Investigation of C1-complex regions reveals new C1Q variants associated with protection from systemic lupus erythematosus, and affects its transcript abundance

Jianping Guo1, Yanyan Gao1,2, Yuxuan Wang3, Yundong Zou3, Yan Du1, Cainan Luo3, Yamei Shi1, Yue Yang1, Xinyu Wu1, Yin Su1, Lijun Wu3, Shi Chen1 & Zhanguo Li1

Although rare variant C1Q deficiency was identified as causative risk for systemic lupus erythematosus (SLE), there are limited and inconsistent reports regarding the common polymorphisms of C1Q genes in SLE susceptibility. Furthermore, there are no reports concerning polymorphisms of C1S, C1R, and C1RL and whether they confer susceptibility to SLE. We therefore evaluated 22 SNPs across six C1-complex genes in two independent case-control cohorts, and identified four novel SNPs that confer protection from SLE. The four SNPs are all located in C1Q. Particularly, the variant rs653286 displayed an independent reduced risk on SLE susceptibility (OR 0.75, P = \(2.16 \times 10^{-3}\)) and anti-dsDNA antibodies (OR 0.68, P = 0.024). By bioinformatics analysis, SNPs rs653286 and rs291985 displayed striking cis-eQTL effects on C1Q genes expression. Individuals homozygous for the ‘protective’ allele at four SNPs had significantly higher levels of serum C1q (rs680123–rs682658: P = 0.0022; rs653286–rs291985: P = 0.0076). To our knowledge, this is the first study to demonstrate that only C1Q polymorphisms are associated with SLE. The C1Q SNP rs653286 confers an independent protective effect on SLE susceptibility and affects transcript abundance.

Systemic lupus erythematosus (SLE) is a chronic and systemic autoimmune disease, clinically characterized by heterogeneous manifestations and production of multiple autoantibodies. The exact etiology of SLE is unknown, but it is generally acknowledged that the disease evolves a complex interplay between multiple environmental and genetic factors.

Complement cascade molecules are the key component in innate immune activation and play an important role in SLE pathogenesis. The C1-complex, composed of three molecules C1q, C1r, and C1s, is the first component in activation of classical complement pathway. The C1q molecule is composed of three similar but different types of chains, namely A-, B-, and C-chains, which are encoded by three distinct genes, i.e. C1QA, C1QB, and C1QC. The genes are arranged tandemly in order of A-C-B on a 24-kb stretch of DNA on human chromosome 1p36.1 Human C1S and C1R are closely located at a distance of approximately 9.5 kb on chromosome 12p13. Both C1S (10.5 kb) and C1R (11 kb) contain 12 exons, and are highly homologous in domain structure and function2,3. Furthermore, the complement C1r-like protein (C1RL) is a recently discovered homologue of C1R, located 2 kb centromeric to C1R4.

Although C1Q and C1S deficiencies were identified as causative genetic risks for SLE (with a disease incidence of 93% and 68%, respectively), such deficiencies are very rare. To date fewer than 90 individuals with C1 deficiencies have been reported worldwide: approximately 70 cases with C1Q deficiency and 19 cases with C1S...
C1S, are protective against SLE susceptibility and transcript abundance. They may affect C1Q polymorphisms in C1R and C1QA, Han population, using a candidate gene approach and focusing only on C1QA.

Weinberg equilibrium; aSNPs in bold: previously known to be associated with SLE or related symptoms; bMinor allele/Major allele.

Table 1. Information of SNPs from HapMap CHB database. CHB: Chinese Han in Beijing; Chr: chromosome; SNPs: single nucleotide polymorphisms; MAF: minor allele frequency; UTR: untranslated region; missense: missense mutation; cds-synon: coding region variant-synonymous mutation; HWpval: p-value for Hardy-Weinberg equilibrium; cSNPs in bold: previously known to be associated with SLE or related symptoms; dMinor allele/Major allele.

| Chr. | Gene | SNPa | Position (Mb) | Allelesb | MAF | Functional alteration | HWpval |
|------|------|------|---------------|----------|-----|----------------------|--------|
| 1    | C1QA | rs680123 | 22635031 | C/T | 0.329 | nearGene-5′ | 0.2733 |
| 2    | C1QA | rs12033074 | 22640116 | G/C | 0.433 | nearGene-3′ | 0.1502 |
| 2    | C1QC | rs4655085 | 22641932 | A/G | 0.151 | nearGene-5′ | 0.5839 |
| 1    | C1QC | rs682658 | 22642322 | G/T | 0.329 | nearGene-5′ | 0.2733 |
| 1    | C1QC | rs655953 | 22644526 | G/A | 0.256 | Intron | 1.0000 |
| 1    | C1QC | rs672693 | 22644953 | A/G | 0.369 | Intron | 0.6547 |
| 1    | C1QC | rs653286 | 22645091 | T/C | 0.300 | Intron | 0.6547 |
| 1    | C1QB | rs12754182 | 22651775 | T/C | 0.211 | nearGene-5′ | 0.2733 |
| 1    | C1QB | rs17433222 | 22652153 | A/G | 0.150 | nearGene-5′ | 0.5839 |
| 1    | C1QB | rs913243 | 22653494 | T/G | 0.386 | intron | 0.3428 |
| 1    | C1QB | rs291985 | 22654446 | T/G | 0.356 | intron | 0.4027 |
| 1    | C1QB | rs629409 | 22660245 | A/G | 0.439 | intron | 0.2683 |
| 12   | CIS  | rs7962629 | 7059466  | G/A | 0.073 | nearGene-5′ | 1.0000 |
| 12   | C1S  | rs12146727 | 7063032  | A/G | 0.073 | missense | 1.0000 |
| 12   | C1S  | rs7183  | 7070715  | T/G | 0.073 | UTR-3′ | 1.0000 |
| 12   | C1R  | rs3813728 | 7088974  | T/C | 0.085 | missense | 1.0000 |
| 12   | C1R  | rs7135975 | 7090972  | G/A | 0.183 | intron | 0.5271 |
| 12   | C1RL | rs7709  | 7094764  | C/A | 0.207 | UTR-3′ | 1.0000 |
| 12   | C1RL | rs3782928 | 7096079  | A/G | 0.159 | UTR-3′ | 0.2733 |
| 12   | C1RL | rs3742089 | 7097002  | G/A | 0.354 | missense | 0.5839 |
| 12   | C1RL | rs12304029 | 7098579  | C/G | 0.364 | intron | 1.0000 |
| 12   | C1RL | rs3742088 | 7102073  | G/T | 0.171 | cds-synon | 1.0000 |

There are limited and inconsistent reports regarding the common polymorphisms in C1Q cluster in relation to SLE susceptibility. One publication evaluated the association of C1Q polymorphisms with SLE in Han population, using a candidate gene approach and focusing only on C1QA. There are no reports on genetic association of C1S, C1R, and C1RL polymorphisms and susceptibility to SLE. We therefore undertook the current study to investigate the possible genetic association(s) between the six C1 genes (C1QA, C1QC, C1QB, C1S, C1R, and C1RL) and SLE susceptibility or clinical/serologic features. We report that C1Q polymorphisms, but not polymorphisms in C1S and C1R, are protective against SLE susceptibility and affect C1Q transcript abundance.

**Results**

A total of 22 SNPs were selected for the discovery screening. These included 18 tag-SNPs spanning C1QA, C1QC, C1QB, C1S, C1R, and C1RL loci and additional 4 SNPs known to be associated with SLE or related symptoms (Table 1). All SNPs were in Hardy-Weinberg equilibrium (HWE) in both patients and controls (P > 0.05, data not shown). In control groups, the allele frequencies of SNPs were similar to the data from HapMap CHB (shown in Tables 1, 2 and Supplementary Table S1).

Discovery screening of C1-complex genomic regions reveals C1Q polymorphisms associated with SLE susceptibility. We first sought to investigate any potential association(s) between the selected SNPs resided in C1-complex and SLE susceptibility in the discovery cohort. As shown in Tables 2 and S2, although at the allele level no association was observed, at the genotype level we found three SNPs that conferred nominal protective effects against SLE (dominant model: C1QA rs680123: OR 0.72, P = 0.028, q = 0.235; C1QC rs682658: OR 0.72, P = 0.032, q = 0.235; and C1QC rs653286: OR 0.70, P = 0.018, q = 0.235, respectively). A suggestive reduced risk effect was also found for C1QB rs291985 (dominant model: OR = 0.75, P = 0.059, q = 0.325).

Notably, the four SNPs are all resided in C1Q genes. No potential association was observed for other polymorphisms outside C1Q in SLE susceptibility (Supplementary Table S1).

Replication study and joint analysis confirms the reduced risk of the four C1Q polymorphisms, but only SNP rs653286 has an independent protective effect on SLE susceptibility. To confirm the possible associations that we observed in the discovery cohort, the top 4 SNPs were replicated in an independent case-control cohort. Joint analysis was then performed by combining results from discovery and replication cohorts.

As shown in Tables 2 and S2, in the replication panel, both rs653286 and rs291985 showed consistent protection from SLE (dominant model: rs653286: OR 0.78, P = 0.045, q = 0.090; rs291985: OR 0.75, P = 0.019, q = 0.076,
### Table 2. Associations between 4 SNPs in C1Q genes with SLE susceptibility, logistic regression adjusting for age and gender. SLE: systemic lupus erythematosus; SNPs: single nucleotide polymorphisms; OR (95% CI): odds ratio (95% confidence interval); *Minor allele frequencies in combined cohort (cases/controls). p-value (Het): p-value for heterogeneity, I²: heterogeneity statistic.

| Gene | SNP    | MAF* Cases/Cons | Models       | Stage I | Stage II | Combined |
|------|--------|-----------------|--------------|---------|----------|----------|
|      |        |                 |              | OR (95% CI) | p-value | OR (95% CI) | p-value | OR (95% CI) | p-value | I² (%) | P-value (Het) |
| C1QA | rs680123 | 0.350/0.369     | Allelic (C/T) | 0.85 (0.69–1.06) | 0.143  | 0.93 (0.78–1.10) | 0.394  | 0.90 (0.79–1.03) | 0.116  | 0      | 0.55      |
|      |        |                 | Recessive (CC/CT + TT) | 1.05 (0.69–1.59) | 0.822  | 1.04 (0.74–1.45) | 0.833  | 1.04 (0.80–1.35) | 0.788  | 0      | 0.39      |
|      |        |                 | Dominant (CC + CT/TT) | 0.72 (0.54–0.97) | 0.028  | 0.85 (0.67–1.08) | 0.186  | 0.80 (0.66–0.96) | 0.016  | 0      | 0.46      |
| C1QC | rs682658 | 0.351/0.372     | Allele (G/T) | 0.87 (0.70–1.07) | 0.187  | 0.94 (0.79–1.12) | 0.489  | 0.91 (0.80–1.04) | 0.187  | 0      | 0.57      |
|      |        |                 | Recessive (GG/GT + TT) | 1.10 (0.73–1.66) | 0.652  | 1.05 (0.75–1.48) | 0.772  | 1.07 (0.82–1.39) | 0.631  | 0      | 0.38      |
|      |        |                 | Dominant (GG + GT/TT) | 0.72 (0.54–0.97) | 0.032  | 0.87 (0.68–1.10) | 0.240  | 0.81 (0.67–0.97) | 0.024  | 0      | 0.47      |
| C1QC | rs653286 | 0.332/0.368     | Allele (T/C) | 0.83 (0.67–1.03) | 0.091  | 0.99 (0.73–1.04) | 0.138  | 0.86 (0.75–0.98) | 0.024  | 0      | 0.44      |
|      |        |                 | Recessive (TT/TC + CC) | 1.03 (0.65–1.62) | 0.905  | 0.99 (0.70–1.40) | 0.960  | 0.99 (0.76–1.31) | 0.993  | 0      | 0.51      |
|      |        |                 | Dominant (TT + TC/CC) | 0.70 (0.52–0.94) | 0.018  | 0.78 (0.62–0.99) | 0.045  | 0.75 (0.62–0.90) | 2.45 × 10⁻³ | 0      | 0.39      |
| C1QB | rs291985 | 0.335/0.368     | Allele (T/G) | 0.87 (0.71–1.08) | 0.218  | 0.86 (0.73–1.03) | 0.098  | 0.87 (0.76–0.99) | 0.035  | 0      | 0.34      |
|      |        |                 | Recessive (TT/TG + GG) | 1.08 (0.69–1.70) | 0.740  | 1.01 (0.72–1.42) | 0.940  | 1.03 (0.79–1.35) | 0.828  | 0      | 0.33      |
|      |        |                 | Dominant (TT + TG/GG) | 0.75 (0.56–1.01) | 0.059  | 0.75 (0.59–0.95) | 0.019  | 0.76 (0.63–0.91) | 2.97 × 10⁻³ | 0      | 0.67      |

respectively). A trend toward a protective effect was also observed for rs680123 and rs682658, although this negative association did not reach statistical significance.

Joint analysis of discovery and replication panels showed that all the four SNPs conferred protective effects on SLE susceptibility (Tables 2 and S2). In particular, the variants rs653286 and rs291985 showed more pronounced protective effects both at allele (rs653286: OR<sub>combined</sub> = 0.86, P = 0.024, q = 0.070; rs291985: OR<sub>combined</sub> = 0.75, P = 0.303, q = 0.070, respectively) and genotype levels (dominant model: rs653286: OR<sub>combined</sub> = 0.75, P = 2.45 × 10⁻³, q = 5.19 × 10⁻³; rs291985: OR<sub>combined</sub> = 0.76, P = 2.97 × 10⁻³, q = 5.94 × 10⁻³, respectively) than the variants rs680123 and rs682658 did (Tables 2 and S2). There was no evidence for heterogeneity between two sample sets in the four variants (allele model: P<sub>het</sub> = 0.032, F<sub>het</sub> = 0.24; genotype model: P<sub>het</sub> ≥ 0.33, F<sub>het</sub> = 0.00%, shown in Supplementary Figure S1).

To test the independence of the four SNPs, a stepwise (forward conditional) logistic regression analysis was performed in the combined cohort. As shown in Table 3, only rs653286 was independently associated with the disease at both allele (P = 0.022) and genotypic (dominant model: P = 2.16 × 10⁻⁵) levels. Subsequent additions of rs291985, rs682658 and rs680123 did not show significant association with SLE susceptibility.

To investigate whether the independent protective SNP rs653286 predispose to any particular disease manifestation(s), we assessed the association(s) between SNP rs653286 and clinical/serologic features in a case-only cohort. Following stratification, we found SNP rs653286 was significantly associated with anti-dsDNA antibodies (dominant model: OR 0.68, P = 0.024). However, no association was observed for other SLE manifestations (Table S4). This finding might be explained by the limited statistical power of this analysis.

**Bioinformatics annotations support potential regulatory function(s) of the four C1Q variants.**

As the four variants are localized in either near 5′-UTR or introns, we performed bioinformatics analysis to access their potential regulatory effects. As annotated in rVarBase database, the four variants all displayed potential regulatory activities, such as location within TF (transcription factor) binding sites or chromatin interactive regions, showing LD-proxies with rSNPs or overlapping with rCNVs, regulating TSS (transcription start site) or transcriptional enhancers, and/or association with mRNA abundance, etc. (Fig. 1A). By searching in the RegulomeDB database, the four variants also showed regulatory potential by affecting protein binding, chromatin structure, and histone modifications (Supplementary Table S5). By searching into the Blood eQTL database, we found SNPs rs653286 and rs291985 displayed striking cis-eQTL (expression Quantitative Trait Loci) effects on expression of C1QB (rs653286: P = 2.91 × 10⁻⁷, FDR = 0.00, Z score = 18.61; rs291985: P = 1.17 × 10⁻⁸, FDR = 0.00, Z score = 18.90), C1QA (rs653286: P = 6.32 × 10⁻⁶, FDR = 0.01, Z score = 4.52; rs291985: P = 1.34 × 10⁻⁵,
support a potential regulatory role for each of the four polymorphisms, are negatively associated with SLE. The SNP, rs653286, has an independent protective effect on SLE susceptibility. Bioinformatics annotations and genotype-dependent expression analyses further confirmed by our candidate SNPs rs629409 (rs631090–rs629409, r2 = 0.84), respectively (shown in Supplementary Figure S2). Among these, rs680123 and rs653286 were shown association with SLE in present study. Interestingly, the two SNPs conferred protective effects in our study. Though rs629409 was in strong LD with rs631090 and was also reported as a risk variant for SLE or subphenotypes 8, no association is observed between this variant and SLE in the present work. We also genotyped three additional SNPs, rs12033074, rs4655085, and rs672693, known as risk variants for SLE or subphenotypes 9. Although the rare deficiencies of C1R and C1S have been reported as causative genetic risks for SLE and the C1Q polymorphisms to SLE susceptibility or sub-phenotypes 8,9,12. C1QA, C1QB, and a novel human complement-related gene C1RL, with C1QA, C1QC, C1QB, CIS, C1R, and CIS polymorphisms, are negatively associated with SLE. The C1QC SNP, rs653286, has an independent protective effect on SLE susceptibility. Bioinformatics annotations and genotype-dependent expression analyses further support a potential regulatory role for each of the four C1Q variants.

Several studies have linked the common C1Q polymorphisms to SLE susceptibility or sub-phenotypes 8,9,12. Among those, Martens et al. 8 reported that C1QB rs631090 was associated with SLE susceptibility, C1QA rs292001 and C1QC rs294183 was associated with more severe disease in 103 patients with SLE and their first degree relatives of a Caucasian cohort. Though the three SNPs were not tested in present study, they were captured by our candidate SNPs rs629409 (rs631090–rs629409, r2 = 0.88), rs680123 (rs292001–rs680123, r2 = 1.0), and rs653286 (rs294183–rs653286, r2 = 0.84), respectively (shown in Supplementary Figure S2). Among these, rs680123 and rs653286 were shown association with SLE in present study. Interestingly, the two SNPs conferred protective effects in our study. Though rs629409 was in strong LD with rs631090 and was also reported as a risk for SLE 9, no association is observed between this variant and SLE in the present work. We also genotyped three additional SNPs, rs12033074, rs4655085, and rs672693, known as risk variants for SLE or subphenotypes 9. However, none of these SNPs demonstrated any association in our study. This finding may indicate that genetic heterogeneity exists among different populations. In addition, C1QA rs172378 has been reported to be associated with photosensitivity in lupus patients in African American and Hispanic populations 8. However, in a previous study that used a candidate gene approach, rs172378 was not shown to have any associations with SLE in a Han Chinese cohort 10. As the polymorphism rs172378 was not a tagSNP according to HapMap phase III CHB panel, it was not included in our study.

As the four polymorphisms reside in near 5′-UTR or introns, their functional consequence and the mecha-nism(s) underlying this genetic association remain unclear. However, by in silico functional annotations, the four variants all displayed potential regulatory activities. SNPs rs653286 and rs291985 also showed strong cis-eQTL effects on C1Q gene expression. Furthermore, several studies have suggested that functionally impaired C1q may contribute to SLE pathogenesis 13,14. Active disease in patients with SLE is often accompanied by low levels of C1q.

Table 3. Independent effects among the identified 4 SNPs in C1Q genes, stepwise logistic regression (forward conditional). B: logistic regression beta coefficients; OR (95% CI): odds ratio (95% confidence interval); m: minor allele; M: major allele.

| Models       | B     | P-value | OR (95% CI) |
|--------------|-------|---------|-------------|
| Allelic (m/M) |       |         |             |
| rs653286     | −0.159| 0.022   | 0.85 (0.75–0.98) |
| rs291985     | 0.219 | —       | —           |
| rs682658     | 0.743 | —       | —           |
| rs680123     | 0.913 | —       | —           |
| Recessive (mm/mM + MM) |       |         |             |
| rs653286     | 0.891 | —       | —           |
| rs291985     | 0.930 | —       | —           |
| rs680123     | 0.798 | —       | —           |
| rs682658     | 0.556 | —       | —           |
| Dominant (mm + mM/MM) |       |         |             |
| rs653286     | −0.291| 2.16 × 10−3 | 0.75 (0.62–0.90) |
| rs291985     | 0.564 | —       | —           |
| rs680123     | 0.444 | —       | —           |
| rs682658     | 0.623 | —       | —           |

FDR = 0.01, Z score = 4.35, and C1QC (rs653286: P = 1.92 × 10−4, FDR = 0.07, Z score = 3.73; rs291985: P = 6.58 × 10−4, FDR = 0.03, Z score = 3.99) (Fig. 1B).

Genotype-dependent expression analyses are further evidence of the ‘protective’ role of the four C1Q variants. Supported by the functional annotations, we next sought to determine whether the ‘protective’ alleles at the four SNPs had any impact on serum C1q levels. As SNPs rs680123–rs682658 and rs653286–rs291985 are almost in complete LD (r2 = 0.96/0.97 in controls/cases, respectively), we defined individuals homozygous for minor alleles of the four SNPs as “m/m” genotype and individuals homozygous for the major alleles as “M/M” genotype, respectively. As shown in Fig. 1C, in concordance with our association data and in silico functional annotations, individuals homozygous the ‘protective’ allele “m/m” at all four SNPs had significantly higher levels of serum C1q expression, compared to individuals homozygous for the major allele “M/M” (rs680123–rs682658: P = 0.0022; rs653286–rs291985: P = 0.0076) or heterozygous “M/m” (rs680123–rs682658: P = 0.0058; rs653286–rs291985: P = 0.0233).

Discussion

Although the rare deficiencies of C1Q and CIS have been reported as causative genetic risks for SLE and the common variants resided in C1Q have been linked to SLE, there are no reports concerning the polymorphisms of C1R and CIS in susceptibility to SLE. In present study, we investigated the possible association(s) of the six C1-complex genes, i.e. C1QA, C1QC, C1QB, CIS, C1R, and C1R, and a novel human complement-related gene C1RL, with SLE susceptibility or its clinical/serologic manifestations. We demonstrate that only C1Q but not C1R, C1RL, and CIS polymorphisms, are negatively associated with SLE. The C1QC SNP, rs653286, has an independent protective effect on SLE susceptibility. Bioinformatics annotations and genotype-dependent expression analyses further support a potential regulatory role for each of the four C1Q variants.

Supported by the functional annotations, we next sought to determine whether the ‘protective’ alleles at the four SNPs had any impact on serum C1q levels. As SNPs rs653286–rs291985 are almost in complete LD (r2 = 0.96/0.97 in controls/cases, respectively), we defined individuals homozygous for minor alleles of the four SNPs as “m/m” genotype and individuals homozygous for the major alleles as “M/M” genotype, respectively. As shown in Fig. 1C, in concordance with our association data and in silico functional annotations, individuals homozygous the ‘protective’ allele “m/m” at all four SNPs had significantly higher levels of serum C1q expression, compared to individuals homozygous for the major allele “M/M” (rs680123–rs682658: P = 0.0022; rs653286–rs291985: P = 0.0076) or heterozygous “M/m” (rs680123–rs682658: P = 0.0058; rs653286–rs291985: P = 0.0233).
and other classical complement components. Restoration of C1q levels by plasma transfusion in C1q-deficient lupus patients resulted in amelioration of the disease. These data are biologically consistent with our findings: the individuals homozygous for the ‘protective’ allele “m/m” at the four SNPs had significantly higher levels of serum C1q. Interestingly, the ‘protective’ T allele at rs653286 also conferred reduced risk for anti-dsDNA antibodies. Anti-dsDNA antibodies are one of the putative serologic markers for diagnosis of SLE. Levels of circulating anti-dsDNA antibodies fluctuate with disease activity in lupus patients. Furthermore, Yang et al. reported that the combination of anti-C1q and anti-dsDNA autoantibodies indicated higher renal disease activity and predicted poor renal outcome in patients with lupus nephritis. The protective effect of rs653286 T allele on this serologic manifestation may explain, at least in part, its consistent association with increased serum C1q expression in SLE patients.

In summary, to best of our knowledge, this is the first report to investigate the genetic association between the six C1-complex genes and susceptibility to SLE. Our data indicate that only C1Q polymorphisms confer susceptibility to SLE. The four novel variants all confer reduced risk against SLE and affect its transcript abundance. The C1QC SNP, rs653286, has an independent protective effect on SLE susceptibility. Future association studies in other populations will be required to further confirm our findings. Future functional characterization of these polymorphisms is warranted to fully understand their contributions to SLE pathogenesis.

Materials and Methods

Study design and study population. A two-stage case-control study was conducted. Two independent cohorts, including 384 SLE patients and 384 healthy controls (discovery cohort), as well as 507 SLE patients and 645 controls (replication cohort), were enrolled in the study. Patients with SLE satisfied 1982 revised American College of Rheumatology classification criteria for a diagnosis of SLE. Autoantibodies, including anti-nuclear antibodies (ANA), anti-double stranded DNA (dsDNA) antibodies, anti-SSA/SSB antibodies, anti-Smith (Sm)
antibodies, anti-cardiolipin antibodies (ACA), and anti-histone antibodies (AHA) were routinely measured and are detailed in the Supplementary Materials and Methods.

The SLE patients in the discovery and replication cohorts were recruited from the Department of Rheumatology at Peking University People's Hospital and People's Hospital of Xinjiang Province, respectively. The healthy controls were recruited from Health Care Centers of People's Hospital. All patients and healthy controls were Han Chinese. The baseline demographic characteristics of patients and healthy controls were summarized in Table 4.

The study was approved by Medical Ethics Committee in Peking University People's Hospital and written informed consent was obtained from all participants. All methods were performed in accordance with the relevant guidelines and regulations.

SNP selection and genotyping. A total of 22 SNPs were selected for the discovery screening, including 18 tag-SNPs spanning the \( C1QA \), \( C1QC \), \( C1QB \), \( C1S \), \( C1R \), and \( C1RL \) loci and an additional 4 SNPs known to be associated with SLE or related symptoms (Table 1). Linkage disequilibrium (LD) tag-SNPs were selected with a threshold of \( r^2 < 0.8 \) and a minor allele frequency (MAF) \( \geq 5\% \) using Haploview v4.2, according to the HapMap phase III Chinese Han Beijing (CHB) panel (http://hapmap.ncbi.nlm.nih.gov/, Figure S2). For the validation analysis, the top 4 SNPs with significant or nominal associations from the discovery cohort were further genotyped in the replication cohort.

All SNPs were genotyped using Sequenom MassArray platform (Sequenom, San Diego, California), and performed at Beijing SequeSci Co., Ltd. Briefly, DNA from study subjects was randomly assigned to the 96 well plates, and genotyping was performed blind to the status of all the samples. Genotyping was repeated in 5% of the samples for validation and quality control. The genotyping error rate was less than 0.1%. Individuals with genotyping success rates less than 90% were excluded from the analyses. Individual SNP markers with more than 10% missing genotypes were also removed from the analyses.

Quantification of human C1q in serum. A total of 342 genotyped SLE patients were quantified for serum C1q when sera were available. All the serum samples were derived from Peking University People's Hospital. Human C1q Platinum ELISA kit (BMS2099) was used to measure serum C1q, according to the manufacturer's
instructions (eBioscience, San Diego, CA). In brief, an anti-human C1q monoclonal antibody is coated and adsorbed onto microtiter plates. The absorbance was measured at 450 nm. The concentration of C1q in a serum sample was determined by matching its absorbance with the corresponding C1q concentration in the standard curve. Samples were run in duplicate and analyzed individually. All cases had genotyping data.

**Bioinformatics analysis.** The variant's potential regulatory features were annotated according to RegulomeDB (http://regulome.stanford.edu/) and rVarBase (http://rv.psych.ac.cn/). RegulomeDB is a database that annotates SNPs with known and predicted regulatory features in the non-coding regions of the human genome. Known and predicted regulatory DNA elements include sites of DNAAse hypersensitivity, binding sites for transcription factors, and promoter regions that have been biochemically characterized to regulate transcription. The sources of these data include public datasets from GEO, the ENCODE project, and published literature. rVarBase annotates a variant's regulatory features in following aspects: chromatin state of the region surrounding the variant, regulatory elements overlapped with the variant, and the variant's potential target genes. rVarBase also provides additional extended annotations for variants, including: LD-proxies of known SNPs, SNP/CNV that are overlapped with or co-localize with the queried variant, and traits (disease and expression quantitative trait) associated with the variant. The blood eQTL data were derived from Blood eQTL browser (http://genenetwork.nl/bloodeqtlbrowser/) and rVarBase.

**Statistical analyses.** The HWE test was performed for each polymorphism, using Pearson's goodness-of-fit chi-square test. The heterogeneity among study cohorts was evaluated using Review Manager 5 software (www.cc-ims.net/RevMan) and carried out with the Mantel-Haenszel method. A significant $I^2$ statistic ($I^2 > 30\%$, $P < 0.05$) indicated heterogeneity for ORs across studies. The fixed-effects model was applied in current heterogeneity analyses.

The frequencies of alleles and genotypes were compared between cases and controls, and were assessed using Pearson chi-square test and logistic regression adjusting for age and sex, respectively. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to estimate the relative risk for developing SLE or clinical/serologic manifestations. A stepwise (forward conditional) logistic regression analysis was performed to test the independence of the identified SNPs. LD and haplotype were calculated using online software SHEsis (http://analysis2.bio-x.cn/myAnalysis.php). The Mann Whitney test was applied for the analysis of serum C1q levels between two genotypic groups. Statistical analyses were conducted using SPSS 13.0 software (SPSS Inc., Chicago, IL). The false discovery rate ($FDR$, q-value) was applied for the multiple testing corrections. $P$-value $\leq 0.05$ was considered statistically significant. A q-value $< 0.10$ was considered statistically significant.

**References**

1. Sellar, G. C., Blake, D. J. & Reid, K. B. Characterization and organization of the genes encoding the A-, B- and C-chains of human complement subcomponent C1q. The complete derived amino acid sequence of human C1q. Biochem. / 274(Pt 2), 481–490 (1991).
2. Tosi, M., Journet, A., Duponchel, C., Couture-Tosi, E. & Meo, T. Human complement C1r and C1s proteins and genes: studies with molecular probes. Behring Inst. Mitt. 65–71 (1989).
3. Nakagawa, M., Yuasa, I., Irizawa, Y. & Umetsu, K. The human complement component C1R gene: the exon-intron structure and the molecular basis of allelic diversity. Ann Hum Genet 67, 207–215 (2003).
4. Ligouidistou, C., Xu, Y., Garnier, G., Circolo, A. & Volanakis, J. E. A novel human complement-related protein, C1r-like protease (C1r-LP), specifically cleaves pro-C1s. Biochem. / 387, 165–173 (2005).
5. Macedo, A. C. & Isaac, L. Systemic Lupus Erythematosus and Deficiencies of Early Components of the Complement Classical Pathway. Front Immunol 7, 55 (2016).
6. Leffler, J., Bengtsson, A. A. & Blom, A. M. The complement system in systemic lupus erythematosus: an update. Ann Rheum Dis 73, 1601–1606 (2014).
7. Raccia, D. M. et al. Homozygous single nucleotide polymorphism of the complement C1QA gene is associated with decreased levels of C1q in patients with subacute cutaneous lupus erythematosus. Lupus 12, 124–132 (2003).
8. Martens, H. A. et al. Analysis of C1q polymorphisms suggests association with systemic lupus erythematosus, serum C1q and CH50 levels and disease severity. Ann Rheum Dis 68, 715–720 (2009).
9. Namjou, B. et al. Evaluation of C1q genomic region in minority racial groups of lupus. Genes Immun 10, 517–524 (2009).
10. Cao, C. W. et al. Association study of C1qA polymorphisms with systemic lupus erythematosus in a Han population. Lupus 21, 502–507 (2012).
11. Rafiq, S., Frayling, T. M., Vys, T. J., Cunningham, G. D. S. & Eggleton, P. Assessing association of common variation in the C1q gene cluster with systemic lupus erythematosus. Clin Exp Immunol 161, 284–289 (2010).
12. Radanova, M. et al. Association of rs172378 C1q gene cluster polymorphism with lupus nephritis in Bulgarian patients. Lupus 24, 280–289 (2015).
13. Stokol, T. et al. C1q governs deposition of circulating immune complexes and leukocyte Fcgamma receptors mediate subsequent neutrophil recruitment. J Exp Med 200, 835–846 (2004).
14. Roumenina, L. T. et al. Functional complement C1q abnormality leads to impaired immune complexes and apoptotic cell clearance. J Immunol 187, 4369–4373 (2011).
15. Walport, M. J., Davies, K. A. & Botto, M. C1q and systemic lupus erythematosus. Immunobiology 199, 265–285 (1998).
16. Moosig, F. et al. Reduced expression of C1q-mRNA in monocytes from patients with systemic lupus erythematosus. Clin Exp Immunol 146, 409–416 (2006).
17. Mehta, P. et al. SLE with C1q deficiency treated with fresh frozen plasma: a 10-year experience. Rheumatology (Oxford) 49, 823–824 (2010).
18. Yang, X. et al. Combination of anti-C1q and anti-dsDNA antibodies is associated with higher renal disease activity and predicts renal prognosis of patients with lupus nephritis. Nephrol Dial Transplant 27, 3552–3559 (2012).
19. Franchin, G. et al. Anti-DNA antibodies cross-react with C1q. J Autoimmun 34–39 (2013).
20. Tan, E. M. et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 25, 1271–1277 (1982).
21. Boyle, A. P. et al. Annotation of functional variation in personal genomes using RegulomeDB. Genome Res 22, 1790–1797 (2012).
22. Guo, L., Du, Y., Qu, S. & Wang, J. rVarBase: an updated database for regulatory features of human variants. Nucleic Acids Res 44, D888–893 (2016).
23. Westra, H. J. et al. Systematic identification of trans eQTLs as putative drivers of known disease associations. Nat Genet 45, 1238–1243 (2013).
24. Gibson, G., Powell, J. E. & Marigorta, U. M. Expression quantitative trait locus analysis for translational medicine. Genome Med 7, 60 (2015).
Acknowledgements

We thank all the members and staff for recruiting patients and healthy controls and the technical assistance from Department of Rheumatology and Immunology, People's Hospital, Peking University. We wish to thank all the patients and healthy volunteers for their cooperation and for giving consent to participate in this study. This work was supported in part by National Key Basic Research Program of China (973 Program) (No. 2014CB541901), National Natural Science Foundation of China (No. 31470875, No. 31670915, No. 31711530023, No. 31270914, No. 31300721, No. 81501388 and No. 31530020), and Beijing Natural Science Foundation (No. 7162192).

Author Contributions

Guo had full access to all of the data in this study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design: Guo, Chen, Li. Acquisition of data: Guo, Gao, Wang, Zou, Du, Luo, Shi, Yang, Wu, Su, Wu. Analysis and interpretation of data: Gao, Wang, Guo. Statistical analysis: Gao, Guo. Manuscript preparation: Guo, Li.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-26380-x.

Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2018