**Introduction**

Systemic sclerosis (Scleroderma, SSc) is a complex systemic autoimmune disease characterized by immune dysregulation, fibrosis, and vasculopathy. It is a rare connective tissue disease, with a prevalence rate per year of 0.0028-0.0253 % in USA [[1](#_ENREF_1)]. Based on the maximum extent of skin involvement, SSc is categorised as limited cutaneous (lcSSc) if skin thickening is limited to the extremities distal to the elbows and knees with or without facial involvement, whereas SSc is categorised as diffuse cutaneous (dcSSc) if skin thickening involves areas proximal to the elbows and knees, including the trunk. Our previous research indicates that chinese patients show 40.3 % lcSSc and 59.7 % dcSSc forms of SSc [[2](#_ENREF_2)]. SSc also can be subgrouped by autoantibody subsets. The most common of the autoantibodies are directed against DNA topoisomerase I (ATA) and centromeric proteins (ACA). ATA was found in 59.9 %, ACA in 13.4 % of chinese patients cohort [[2](#_ENREF_2)]. The pathogenesis of SSc is not completely understood despite vigorous research efforts. A multitude of genetic studies, ranging from candidate-gene studies to genome-wide association studies, have identified a large number of genetic susceptibility factors for SSc and its clinical phenotypes[[3-15](#_ENREF_3)], but the contribution of these factors to disease susceptibility is only modest.[[16](#_ENREF_16)] However, mounting evidences of gene-specific epigenetic alteration in SSc has occurred [[17-19](#_ENREF_17)].

As an autoimmune disease, immune cells, especially T cells play a core role in the aetiology of SSc.[[20-23](#_ENREF_20)] In early SSc, activated CD4+ and CD8+ T lymphocytes and monocytes/macrophages, and less commonly B cells, eosinophils, mast cells and NK cells, are observed in perivascular regions in the lesional skin, lungs, and other affected organs; these inflammatory cell infiltrates are detectable before the appearance of fibrosis. [[24](#_ENREF_24)]In situ hybridization studies show prominent procollagen gene expression in early-stage SSc skin in fibroblasts that are adjacent to inflammatory cells, suggesting a role for the inflammatory cells or their soluble products in inducing fibroblast activation. [[25](#_ENREF_25)] The extent of lymphocytic tissue infiltration correlates with the severity and progression of skin fibrosis. However, the exact roles of these immune cells, especially CD4+ and CD8+ T cells, are not clear.

DNA methylation is an important epigenetic mechanism that can cause durable changes of gene expression that are heritable during cell division. So far, DNA methylation status of CD4+ and CD8+ T cells in SSc and their methylation influence to the [occurrence](javascript:void(0);) [and](javascript:void(0);) [development](javascript:void(0);) of SSc are also unknown. Herein, we used whole-genome DNA methylation chip and methylation sequence technology to determine the methylation status of CD4+ and CD8+ T cells from peripheral blood of SSc patients attempting to explore epigenetic evidence of its aetiology.

**Materials and methods**

**Study subjects**

54 patients with SSc (mean ± SD age 47.4 ±14.1 years; 17 women, 7 men) were recruited from the outpatient dermatology clinic and the inpatient ward of Shanghai TCM-integrated Hospital wherein the first 24 cases (mean ± SD age 47.4 ±14.1 years; 17 women, 7 men) for whole-genome DNA methylation chip test and another 42 patients (P13-P54; mean ± SD age 47.4 ±14.1 years; 17 women, 7 men) for methylation sequencing. All patients met the criteria for SSc established by the American College of Rheumatology [[26](#_ENREF_26)]. The clinical profiles of the patients with SSc are shown in Table 1. The first 24 control subjects were 19 healthy women and 5 healthy men (mean ± SD age 46.8± 8.2 years) recruited from the handymen at Shanghai Changning District Tongren Hospital and another 30 control subjects were 19 healthy women and 5 healthy men (mean ± SD age 46.8± 8.2 years) recruited from the staff at Affiliated Hospital of Nantong University. None of the control subjects had a family history of autoimmune disorders. Study protocol was approved by the human ethics committee of Fudan University, and all subjects signed an informed consent form.

**Separation of CD4+ and CD8+ T cells from peripheral blood mononuclear cells (PBMCs) of study subjects**

Venous peripheral blood (about 10 ml) was collected from all study subjects and preserved with 2 mg/ml EDTA·K2. PBMCs were isolated by density-gradient centrifugation (Ficoll-PaqueTM PLUS; GE Healthcare Bio, [Buckinghamshire](https://en.wikipedia.org/wiki/Buckinghamshire), UK). Then CD4+ and CD8+ T cells were successively separated by positive selection, using CD4 and CD8 magnetic microbeads according to the manufacturer’s protocol (Miltenyi Biotec, Bergisch Gladbach, Germany). Extraction of genomic DNA was performed using the Puregene Blood Core Kit (Qiagen, Valencia, California, and USA) as described in the manufacturer’s protocol.

**DNA methylation studies**

Genome-wide DNA methylation in CD4+ and CD8+ T cells from SSc patients and controls included in this study was assessed using the Illumina Infinium HumanMethylation450 BeadChip array, which allows for the interrogation of over 485 000 methylation sites within the entire genome. This array covers 99% of RefSeq genes, with an average of 17 CpG sites per gene across the promoter region, 5’ untranslated region (5’-UTR), first exon, gene body and 3’-UTR. It also covers 96% of CpG islands. Non-CpG-methylated sites recently identified in human stem cells are also covered as well as microRNA promoter regions.

DNA methylation analysis was performed using Rnbeads …

**Validation of differential methylation regions (DMRs) using MethylTarget sequence technique**

Genomic DNA was converted with bisulfite treatment. Then PCR was performed to amplify the interested DNA sequence involved differential methylation regions (DMR). Finally, the interested DNA fragments were sequenced by Illumina Miseq. Sequencing depth was about 200×.

**RNA isolation and genes mRNA quantitation**

CD4+ and CD8+ T cells were from 26 patients among the above patients. Total RNA was isolated by TRIzol method. First-strand complementary DNA (cDNA) was synthesized with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Gene transcripts that had significant DMRs were assayed by quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) in an ABI 7900 Sequence Detector System (Applied Biosystems). The primers sequences for each gene (Col1a1, Col1a2, Col3a1, Ctgf, Sparc, Ccr2, Mmp3, Il-6, Il-5, α-Sma, Il-1β) were shown in supplementary table 1. GAPDH gene was as an internal reference control.

**Results**

**Discussion**

**Aknowledgement**

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