Polymorphisms in COL15 Gene Are Not Associated with Systemic Sclerosis

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ABSTRACT. ***Objective.*** Systemic sclerosis (SSc) is marked by microvascular abnormalities leading to ischemic features such as Raynaud's phenomenon and fingertip ulcers. Digital ischemia in turn results in hypoxia, which is expected to drive compensatory angiogenesis; however, this phenomenon is deregulated in SSc. Vascular basement membrane (VBM) that consists of type IV, XV, and XVIII collagens supports the growth and survival of vascular endothelial cells and plays a key role in regulating angiogenesis. Recent gene expression analyses of skin tissue and dermal fibroblasts from patients with SSc revealed *COL15* to be one of the significantly differentially regulated genes. We undertook an association study to explore the role of *COL15* single-nucleotide polymorphisms (SNP) in SSc disease development.

Methods. Eleven SNP across *COL15* were genotyped in a cohort of 175 UK Caucasian patients with SSc and 190 population-matched unrelated healthy subjects using 2 methods: TaqMan and SNaPshot. Statistical analysis was performed by Pearson's chi-square test and HelixTree software was utilized for haplotype analysis.

Results. No difference in genotype or allele frequencies were detected between patients with SSc and controls. None of the haplotype frequencies were found to differ between patients and controls.

Conclusion. Failure to detect an association may reflect a true lack of association or could be a false-negative result arising as a result of low power of the study. Our study had sufficient power to detect an effect size of 2.1 ($p = 0.05$); however, larger patient cohorts may be needed for exclusion of *COL15* from a possible candidacy in SSc. (First Release Jan 15 2008; J Rheumatol 2008;35:251–3)

Key Indexing Terms:
SYSTEMIC SCLEROSIS SCLERODERMA GENE POLYMORPHISMS
COL15 VASCULAR BASEMENT MEMBRANE ANGIOGENESIS

Abnormalities of the microvasculature are an integral part of the systemic sclerosis (SSc) disease process and are well demonstrated by nailfold capillary microscopy: typical features include widening of capillary loops and areas of avascularity. These microvascular abnormalities lead to ischemic features such as Raynaud's phenomenon, fingertip ulcers, and gangrene. Digital ischemia in turn results in hypoxia, which is expected to drive compensatory angiogenesis[1]; however, this phenomenon does not happen in SSc[2]. Angiogenesis is a complex physiological process modulated by a number of factors, including the balance between pro- and anti-angiogenic factors. A study by Distler, *et al*[3] did not find any difference in the levels of serum basic fibroblast growth factor (pro-angiogenic) and endostatin (anti-angiogenic) between patients with SSc and controls, but observed a significant increase in the levels of vascular endothelial growth factor (VEGF), a pro-angiogenic factor. This increase in VEGF is expected to stimulate subepidermal angiogenesis in SSc skin; however, VEGF-driven compensatory angiogenesis is not observed in SSc. The faint immunoreactivity observed with αvß3 integrin receptor, a mediator of VEGF-regulated angiogenesis, in SSc skin has been suggested by Konttinen, *et al* to be one of the reasons for failure of angiogenesis in SSc[2]. Recently the role of vascular basement membrane (VBM) in regulating angiogenesis has been highlighted by several investigators. VBM is located between the endothelial cell (EC) lining and pericytes that make up the outer vessel wall and regulates cell growth, cell differentiation, and cell-matrix interactions, as well as apoptosis. It is mainly composed of type IV, XV, and XVIII collagens, laminin, heparin-sulfate proteoglycans, fibulins, and osteonectin. These VBM components support the growth and survival of vascular EC, thereby critically regulating angiogenesis[4]. The exact role of each of these components in angiogenesis remains largely unknown; however, domains of collagen IV, collagen XV, and collagen XVIII are known to have anti-angiogenic functions[5]. The various collagen-derived

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fragments include arresten (NC1 domain of collagen IV α1 chain), canstatin (NC1 domain of collagen IV α2 chain), tumstatin (NC1 domain of collagen IV α3 chain), endostatin (NC1 domain of collagen XVIII α1 chain), and the endostatin homolog of collagen XV α1 chain. While collagen XVIII regulates retinal vascular development[6], collagen XV knockout in mice leads to collapsed capillaries and EC degeneration[7]. Upregulation of *COL15* gene was also found to accompany fibrotic conditions such as radiation-induced fibrosis[8] and renal fibrosis[9]; however, whether this is independent from its role in angiogenesis is unknown. Interestingly, recent gene expression analyses of skin tissue as well as dermal fibroblasts from patients with SSc revealed *COL15* to be one of the significantly differentially regulated genes[10,11] (and our unpublished observation).

COL15 is therefore a strong candidate gene for SSc. We investigated the association between single-nucleotide polymorphisms (SNP) in this gene and SSc. To our knowledge, ours is the first study to explore the role of *COL15* polymorphisms in any pathological condition including SSc.

MATERIALS AND METHODS

Our study population consisted of 175 Caucasian patients with SSc recruited from one center in the North West of England (Hope Hospital, Salford). One hundred thirty-seven patients had limited cutaneous SSc (lcSSc) and 38 had diffuse cutaneous SSc (dcSSc), as defined by LeRoy, *et al*[12]. Of these 175 patients, 76 were anticentromere-positive. The control cohort included 190 unrelated healthy subjects of the same ethnic origin, recruited as part of a prospective study in inflammatory arthritis[13]. Ethical approval was obtained from the relevant research ethics committees. Eleven SNP spread across the whole gene were selected (Figure 1) and genotyped using a primer extension-based assay, SNaPshot (Applied Biosystems, 3100) and an allelic discrimination assay, TaqMan (Applied Biosystems, 7700). Pearson's chi-square test was used to compare distribution of genotypes and alleles for each SNP between patients and controls. HelixTree software program (GoldenHelix) was utilized for pairwise linkage disequilibrium (LD) analysis as well as for inferring haplotype frequencies in patients and controls.

RESULTS

Genotypes for all but one SNP were in Hardy-Weinberg equilibrium in the control cohort; this SNP was excluded from further analysis. No differences in genotype or allele frequencies were detected between patients with SSc and controls (Table 1). Pairwise LD analysis revealed 2 different blocks of LD; however, none of the haplotype frequencies differed between patients and controls (Table 2). SSc being a clinically heterogeneous disease, we undertook a stratified analysis of our sample cohort, comparing genotype and allele frequencies for limited, diffuse, and anticentromere-positive subgroups against that of controls (data not shown). These analyses did not yield any significant association with any of the *COL15* SNP either; however, it is to be noted that our subset analysis lacked sufficient power owing to small sample numbers in certain subsets.

DISCUSSION

Failure to detect an association may reflect a true lack of association or could be a false-negative result arising as a result of low power of the study. Our study had sufficient power to detect an odds ratio (OR) of 2.1 at 5% significance level ($p = 0.05$); however, if the effect size of *COL15A1* SNP in SSc were to be lower than this, then our study would have missed it. It is to be noted, though, that designing studies with sufficient power to exclude an effect is difficult, particularly in a rare disease like SSc. To exclude a modest effect size (for example, OR = 1.2 at 5% significance level, given the minor allele frequency ≥ 10%), we would require a patient sample size of 4107, which is clearly prohibitive for SSc. *COL15* is a large gene (145 kb) and it may be that in our study we failed to investigate the causal marker or those in LD with it. To exclude this possibility, we compared our own haplotypes with that observed by the HapMap project[14] for the same region. This comparison revealed our study recorded all the major haplotypes identified by the HapMap project, suggesting our study had good, if not complete coverage of the gene. It is to be noted that 2 studies that reported upregulation of *COL15* previously[10,11] had utilized skin biopsies or fibroblasts obtained from patients with dcSSc. We did not observe an association between dcSSc and any of the *COL15* SNP by stratified analysis; however, our subset analysis lacked suffi-

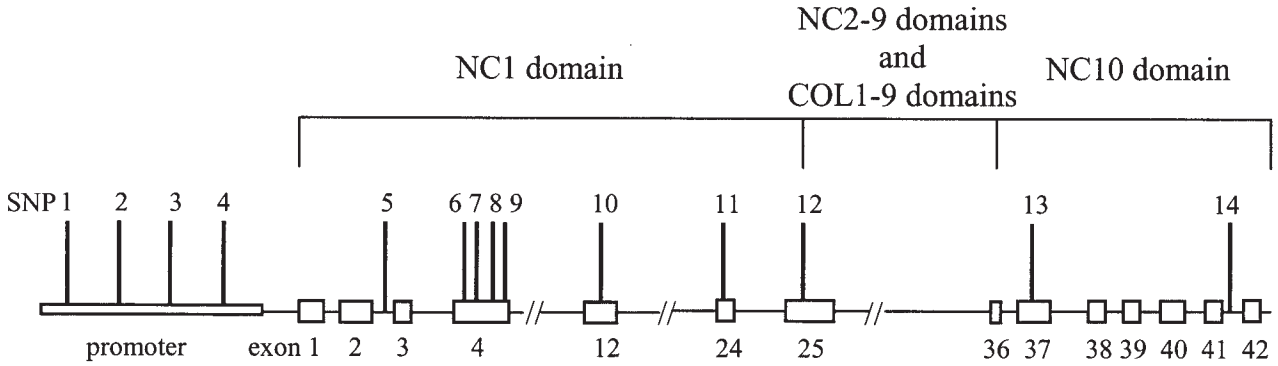

Figure 1. Representation of SNP selected in *COL15A1* in the context of various domains. Boxes indicate exons and black vertical lines indicate SNP selected. Exons 1–11 encode the N-terminal noncollagenous (NC1) domain, exons 12–36 encode both noncollagenous (NC2-9) and collagenous (COL1-9) sequences, and exons 37–42 encode the C-terminal noncollagenous domain.

Table 1. Minor allele and genotype frequencies for *COL15A1* SNP in patients with SSc and controls.

SNP	SNP Location	Minor Allele Frequency, % Controls, n = 190	SSc Patients, n = 175	p	Differences* in Genotype Frequencies, p
rs911932	Promoter	122 (32.8)	115 (32.8)	0.9	0.9
rs7042257	Promoter	123 (32.7)	113 (32.8)	0.9	0.7
rs3758312	Promoter	136 (36.3)	114 (32.5)	0.2	0.5
rs3824515	Promoter	132 (35.2)	112 (32.1)	0.3	0.5
rs911933	Intron 2	110 (29.7)	93 (26.5)	0.3	0.2
rs2075662	Exon 4	55 (14.5)	64 (18.2)	0.1	0.1
rs2075663	Exon 4	151 (39.9)	140 (40.4)	0.8	0.9
rs2297603	Exon 12	48 (13.0)	37 (10.6)	0.3	0.6
rs7854112	Exon 25	59 (15.8)	47 (13.5)	0.3	0.5
rs4480177	Exon 37	78 (21.2)	70 (20.0)	0.6	0.9
rs1051105	3′ UTR	80 (22.8)	73 (21.1)	0.5	0.7

p values were generated by chi-square analysis. * Comparison with genotype frequencies in controls. SNP: single-nucleotide polymorphism; SSc: systemic sclerosis.

Table 2. Haplotype frequencies in patients with SSc and controls.

Haplotype Block	Alleles	Frequency in Controls	Frequency in SSc Patients	p
	1111	0.32	0.34	0.4
I	2211	0.30	0.32	0.5
	1122	0.32	0.31	0.7
	111	0.74	0.76	0.7
II	222	0.15	0.12	0.1
	122	0.04	0.06	0.2
	112	0.03	0.02	0.5

Haplotype block I is defined by *COL15A1* SNP markers rs911932, rs7042257, rs3758312, and rs3824515. Haplotype block II is defined by *COL15A1* markers rs7854112, rs4480177, and rs1051105. The major allele was always referred to as allele 1, the minor allele as allele 2. p values were generated by chi-square analysis. SNP: single-nucleotide polymorphism; SSc: systemic sclerosis.

cient power owing to small sample numbers. Hence an association between dcSSc and *COL15* SNP could not be excluded. To convincingly exclude this gene from a possible candidacy in SSc would require further investigation using larger patient cohorts and for this relatively rare disease this would require the collaboration of a number of groups internationally.

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