Variants in CCR6 are associated with susceptibility to lupus nephritis in Chinese

Authors:
Xu-jie Zhou¹, MD & PhD; Rong Mu², MD & PhD; Chun Li², MD; Swapan K Nath³, PhD; Yue-miao Zhang¹, MD; Yuan-yuan Qi¹, MD; Zhan-guo Li¹, MD & PhD; Ming-hui Zhao¹, MD & PhD; Hong Zhang¹, MD & PhD

Author affiliations:
1, Renal Division, Peking University First Hospital; Peking University Institute of Nephrology; Key Laboratory of Renal Disease, Ministry of Health of China; and Key Laboratory of Chronic Kidney Disease Prevention and Treatment (Peking University), Ministry of Education; Beijing, 100034, People's Republic of China
2, Department of Rheumatology and Immunology, Peking University People's Hospital, Beijing, 100044, China.
3, Arthritis and Clinical Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA

Correspondence to: Prof. Hong Zhang, MD, PhD
Renal Division, Peking University First Hospital,
Peking University Institute of Nephrology
No.8 Xi Shi Ku Street, Xi Cheng District,
Beijing 100034 (China)
Tel: +86-10-83572388,
Fax: +86-10-66551055,
E-Mail: hongzh@bjmu.edu.cn

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Systemic lupus erythematosus (SLE) is a common autoimmune disease with a complex genetic etiology. In the past two years, exciting insights supporting the proinflammatory activity of IL-17-producing T-helper 17 subset (Th17 cells) in the pathogenesis of several autoimmune or allergic diseases, including asthma, atopic dermatitis, rheumatoid arthritis (RA) and SLE, have emerged (1,2). High production of IL-17 is observed in SLE patients and several compounds targeting IL-17 have demonstrated marked clinical efficacy (1,2). Genetic studies have reproducibly demonstrated an association between susceptibility to RA and polymorphisms of the CCR6 gene, a surface marker for Th17 cells (3,4). However, to date, no direct link between CCR6 and SLE, has been found from genetic studies, although higher percentages of CCR6+ T helper cells were observed in patients with SLE especially in lupus nephritis (LN) compared to controls (5,6). Thus the current study sought to investigate whether there are any associations between CCR6 polymorphisms and susceptibility to LN.

In the current study, discovery cohort comprised of 500 LN patients (age 32.0±11.5 years, female 83.6%) and 900 unrelated healthy individuals (31.5±8.4 years, female 40.1%). And replication cohort comprised of 626 LN patients (32.5±12.8 years, female 84.0%) and 1932 healthy individuals (40.9±12.7 years, female 47.7%) as well as 1063 SLE patients without indicators of renal involvement (36.6±13.4 years, female 90.5%) as controls. All the patients met the revised SLE criteria of the American College of Rheumatology (7). The study was approved by the medical ethics committee of Peking University. All patients gave informed consent. Two intronic SNPs rs3093023 and rs3093024 known to be associated with RA with top association signals were selected (3, 4) and genotyping was undertaken by TaqMan allele discrimination assays (Applied Biosystems, FosterCity, California, USA) as previously reported (8, 9). Direct sequencing was performed in randomly selected 45 samples on the basis of rs3093024 genotype (15 subjects with A/A, 15 with G/A, and 15 with G/G genotype) to determine whether rs3093024 could tag the recently identified functional CCR6DNP (a triallelic dinucleotide polymorphism of CCR6) (3).

Power of the study was calculated by CaTS.
As rs3093023 and rs3093024 are in high linkage disequilibrium (r^2 0.98), indicating statistical tests performed on each SNP are actually highly dependent, no multiple correction was applied. Statistical analyses were performed with SPSS16.0 software (SPSS Inc., Chicago, IL). Functional annotations of variants were obtained from HaploReg and regulomeDB databases.

A total 5021 Chinese were included and the call rate for rs3093024 and rs3093023 were 99.82% and 98.94%. Both SNPs studied were in Hardy-Weinberg equilibrium in controls and patients (p>0.05). With the expected frequency of rs3093024 and rs3093023 minor allele (40%), we had a power of 96-99% to detect a 1.20 fold increased risk at separated stages and in joint. The frequency of the A allele of rs3093024 was significantly higher in LN both in discovery cohort and replication cohort as compared with healthy controls (Table 1, p= 1.32×10^-2 and p = 4.15×10^-2, respectively). This difference became more significant for the combined groups (p= 1.42×10^-3, OR 1.18, 95% CI 1.06-1.30). Comparing with patients of SLE without LN, increased frequencies of rs3093024A and rs3093023A were also observed in LN patients (Table 2, p<0.05). Dominant models showed better fit whereas additive models also showed significant associations between CCR6 variants and SLE (Table 2), consistent with the data from rheumatoid arthritis. And logistic regression analysis adjusted by sexes and ages also suggested that risk genotypes of rs3093024 (AA+AG, p= 0.02, OR 1.21, 95% CI 1.03-1.46) and rs3093023 (AA+AG, p= 0.01, OR 1.24, 95% CI 1.05-1.49) were associated with LN compared to controls. As the two SNPs were in high LD, risk haplotype AA also showed significant associations with LN compared to controls (p= 1.78×10^-2, OR 1.08, 95% CI 1.02-1.13). However, no other phenotypic (serum creatinine, complement level, SLEDAI, proteinuria, and type of LN) correlations were observed (p>0.05). By searching ENCODE data, the rs3093024 was observed to locate in region of promoter histone marks in GM12878 (B lymphoblastoid) and rs3093023 is a cis-expression SNP for CCR6 in monocyte. In addition, direct sequencing of the functional dineucleotide polymorphism of CCR6, CCR6DNP, revealed the presence of 5 genotypes (CA/CG, CG/CG, TG/CA, TG/CG
and TG/TG). Presence of TG/TG, TG/CG and CG/CG was correlated with presence of A/A(12/15), A/G(11/15) and G/G(11/15) genotype of rs3093024, suggesting that rs3093024 and rs3093023 were in linkage disequilibrium with CCR6DNP, which has been shown to correlate with the expression level of CCR6(3).

In conclusion, the current data demonstrate a genetic association between CCR6 variants and susceptibility to LN, further demonstrating a potential role of Th17 cells in SLE pathogenesis. However, more widespread replications and functional assays are still needed.
References:


Table legends:

Table 1. Associations between *CCR6* variants and susceptibility to lupus nephritis (LN).

Table 2. Associations between *CCR6* variants and susceptibility to SLE stratified by renal involvement.
Table 1. Associations between CCR6 variants and susceptibility to lupus nephritis (LN).

<table>
<thead>
<tr>
<th>SNP</th>
<th>Pos (hg19)</th>
<th>Minor allele</th>
<th>Discovery 500 LN /900 Controls</th>
<th>Replication 626 LN /1932 Controls</th>
<th>Total 1126 LN /2832 Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3093024</td>
<td>167532793</td>
<td>A</td>
<td>MAF case/ control</td>
<td>MAF case/ control</td>
<td>MAF control model p (95% CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.32×10^{-2} 1.22</td>
<td>4.15×10^{-2} 1.14</td>
<td>1.42×10^{-3} 1.18</td>
</tr>
<tr>
<td>rs3093023</td>
<td>167534290</td>
<td>A</td>
<td>44.5/39.7</td>
<td>43.0/42.9 0.97</td>
<td>43.6/41.7 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7.71×10^{-3} 1.24</td>
<td>1.00</td>
<td>1.08</td>
</tr>
</tbody>
</table>

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Table 2. Associations between CCR6 variants and susceptibility to SLE stratified by renal involvement.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genetic model</th>
<th>Risk factor</th>
<th>SLE vs. Healthy control (2189/2832)</th>
<th>LN vs. Healthy control (1126/2832)</th>
<th>LN vs. SLE without LN (1126/1063)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Frequency of risk factor</td>
<td>$p$</td>
<td>OR (95% CI)</td>
<td>Frequency of risk factor</td>
</tr>
<tr>
<td>rs3093024</td>
<td>Allele model</td>
<td>A</td>
<td>42.2/39.9</td>
<td>1.57×10^{-2}</td>
<td>1.10(1.02-1.20)</td>
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<td></td>
<td>Dominant model</td>
<td>AA+GA</td>
<td>66.6/60.7</td>
<td>1.72×10^{-5}</td>
<td>1.29(1.15-1.45)</td>
</tr>
<tr>
<td></td>
<td>Additive model</td>
<td>GA, AA</td>
<td>9.71×10^{-5}</td>
<td>3.29×10^{-5}</td>
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</tr>
<tr>
<td></td>
<td>Recessive model</td>
<td>AA</td>
<td>17.9/16.3</td>
<td>0.13</td>
<td>2.72×10^{-2}</td>
</tr>
<tr>
<td>rs3093023</td>
<td>Allele model</td>
<td>A</td>
<td>42.0/41.7</td>
<td>0.77</td>
<td>43.6/41.7</td>
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<tr>
<td></td>
<td>Dominant model</td>
<td>AA+GA</td>
<td>66.5/60.6</td>
<td>1.86×10^{-5}</td>
<td>1.29(1.15-1.45)</td>
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<td></td>
<td>Additive model</td>
<td>GA, AA</td>
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<td>2.49×10^{-5}</td>
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</tr>
<tr>
<td></td>
<td>Recessive model</td>
<td>AA</td>
<td>17.6/18.3</td>
<td>0.53</td>
<td>19.0/18.3</td>
</tr>
</tbody>
</table>

Significant associations were marked in bold and OR values were presented for the most fitted genotypic models.