

## Review

# Genetic epidemiology Systemic sclerosis

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## Abstract

Systemic sclerosis (SSc) is a multisystem connective tissue disease characterised phenotypically by fibrosis and ischaemic atrophy. Its aetiology is most likely multifactorial. A genetic predisposition to the condition is suggested by reports of familial SSc (a positive family history is the strongest risk factor yet identified), by animal models, and by disease-association studies, in which researchers have examined a wide variety of genes including those involved in fibrosis, in vascular function and structure, and in autoimmunity – the relative rarity of SSc has precluded linkage studies, except in the Choctaw Indians. Recent advances in genetic methodologies should further our understanding of this complex disease process.

**Keywords:** candidate genes, genetic epidemiology, scleroderma, systemic sclerosis.

## Introduction

Systemic sclerosis (SSc), also termed 'scleroderma', is a multisystem connective tissue disease characterised by excessive fibrosis, vascular abnormalities, and immune dysfunction. There is a strong predominance of affected females over males (3:1), and the peak age of onset is 30 to 50 years. There are two disease subtypes, defined on the basis of the extent of skin involvement: limited cutaneous and diffuse cutaneous [1]. These two subtypes have differing natural histories, prognoses, and autoantibody associations. Patients with limited cutaneous disease have prominent vascular features, often with severe digital ischaemia and, later on in their disease, pulmonary arterial hypertension.

In recent years, there has been considerable interest in the concept of SSc, similar to that in other rheumatic and autoimmune diseases, as a multifactorial disease, possibly

triggered by environmental factors in a genetically predisposed host. A genetic predisposition to SSc is suggested by the following: familial SSc (families with multiple cases, although rare, have been reported); animal models; and associations between SSc and polymorphisms in what is now a sizable number of genes.

## Familial systemic sclerosis

Recent large cohort studies from Australia and the USA reported that SSc occurred in one or more first-degree relatives in 1.4% [2] and 1.6% [3] of the families of patients with SSc. These figures compare with an estimated prevalence of SSc in the USA of 2.6 cases/10,000 (0.026%) [3]. Familial risk can be quantified as a recurrence rate ( $\lambda$ ), and on the basis of these figures,  $\lambda_r$  (for first-degree relatives) is 54. This is a high value in comparison with the values for many other complex diseases, and the conclusion from these studies is that although the

absolute risk for each family member is less than 1%, a positive family history is the strongest risk factor yet identified for SSc. Comparison of concordance rates in monozygotic and dizygotic twins can also be used to quantify the genetic component of susceptibility, but in a condition as rare as SSc, collecting sufficient twin pairs is likely to be extremely difficult. Not surprisingly, therefore, relatively few twin studies have been reported: De Keyser *et al.* recently reported concordance for scleroderma in two pairs of identical female twins – one pair with SSc, the other pair with localised scleroderma [4].

### Animal models

Two of the best-known animal models for SSc are the tight skin 1 (*tsk1*) mouse and the University of California at Davis line 200 (UCD 200) chicken. Of these two models, only the UCD 200 chicken develops vascular injury [5]. The *tsk1* mouse possesses a duplication within the fibrillin1 (*FBN1*) gene [6], which encodes a glycoprotein that is a major constituent of 10–12 nm microfibrils in the extracellular matrix. The UCD 200 chickens show early endothelial-cell apoptosis, followed by perivascular infiltration of mononuclear cells and collagen deposition [7]. Thus these animal models provide insight into how genetic abnormalities that are presumably different can lead to a fibrosing phenotype. Recently, Yamamoto *et al.* have described a bleomycin-induced mouse model for scleroderma – certain strains of mouse (Ch3/He and B10.A) were particularly susceptible to bleomycin-induced dermal sclerosis, emphasising how genetic susceptibility and an environmental trigger may both play a role in the pathogenesis of SSc [8].

### Disease associations

Over the past 10 years, the genetic basis of an ever-increasing number of complex or multifactorial conditions has been investigated through the approach of non-parametric analysis of linkage in affected sibling-pair families. Such studies screen the whole genome in search of loci linked to disease. Hundreds of families are required for adequate power and the approach is not likely to be feasible for SSc except in an internationally coordinated study. Such a study, in turn, could be complicated by genetic heterogeneity across populations: a recognised feature of SSc. The highest disease prevalence observed to date was in a genetically isolated population of Choctaw American Indians. This population offers a rare opportunity to study large, extended pedigree(s) with many affected individuals. With the exception of studies in this population, all investigations of the genetic basis of SSc have been disease-association studies, in which allele or genotype frequencies of polymorphisms in potential disease genes are compared in cases and controls. This approach has advantages, but there are many examples in the literature, in all diseases, of both false-positive and false-negative results arising from poor study

design. Ideally, cases and controls should be well matched, power calculations should be used to determine the appropriate number of samples to be analysed, and positive associations should be replicated in independent cohorts. Recent data suggest that the selection of markers for association studies is critical, because old assumptions about linkage disequilibrium do not necessarily hold true [9].

The complex pathophysiology of SSc means that there are a great many genes that are potential 'culprits', either singly or, more likely, together, in driving the disease process. Thus, genes involved in fibrosis, in vascular structure and function, and in autoimmunity all warrant investigation. The genes discussed below are among those that have already been examined in recent years.

### Genes primarily involved in fibrosis/excessive extracellular matrix accumulation

#### *Fibrillin 1*

Tan *et al.*, in an extension of their previous work on fibrillin 1 [10], reported that a single-nucleotide polymorphism in the 5'-untranslated region of *FBN1* was strongly associated with SSc in Choctaw Indians (who have a very high prevalence of SSc) and that the two haplotypes in Choctaws containing this polymorphism have associations with SSc in Japanese patients [11]. *FBN1* had been chosen as a candidate gene because it mapped to the region of linkage on chromosome 15q identified in the earlier study and because of the duplication of *FBN1* gene in the *tsk1* mouse.

#### *COL1A2*

Hata *et al.* reported the association with SSc of a specific combination of functional dinucleotide repeats (13,6,8)-12 in the human type-I-collagen  $\alpha 2$  chain (*COL1A2*) gene, especially in male patients with disease-specific autoantibodies [12].

#### *Transforming growth factor- $\beta$ (TGF- $\beta$ )*

TGF- $\beta 1$  has been implicated in the pathogenesis of fibrosis and its expression is increased in sclerodermatous skin. It therefore seems an obvious candidate gene. However, Zhou *et al.*, using microsatellites and intra-genic markers, found no significant associations between TGF- $\beta 1$ , TGF- $\beta$  receptors I and II, latent TGF- $\beta 1$ -binding protein, platelet-derived growth factors A and B (and their receptors), and SSc in Choctaw Indians; although one microsatellite near the TGF- $\beta 1$  receptor I showed a difference in allele frequency between SSc patients and controls, this result was thought to be a false positive [13]. Similarly, we found no association between SSc and microsatellite markers for TGF- $\beta 1$  or for platelet-derived growth factor B, but we did find associations between SSc and markers for TGF- $\beta 3$ , TGF- $\beta 2$ , and (in males only) tissue inhibitor of metallo-

proteinase-1 [14]. The TGF- $\beta$  associations were dependent on disease subtype: limited cutaneous disease was associated with TGF- $\beta$ 2 and diffuse cutaneous disease, with TGF- $\beta$ 3 [14].

### Genes implicated in pulmonary fibrosis

The most life-threatening aspect of excessive fibrosis is fibrosing alveolitis. Investigators have recently reported polymorphisms in the fibronectin gene in SSc-related pulmonary fibrosis [15]. The same investigators went on to examine polymorphisms of the IL-8 and IL-8-receptor genes *CXCR1* (IL8RA) and *CXCR2* (IL8RB) in patients with SSc (subclassified as those with and those without fibrosing alveolitis), in patients with cryptogenic fibrosing alveolitis, and in healthy control subjects: while there was an association between SSc and two polymorphisms of the *CXCR2* gene, this association was independent of the presence or absence of fibrosing alveolitis [16].

### Genes primarily involved in vascular function and structure

The recent developments in the genetics of pulmonary arterial hypertension have been of considerable interest to those with an interest in SSc [17]. Mutations in the gene for bone morphogenetic protein receptor II, a member of the TGF- $\beta$ -receptor family, have been identified in patients with familial primary pulmonary hypertension [18], and mutations in activin-receptor-like kinase I, a TGF- $\beta$  receptor, have been identified in patients with pulmonary arterial hypertension associated with the inherited disease hereditary haemorrhagic telangiectasia [19]. Elucidation of the molecular mechanisms by which these mutations lead to pulmonary vascular change may provide new insights into the pathophysiology of SSc as well as of pulmonary arterial hypertension.

### Genes involved in autoimmunity

There have been many studies investigating genes for human leukocyte antigen (HLA) in patients with SSc: several HLA genes have been weakly associated with SSc in different ethnic groups. Much stronger associations, however, have been observed with specific autoantibodies and/or disease subsets (reviewed by Tan and Arnett [20]). For example, anticentromere antibodies have been associated with HLA-DQB1 alleles [21], and antitopoisomerase antibodies, with an HLA-DRw11 allele as well as with a particular HLA-DQB1 sequence [22]. Anti-Th/To antibodies have also been associated with HLA-DR11, as well as with a reduced frequency of HLA-DR7, this latter association found also in patients with anti-centromere antibodies [23]. These immunogenetic associations are thought to reflect T-cell involvement in autoimmunity.

One of the most interesting novel hypotheses to be investigated in recent years is that SSc might result from a graft-versus-host reaction as a result of retained and per-

sistent fetal cells in mothers – so-called microchimerism. This concept can be expanded to explain SSc in males, as the exchange of cells at birth can be two-way. The investigation of this hypothesis is technically difficult, because PCR contamination must be completely avoided. Both HLA and Y-chromosome sequences have been investigated, but from the results to date it is difficult to conclude that this concept is specific to SSc [24,25]. This recent interest in microchimerism in the pathogenesis of SSc has extended to its link with HLA. Lambert *et al.* reported that persistent fetal microchimerism (in both healthy women and those with SSc) is associated with DQA1\*0501 [26] and that this allele is associated with SSc in male patients [27]. These observations suggest that HLA may confer susceptibility to SSc via many different mechanisms.

Finally, on autoimmunity and the immune response, tumour necrosis factor (TNF) has recently received considerable interest, because its actions can be blocked therapeutically. While an association between the microsatellite TNF- $\alpha$ 13 and SSc has recently been reported in Japanese patients, the fact that TNF alleles are in linkage disequilibrium with HLA class II alleles reduced the importance of this association [28].

### Conclusion

Evidence is accumulating that genetic susceptibility plays a role in the pathogenesis of SSc. However, the situation is highly complex and association studies suggest that susceptibility may be determined by a number of different genes, with different genes interacting to produce a phenotype that is predominantly fibrotic or is predominantly associated with vascular abnormality, depending upon the patient's disease subtype. With the advent of new technologies, our ability to look for genetic abnormalities in different clinical and serological subgroups of patients, and the functional correlates of these, is rapidly expanding. The combination of developments in microarray technology and knowledge of the human genome will ultimately mean that the gene-expression profiles of cells will be intimately mapped and thus potential disease genes and pathways will be identified for genetic investigation and perhaps, ultimately, therapeutic intervention. This approach will be ideal for comparing processes in clinically affected and unaffected skin from SSc patients; in an early application of this technology, Feghali and Wright found that many RNAs, including fibronectin, were differentially expressed in sclerodermatous skin [29]. Such experiments require careful interpretation and there is also growing evidence that it will be necessary to study expression at the protein rather than the RNA level, because the two are not necessarily correlated. Understanding more about gene regulation and gene expression in different tissues, and thus about genetic susceptibility, will help us to unravel the pathophysiology of this complex and fascinating disease process.

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