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Association of C-reactive protein and Complement Factor H gene polymorphisms with Risk of Lupus Nephritis in a Chinese Population

Running Title: *CRP and CFH SNPs in LN*

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Abstract

Complement overactivation is a major driver of lupus nephritis (LN). Impaired interactions of C-reactive protein (CRP) with complement factor H (CFH) have been shown as a pathogenic mechanism that contributes to the overactivation of complement in LN. However, genetic variations of neither CRP nor CFH show consistent influences on the risk of LN. Here using a case control study, we examined whether genetic variations of CRP and CFH in combination can improve the risk stratification in a Chinese population. We genotyped six CRP SNPs (rs1205, rs3093062, rs2794521, rs1800947, rs3093077, and rs1130864) and three CFH SNPs (rs482934, rs1061170, and rs1061147) in 270 LN patients and 303 healthy subjects. No linkage was found among CRP and CFH SNPs, indicating lack of genetic interactions between the two genes. Moreover, CRP and CFH SNPs, neither individually nor in combination, are associated with the risk or clinical manifestations of LN. Given the unambiguous pathogenic roles of the two genes, these findings suggest that the biological effects of most genetic variations of CRP and CFH on their expressions or activities are not sufficient to influence the disease course of LN.

Keywords: Systemic lupus erythematosus, Lupus nephritis, C-reactive protein, Complement factor H, Single nucleotide polymorphism.

Introduction

As an autoimmune disease, the pathogenesis of systemic lupus erythematosus (SLE) involves clearance defect of apoptotic cells, uncontrolled activation of complement and massive production of autoantibodies [1, 2]. Previously studies have shown that 40% of SLE patients have clinical manifestations of renal dysfunction and that about 80% of SLE patients have different degrees of pathological renal damage, including lupus nephritis (LN), a common and severe complication of SLE, which is regarded as the main factor for the poor prognosis of patients [3-5].

C-reactive protein (CRP) is an acute phase reactant and a commonly used clinical marker of inflammation [6-11]. Besides, CRP could promote the elimination of damaged cells or pathogens by activating and regulating the complement system. Previous clinical studies suggest that CRP and SLE/LN have certain correlations. Firstly, CRP gene locates in the chromosome 1q23, an SLE linkage region [12, 13]. Secondly, elevation of CRP levels is generally impaired in SLE patients especially those with kidney and skin involvement [14]. Thirdly, CRP autoantibodies can be detected in a considerable number of patients, which may relate to abnormal plasma levels in SLE [15-17]. Lastly, several animal studies on lupus-prone mouse strain (NZB/NZW) revealed that human CRP could help to produce less proteinuria and prolonged survival [18, 19]. In the past decades, studies have been made to clarify the associations of CRP SNPs with SLE susceptibility, while conclusions remained inconsistent [20-27]. Moreover, no CRP SNPs studies have been performed in the Chinese population.

In addition to CRP, complement factor H (CFH), a factor that negatively regulates alternative complement pathway, is another candidate associated with SLE. Firstly, CFH deficiency has been proven to accelerate the development of LN [28], and serum CFH level was observed to be associated with clinical and pathological activities of SLE patients with LN [29]. Secondly, several families suffering from SLE were reported to possess CFH deficiency or mutations [30, 31]. Genetic variations of CFH can sometimes affect its bioactivities, in which several exotic SNPs were found to be related to various human diseases [32]. Among CFH SNPs, rs1061170 corresponds to a CFH

variant Tyr402His, which exhibits impaired CFH-CRP binding efficiency. Because CRP could inhibit the complement overactivation by recruiting CFH, so Tyr402His theoretically results in dysregulated complement activation [33].

Despite that LN is a complement-related disease, and that both CRP and CFH are involved in complement regulation, it remains unclear whether CRP and CFH SNPs directly impact the pathogenesis of LN. In this scenario, we carried out the present study, in which six CRP SNPs and three CFH SNPswere genotyped in 270 LN patients and 303 healthy controls of a Chinese cohort. Association analysis was subsequently performed for these SNPs and LN risk from the perspectives of allele, genotype, combined SNPs and haplotype. As far as we know, this is the first study to consider SNPs of CRP and CFH together when evaluating their relationship with LN risk in the Chinese population. Our data show that SNPs of both genes have no significant association with LN risk. Given the unambiguous pathogenic role of the two genes, these findings suggest that the biological effects of genetic variations of CRP and CFH on their expression or activities are not sufficient to influence the disease course of LN in the Chinese population.

Materials and Methods

Participators. Renal histopathological data of 270 patients with renal biopsy-proven lupus nephritis, diagnosed between January 2000 and July 2017 in Peking University First Hospital, were reviewed and reclassified according to the International Society of Nephrology and Renal Pathology Society (ISN/RPS) 2003 classification [34]. 303 age and gender matched healthy controls were collected. The work was approved by the Ethics Committee of Peking University First Hospital (Approval No. 2017(1333)).

DNA Preparation and SNP Genotyping. Blood samples of the 270 SLE patients and 303 healthy controls of Chinese Han individuals were collected with the approval of the Ethics Committee of Peking University First Hospital. Human genomic DNA was extracted using Qiagen Blood DNA Kit (QIAGEN China, Shanghai) according to the manufacturer's instructions. Subsequently, the CRP SNPs (rs1205, rs3093077, rs3091244, rs1130864, rs1800947, rs2794521), and CFH SNPs (rs1061170, rs482934 and rs1061147) was genotyped by SNaPshot (ABI PRISM® SNaPshot™ Multiplex Kit, ABI) with specific primers.

Evaluation of Clinical, Laboratory and Renal Pathological Indexes of LN Patients. For clinical evaluation, the disease activities of all patients were assessed by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [35, 36]. Briefly, the following items were collected and analyzed: sex, fever, malar rash, photosensitivity, oral ulcer, alopecia, arthritis, serositis, neurologic disorder, anemia, leukocytopenia, thrombocytopenia, hematuria, and leukocyturia. For laboratory assessment, the following items were collected as we previously reported [37]: complete blood count, plasma lactate dehydrogenase, liver enzymes, peripheral blood smear, urine analysis, serum creatinine, serum antinuclear antibodies (ANA), anti-double-stranded DNA (dsDNA) antibodies, anti-extractable nuclear antigen (ENA) antibodies, anti-cardiolipin antibodies and C3. For renal histopathology, all renal biopsy specimens were examined by light microscopy, direct immunofluorescence, and electron

microscopy techniques as our previous reports [38]. All samples were double-blind reviewed by two experienced pathologists based on the 2003 ISN/RPS recommendation on lupus nephritis classification [39]. The pathologists classified and scored the biopsies separately, especially for the activity indices (AI), chronicity indices (CI). Differences in scoring between pathologists were resolved by re-reviewing the biopsies and thus reaching a consensus. Renal histopathological data of 270 LN patients was classified according to the International Society of Nephrology and Renal Pathology Society (ISN/RPS) 2003 classification, which was an improved version of WHO [39].

Statistical Analysis. Hardy-Weinberg equilibrium testing was performed for all healthy controls using a chi-squared test. Distributions of genotype, allele and haplotype were compared between control and case groups using Pearson's chi-squared test or Fisher's exact test. For comparison of clinical, laboratory and pathological features of patients, student's t-test and one way ANOVA analysis of variance were used. A p-value less than 0.05 was considered to be significant. Pairwise Linkage Disequilibrium (LD) and haplotype analysis were both conducted using the SHEsis platform [40]. SNP pairs with D' value great than 0.8 and r^2 value great than 0.33 were considered to be in significant LD. Meta-analysis was performed by using Stata 15 software. Relative risks of SLE/LN were estimated according to odds ratios (ORs) with 95% confidence intervals (CIs). The inconsistency index I^2 was calculated to quantify the heterogeneity: if $I^2<50\%$, suggesting that the degree of heterogeneity was low, and the meta-analysis was performed using the fixed effect model (FE); otherwise, the random effects model (RE) was used. $p>0.05$ means no statistical significance.

Results

Association of CRP SNPs and haplotypes with LN risk

Six CRP SNPs (rs1205, rs3093077, rs3091244, rs1130864, rs1800947 and rs2794521) were genotyped in 270 LN patients and 303 healthy controls (Figure 1). Chi-squared test showed that genotype frequency distributions of CRP SNPs in all healthy controls were all in Hardy-Weinberg equilibrium (Table S1). In subsequent comparisons of case and control groups (Table 1), none of the alleles or genotypes was observed to be significantly associated with the SLE risk ($p > 0.05$). In further analysis, we examined the effects of CRP SNP combinations, and again failed to observe any significant difference in genotype distributions of LN patients and healthy controls (Table S2).

Subsequently, pairwise linkage disequilibrium (LD) analysis was conducted for CRP SNPs in healthy controls. Of all SNP pairs, rs3091244/rs3093077 and rs3091244/rs1205 pairs were found to be in significant LD (Table 2). Considering that SNP haplotype may provide more informative details, CRP haplotypes were thus included for further investigation. Given the acceptable number, we included all 6 CRP SNPs in the haplotype analysis. Finally, 6 CRP haplotypes were observed at frequencies greater than 3.0% in both healthy controls and LN patients. However, no significant differences were found in the distribution frequencies of those haplotypes between the two groups (Table 3).

To further confirm whether these CRP SNPs are indeed unrelated to LN in the present population, we thus further checked the association of these SNPs with clinical, laboratory and pathological features of all patients (Tables S3-6). In line with the conclusions above, most indexes exhibited no significant differences between genotypes of these SNPs. Notably, WHO classification for all LN patients was performed and association between pathological subclass and SNPs were further analyzed, whereas no significant differences were observed. However, several items showed differences, which might imply potential relevance of these SNPs with LN to

some extent, suggesting that conclusion should cautiously draw.

Association of CFH SNPs and haplotypes with LN risk

Similarly, we subsequently genotyped 3 CFH SNPs, namely rs1061170, rs482934 and rs1061147, in 270 LN patients and 303 healthy controls (Figure 1). After Hardy-Weinberg equilibrium was checked for all genotype frequencies of controls (Table S7), association of CFH polymorphism with LN risk were examined. Of note, no significant enrichment or depletion of allele and genotype distribution has been observed in LN patients (Table 4). In further exploration, pairwise LD for those CFH SNPs was examined as before. Dramatically, all three SNP pairs were found to be in strong LD (Table 5). Based on this, haplotype analysis was conducted, in which two CFH haplotypes were observed at frequencies greater than 3.0% in both healthy controls and LN patients. However, no significant associations were found between those two haplotypes and LN risk (Table 6).

In further analysis, we checked the association of rs1061170 with clinical, laboratory and pathological features of all LN patients (Table S8). Similar to CRP SNPs, except for a few items, most indexes exhibited no significant differences between genotypes of these SNPs.

Association of CRP-CFH SNP combinations with LN risk

Given the key roles of complement overactivation in SLE pathogenesis and the capacity of CRP to inhibit this process via interaction with CFH, we next asked whether any potential associations could be found in SNP combinations of CRP and CFH. Specifically, CFH SNP rs1061170, which corresponds to a variant Tyr402His with impaired capacity to bind CRP [32,41,42], was combined with 6 CRP SNPs and evaluated individually. However, we failed to observe any significant associations in all SNP combinations included (Table 7). Besides, cross pairs of the CRP and CFH SNPs were further included for pairwise LD evaluation, in which no significant LD was observed (Table S9).

Discussion

In the past decades, studies have been focused on revealing the associations of CRP/CFH genetic variations with SLE/LN[21-28, 33, 43, 44]. However, those studies were mainly based on the European or American populations, and often gained inconsistent conclusions. Moreover, although CRP/CFH interaction theoretically plays a role in LN pathogenesis, they have not been considered together when evaluating the association of their genetic variations with the LN risk.

In this study, we enrolled 6 CRP SNPs and 3 CFH SNPs of a Chinese cohort, and studied their relationship with LN risk, which has not yet been systematically reported. Our study revealed that there were no significant associations between these SNPs and LN susceptibility in the Chinese population. All patients in this study were selected from the same center and their diagnosis were all confirmed by renal biopsy. Moreover, the complete clinical, laboratory and pathological indexes were also included to test the results. Therefore, although no statistical associations were observed in our study, valid and useful information could still be revealed.

In addition, these negative results are generally consistent with the conclusions of previous researches to a large extent. Among the 6 CRP SNPs, rs1800947 [21, 25-27] and rs2794521 [24, 26] were included in several studies, which were repeatedly reported to be unrelated to SLE in various populations, consistent with our results. For rs1205 [21, 22, 25-27], rs3091244 [21, 22, 25, 26] and rs1130864 [21-23], conclusions remained inconsistent among these studies, which might rationalize the existence of our negative results to some extent. For the CFH SNPs, Zhao *et al* evaluated an Asian group involving 200 Chinese SLE cases and found no significant association between rs1061147 and SLE (without LN) [33]. Tan *et al* enrolled 334 LN patients, 269 SLE patients without LN and 350 healthy controls from China, but failed to observe any significant differences in allele and genotype frequencies of rs1061170 among groups [45]. Both conclusions were consistent with our present findings.

Given that interaction of CRP and CFH would theoretically help to regulate

complement and therefore play roles in SLE/LN pathogenesis, we combined 6 CRP SNPs individually with CFH SNP rs1061170, which corresponds to a CFH variant with impaired capacity in CRP binding. To our knowledge, this is the first study to combine these two genes when performing a correlation analysis with LN risk. However, we still failed to observe any significant associations from this perspective.

Overall, our results suggest that CRP and CFH genetic variation and interaction do not affect the occurrence of LN at the gene level in a Chinese population. In future studies, multiple-center sampling is needed to expand the study scale, whereas SLE patients without LN from other rheumatism departments should also be included. Moreover, more SNPs should be examined for these two genes, while other molecules along the pathogenesis pathway of CRP and CFH should be involved for a joint analysis.

Conclusion

In spite of the unambiguous pathogenic roles of CRP and CFH in LN, our present study involving a Chinese population has failed to reveal any significant associations of their genetic variations with LN risk. These findings suggest that most genetic variations of CRP and CFH might possess limited biological effects on their expressions or activities, and are thus not sufficient to influence the disease course of LN. Overall, we concluded that genetic variations of CRP and CFH could not be used to improve the risk stratification of LN in Chinese population.

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