A profibrotic polymorphism (of TGFβ1) in systemic sclerosis

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TGFβ1 and several other polymorphic determinants are risk factors for SSc

In this issue, Crilly *et al* demonstrate polymorphisms of transforming growth factor β1 (TGFβ1) in subjects with systemic sclerosis (SSc) which might have functional significance.¹ Much remains unknown about the pathogenesis of SSc, the generalised form of scleroderma, which comprises prominent autoimmune, vascular/microvascular, and ultimately fibrotic features. This combination substantially reduces both quality of life and life expectancy, depending on the site and a highly variable rate of involvement. Underlying the unregulated deposition of extracellular matrix (ECM) in skin, lung, heart, gut, and kidney, there is a fibroproliferative intimal vascular lesion which extends into the microcirculation, seems to be progressive, and remains poorly understood, despite attempts to isolate abnormalities of platelets, and of endothelial, immune, and vascular wall smooth muscle cells, among others (for example, pericytes and myofibroblasts).

ECM depositions by immune/inflammatory reactions have in common the expression of profibrogenic cytokines, including platelet derived growth factor (PDGF), connective tissue growth factor (CTGF), vascular endothelial growth factor (VEGF), and perhaps the master profibrogenic cytokine, TGFβ, a ligand-receptor system with many family members, which is highly conserved among vertebrates. Biologically, each of three ligands is expressed in inactive form and rapidly becomes enveloped in a separate second inhibitor, after which the entire complex can be attached covalently to the ECM by a transglutaminase dependent mechanism. Proteases, including plasmin, cleave the inhibitors; mannose-6-phosphate transport mechanisms have been implicated in ligand transfer; there are at least three cell membrane receptors which interact to activate SMAD signalling pathways to up regulate ECM gene expression, reduce metalloproteinase expression, and increase protease inhibitor expression. In addition, TGFβ1 can up regulate PDGF, CTGF, and VEGF, as well as itself (autocrine self up regulation). It is no

coincidence that, for more than a decade, sclerodermologists have been interested in TGFβ1.²

The TGFβ1 gene, localised on human chromosome 19q13, is very polymorphic.³ To date, seven single nucleotide polymorphisms (SNP) have been described—three in the promoter region, one in a non-translated region, and one each in codons 10, 25, and 263. Crilly *et al* found that the allelic variation in codon 10 (position +869) was significantly associated with SSc.¹ The frequency of the gene with C at this position was significantly higher in patients than in controls. In addition, patients were more likely to be heterozygous (C/T) at this position than controls. This is an important finding, as the implicated allele affects the gene product in a physiologically meaningful way: the T to C substitution in codon 10 is associated with higher TGFβ1 mRNA and protein levels.⁴

"Host genotype, viruses, and environmental factors influence disease risk"

Despite a considerable body of evidence supporting the contribution of TGFβ1 to the fibrosis of skin and visceral organs, a hallmark feature of SSc, there is a paucity of studies to assess whether the TGFβ1 gene is a candidate gene for this disorder. There is only one other study to our knowledge which has examined the role of TGFβ1 polymorphism in SSc.⁵ In marked contrast with the results reported by Crilly *et al*,¹ these investigators found no significant association between the SNP in codon 10 and SSc. The reasons for this discordance are not clear; possible explanations include the differences in the ethnic background of the patients in the two studies. The genetic background of the relatively isolated population studied by Zhou *et al*⁶ was much more homogeneous—all descendants of a founding family of Oklahoma Choctaw in the mid-1700s—compared with the unrelated Scottish subjects in the investigation by Crilly *et al*.¹ Also, goodness-of-fit tests for the

SNP in codon 10 show that the genotype frequencies for the data presented by Zhou *et al* are in Hardy-Weinberg equilibrium,³ which is not the case for those reported by Crilly *et al*.¹ In addition to the possibility of genetic heterogeneity in SSc mentioned by Zhou *et al*,⁶ potential differences in linkage disequilibrium between the two populations may also contribute to these ethnically restricted TGFβ1 genotype associations.⁶ Furthermore, SSc is thought to be a polygenic disease and it is likely that alleles at several loci epistatically interact to cause SSc; racial differences in gene frequencies at these loci may result in differences in the relative risk of developing the disease in various groups. In addition to the host genetic factors, there is growing evidence for the involvement of viruses and exposure to various occupational and environmental chemicals in SSc pathogenesis.⁷⁻¹⁰ Population differences in exposure to these environmental risk factors—coupled with the interindividual differences in the ability to respond to these external factors—may also contribute to the differences in disease risk.

In addition to the functional SNP in codon 10, other TGFβ1 SNPs also influence the protein level.¹¹ Given the strong linkage disequilibrium among the SNPs in this gene—almost absolute between determinants at positions +72 and +915 (codon 25)—it is difficult, if not impossible, to assess which, if any, of these SNPs is truly responsible for the quantitative variation in TGFβ1 level. Possibly, particular alleles at these loci additively (or interactively) influence the quantitative (and possibly qualitative) expression of this cytokine. Therefore it is essential to construct a haplotype map of these SNPs for future association studies. Also, it is important to identify additional functional polymorphisms in this gene which might have been missed by the initial screening strategy.¹²

In addition to TGFβ1, several other polymorphic determinants—which are either risk factors for the development of SSc or influence disease associated autoimmune responsiveness—have been identified. These include HLA,¹³ tumour necrosis factor,^{14,15} fibrillin-1,¹⁶ and GM and KM allotypes—genetic markers of γ and κ chains, respectively.^{17,18} Simultaneous examination of all known candidate genes in a large study population may provide a unique genetic profile of subjects at risk of developing SSc. This knowledge may be useful in developing possible therapeutic interventions in the future.

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