

A single-nucleotide polymorphism of the TNFSF4 gene is associated with systemic lupus erythematosus in Chinese Han population

Sheng-Quan Zhang · Jian-Wen Han · Liang-Dan Sun · Wen-sheng Lu · Xian-Yong Yin · Xue-Jun Zhang · Sen Yang

Received: 29 June 2009 / Accepted: 27 November 2009 / Published online: 13 December 2009
© Springer-Verlag 2009

Abstract Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease with complex genetic inheritance. Recently, single-nucleotide polymorphisms (SNPs) in tumor necrosis factor (TNF) superfamily gene TNFSF4 have been shown to be associated with SLE in European and Hong Kong Chinese populations. But it is unknown whether it is also associated with the disease in Mainland Chinese Han population. We genotyped the SNPs rs1234315 near the TNFSF4 gene in 1,344 SLE patients and 4,315 controls of Chinese Han population and confirmed the association between the SNP and the SLE [odds ratios (ORs) of 1.45 and P values of 1.5×10^{-16}]. The stratification analyses showed that rs1234315 was more strongly associated with SLE patients with arthritis. Our study not only suggested that the TNFSF4 gene was associated with SLE in Chinese Han population, but also implied

that it might be a common genetic factor predisposing to the development of SLE in multiple populations.

Keywords SLE · TNFSF4 · Chinese Han · Genetic association

Introduction

Systemic lupus erythematosus (SLE) (OMIM 152700) is a multisystem, autoimmune inflammatory disease characterized by antinuclear autoantibodies (ANA), complement and interferon activation [1–3]. Autoantibody production leads to immune complex formation, resulting in local and systemic inflammation and organ failure. The clinical features of SLE are highly pleomorphic, affecting skin, joints, serosa, central nervous system and kidney [1]. SLE has been estimated to affect 60 per 100,000 people in China with a 9:1 female-to-male ratio [2]. Several lines of evidence show the importance of genetic factors in SLE [3, 4], for example, the disease exhibits familial clustering, with 10–12% of SLE patients having a first-degree relative; the available data indicate a concordance rate for SLE between 24 and 69% for monozygotic (MZ) twins and from 2 to 9% for dizygotic (DZ) twins [5].

During the past 20 years, many linkage and candidate-gene studies have been performed to identify genetic factors predisposing to SLE [6]. Despite enormous efforts, the results of SLE genetic studies have not been very satisfactory. Recent technological advances have allowed rapid efficient analysis of single-nucleotide polymorphisms (SNPs) in patients with complex diseases and appropriate control subjects [1]. To date, five genome-wide association (GWA) studies in SLE have identified many risk factors for SLE including the major histocompatibility complex

S.-Q. Zhang · J.-W. Han · L.-D. Sun · W. Lu · X.-Y. Yin · X.-J. Zhang · S. Yang (✉)
Department of Dermatology, Institute of Dermatology,
No. 1 Hospital, Anhui Medical University,
69 Meishan Road, 230032 Hefei, Anhui, China
e-mail: yangsen@medmail.com.cn

S.-Q. Zhang · J.-W. Han · L.-D. Sun · W. Lu · X.-Y. Yin · X.-J. Zhang · S. Yang
Key Laboratory of Dermatology,
Anhui Medical University, Ministry of Education,
Hefei, China

S.-Q. Zhang · J.-W. Han · L.-D. Sun · W. Lu · X.-Y. Yin · X.-J. Zhang · S. Yang
Department of Dermatology and Venereology,
Anhui Medical University, 230032 Hefei, Anhui, China

S.-Q. Zhang
Department of Biochemistry and Molecular Biology,
Anhui Medical University, 230032 Hefei, Anhui, China

(MHC), IRF5, ITGAM, STAT4, BLK, BANK1, PDCD1, PTPN22, TNFSF4, TNFAIP3, SPPI1, C1q, C4 and C2 and some of the Fc gamma receptors [6–10].

To be worthy of mention, among all the risk factors mentioned above, TNFSF4 genes were identified in a study of two SLE case–control cohorts and two independent family-based cohorts with high significant-associated evidence across the combined data sets in European populations [10]. TNFSF4, also known as TNFRSF4/OX40 ligand, belongs to the tumor necrosis factor (TNF) ligand family, and is found to be involved in T cell antigen-presenting cell (APC) interactions. This cytokine and its receptor are reported to directly mediate adhesion of activated T cells to vascular endothelial cells. There is also good evidence that signaling through OX40L can induce B cell activation and differentiation [8, 11–13].

Despite the convincing evidence of its association with SLE in populations of European ancestry and Hong Kong Chinese, it is not yet known whether TNFSF4 plays a role in the development of SLE in other populations such as Mainland Chinese Han. The merits of its replication in different populations should not be overlooked [14]. China has a much higher SLE prevalence and more severe disease manifestations than the Europe [15]. So it is important to further explore whether rs1234315 is also associated with SLE in Mainland Chinese Han population. Here, we examine whether the SNP rs1234315 in the gene TNFSF4 is associated with SLE in Mainland Chinese Han population by analyzing 1,344 patients and 4,135 controls.

Materials and methods

Patients and control subjects

The present study included 1,344 patients with SLE and 4,135 control subjects enrolled by doctors from the Chinese Han population through collaboration with multiple hospitals. All patients fulfilled the American College of Rheumatology classification criteria for SLE. The clinical diagnosis of all subjects was confirmed by at least two rheumatologists or dermatologists. The mean age of the subjects was 31.8 and 93.1% were females. All the controls used in this study were provided after a written informed consent. The study was approved by Institutional Ethical Committees of each hospital and was conducted according to Declaration of Helsinki Principles.

Genotyping

Genomic DNA was extracted from PBMCs. Genotyping of SNP (rs1234315) within TNFSF4 was performed with a Sequenom MassArray system (Sequenom iPLEX assay). In

brief, approximately 15 ng of genomic DNA was used to genotype each sample. Locus-specific PCR primers were designed using the MassARRAY Assay Design 3.0 software (Sequenom). The sample DNA was amplified by multiplex PCR reaction and the PCR products were then used for locus-specific single-base extension reaction. The resulting products were desalted and transferred to a 384-element SpectroCHIP array. Allele detection was performed using MALDI-TOF MS. The mass spectrograms were analyzed by the MassARRAY TYPER software (Sequenom).

Real-time PCR analysis

To analyze the mRNA level of the TNFSF4 in PBMCs, we isolated the PBMCs from the fresh blood of the cases and controls. Then we extracted total cellular RNA by using Trizol purchased from Invitrogen. After quantification, 1 µg of total RNA was used to conduct reverse transcription with a Promega RT kit (A3800) and an oligo(dT) primer. The PCR was completed in a 50 µl reaction system containing 200 nM of primers (TNFSF4 primers: Forward 5'-GGT ATCACATCGGTATCCTCGA, Reverse 5'-TGAGTTGT TCTGCACCTTCATG; GAPDH primers: Forward 5'-AG ATCATCAGCAATGCCTCCTG, Reverse: 5'-ATGGCAT GGACTGTGGTCATG), SYBR[®] Green PCR Core Mix from Roche (New Jersey, USA). Samples were amplified in the Applied Biosystem 7500 Real-Time PCR System for 40 cycles with the following conditions: denaturation for 15 s at 95°C; annealing and extension for 40 s at 60°C. All primers were purchased from Takara Company (DaLian, China).

Statistical analysis

The genotype frequencies of all SNPs were tested for Hardy–Weinberg equilibrium in controls ($P > 0.05$). Disease associations were analyzed by allelic test, as well as logistic regression. The values of $P < 0.05$ (two-tailed) were regarded as significant. Odds ratio (OR) with 95% CI were calculated using PLINK. The expression of the gene was analyzed by SPSS10.0 software.

Results

Association of SLE with SNP rs1234315

We genotyped the SNPs rs1234315, which is about 15 kb away from 5' of the TNFSF4 gene, in 1,344 SLE patients and 4,315 controls of Chinese Han population and confirmed its association with SLE, showing OR of 1.45 and P values of 1.5×10^{-16} . Genotype and allele data were

Table 1 Distribution of alleles and genotypes for the rs1234315 T/C polymorphism in patients with systemic lupus erythematosus and in control subjects

Allele	Controls (<i>n</i> = 4,135)	Case (<i>n</i> = 1,344)	<i>P</i>	OR (95% CI)
T	3,265 (39.5%)	1,377 (51.2%)	4.175E−17 ^a	1.45 (1.335–1.592)
C	5,005 (70.5%)	1,311 (48.8%)		
Genotype				
CC	1,445 (33.1%)	308 (22.9%)	3.318E−16 ^b	
CT	2,115 (48.5%)	695 (51.7%)		
TT	755 (17.3%)	341 (25.3%)		
Recessive model				
T/T (vs. C/T + C/C)	755 (17.3%)	341 (25.3%)	1.779E−10	1.603 (1.386–1.855)
Dominant model				
T/T + C/T (vs. C/C)	2,870 (65.8%)	1,036 (77%)	2.506E−10	1.694 (1.469–1.952)

OR odds ratio, 95% CI 95% confidence intervals

^a Patients versus controls using a 2 × 2 contingency table

^b Patients versus controls using a 3 × 2 contingency table

shown in Table 1. Risk allele T was the minor allele in Chinese Han population. The association was compatible with a dominant model with a higher OR (Table 1).

Stratification analysis

Because the SLE manifestations are highly diverse, we performed the stratification analysis. Table 2 illustrates a spectrum of subphenotypes of SLE and the analysis of subphenotype stratification. The results of stratification analysis showed that rs1234315 was more strongly associated with SLE patients with arthritis ($P = 0.0325$; Table 2).

Correlation of variant in TNFSF4 gene with expression level in PBMC

In order to detect whether the variant of the SNP rs1234315 is associated with the TNFSF4 expression, we determined the relative mRNA expression of the TNFSF4 by quantitative real-time RT-PCR on total RNA purified from human PBMCs. The results shown in Fig. 1 reveal that it was not associated with the levels of messenger RNA expression of TNFSF4.

Discussion

In our study, we confirmed that SNP rs1234315 in the gene TNFSF4 was associated with SLE in the Mainland Chinese Han population, which replicated the findings of European and Hong Kong Chinese populations. Cunninghame Graham et al. [10] had analyzed the association of SLE with several SNPs within or near TNFSF4 gene, and confirmed its association with SLE in European ancestry. But the

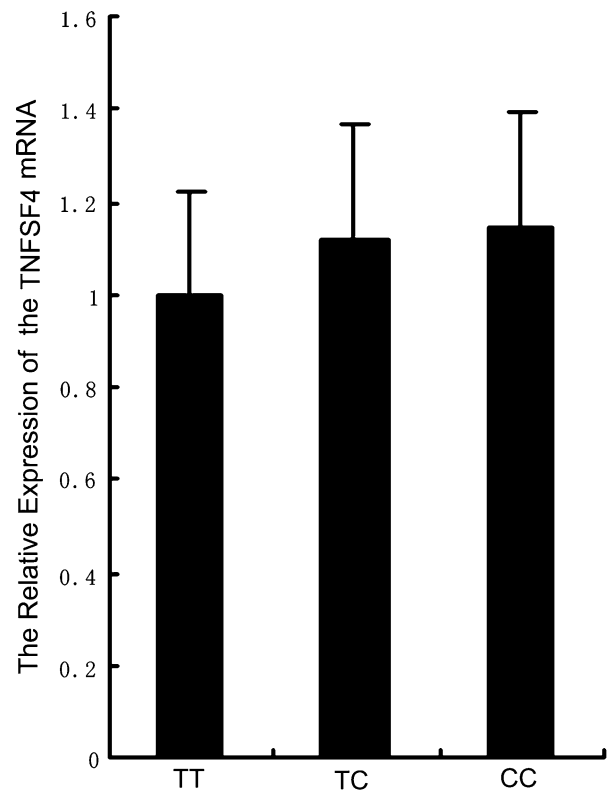


Fig. 1 Correlation of variant in TNFSF4 gene with expression level in PBMC. Relative mRNA expression of the TNFSF4, as determined by quantitative real-time RT-PCR on total RNA purified from human PBMCs of healthy controls and cases. Data represent mean ± SD. We analyzed 6 individuals with TT, 20 with TC and 12 with CC. The TNFSF4 expression did not significantly correlate with genotypes of rs1234315. TT versus CC ($P = 0.257$)

strongest association of SNP within TNFSF4 was rs844648 ($P = 1.0 \times 10^{-5}$) instead of rs1234315. Chang [15] found that rs1234315 (OR = 1.46, $P = 4.89 \times 10^{-5}$) was associated

Table 2 Association of rs1234315 with SLE analyzed by subphenotype stratification

Subphenotype	Comparison	<i>P</i>	rs1234315 OR	95% CI
Neurologic disorder	SLE(+) (<i>N</i> = 77) versus control (<i>N</i> = 4,315)	0.0006162	1.54	1.201–1.976
	SLE(−) (<i>N</i> = 1,267) versus control (<i>N</i> = 4,315)	8.43E−16	1.438	1.316–1.572
	SLE(+) (<i>N</i> = 77) versus SLE(−) (<i>N</i> = 1,267)	0.387	0.8633	0.6186–1.205
Arthritis	SLE(+) (<i>N</i> = 841) versus control (<i>N</i> = 4,315)	3.35E−09	1.365	1.231–1.513
	SLE(−) (<i>N</i> = 503) versus control (<i>N</i> = 4,315)	4.55E−13	1.6	1.408–1.819
	SLE(+) (<i>N</i> = 841) versus SLE(−) (<i>N</i> = 503)	0.0325	1.191	1.015–1.398
Photosensitivity	SLE(+) (<i>N</i> = 721) versus control (<i>N</i> = 4,315)	4.04E−10	1.418	1.27–1.583
	SLE(−) (<i>N</i> = 623) versus control (<i>N</i> = 4,315)	2.62E−11	1.487	1.323–1.672
	SLE(+) (<i>N</i> = 721) versus SLE(−) (<i>N</i> = 623)	0.5119	1.053	0.9021–1.23
Sm	SLE(+) (<i>N</i> = 470) versus control (<i>N</i> = 4,315)	1.45E−14	1.477	1.337–0.632
	SLE(−) (<i>N</i> = 874) versus control (<i>N</i> = 4,315)	6.67E−14	1.429	1.301–1.569
	SLE(+) (<i>N</i> = 470) versus SLE(−) (<i>N</i> = 874)	0.3265	0.892	0.71–1.121
Anti-dsDNA autoantibodies	SLE(+) (<i>N</i> = 733) versus control (<i>N</i> = 4,315)	2.94E−14	1.448	1.316–1.594
	SLE(−) (<i>N</i> = 611) versus control (<i>N</i> = 4,315)	2.99E−13	1.441	1.306–1.591
	SLE(+) (<i>N</i> = 733) versus SLE(−) (<i>N</i> = 611)	0.9179	0.9887	0.7967–1.227
Hematologic disorder	SLE(+) (<i>N</i> = 980) versus control (<i>N</i> = 4,315)	3.43E−14	1.458	1.322–1.607
	SLE(−) (<i>N</i> = 364) versus control (<i>N</i> = 4,315)	2.21E−06	1.415	1.225–1.635
	SLE(+) (<i>N</i> = 980) versus SLE(−) (<i>N</i> = 364)	0.7762	0.9751	0.8196–1.16
Malar rash	SLE(+) (<i>N</i> = 840) versus control (<i>N</i> = 4,315)	1.45E−12	1.453	1.31–1.612
	SLE(−) (<i>N</i> = 504) versus control (<i>N</i> = 4,315)	1.23E−08	1.444	1.272–1.639
	SLE(+) (<i>N</i> = 840) versus SLE(−) (<i>N</i> = 504)	0.9322	0.9931	0.8469–1.165
Oral ulcers	SLE(+) (<i>N</i> = 401) versus control (<i>N</i> = 4,315)	1.17E−05	1.362	1.186–1.565
	SLE(−) (<i>N</i> = 943) versus control (<i>N</i> = 4,315)	2.44E−15	1.492	1.351–1.648
	SLE(+) (<i>N</i> = 401) versus SLE(−) (<i>N</i> = 943)	0.2418	1.106	0.9345–1.308
Serositis	SLE(+) (<i>N</i> = 82) versus control (<i>N</i> = 4,315)	0.02551	1.32	1.034–1.685
	SLE(−) (<i>N</i> = 1,262) versus control (<i>N</i> = 4,315)	3.67E−17	1.464	1.339–1.6
	SLE(+) (<i>N</i> = 82) versus SLE(−) (<i>N</i> = 1,262)	0.3286	1.173	0.8515–1.616
Renal disorder	SLE(+) (<i>N</i> = 665) versus control (<i>N</i> = 4,315)	3.17E−10	1.437	1.283–1.61
	SLE(−) (<i>N</i> = 679) versus control (<i>N</i> = 4,315)	7.56E−11	1.453	1.298–1.627
	SLE(+) (<i>N</i> = 665) versus SLE(−) (<i>N</i> = 679)	0.8805	1.012	0.8671–1.181
ANA	SLE(+) (<i>N</i> = 1,301) versus control (<i>N</i> = 4,315)	1.51E−16	1.445	1.324–1.578
	SLE(−) (<i>N</i> = 43) versus control (<i>N</i> = 4,315)	0.03882	1.33	1.014–1.745
	SLE(+) (<i>N</i> = 1,301) versus SLE(−) (<i>N</i> = 43)	0.4331	1.225	0.7369–2.036
Discoid rash	SLE(+) (<i>N</i> = 194) versus control (<i>N</i> = 4,315)	3.96E−07	1.603	1.334–1.926
	SLE(−) (<i>N</i> = 1,150) versus control (<i>N</i> = 4,315)	7.76E−14	1.419	1.295–1.556
	SLE(+) (<i>N</i> = 194) versus SLE(−) (<i>N</i> = 1,150)	0.1702	0.858	0.6893–1.068

with SLE in Hong Kong Chinese population. In this study, we had found SNP rs1234315 in TNFSF4 was significantly associated with SLE in Mainland Chinese Han population ($P = 10 \times 10^{-16}$), which suggested that TNFSF4 might be a common genetic factor for the development of SLE within multiple populations in terms of Mainland Chinese Han, European and Hong Kong Chinese, although the most significant SNPs were different among them. Biologically, the TNFSF4 gene mediates adhesion of activated T cells to vascular endothelial cells, which can induce B cell

activation and differentiation, suggesting that TNFSF4 may play a role in the pathogenesis of SLE. Taken together, all the factors mentioned above supports that TNFSF4 should be involved the development of SLE.

By performing stratification analysis, we found that TNFSF4 may be involved in the SLE patients with arthritis ($P = 0.0325$), and further study is needed to explore the specific mechanism. Although we showed that the variant of rs1234315 was not associated with the levels of mRNA expression of TNFSF4, which might be due to the small

sample size used in this study or some unknown reason yet to be identified. Further investigation is warranted to explore its exact role in the development of SLE.

In summary, our study independently replicates and confirms the strong association of SNP rs1234315 in TNFSF4 with the risk of SLE in Mainland Chinese Han population, which implies that there exist some common genetic factors shared in different populations, although the importance of genetic heterogeneity might not be ignored. Exploring the full genetic basis of SLE in different ethnic populations might help advance our overall understanding of the disease pathogenesis.

Acknowledgments We thank all the participants of this study, which was supported by the Key Project of National Natural Science Foundation (nos 30530670 and 30830089).

Conflict of interest statement The authors declared no competing financial interests.

References

- International Consortium for Systemic Lupus Erythematosus Genetics (SLEGEN), Harley JB, Alarcón-Riquelme ME, Criswell LA, Jacob CO, Kimberly RP et al (2008) Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXX1, KIAA1542 and other loci. *Nat Genet* 40:204–210
- Tikly M, Navarra SV (2008) Lupus in the developing world—is it any different? *Best Pract Res Clin Rheumatol* 22:643–655
- Criswell LA (2008) The genetic contribution to systemic lupus erythematosus. *Bull NYU Hosp Jt Dis* 66:176–183
- Crow MK (2008) Collaboration, genetic associations, and lupus erythematosus. *N Engl J Med* 358:956–961
- Deapen D, Escalante A, Weinrib L, Horwitz D, Bachman B, Roy-Burman P (1992) A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum* 35:311–318
- Hom G, Graham RR, Modrek B, Taylor KE, Ortmann W, Garnier S (2008) Association of systemic lupus erythematosus with C8orf13–BLK and ITGAM–ITGAX. *N Engl J Med* 358:900–909
- Moser KL, Kelly JA, Lessard CJ, Harley JB (2009) Recent insights into the genetic basis of systemic lupus erythematosus. *Genes Immun* 10(5):373–379
- Rhodes B, Vyse TJ (2008) The genetics of SLE: an update in the light of genome-wide association studies. *Rheumatology (Oxford)* 47:1603–1611
- Harley IT, Kaufman KM, Langefeld CD, Harley JB, Kelly JA (2009) Genetic susceptibility to SLE: new insights from fine mapping and genome-wide association studies. *Nat Rev Genet* 10:285–290
- Cunningham Graham DS, Graham RR, Manku H, Wong AK, Whittaker JC, Gaffney PM, Moser KL, Rioux JD (2009) Polymorphism at the TNF superfamily gene TNFSF4 confers susceptibility to systemic lupus erythematosus. *Nat Genet* 40:83–89
- Croft M (2009) The role of TNF superfamily members in T-cell function and diseases. *Nat Rev Immunol* 9:271–285
- Ito T, Wang YH, Duramad O, Hanabuchi S, Perng OA, Gilliet M et al (2006) OX40 ligand shuts down IL-10-producing regulatory T cells. *Proc Natl Acad Sci USA* 103:13138–13143
- Lane P (2000) Role of OX40 signals in coordinating CD4T cell selection, migration, and cytokine differentiation in T helper (Th)1 and Th2 cells. *J Exp Med* 191:201–206
- Lettre G, Rioux JD (2008) Autoimmune diseases: insights from genome-wide association studies. *Hum Mol Genet* 17(R2):R116–R121
- Chang YK, Yang W, Zhao M, Mok CC, Chan TM, Wong RW, Lee KW, Mok MY, Wong SN, Ng IO, Lee TL, Ho MH, Lee PP, Wong WH, Lau CS, Sham PC, Lau YL (2009) Association of BANK1 and TNFSF4 with systemic lupus erythematosus in Hong Kong Chinese. *Genes Immun* 10:414–420