasian population consisting of 1,031 SSc patients and 1,014 controls (33). This study confirmed the association of *CD247* rs2056626-G allele with SSc susceptibility under a dominant model of inheritance (p =7.22×10^{-5}; OR 0.69; 95% CI [0.58–0.83]) (33).

Tumour necrosis factor alpha-induced protein 3 (TNFAIP3): *TNFAIP3* gene encodes the ubiquitin-modifying enzyme, a key regulator of inflammatory signalling pathways. Polymorphisms in *TNFAIP3* have been associated with multiple autoimmune diseases (34). In a recent study, three SNPs, two intergenic (rs10499194 and rs6920220) and one located in intron 2 (rs5029939), were genotyped in a set of 1,018 SSc patients and 1,012 controls of French European Caucasian ancestry (34). *TNFAIP3* rs5029939 was found associated with SSc in the French population, and then the association was replicated in a second set of 465 SSc patients and 182 controls from Germany and 184 SSc patients and 124 controls from Italy. In the combined populations, the rs5029939-G allele was found to be significantly associated with SSc susceptibility (pooled OR=2.08; 95% CI [1.59–2.72]; p=1.16×10^{-7}) and in particular with dcSSc (pooled OR 2.71; CI [1.94–3.79], p=5.2×10^{-9}), SSc-related fibrosing alveolitis (pooled OR 2.26; CI [1.61–3.17], p=2.5×10^{-6}) and pulmonary arterial hypertension (PAH) (pooled OR=3.11; CI [1.86–5.17], p=1.3×10^{-5}). No association was found for the rs10499194 and rs6920220 variants (34). These results suggest that *TNFAIP3* may contribute to the autoimmune component of SSc pathogenesis.

CD226: *CD226* gene encodes DNAX accessory molecule 1, which is involved in T cell co-stimulation pathways. The non-synonymous rs763361 polymorphism in the *CD226* gene has recently been identified as a genetic risk factor for different autoimmune disorders. The rs763361 SNP has been genotyped in 3,632 European Caucasian individuals, consisting of a discovery sample (991 SSc patients and 1,008 controls) and a replication sample (999 SSc patients and 634 controls) (35). The *CD226* rs763361-T allele was found to be associated with SSc in both the discovery and the replication samples, reaching an OR of 1.22 (95% CI [1.10–1.34], p=5.69×10^{-5}) in the combined population. The most remarkable associations of the *CD226* TT risk genotype were observed with the dcSSc subtype (OR 1.86, 95% CI [1.42–2.43], p=5.15×10^{-6}), the anti-topo I antibody-positive (OR 1.82, 95% CI [1.38–2.40], p=2.16×10^{-5}), and SSc-related fibrosing alveolitis subsets (OR 1.61, 95% CI [1.25–2.08], p=2.73×10^{-4}) (35). These results suggest that co-stimulation pathways may be involved in the immune abnormalities of SSc. However, postgenomic functional studies on peripheral blood mononuclear cells did not reveal any correlation between *CD226* rs763361 genotypes and CD226 expression in all the T cell subtypes investigated (35). Therefore, further studies will be necessary to define the causal variant at the CD226 locus as well as the functional consequences.

FAS: FAS antigen (Apo-1/CD95) is a key molecule involved in the apoptosis of a wide variety of cell types, including activated immune cells and fibroblasts. A SNP in the enhancer region of the *FAS* gene promoter, *FAS*-670G>A SNP (rs1800682), has been described (36). SSc is characterized by sustained activation and clonal expansion of B and T lymphocytes, and different subsets of circulating T cells from SSc patients have been shown to be resistant to FAS-mediated apoptosis (37). In a large cohort of Italian Caucasian patients, an association between the *FAS*-670G>A polymorphism and SSc was described (38). Moreover, *FAS*-670 genotype was found to be correlated with serum levels of antiapoptotic soluble FAS, which competes with membrane-bound FAS for the binding to FAS-ligand (38). In another study, the role of *FAS*-670G>A polymorphism has been analysed in 9 distinct ethnic cohorts, 6 of European Caucasian ancestry and 3 distinct ethnic cohorts from the US (white, black and hispanic) (39). A meta-analysis comprising all 9 cohorts revealed an association of both the *FAS*-670G allele (OR 1.10) and the *FAS*-670GG genotype (OR 1.13) with the lcSSc phenotype. In addition, the -670GG genotype was associated with ACA-positive lcSSc (39). Dysregulation of the FAS-mediated proapoptotic signalling might play a role in SSc pathogenesis through the genetic control of apoptosis resistance

in different cell types, such as activated lymphocytes and fibroblasts.

Interleukin-23 receptor (IL23R): Multiple studies suggest a role for mediators of Th17 pathway, such as IL-17 and IL-23, in different autoimmune diseases, including SSc. In particular IL-23 is essential for the amplification and/or stabilisation of Th17 phenotype, and IL-23 protein levels have been found to be increased in patients with SSc. IL-23 activity is mediated by binding to the IL-23 receptor complex, which is composed of an IL-12Rβ1 and a unique cytokine receptor subunit termed IL-23R. Recent results pointed toward the important role of *IL23R* gene as a genetic marker for autoimmunity (40). In particular, a non-synonymous polymorphism (rs11209026, Arg381Gln) has been associated with susceptibility to different autoimmune conditions such as inflammatory bowel disease, ankylosing spondylitis or psoriasis. Seven SNPs spanning the *IL23R* gene, including rs11209026, were genotyped in two cohorts of European Caucasian patients, but no association was found between any *IL23R* genetic variant and SSc susceptibility and clinical phenotypes (40). In a recent study on 1,402 SSc cases and 1,038 controls of Caucasian, African American and Hispanic ethnicity from US, the *IL23R* rs11209026-GG and rs11465804-TT genotypes were found to be associated with anti-topo I antibody-positive SSc (p=0.001 and p=0.0001 respectively) (41). Moreover, the rs11465804-TT genotype was significantly associated with anti-topo I antibody-positivity in the dcSSc subset (p=0.0026). Wild-type genotype at both rs11209026 and rs11465804 showed significant protection against the developement of PAH (p=3x10^{-5}, p=1x10^{-5}, respectively) (41).

Allograft inflammatory factor-1 (AIF-1): AIF-1 is involved in the immune response and proliferative vasculopathy that occours during allograft rejection. It is encoded within the *HLA class III* genomic region on chromosome 6p21. AIF-1 expression was found increased in affected blood vessels of the lung and skin of SSc patients (42). A non-synonymous polymorphism (rs2269475) located in exon 3 and causing a tryptophan to arginine amino acid substitution has been identified in the *AIF1* gene. The rs2269475 SNP has been investigated in two large independent cohorts of SSc patients of Caucasian, African American and Hispanic origin (43). T and CT/TT rs2269475 frequencies were significantly increased in ACA-positive Caucasian SSc patients, and in all ACA-positive SSc patients (the three ethnic groups combined), when compared with both ACA-negative SSc patients and healthy controls. In another study, the rs2269475 SNP was significantly associated with SSc (p=0.0009), and in particular with the dcSSc subset (p=0.002) in a Caucasian cohort (44). The discrepancies between these two studies indicate that further investigations and meta-analyses will be necessary to determine the exact role that *AIF1* gene may play in the genetic susceptibility to SSc phenotype.

FCG receptor (FCGR): FCGRs recognise the Fc portion of IgG and are important in determining the response of leucocytes to deposited immune complexes. FCGRs also provide positive and negative regulation of immune cell responses (45). The *FCGR2A* 519A>G and *FCGR3A* 559A>C functional variants have been recently analysed in 6 independent European Caucasian cohorts (for a total of 1,566 SSc patients and 2,271 controls). Neither *FCGR2A* 519A>G nor *FCGR3A* 559A>C was significantly associated with susceptibility to SSc, and no association was found with specific disease phenotypes, limited or diffuse cutaneous involvement, autoantibody profiles, or pulmonary involvement (45).

Tumour necrosis factor ligand superfamily member 4 (TNFSF4, OX40L): *TNFSF4* gene encodes for OX40L, a protein expressed on dendritic cells, macrophages, B and T cells, natural killer cells as well as non-immune cells such as endothelial cells and smooth muscle cells. It is the ligand for the OX40 which is expressed on CD4+ and CD8+ T cells, where it provides a co-stimulatory signal resulting in T cell proliferation, survival and cytokine production. Studies have suggested that OX40–OX40L interaction may preferentially promote Th2 cytokines and may be a negative regulatory signal for IL-17 production (46). A total of 9 SNPs in the *TNFSF4* gene, previously associated with susceptibility to SLE, were tested for association with SSc in a population of 1,059 patients with SSc and 698 controls of North American Caucasian origin (46). Case-control comparisons revealed a significant association between SSc susceptibility and rs1234314-G (OR 1.20, 95% CI [1.04–1.4], p=0.019), rs2205960-T (OR 1.24, 95% CI [1.10–1.50], p=0.019) and rs844648-A (OR 1.16, 95% CI [1.01–1.30], p=0.032) minor alleles. Instead, the rs844644-A minor allele was protective (OR 0.84, 95% CI [0.70–0.97], p=0.038). Moreover, the rs1234314-G and rs2205960-T minor alleles were more common in lcSSc compared to controls, while an increased frequency of the minor alleles at *TNFSF4* rs1234314, rs2205960 and rs844648 SNPs, as well as a decreased frequency of the minor allele at rs844644, were found in dcSSc. A significant association was also observed with rs1234314 and both ACA-positive and anti-topo I-positive SSc patients. The minor allele at rs2205960 was more frequent in anti-topo I-positive SSc. The rs844648 SNP was associated with anti-RNA polymerase III-positive SSc (46). In a second study, four genetic variants of *TNFSF4* gene promoter (rs1234314, rs844644, rs844648 and rs12039904) were analysed in a total of 8 European populations of Caucasian ancestry (3,014 SSc patients and 3,125 healthy controls) (47). A pooled analysis found a significant association of rs1234314 and rs12039904 polymorphisms with SSc. All four tested variants were found to be significantly associated with lcSSc subset. In addition, rs1234314, rs844648 and rs12039904 minor alleles were associated with ACA-positive SSc (47). Collectively, these data confirm the influence of *TNFSF4* gene in SSc susceptibility (46,47). However, the two studies highlight some discrepancies concerning the specific SSc subsets associated with different *TNFSF4* SNPs. Therefore, additional meta-analytic studies will be required.

Table I summarises the confirmed associations with SSc found in some of the genes involved in immune regulation.

Table I. Immune genes associated with SSc susceptibility and phenotypes.

Gene	Polymorphism	Population	Associated phenotype	Study
STAT4	rs7574865	European Caucasian	lcSSc	Rueda *et al*. (12)
		Japanese	lcSSc, ACA	Tsuchiya *et al*. (13)
		French Caucasian	SSc	Dieude *et al*. (14)
	rs11889341	US Caucasian	SSc	Gourh *et al*. (15)
	rs3821236	European/US Caucasian	SSc	Radstake *et al*. (11)
IRF5	rs2004640	French Caucasian	SSc,ANA, fibrosing alveolitis	Dieude *et al*. (19)
		Japanese	dcSSc, ATA	Ito *et al*. (20)
		French Caucasian	dcSSc, fibrosing alveolitis	Dieude *et al*. (21)
	rs3757385	French Caucasian	dcSSc, fibrosing alveolitis	Dieude *et al*. (21)
	rs10954213	French Caucasian	dcSSc, fibrosing alveolitis	Dieude *et al*. (21)
	rs10488631	European/US Caucasian	SSc	Radstake *et al*. (11)
	rs12537284	European/US Caucasian	SSc	Radstake *et al*. (11)
	rs4728142	European/US Caucasian	SSc	Radstake *et al*. (11)
TBX21	rs11650354	US Caucasian	SSc	Gourh *et al*. (15)
PTPN22	rs2476601	US mixed background	ACA, ATA	Gourh *et al*. (24)
		European Caucasian	SSc, ATA	Dieude *et al*. (25)
		European/US Caucasian	ACA	Diaz-Gallo *et al*. (26)
BANK1	rs3733197	French/German Caucasian	dcSSc	Dieude *et al*. (27)
		European/US Caucasian	dcSSc, ATA	Rueda *et al*. (28)
	rs10516487	French/German Caucasian	dcSSc	Dieude *et al*. (27)
		European/US Caucasian	dcSSc, ATA	Rueda *et al*. (28)
BLK	rs13277113	European/US Caucasian	lcSSc, ACA	Gourh *et al*. (30)
		Japanese	SSc	Ito *et al*. (31)
		French Caucasian	dcSSc	Coustet *et al*. (32)
	rs2736340	European/US Caucasian	lcSSc, ACA	Gourh *et al*. (30)
CD247	rs2056626	European/US Caucasian	SSc	Radstake *et al*. (11)
		French Caucasian	SSc	Dieude *et al*. (33)
TNFAIP3	rs5029939	European Caucasian	SSc, dcSSc, fibrosing alveolitis, PAH	Dieude *et al*. (34)
CD226	rs763361	European Caucasian	SSc, dcSSc, ATA, fibrosing alveolitis	Dieude *et al*. (35)
TNFSF4	rs1234314	US Caucasian	SSc, dcSSc	Gourh *et al*. (46)
		European Caucasian	SSc, lcSSc, ACA	Bossini-Castillo *et al*. (47)
	rs2205960	US Caucasian	SSc, dcSSc	Gourh *et al*. (46)
	rs844648	US Caucasian	SSc, dcSSc	Gourh *et al*. (46)
		European Caucasian	lcSSc, ACA	Bossini-Castillo *et al*. (47)
	rs12039904	European Caucasian	SSc, lcSSc, ACA	Bossini-Castillo *et al*. (47)
	rs844644	European Caucasian	lcSSc	Bossini-Castillo *et al*. (47)
FAS	rs1800628	Italian Caucasian	SSc	Liakouli *et al*. (38)
		European/US Caucasian	lcSSc, ACA	Broen *et al*. (39)

ACA: anticentromere antibodies; ANA: antinuclear antibodies; ATA: anti-topoisomerase I antibodies; dcSSc: diffuse cutaneous systemic sclerosis; lcSSc: limited cutaneous systemic sclerosis; PAH: pulmonary arterial hypertension; SSc: systemic sclerosis.

Fibrosis

Fibrosis is the most pathological hallmark of SSc. Progressive replacement of tissue architecture by collagen-rich extracellular matrix (ECM) results in functional impairment of affected organs. The ECM consists of a cellular compartment of resident and infiltrating cells, and a connective tissue compartment composed of collagens, proteoglycans, fibrillins and adhesion molecules. The ECM also functions as a reservoir for transforming growth factor β (TGFβ), connective tissue growth factor (CTGF), other growth factors and matricellular proteins that, together with the connective tissue compartment, control mesenchymal cell differentiation, function and survival (48). Excessive connective tissue accumulation in fibrotic disorders is due to overproduction by activated fibroblasts, myofibroblasts and related interactions. Impaired ECM degradation and turnover, and expansion of the pool of mesenchymal cells in lesional tissues further contribute to ECM accumulation (48). Genes involved in the fibrotic process encode proteins that make up or regulate the ECM composition, cytokines and growth factors.

Connective tissue growth factor (CTGF): CTGF (also known as CCN2) has a critical role in the development and maintenance of fibrosis (49-51). The expression of *CTGF* gene has been found to be up-regulated in gene-expression–profiling studies of skin-biopsy specimens and of fibroblasts cultured from SSc skin (49, 50). Moreover, CTGF has been associated with key biologic functions, including fibroblast proliferation, the production of ECM, and the formation of granulation tissue, which are all processes relevant in the field of SSc (49-51). A polymorphism (G–945C) (rs6918698) in the promoter of the *CTGF* gene was found to be associated with SSc in a UK population (52) and in a Japanese population (53). The homozygosity for the G allele was significantly associated with SSc, anti-topo I antibody-positivity and SSc-related fibrosing alveolitis (52). Functional studies demonstrated that the rs6918698-C allele had higher affinity for the transcriptional regulator Sp3 resulting in reduced transcriptional activity of the *CTGF* gene (52). However, this association was not replicated in two large studies in American and European Caucasian populations (54, 55). Recently, an association between another SNP in *CTGF* gene (rs9399005 C/T) and both dcSSc and lcSSc has been found in a French European Caucasian population (56). These authors also provided evidence that the rs9399005 SNP may affect the structure of CTGF messenger RNA (56).

Serotonin 5-HT2A receptor: Platelet aggregation may contribute to the pathogenesis of SSc. Indeed, following activation, platelets release significant amounts of serotonin which promotes vasoconstriction and fibrosis. The C+1354T polymorphism in the exonic region of the *serotonin 5-HT2A receptor* gene, which determines a His452Tyr substitution, was associated with blunted intracellular responses after serotonin stimulation, and may have a role in susceptibility to SSc. In an Italian study, this polymorphism resulted associated with a three-fold reduction in the

risk for SSc (57), but this was not replicated in a successive population-based replication study in a German Caucasian SSc cohort (58). Another study on serotonin transporter gene (*SLC6A4*) polymorphisms (rs1042173, rs2066713 and a long/short 44 base pair insertion within the promoter region), performed in a large cohort of European Caucasian SSc patients, showed no association between these polymorphisms and SSc or with SSc-related PAH and/or digital ulcerations (59).

Interleukin-1 alpha (IL1-α) and Interleukin-1 beta (IL1-β): IL1-α and IL1-β have been implicated in the fibrogenic phenotype of SSc fibroblasts. Three *IL1-α* gene SNPs (-889C/T, +4845G/T, and +4729T/C) were associated with SSc-related pulmonary fibrosis in a Japanese population (60). However, the association between *IL1-α*-889T polymorphism and SSc resulted controversial in subsequent studies (61-64). Three polymorphisms in the *IL1-β* gene (C-511T; C-31T; C+3962T) have been studied in Italian Caucasian SSc patients (62). The two polymorphic alleles *IL1-β* -511-T and *IL1-β* -31-C were significantly more frequent in SSc patients compared to a control population from the same geographic area (62), while the *IL1-β* C+3962T SNP resulted associated with the presence of severe restrictive pulmonary function in other studies (64,65). The important role of *IL1-β* in SSc has been confirmed in a recent study on *NLRP1* gene which codifies a molecule that provides a scaffold for inflammasome assembly, promoting pro-IL1-β processing and maturation (66). In this study, performed in two large sets of European Caucasian individuals, the *NLRP1* rs8182352 variant was found to be associated with both anti-topo I-positive SSc and SSc-related fibrosing alveolitis subsets (p=0.0042 and p=0.0065 respectively) (66). Furthermore, *NLRP1* rs8182352, *IRF5* rs2004640 and *STAT4* rs7574865 risk alleles showed an additive effect on SSc-related fibrosing alveolitis (66). These findings provide new insights into the pathogenesis of SSc, underlining the potential role of innate immunity in particular in the fibrosing alveolitis-positive SSc phenotype, which represents one of the most severe subsets of the disease.

Matrix metalloproteinase (MMP): ECM remodelling involves the complex interplay between ECM component synthesis, deposition, and degradation. MMPs are enzymes able to degrade ECM participating in physiologic and pathologic processes. In an Italian case-control study performed on 513 Caucasian subjects, the rs2276109 A/G functional polymorphism in the *MMP12* gene promoter region was found to contribute to SSc susceptibility (67). The rs2276109-A allele has a higher affinity for AP-1 transcription factors, resulting in increased *MMP12* gene expression. The *MMP12* rs2276109-A allele was found to be significantly associated with dcSSc subset, anti-topo I antibody-positivity and SSc-related pulmonary fibrosis under a recessive model of inheritance (67). Studies performed on *MMP1*, *MMP2*, *MMP9* and *MMP14* gene polymorphisms revealed no association with SSc susceptibility or clinical phenotypes (68-70).

Vascular disease

Vascular damage is a primary event in the pathogenesis of SSc. The progressive vascular injury includes persistent endothelial cell activation/damage and apoptosis, intimal thickening, delamination, vessel narrowing and obliteration. These changes lead to vascular tone dysfunction and reduced capillary blood flow, with consequent tissue ischaemia and severe clinical manifestations, such as digital ulceration or amputation, pulmonary arterial hypertension, and scleroderma renal crisis (71). Nevertheless, in SSc patients there is no evidence of significant angiogenesis and the disease evolves towards chronic tissue ischaemia, with progressive and irreversible structural changes in multiple vascular beds culminating in the loss of capillaries (71). PAH is the main pulmonary vascular complication of SSc; it occurs in about 10% of patients and leads to major excess mortality (72). Many mediators that modulate arterial tone and remodelling are involved in the genesis of PAH, and all the genes encoding these mediators are potential candidate genes.

Endoglin (ENG): Among the factors that maintain vascular integrity, endoglin (CD105) is a component of the TGF-β receptor complex and is predominantly expressed on the cell surface of endothelial cells. A 6-base insertion in intron 7 (6bINS) of *ENG* gene has been reported to be associated with microvascular disturbance (73). In a multicentric cohort of European Caucasian SSc patients, the 6bINS allele resulted associated with a decreased risk of PAH (74). However, these results need independent replication in other populations.

Stromal cell-derived factor 1 (SDF-1/CXCL12): The chemokine SDF-1 and its receptor CXCR4 regulate specific steps in new vessel formation and play a role in CD34+ endothelial progenitor cells mobilisation into peripheral blood and homing to ischaemic tissues (75, 76). The *SDF1* gene, located on chromosome 10q11, is polymorphic, with a G-to-A transition at position 801 in the 3'-untraslated region of cDNA encoding SDF-1β, one of the two isoforms of SDF-1 protein, referred as *SDF1-3*' polymorphism (rs1801157). This SNP has been correlated with the mobilisation of bone marrow CD34+ endothelial progenitor cells and the levels of SDF-1 transcript (77). In an Italian cohort, the *SDF1-3*'A allele was found to influence the predisposition to SSc-related PAH (OR=2.52, 95% CI [1.11–5.69], p=0.02) and skin ulcers (OR=2.31, 95% CI [1.18–4.52], p=0.01). After adjustment for age and gender, the *SDF1-3*'A allele remained a susceptibility factor for the SSc-related vascular manifestations (PAH: OR=2.37, 95% CI [1.04–5.42], p=0.04; ulcers: OR=2.33, 95% CI [1.78–4.62], p=0.01) (77). Further studies will be required to confirm these associations in independent cohorts of SSc patients.

Hypoxia-inducible factor 1A (HIF1A): HIF-1A is a transcription factor that plays an essential role in cellular and systemic responses to hypoxia, and is involved in the ischaemia-induced angiogenic response. Three SNPs in the *HIF1A* gene (rs12434438 A/G, rs1957757 C/T and rs11549465 C/T) have been studied in a large French Caucasian population. The heterozygous

rs12434438-AG genotype was significantly associated with SSc susceptibility, lcSSc and ACA-positive subsets, suggesting a role for *HIF1* gene in SSc phenotype (78).

Urokinase-type plasminogen activator receptor (uPAR): uPAR (also known as CD87) is a glycosylphosphatidylinositol-anchored 3-extracellular domain receptor that has been implicated in SSc-related microvascular abnormalities and impaired angiogenesis, as well as in the fibrotic process (71, 79-83). Two *uPAR (PLAUR)* gene promoter polymorphisms (rs344781 and rs4251805) were investigated in two European Caucasian populations: an Italian discovery cohort and a French replication cohort. The combined analysis of the pooled samples (732 SSc patients and 607 controls) showed a strong association of the rs344781-G allele and -GG genotype with SSc-related digital ulcers (allele OR 1.41; 95%CI [1.11–1.78]; p=0.005; genotype OR 2.15; 95%CI [1.25–3.72]; p=0.005), SSc-related PAH (allele OR 1.65; 95%CI [1.17–2.32]; p=0.004; genotype OR 3.16; 95%CI [1.58–6.32]; p=0.0006), ACA-positive SSc (allele OR 1.47; 95%CI [1.15–1.89]; p=0.002; genotype OR 2.40; 95%CI [1.38–4.19]; p=0.001) and lcSSc (allele OR 1.34; 95%CI [1.09–1.64]; p=0.004; genotype OR 1.77; 95%CI [1.08–2.91]; p=0.02). Furthermore, in a multivariate logistic regression analysis model including the above associated phenotypes of SSc patients, the rs344781-GG genotype remained an independent risk factor for SSc-related digital ulcers (OR 1.96; 95%CI [1.01–3.85]; p=0.04) and SSc-PAH (OR 2.68; 95%CI [1.25–5.75]; p=0.01). Further functional studies are necessary to elucidate the exact molecular mechanisms by which the *uPAR* rs344781 SNP may be implicated in different aspects of SSc vascular phenotype, such as the lack of angiogenesis and proliferative vasculopathy (83).

Potassium voltage-gated channel, shaker related subfamily, member 5 (KCNA5): Functional *KCNA5* SNPs have been reported to be associated with idiopathic PAH, including SNPs located in exons or the promoter region that may underlie the altered function and/or expression of voltage-gated K+ channel 1.5 (Kv1.5) channels observed in pulmonary arteriolar smooth muscle cells from patients with idiopathic PAH (84). Four *KCNA5* SNPs (rs10744676, rs1860420, rs3741930, and rs2284136) were genotyped in a discovery set of 638 SSc patients and 469 controls of French Caucasian ancestry. In the same study, the rs10744676 was further genotyped in an independent replication sample (938 SSc patients and 564 controls) of German and Italian Caucasian ancestry, as well as in a cohort of 168 patients with different PAH subtypes (85). The rs10744676 SNP showed an association with SSc-PAH in the French population, and this finding was replicated in the second set. The other *KCNA5* SNPs tested were not associated with any SSc subset. In the independent cohort of patients with different PAH subtypes, only the rs10744676 SNP showed an association with SSc-related PAH (85). This work suggests that *KCNA5* gene may contribute to the development of proliferative vasculopathy in SSc.

Vascular endothelial growth factor (VEGF): VEGF is an endothelial cell-specific mitogen glycoprotein that promotes angiogenesis. Previous studies have implicated VEGF and VEGF receptors (VEGFRs) in the microvascular abnormalities and impaired angiogenesis that characterise SSc (71). In a multinational study involving European Caucasian individuals, three functional *VEGF* gene polymorphisms were studied (634 C/T, 936 C/G, and insertion/deletion of 18 base pairs at -2549 position in the promoter) (86). Moreover, in another case-control study on 1,170 European Caucasian subjects, eight SNPs covering the entire *VEGFR1* gene and five SNPs in the *VEGFR2* gene were analysed (87). Neither *VEGF* nor *VEGFR1* and *VEGFR2* polymorphisms were significantly associated with susceptibility to SSc or with specific disease phenotypes (86, 87). However, independent replication should be performed to definitely roule out the possible influence of *VEGF*, *VEGFR1* and *VEGFR2* genes in SSc phenotype.

Angiotensin-converting enzyme (ACE): The renine angiotensin system (RAS) is closely implicated in vascular disease. ACE, a key enzyme in the RAS, is encoded by the *ACE* gene, mapping to the 17q23 region. An insertion/deletion (I/D) polymorphism in intron 16 of the *ACE* gene correlates with high circulating levels of ACE enzyme in the general population. However, studies of its possible role in SSc susceptibility produced conflicting results: an association between the D allele of *ACE* I/D and SSc was firstly detected in an Italian cohort (88), but this finding was not replicated in North American (89), Greek (90), Korean (91) and French populations (92). These discrepancies may be explained by low statistical power or population stratification because of the heterogeneous genetic background.

Endothelial nitric oxide synthase (eNOS/NOS3) and inducible NOS (iNOS/NOS2): Endothelium derived nitric oxide (NO) plays a key role in the regulation of vascular tone and has vasoprotective effects by scavenging superoxide radicals and suppressing platelet aggregation, leucocyte adhesion and smooth muscle cell proliferation. The relative underproduction of the vasodilator NO, by eNOS, could contribute to the vascular damage typical of SSc. In an Italian cohort of SSc patients, the *eNOS* 894G>T polymorphism was more common in patients than in controls (88). In a successive study, the same group showed a role of *eNOS* -786T>C and 894G>T polymorphisms in influencing the hemorheologic behavior in SSc (93). Subsequent studies failed to confirm the association between *eNOS* 894G>T polymorphism and SSc (89, 94). In a recent Turkish study, three *eNOS* gene polymorphisms (tandem 27-bp repeats (VNTR) in intron 4, -786T>C in promoter region and 894G>T in exon 7) were investigated (95). Genotype distribution was significantly different between SSc patients and controls for intron 4aa (alleles for four repeats), genotype frequency being 3.4% and 17.1%, respectively. The -786CC genotype of the promoter was significantly higher in frequency in the SSc patients (16.9%) compared to controls (7.3%) (95). Another study investigated two SNPs in the promoter region (-1,026 and -277) and a pen-

tanucleotide repeat (CCTTT) at -2.5 kilobases of the *NOS2* gene (96). The -1,026 and -277 SNPs were found to be associated with SSc-related PAH. The CCTTT repeat was significantly shorter in SSc patients with PAH than in those without PAH or healthy controls. Functional studies demonstrated that a short allele of the CCTTT repeat determined a lower transcriptional activity of *NOS2* gene (96).

Endothelin (ET-1) and receptors: ET-1 plays a crucial role in vascular damage, both directly through its potent vasoconstrictive effect and indirectly through the induction of genes implicated in vascular dysfunction and inflammatory response. ET-1 can also promote tissue remodeling and fibrosis by induction of fibroblast activation (97). The two endothelin receptors, ETRA and ETRB, belong to the superfamily of G protein-coupled receptors. Polymorphisms within the genes encoding ET-1 (*EDN1* at 6p24), and its receptors (*EDNRA* at 4q31, and *EDNRB* at 13q22) have been analysed (97). No significant differences between the SSc group and control subjects were observed for any of the investigated polymorphisms. However, the presence of anti-RNA polymerase autoantibodies was associated with the *EDNRA* +69 C/T and +105 A/G SNPs in exon 6, while *EDNRB* -2446 C/A (exon 3), +2841 G/A (exon 2), and -2547 A/G (exon 3) SNPs were associated with dcSSc (97). These findings and their functional significance need to be confirmed and investigated in future studies.

Fibrinogen: Fibrinogen is a key protein involved in blood clotting. It increases blood viscosity, erythrocyte aggregation, vasoreactivity and endothelial permeability, thereby promoting intravascular and parietal thrombosis in individuals with an underlying vascular injury, as occurs in SSc patients. The -455G>A polymorphism in the proximal promoter region of the *β-fibrinogen* gene has been reported to be an independent risk factor for thrombosis in both homo-(AA) and heterozygosity (GA) and to increase plasma fibrinogen levels (98). A preliminary study performed on 47 Italian SSc patients and 66 controls showed that the *β-fibrinogen* -455A allele was more frequent in SSc patients with digital lesions (pitting scars/ulcers) than in those with uncomplicated Raynaud's phenomenon (13/17 *vs*. 6/30) (OR=13.000; 95% CI [3.098–54.557]; p=0.0002). Moreover, SSc patients carrying the *β-fibrinogen* -455GA genotype showed an increase in fibrinogen plasma levels (98). These preliminary data suggest an association between the *β-fibrinogen* -455A allele and peripheral vascular damage in SSc patients, and need to be extended and replicated in larger studies.

Conclusions and future perspectives

To date, most of the studies that have provided evidence for the contribution of various genes or gene regions to SSc pathogenesis are based on a candidate gene approach, on the basis of a shared autoimmune genetic background with other autoimmune diseases, notably SLE. Recently, the availability of GWAS, which makes it possible to screen SNPs across the entire genome without previous knowledge of candidate regions or genes, has yielded a wealth of new genetic susceptibility loci leading to the identification of new pathogenetic mechanisms of complex genetic disorders, including SSc. Collectively, a major role of autoimmunity in SSc pathogenesis is supported by the recent identification of multiple genetic markers related to the innate and adaptive immune regulation, such as *HLA class II* gene region, *STAT4*, *IRF5*, *BANK1*, *BLK*, *TNFSF4* and *CD247* genes, which have been firmly established as SSc susceptibility genes through replication in independent studies. Recent meta-analytic studies have also proved to be useful tools in solving previously reported conflicting data on the association of immune genes with specific clinical and immunological subsets of the disease. Moreover, increasing evidence suggests that multiple immune genes may show an additive effect on SSc susceptibility and phenotypes. However, SSc is an heterogeneous disease likely regulated by complex mechanisms that are mostly unknown, but probably unique, and that involve a cross-talk among immune, vascular and fibrotic pathways. Controversial results have been reported for genes involved in the development of fibrosis and vascular disease. However, those studies were often limited by small sample sizes and clinical heterogeneity of patients. Therefore, larger studies based on a candidate gene or GWAS approach, will be necessary to clarify the role of genes involved in vascular and fibrotic pathways in SSc genetic susceptibility. Future studies should also investigate whether gene-gene interactions between multiple common immune, vascular and fibrotic risk variants, as well as gene-environmental interactions, may influence the susceptibility to SSc and its clinical manifestations. Finally, postgenomic functional studies are now required to unravel the exact cellular and molecular mechanisms by which the multiple risk genetic components to date identified may participate in the pathogenesis of SSc.

References

1. ALLANORE Y, WIPFF J, KAHAN A, BOILEAU C: Genetic basis for systemic sclerosis. *Joint Bone Spine* 2007; 74: 577-83.
2. ARNETT FC, CHO M, CHATTERJEE S, AGUILAR MB, REVEILLE JD, MAYES MD: Familial occurrence frequencies and relative risks for systemic sclerosis (scleroderma) in three United States cohorts. *Arthritis Rheum* 2001; 44: 1359-62.
3. FEGHALI-BOSTWICK C, MEDSGER JR TA, WRIGHT TM: Analysis of systemic sclerosis in twins reveals low concordance for disease and high concordance for the presence of antinuclear antibodies. *Arthritis Rheum* 2003; 48: 1956-63.
4. KUWANA M, INOKO H, KAMEDA H *et al*.: Association of human leucocyte antigen class II genes with autoantibody profiles, but not with disease susceptibility in Japanese patients with systemic sclerosis. *Intern Med* 1999; 38: 336-44.
5. GILCHRIST FC, BUNN C, FOLEY PJ *et al*.: Class II HLA associations with autoantibodies in scleroderma: a highly significant role for HLA-DP. *Genes Immun* 2001; 2: 76-81.
6. UEKI A, ISOZAKI Y, TOMOKUNI A *et al*.: Different distribution of HLA class II alleles in anti-topoisomerase I autoantibody responders between silicosis and systemic sclerosis patients, with a common distinct sequence in the HLA-DQB1 domain. *Immunobiology* 2001; 204: 458-65.
7. REVEILLE JD, FISCHBACH M, MCNEARNEY T *et al*.: Systemic sclerosis in 3 US ethnic groups: a comparison of clinical, sociodemographic, serologic, and immunogenetic determinants. *Semin Arthritis Rheum* 2001; 30: 332-46.
8. GREGERSEN PK, OLSSON LM: Recent ad-

vances in the genetics of autoimmune disease. *Annu Rev Immunol* 2009; 27: 363-91.
9. ARNETT FC, GOURH P, SHETE S *et al*.: Major Histocompatibility Complex (MHC) class II alleles, haplotypes, and epitopes which confer susceptibility or protection in the fibrosing autoimmune disease systemic sclerosis: analyses in 1300 Caucasian, African–American and Hispanic cases and 1000 controls. *Ann Rheum Dis* 2010; 69: 822-7.
10. ZHOU X, LEE JE, ARNETT FC *et al*.: HLA-DPB1 and DPB2 are genetic loci for systemic sclerosis: a genome-wide association study in Koreans with replication in North Americans. *Arthritis Rheum* 2009; 60: 3807-3814.
11. RADSTAKE TR, GORLOVA O, RUEDA B *et al*.: Genome-wide association study of systemic sclerosis identifies CD247 as a new susceptibility locus. *Nat Genet* 2010; 42: 426-29.
12. RUEDA B, BROEN J, SIMEON C *et al*.: The STAT4 gene influences the genetic predisposition to systemic sclerosis phenotype. *Hum Mol Genet* 2009; 18: 2071-7.
13. TSUCHIYA N, KAWASAKI A, HASEGAWA M *et al*.: Association of STAT4 polymorphism with systemic sclerosis in a Japanese population. *Ann Rheum Dis* 2009; 68: 1375-76.
14. DIEUDÉ P, GUEDJ M, WIPFF J *et al*.: STAT4 is a genetic risk factor for systemic sclerosis having additive effects with IRF5 on disease susceptibility and related pulmonary fibrosis. *Arthritis Rheum* 2009; 60: 2472-9.
15. GOURH P, AGARWAL SK, DIVECHA D *et al*.: Polymorphisms in TBX21 and STAT4 increase the risk of systemic sclerosis: evidence of possible gene-gene interaction and alterations in Th1/Th2 cytokines. *Arthritis Rheum* 2009; 60: 3794-806.
16. AVOUAC J, FÜRNROHR BG, TOMCIK M *et al*.: Inactivation of the transcription factor STAT4 prevents inflammation-driven fibrosis in systemic sclerosis animal models. *Arthritis Rheum* 2011; 63 :800-9.
17. BAECHLER EC, BATLIWALLA FM, KARYPIS G *et al*.: Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc Natl Acad Sci USA* 2003; 100: 2610-5.
18. GOTTENBERG JE, CAGNARD N, LUCCHESI C *et al*.: Activation of IFN pathways and plasmacytoid dendritic cell recruitment in target organs of primary Sjögren's syndrome. *Proc Natl Acad Sci USA* 2006; 103: 2770-5.
19. DIEUDÉ P, GUEDJ M, WIPFF J *et al*.: Association between the IRF5 rs2004640 functional polymorphism and systemic sclerosis: A new perspective for pulmonary fibrosis. *Arthritis Rheum* 2009; 60: 225-33.
20. ITO I, KAWAGUCHI Y, KAWASAKI A *et al*.: Association of a functional polymorphism in the IRF5 region with systemic sclerosis in a Japanese population. *Arthritis Rheum* 2009; 60: 1845-50.
21. DIEUDE P, DAWIDOWICZ K, GUEDJ M *et al*.: Phenotype-haplotype correlation of IRF5 in systemic sclerosis: role of 2 haplotypes in disease severity. *J Rheumatol* 2010; 37: 987-92.
22. WIPFF J, ALLANORE Y, KAHAN A *et al*.: Lack of association between the protein tyrosine phosphatase non-receptor 22 (PTPN22)* allele 620W and systemic sclerosis in the French Caucasian population. *Ann Rheum Dis* 2006; 65: 1230-2.
23. BALADA E, SIMEON-AZNAR CP, SERRANO-ACEDO S *et al*.: Lack of association of the PTPN22 gene polymorphism R620W with systemic sclerosis. *Clin Exp Rheumatol* 2006; 24: 321-4.
24. GOURH P, TAN FK, ASSASSI S *et al*.: Association of the PTPN22 R620W polymorphism with antitopoisomerase I- and anticentromere antibody-positive systemic sclerosis. *Arthritis Rheum* 2006; 54: 3945-53.
25. DIEUDÉ P, GUEDJ M, WIPFF J *et al*.: The PTPN22 620W allele confers susceptibility to systemic sclerosis: findings of a large case-control study of European Caucasians and a meta-analysis. *Arthritis Rheum* 2008; 58: 2183-8.
26. DIAZ-GALLO LM, GOURH P, BROEN J *et al*.: Analysis of the Influence of *PTPN22* Gene Polymorphisms in Systemic Sclerosis. *Ann Rheum Dis* 2011; 70: 454-62.
27. DIEUDÉ P, WIPFF J, GUEDJ M *et al*.: BANK1 is a genetic risk factor for diffuse cutaneous systemic sclerosis and has additive effects with IRF5 and STAT4. *Arthritis Rheum* 2009; 60: 3447-54.
28. RUEDA B, GOURH P, BROEN J *et al*.: BANK1 functional variants are associated with susceptibility to diffuse systemic sclerosis in Caucasians. *Ann Rheum Dis* 2010; 69: 700-5.
29. VAN LAAR JM: B-cell depletion with rituximab: a promising treatment for diffuse cutaneous systemic sclerosis. *Arthritis Res Ther* 2010; 12: 112.
30. GOURH P, AGARWAL SK, MARTIN E *et al*.: Association of the C8orf13-BLK region with systemic sclerosis in North-American and European populations. *J Autoimmun* 2010; 34: 155-62.
31. ITO I, KAWAGUCHI Y, KAWASAKI A *et al*.: Association of the FAM167A-BLK region with systemic sclerosis. *Arthritis Rheum* 2010; 62: 890-5.
32. COUSTET B, DIEUDÉ P, GUEDJ M *et al*.: C8orf 13/BLK is a genetic risk locus for systemic sclerosis and has additive effect with BANK1: Results from a large French cohort and meta-analysis. *Arthritis Rheum* 2011 Apr 7 [Epub ahead of print].
33. DIEUDÉ P, BOILEAU C, GUEDJ M *et al*.: Independent replication establishes the *CD247* gene as a genetic systemic sclerosis susceptibility factor. *Ann Rheum Dis* 2011 [Epub ahead of print]
34. DIEUDÉ P, GUEDJ M, WIPFF J *et al*.: Association of the TNFAIP3 rs5029939 variant with systemic sclerosis in the European Caucasian population. *Ann Rheum Dis* 2010; 69: 1958-64.
35. DIEUDÉ P, GUEDJ M, TRUCHETET ME *et al*.: Association of the CD226 Ser(307) variant with systemic sclerosis: evidence of a contribution of costimulation pathways in systemic sclerosis pathogenesis. *Arthritis Rheum* 2011; 63: 1097-105.
36. HUANG QR, MORRIS D, MANOLIS N: Identification and characterization of polymorphisms in the promoter region of human Apo-1/Fas (CD95) gene. *Mol Immunol* 1997; 34: 577-82.
37. CIPRIANI P, FULMINIS A, PINGIOTTI E *et al*.: Resistance to apoptosis in circulating α/β and γ/δ T lymphocytes from patients with Sistemic Sclerosis. *J Rheumatol* 2006; 33: 2003-14.
38. LIAKOULI V, MANETTI M, PACINI A *et al*.: The -670G>A polymorphism in the FAS gene promoter region influences the susceptibility to systemic sclerosis. *Ann Rheum Dis* 2009; 68: 584-90.
39. BROEN J, GOURH P, RUEDA B *et al*.: The FAS -670A>G polymorphism influences susceptibility to systemic sclerosis phenotypes. *Arthritis Rheum* 2009; 60: 3815-20.
40. RUEDA B, BROEN J, TORRES O *et al*.: The interleukin 23 receptor gene does not confer risk to systemic sclerosis and is not associated with systemic sclerosis disease phenotype. *Ann Rheum Dis* 2009; 68: 253-6.
41. AGARWAL SK, GOURH P, SHETE S *et al*.: Association of interleukin 23 receptor polymorphisms with anti-topoisomerase-I positivity and pulmonary hypertension in systemic sclerosis. *J Rheumatol* 2009; 36: 2715-23.
42. DEL GALDO F, MAUL GG, JIMÉNEZ SA, ARTLETT CM: Expression of allograft inflammatory factor 1 in tissues from patients with systemic sclerosis and in vitro differential expression of its isoforms in response to transforming growth factor beta. *Arthritis Rheum* 2006; 54: 2616-25.
43. ALKASSAB F, GOURH P, TAN FK *et al*.: An allograft inflammatory factor 1 (AIF1) single nucleotide polymorphism (SNP) is associated with anticentromere antibody positive systemic sclerosis. *Rheumatology* (Oxford) 2007; 46: 1248-51.
44. OTIENO FG, LOPEZ AM, JIMENEZ SA, GENTILETTI J, ARTLETT CM: Allograft inflammatory factor-1 and tumor necrosis factor single nucleotide polymorphisms in systemic sclerosis. *Tissue Antigens* 2007; 69: 583-91.
45. ALIZADEH BZ, BROEN J, RUEDA B *et al*.: Functional variants of Fc gamma receptor (FCGR2A) and FCGR3A are not associated with susceptibility to systemic sclerosis in a large European Study (EUSTAR). *J Rheumatol* 2010; 37: 1673-9.
46. GOURH P, ARNETT FC, TAN FK *et al*.: Association of TNFSF4 (OX40L) polymorphisms with susceptibility to systemic sclerosis. *Ann Rheum Dis* 2010; 69: 550-5.
47. BOSSINI-CASTILLO L, BROEN JC, SIMEON CP *et al*.: A replication study confirms the association of TNFSF4 (OX40L) polymorphisms with systemic sclerosis in a large European cohort. *Ann Rheum Dis* 2011; 70: 638-41.
48. WYNN TA: Cellular and molecular mechanisms of fibrosis. *J Pathol* 2008; 214: 199-210.
49. IGARASHI A, NASHIRO K, KIKUCHI K *et al*.: Significant correlation between connective tissue growth factor gene expression and skin sclerosis in tissue sections from patients with systemic sclerosis. *J Invest Dermatol* 1995; 105: 280-4.
50. SHI-WEN X, PENNINGTON D, HOLMES A *et al*.: Autocrine overexpression of CTGF maintains fibrosis: RDA analysis of fibrosis genes in systemic sclerosis. *Exp Cell Res* 2000; 259: 213-24.

51. SATO S, NAGAOKA T, HASEGAWA M *et al*.: Serum levels of connective tissue growth factor are elevated in patients with systemic sclerosis: association with extent of skin sclerosis and severity of pulmonary fibrosis. *J Rheumatol* 2000; 27: 149-54.
52. FONSECA C, LINDAHL GE, PONTICOS M *et al*.: A polymorphism in the CTGF promoter region associated with SSc. *N Engl J Med* 2007; 357: 1210-20.
53. KAWAGUCHI Y, OTA Y, KAWAMOTO M *et al*.: Association study of a polymorphism of the CTGF gene and susceptibility to systemic sclerosis in the Japanese population. *Ann Rheum Dis* 2009; 68: 1921-4.
54. GOURH P, MAYES MD, ARNETT FC: CTGF polymorphism associated with systemic sclerosis. *N Engl J Med* 2008; 358: 308-9.
55. RUEDA B, SIMEON C, HESSELSTRAND R *et al*.: A large multicentre analysis of CTGF -945 promoter polymorphism does not confirm association with systemic sclerosis susceptibility or phenotype. *Ann Rheum Dis* 2009; 68: 1618-20.
56. GRANEL B, ARGIRO L, HACHULLA E *et al*.: Association between a *CTGF* gene polymorphism and systemic sclerosis in a French population. *J Rheumatol* 2010; 37: 351-8.
57. BERETTA L, COSSU M, MARCHINI M *et al*.: A polymorphism in the human serotonin 5-HT2A receptor gene may protect against systemic sclerosis by reducing platelet aggregation. *Arthritis Res Ther* 2008; 10: R103.
58. KIRSTEN H, BURKHARDT J, HANTMANN H *et al*.: 5HT2A polymorphism His452Tyr in a German Caucasian sistemic sclerosis population. *Arthritis Res Ther* 2009; 11: 403.
59. WIPFF J, BONNET P, RUIZ B *et al*.: Association study of serotonin transporter gene (SLC6A4) in systemic sclerosis in European Caucasian populations. *J Rheumatol* 2010; 37: 1164-7.
60. KAWAGUCHI Y, TOCHIMOTO A, ICHIKAWA N *et al*.: Association of IL1A gene polymorphisms with susceptibility to and severity of systemic sclerosis in the Japanese population. *Arthritis Rheum* 2003; 48: 186-92.
61. HUTYROVA B, LUKAC J, BOSAK V, BUC M, DU BOIS R, PETREK M: Interleukin 1 alpha single-nucleotide polymorphism associated with systemic sclerosis. *J Rheumatol* 2004; 31: 81-4.
62. MATTUZZI S, BARBI S, CARLETTO A *et al*.: Association of polymorphisms in the IL1B and IL2 genes with susceptibility and severity of systemic sclerosis. *J Rheumatol* 2007; 34: 997-1004.
63. BERETTA L, CAPPIELLO F, BARILI M, BERTOLOTTI F, SCORZA R: T-889C IL-1alpha promoter polymorphism influences the response to oral cyclophosphamide in scleroderma patients with alveolitis. *Clin Rheumatol* 2007; 26: 88-91.
64. BERETTA L, BERTOLOTTI F, CAPPIELLO F *et al*.: Interleukin-1 gene complex polymorphisms in systemic sclerosis patients with severe restrictive lung physiology. *Hum Immunol* 2007; 68: 603-9.
65. BERETTA L, CAPPIELLO F, MOORE JH, SCORZA R: Interleukin-1 gene complex single nucleotide polymorphisms in systemic sclerosis: a further step ahead. *Hum Immunol* 2008; 69: 187-92.
66. DIEUDÉ P, GUEDJ M, WIPFF J *et al*.: NLRP1 influences the systemic sclerosis phenotype: a new clue for the contribution of innate immunity in systemic sclerosis-related fibrosing alveolitis pathogenesis. *Ann Rheum Dis* 2011; 70: 668-74.
67. MANETTI M, IBBA-MANNESCHI L, FATINI C *et al*.: Association of a functional polymorphism in the matrix metalloproteinase-12 promoter region with systemic sclerosis in an Italian population. *J Rheumatol* 2010; 37: 1852-7.
68. JOHNSON RW, REVEILLE JD, MCNEARNEY T *et al*.: Lack of association of a functionally relevant single nucleotide polymorphism of matrix metalloproteinase-1 promoter with systemic sclerosis (scleroderma). *Genes Immun* 2001; 2: 273-5.
69. JOUNG CI, NA YI, SHIN ES, SUNG YK, YOO DH, JUN JB: The single nucleotide polymorphisms of matrix metalloproteinase-1 in patients with systemic sclerosis. *Rheumatol Int* 2008; 28: 1183-5.
70. WIPFF J, DIEUDE P, AVOUAC J *et al*.: Association of metalloproteinase gene polymorphisms with systemic sclerosis in the European Caucasian population. *J Rheumatol* 2010; 37: 599-602.
71. MANETTI M, GUIDUCCI S, IBBA-MANNESCHI L, MATUCCI-CERINIC M: Mechanisms in the loss of capillaries in systemic sclerosis: angiogenesis versus vasculogenesis. *J Cell Mol Med* 2010; 14: 1241-54.
72. GALIE N, MANES A, FARAHANI KV *et al*.: Pulmonary arterial hypertension associated to connective tissue diseases. *Lupus* 2005; 14: 713-7.
73. TAKENAKA K, SAKAI H, YAMAKAWA H *et al*.: Polymorphism of the endoglin gene in patients with intracranial saccular aneurysms. *J Neurosurg* 1999; 90: 935-8.
74. WIPFF J, KAHAN A, HACHULLA E *et al*.: Association between an endoglin gene polymorphism and systemic sclerosis-related pulmonary arterial hypertension. *Rheumatology* (Oxford) 2007; 46: 622-5.
75. MOHLE R, BAUTZ F, RAFII S, MOORE MA, BRUGGER W, KANZ L: The chemokine receptor CXCR4 is expressed on CD34+ hematopoietic progenitors and leukemic cells and mediates transendothelial migration induced by stromal cell-derived factor-1. *Blood* 1998; 91: 4523-30.
76. SCHAPER W, SCHOLZ D: Factors regulating arteriogenesis. *Arterioscler Thromb Vasc Biol* 2003; 23: 1143-51.
77. MANETTI M, LIAKOULI V, FATINI C *et al*.: Association between a stromal cell-derived factor 1 (SDF-1/CXCL12) gene polymorphism and microvascular disease in systemic sclerosis. *Ann Rheum Dis* 2009; 68: 408-11.
78. WIPFF J, DIEUDE P, AVOUAC J *et al*.: Association of hypoxia-inducible factor 1A (HIF1A) gene polymorphisms with systemic sclerosis in a French European Caucasian population. *Scand J Rheumatol* 2009; 20: 1-4.
79. BLASI F, CARMELIET P: uPAR: a versatile signalling orchestrator. *Nat Rev Mol Cell Biol* 2002; 3: 932-43.
80. RAGNO P: The urokinase receptor: a ligand or a receptor? Story of a sociable molecule. *Cell Mol Life Sci* 2006; 63: 1028-37.
81. D'ALESSIO S, FIBBI G, CINELLI M *et al*.: Matrix metalloproteinase 12-dependent cleavage of urokinase receptor in systemic sclerosis microvascular endothelial cells results in impaired angiogenesis. *Arthritis Rheum* 2004; 50: 3275-85.
82. MARGHERI F, MANETTI M, SERRATÌ S *et al*.: Domain 1 of the urokinase-type plasminogen activator receptor is required for its morphologic and functional, β2 integrin-mediated connection with actin cytoskeleton in human microvascular endothelial cells: failure of association in systemic sclerosis endothelial cells. *Arthritis Rheum* 2006; 54: 3926-38.
83. MANETTI M, ALLANORE Y, REVILLOD L *et al*.: A genetic variation located in the promoter region of the UPAR (CD87) gene is associated with the vascular complications of systemic sclerosis. *Arthritis Rheum* 2011; 63: 247-56.
84. REMILLARD CV, TIGNO DD, PLATOSHYN O *et al*.: Function of Kv1.5 channels and genetic variations of KCNA5 in patients with idiopathic pulmonary arterial hypertension. *Am J Physiol Cell Physiol* 2007; 292: 1837-53.
85. WIPFF J, DIEUDÉ P, GUEDJ M *et al*.: Association of a KCNA5 gene polymorphism with systemic sclerosis-associated pulmonary arterial hypertension in the European Caucasian population. *Arthritis Rheum* 2010; 62: 3093-100.
86. ALLANORE Y, BORDERIE D, AIRO P *et al*.: Lack of association between 3 vascular endothelial growth factor gene polymorphisms and systemic sclerosis: results from a multicenter EUSTAR study of European Caucasian patients. *Ann Rheum Dis* 2007; 66: 257-9.
87. AVOUAC J, WIPFF J, GOLDMAN O *et al*.: Angiogenesis in systemic sclerosis: impaired expression of vascular endothelial growth factor receptor 1 in endothelial progenitor-derived cells under hypoxic conditions. *Arthritis Rheum* 2008; 58: 3550-61.
88. FATINI C, GENSINI F, STICCHI E *et al*.: High prevalence of polymorphisms of angiotensin-converting enzyme (I/D) and endothelial nitric oxide synthase (Glu298Asp) in patients with systemic sclerosis. *Am J Med* 2002; 112: 540-4.
89. ASSASSI S, MAYES MD, MCNEARNEY T *et al*.: Polymorphisms of endothelial nitric oxide synthase and angiotensin-converting enzyme in systemic sclerosis. *Am J Med* 2005; 118: 907-11.
90. GUIDUCCI S, FATINI C, GEORGOUNTZOS A *et al*.: Etrurians vs Greeks: May ACE I/D polymorphism still be considered as a marker of susceptibility to SSc? *Clin Exp Rheumatol* 2006; 24: 432-4.
91. JOUNG CI, PARK YW, KIM SK, UHM WS, KIM TH, YOO DH: Angiotensin-converting enzyme gene insertion/deletion polymorphism in Korean patients with systemic sclerosis. *J Korean Med Sci* 2006; 21: 329-32.
92. WIPFF J, GALLIER G, DIEUDE P *et al*.: Angiotensin-converting enzyme gene does not contribute to genetic susceptibility to systemic sclerosis in European Caucasians. *J Rheumatol* 2009; 36: 337-40.

93. FATINI C, MANNINI L, STICCHI E *et al*.: Hemorheologic profile in systemic sclerosis: role of NOS3 -786T>C and 894G >T polymorphisms in modulating both the hemorheologic parameters and the susceptibility to the disease. *Arthritis Rheum* 2006; 54: 2263-70.
94. ALLANORE Y, BORDERIE D, LEMARÉCHAL H, EKINDJIAN OG, KAHAN A: Lack of association of eNOS (G894T) and p22phox NADPH oxidase sub-unit (C242T) polymorphisms with systemic sclerosis in a cohort of French Caucasian patients. *Clin Chim Acta* 2004; 350-51.
95. SINICI I, KALYONCU U, KARAHAN S, KIRAZ S, ATALAR E: Endothelial nitric oxide gene polymorphism and risk of systemic sclerosis: predisposition effect of T-786C promoter and protective effect of 27 bp repeats in Intron 4. *Clin Exp Rheumatol* 2010; 28: 169-75.
96. KAWAGUCHI Y, TOCHIMOTO A, HARA M *et al*.: NOS2 polymorphisms associated with the susceptibility to pulmonary arterial hypertension with systemic sclerosis: contribution to the transcriptional activity. *Arthritis Res Ther* 2006; 8: R104.
97. FONSECA C, RENZONI E, SESTINI P *et al*.: Endothelin axis polymorphisms in patients with scleroderma. *Arthritis Rheum* 2006; 54: 3034-42.
98. VETTORI S, CIRILLO F, CUOMO G, DI MINNO G, VALENTINI G: The β-fibrinogen -455G>A gene polymorphism is associated with peripheral vascular injury in systemic sclerosis patients. *Clin Exp Rheumatol* 2010; 28: 923-4.