Common susceptibility loci in both systemic sclerosis and localized scleroderma identified using genetic analysis

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Scleroderma is an autoimmune fibrosing disorder that can be further subclassified as localized scleroderma (LSc) and systemic sclerosis (SSc). LSc is characterized by sclerotic and pigmented skin lesions, while SSc is a more generalized disorder of the connective tissue involving a number of organs. SSc is characterized by the thickening of dermal collagen bundles, fibrosis, and vascular abnormalities in the visceral organs.[1] Despite the differences in their morphologic features and clinical presentation, these two diseases do share some characteristics including endothelial cell dysfunction, T helper 2 (Th2) cell dominance during immune activation and excess fibrosis of the skin with similar pathologic findings, leading to the hypothesis that SSc and LSc share an underlying mechanism of pathogensis. Currently, multiple lines of evidence suggest that genetic factors may contribute to SSc susceptibility.[2] Human leukocyte antigen (*HLA*) genes are closely linked to SSc susceptibility, with studies describing some correlation between SSc and *HLA-B*, *HLA-C*, *HLA-DRA*, *HLA-DRB1*, *HLA-DRB5*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DMB*, *HLA-DOA*, *HLA-DPA1*, *HLA-DPB1*, and *HLA-DPB2*.[2] A large number of non-*HLA* genes have also been reported to be associated with the development of SSc, these include connective tissue growth factor gene (*TGF*), signal transducer and activator of transcription gene (*STAT4*) amongst others [Supplementary Table 1, http://links.lww.com/CM9/A294]. However, only some of these results could be verified in additional studies and similar studies in the Chinese population remain extremely limited. Interestingly, genes like interleukin 1 (*IL-1*), ras homolog family member B (*RHOB*), family with sequence similarity 167-member A-BLK proto-oncogene (*FAM167A-BLK*), *STAT4*, interferon regulatory factor 5 gene (*IRF5*), *HLA-DPB1*, and keratin1 (*KRT1*) have all been linked to SSc susceptibility in the Chinese population.[3] Although it has been reported that LSc tends to exhibit some form of familial inheritance, there have been no clearly identified heritable traits for this disease. Additionally, no study, to our knowledge, has investigated the common genetic factors for LSc and SSc. Thus, it would be of significant interest to examine the susceptibility of LSc and determine whether there are any correlations between the genetic profiles of SSc and LSc. This study aimed to identify the genetic profile of LSc patients and any shared genetic features of SSc and LSc in the Chinese population. Furthermore, this study evaluated the occurance of previously described SSc loci in the Chinese population and compared their frequency with those reported in other ethnic communities.

This study was approved by the Institutional Ethics Committee of the Peking Union Medical College Hospital (PUMCH) (No. S-K007) and written informed consent was obtained from each participant. A total of 697 Chinese scleroderma patients undergoing treatment at PUMCH were recruited to this study between January 2009 and January 2015 (562 females, 135 males; aged 34.3 ± 17.3 years), including 464 SSc patients and 233 LSc patients, as well as 3349 healthy controls (2755 females, 594 males; aged 38.0 ± 14.9 years). These patients were diagnosed based on the criteria described by the American College of Rheumatology.[4] Genomic DNA was obtained from the peripheral blood cells of the participants using

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Flexi Gene DNA kits (Qiagen, Germany). A total of 29 single-nucleotide polymorphisms (SNPs) were obtained from the previous genome-wide association studies, and 19 SNPs were selected based on other candidate gene studies using a $P < 0.05$ within each of the subphenotypes before 2015. These samples were genotyped using the Sequenom MassArray system (Sequenom, San Diego, CA, USA) [Supplementary Table 1, http://links.lww.com/CM9/A294]. And case-control analyses were performed to examine the association of each SNP with LSc or SSc susceptibility, respectively. Allelic associations for each SNP were estimated using an odds ratio (OR) with a 95% confidence interval calculated using the Chi-squared test. This combined analysis was performed using meta-analysis software package PLINK 1.07. The fixed-effect model (Mantel-Haenszel) was implemented when I^2 was <30%. Otherwise, the random effect model (DerSimonian-Laird) was applied. Multiple testing corrections were used to modify the statistical significance, and P values of $<1.04 \times 10^{-3}$ were considered statistically significant. The level of statistical significance was set at $P < 0.05$ for the regression analysis. The statistical power for each of the SNPs was estimated using Power and Sample Size Program software (Version 3.1.2, HyLown Consulting LLC, GA, USA).

The combined analysis of the LSc and SSc samples revealed significant associations for SNPs rs7574865, rs10168266, rs3821236 within the *STAT4* gene and SNP rs9303277 in the Ikaros family zinc finger protein 3 gene (*IKZF3*) ($P_{meta} = 1.97 \times 10^{-7} - 8.92 \times 10^{-6}$, OR = 1.30–1.35). Because SNPs rs7574865, rs10168266, and rs3821236 were all located within chromosome 2, we went on to perform linkage disequilibrium analysis which uncovered a weak correlation between rs7574865 and rs3821236 ($r^2 = 0.33$) and a strong correlation between rs7574865 and rs10168266 ($r^2 = 0.80$). Regression analysis of these three SNPs was then carried out and after controlling for the genetic effect of rs7574865, the P value of rs3821236 reached nominal significant ($P_{comditional} = 3.79 \times 10^{-2}$), while no significance ($P_{comditional} = 3.37 \times 10^{-1}$) was found for rs10168266. In addition after SNP rs3821236 was subjected to conditioning the P value for rs7574865 was recalculated and reached 2.92×10^{-3}, which suggests that rs7574865 and rs3821236 are independent SNPs. SNP rs12711490 in *IRF8* was shown to have a suggestive association with scleroderma ($P_{meta} = 3.60 \times 10^{-2}$, OR = 0.81). In the sub-phenotype studies, four SNPs were shown to exhibit significant associations with SSc: rs7574865 ($P = 1.57 \times 10^{-5}$, OR = 1.37), rs10168266 ($P = 2.69 \times 10^{-5}$, OR = 1.35), rs3821236 at *STAT4* ($P = 8.91 \times 10^{-4}$, OR = 1.26), and rs7172677 at c-src tyrosine kinase gene (*CSK*) ($P = 8.34 \times 10^{-4}$, OR = 1.29). Other SNPs identified within the *STAT4* gene did not show any significant association when conditioned on rs7574865. Four SNPs showed suggestive associations with SSc: rs9303277 in the *IKZF3* gene ($P = 1.71 \times 10^{-3}$, OR = 1.26), rs1133906 in the sterile alpha motif domain containing 9 like (*SAMD9L*) gene ($P = 8.19 \times 10^{-3}$, OR = 0.83), rs12711490 in the *IRF8* gene ($P = 3.81 \times 10^{-2}$, OR = 0.76), and rs16860537 in the nicotinamide nucleotide adenylyltransferase 2 (*NMNAT2*) gene ($P = 4.26 \times 10^{-2}$, OR = 0.75). Similarly, rs3821236 in the *STAT4* gene was shown to be significantly associated with LSc ($P = 4.22 \times 10^{-4}$, OR = 1.40), while five SNPs showed only suggestive associations: rs9303277 in the *IKZF3* gene ($P = 1.16 \times 10^{-3}$, OR = 1.38), rs7601754 ($P = 1.75 \times 10^{-3}$, OR = 0.60), rs10168266 ($P = 2.97 \times 10^{-3}$, OR = 1.34), and rs7574865 in the *STAT4* gene ($P = 3.38 \times 10^{3}$, OR = 1.33) and rs231775 in the cytotoxic T-lymphocyte-associated antigen-4 gene (*CTLA-4*) gene ($P = 3.11 \times 10^{-2}$, OR = 0.79).

Scleroderma is an autoimmune disease of the connective tissue, characterized by the creation of scar tissue (fibrosis) in the skin and organs. To date, the underlying pathologic mechanisms of this disease remain largely unknown. One interesting question is whether LSc might progress to SSc and whether they share any underlying genetic indicators for disease. Our genetic study identified a significant association between both LSc and SSc and the *STAT4* gene, suggesting that there may be a common mechanism of pathogenesis underlying both of these conditions. The *STAT4* gene encodes a transcription factor that plays an essential role in the regulation of innate and adaptive immunity. Interestingly, several SNPs in the *STAT4* gene have been reported to be significantly associated with rheumatoid arthritis, systemic lupus erythematosus, and SSc.[5] *STAT4*-deficient mice have been shown to exhibit reduced fibrosis, supporting the hypothesis that *STAT4* may play a role in scleroderma pathogenesis. When the SSc and LSc data were evaluated independently, our study found that mutations in the *CSK* gene are associated with the development of SSc but not LSc. *CSK* is involved in the development of fibrosis via its regulation of focal adhesion kinase (*FAK*), which is needed to transmit integrin signaling when fibroblasts adhere to the extracellular matrix. Thus, polymorphisms in the *CSK* gene can be linked to SSc pathogenesis making *CSK* mutations another potential risk factor for SSc. It seems that *CSK* is only involved in the pathogenesis of SSc and not LSc. *CTLA-4* expression is critical for T-cell identity and several reports have demonstrated a possible connection between *CTLA-4* mutations and the development of various autoimmune diseases, so it has been suggested that *CTLA-4* may play a role in the regulation of immune system self-tolerance and autoreactive T cells and that changes in its expression and regulation may be linked to the pathogeneses of various autoimmune disorders. Our analysis showed that the *CTLA-4* polymorphism had a suggestive association with LSc. Taken together these results suggest that LSc and SSc may share a common pathology mediated by the activation or inhibition of several immune-related genes including *IKZF3* and *STAT4*. This suggests that there may be some common therapeutic strategies to combat both diseases. However, additional genetic risks such as polymorphisms in *SAMD9L*, *NMNAT2*, and *CSK* genes only found in SSc, might be necessary in order for LSc to progress to SSc, while mutation rs231775 in *CTLA-4*, which was only found in LSc, may provide some protection against SSc progression. Our study describes the genetic diversity of SSc and LSc associated loci and the associations between the SNP profiles of these diseases within in the Han population of China. However, our study was limited by the relatively small number of SSc and LSc cases. Thus, our future research should be expanded to include a larger

group of patients to allow for the validation of these SNPs and to determine if these SNPs are causative for LSc or SSc. Future studies should also be designed to evaluate whether these genetic changes will affect a patients' response to therapy. Additionally, future studies should evaluate the transcriptional effect of these SNPs on the expression of these genes to determine their regulatory effect.

Conflicts of interest

None.

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