The novel adipokine C1q-TNF related protein 9 (CTRP9) is elevated in systemic sclerosis-associated interstitial lung disease

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Systemic sclerosis (SSc) is a multisystem disease with variable presentations, organ involvement and rates of progression (1). The emerging role of adipose tissue in fibrosis has focused attention on adipokines as potential mechanistic disease markers in SSc (2). In addition to their well-established role in metabolic regulation, adipokines exert profound effects on immune, vascular and connective tissue homeostasis. The C1q/TNF (CTRP) family is comprised of 15 proteins implicated in metabolism and inflammation. The best characterised members, CTRP3 and CTRP9, have anti-fibrotic effects, but their expression in SSc or autoimmune diseases are still unknown (3). In view of their structural homology to adiponectin and putative roles in inflammation and fibrosis, we sought to investigate CTRP expression in SSc. Patients with SSc (n=126) enrolled in the Northwestern Scleroderma Registry and Biorepository were studied. Twenty-nine healthy individuals with no personal or family history of autoimmune disease were included as controls. The study was approved by the Northwestern University Institutional Review Board, and patients provided written informed consent. Fifty-six SSc patients (44.4%) were classified as dcSSc and 70 (55.6%) as lcSSc (4). The mean disease duration was 9.7 (±8.4) years, MRSS 11.0 (±10.3) and FVC 77.7 (±18.6) percent predicted. Demographic and clinical characteristics are summarised in Supplementary Table I. Serum obtained at baseline were analysed for levels of CTRP3 and CTRP9 by ELISA (Aviscera Bioscience, Santa Clara, CA). In contrast to CTRP3, levels of serum CTRP9 were dramatically elevated in SSc compared to controls (213.8±685.9 *vs*. 19.1±31.0 ng/mL; p=0.0006; Fig. 1A). Of note, CTRP9 was below the limit of detection (<8 ng/mL) in 79.3% of controls, compared to 38.9% of SSc patients (χ^2 p-value 7.2x10-5, O.R. 6.1, range 2.5–14.9). CTRP9 levels > than two SD above the mean in controls (81 ng/mL) was seen in 34.1% of SSc patients but only 3% of controls. Elevated CTRP9 was associated with significantly lower FVC (66.8±18.3 *vs*. 80.1±17.9% of predicted, p=0.01; Fig. 1B) and FEV1 (71.1±17.9 *vs*. 81.0±17.4% of predicted, p=0.02; data not shown). Stratification of low/high CTRP9 by antibody status and ESR showed significant differences in FVC across subgroups (ANOVA p<0.01 for all comparisons; Fig. 1B). Importantly, elevated CTRP9 was associated with radiologic evidence of lung fibrosis on HRCT (p=0.04, Fig. 1C).

The present study is the first to investigate CTRP in SSc. The results demonstrate that levels of CTRP9 are elevated in SSc, and are significantly associated with SSc-ILD. Originally described as an adipokine that

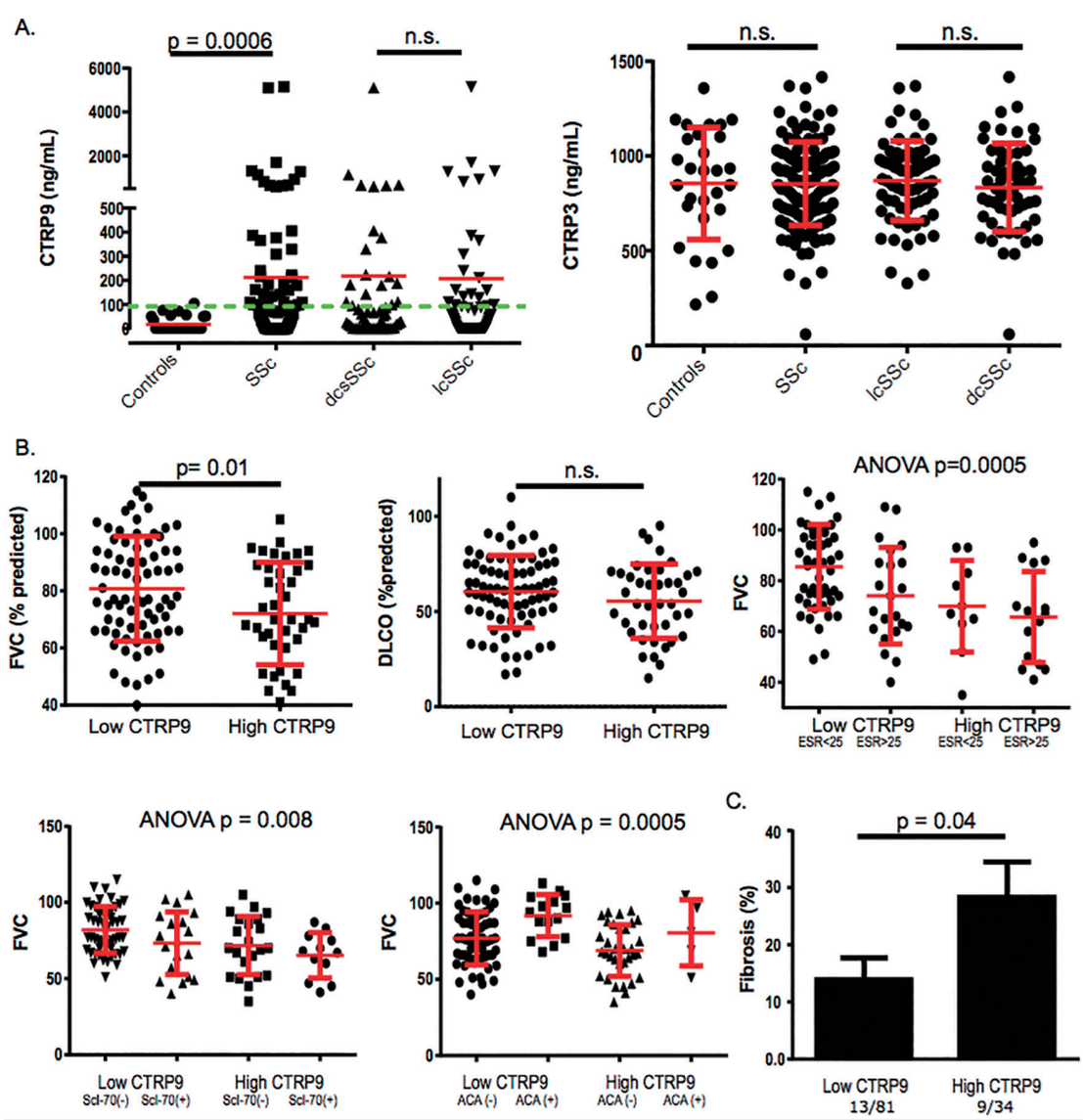

Fig. 1. Elevated serum CTRP9 in systemic sclerosis is associated with SSc-interstitial lung disease.
A. Serum levels of CTRP9 and CTRP3 were determined in 126 SSc patients and 29 controls. Dotted horizontal green line represents cutoff (mean + 2 standard deviations in controls). Each dot represents a single individual. Horizontal bars are the means for each group. Next, patients were stratified by CTRP9 levels for **B.** Association with forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DLCO). Stratified associations of FVC by antibody status and ESR. **C.** Prevalence of fibrotic changes on chest HRCT.
Results are shown as mean ± standard deviation. Mann-Whitney U test was used to assess the rank-order difference between groups. Student t test was performed to assess differences between FVC (overall), DLCO. Analysis of variance (ANOVA) was used to analyse FVC stratified by antibodies and ESR status. Fisher exact test was used to assess association between HRCT and CTRP9 levels. p<0.05 was considered significant. n.s.: non significant.

lower serum glucose and forms complexes with adiponectin (5), CTRP9 was recently shown to be protective in cardiac disease, pulmonary hypertension and hepatic steatosis (6, 7). On the other hand, in diabetic patients, elevated CTRP9 levels are associated with renal involvement (8).

Our results indicate that in sharp contrast to adiponectin, CTRP9 is elevated in patients with SSc and associated ILD, independent of disease duration. CTRP9 may be part of a protective response in lung injury. To our knowledge, CTRP9 has not been studied in other ILD. Although ILD is a leading cause of mortality in SSc, there are no validated serum biomarkers to assess severity or progression (9, 10). The present results add CTRP9 to the list of putative markers which may identify ILD, and play a role in disease.

In conclusion, we show for the first time that the novel adipokine CTRP9 is elevated in SSc and SSc-related ILD. The results further contribute to the emerging roles of adipokines in SSc. CTRP9 may represent a biomarker for SSc-related lung disease, and potential play a role in disease pathogenesis.

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