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*Original Article*

# High-sensitivity C-reactive protein (hs-CRP) gene polymorphisms (rs1205 and rs2794520) associated with hs-CRP serum levels in periodontitis-associated chronic kidney disease patients from Uyghur adults

Li Zhang[1], Suhua Li[1], Jing Li[1], Xiaohong Sang[1], Rasheeda Zakir[1], Jian Liu[1], Zehui Li[2]

*Departments of [1]Nephrology, [2]Stomatology, The First Affiliated Hospital of Xinjiang Medical University, Urumqi 830054, China*

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**Abstract:** Chronic inflammation may play a role in chronic kidney disease (CKD) and chronic periodontitis (CP) progression. *CRP* gene polymorphisms are associated with serum C-reactive protein (CRP) concentrations. It is unknown if *CRP* polymorphisms are associated with CKD and CP progression. 181 CKD patients with 161 PC and 197 Non-CKD subjects with 145 CP were included in this study. Genotyping was conducted using SNaPshot assays. The significance of differences in allele and genotype frequencies between groups was assessed by the $\chi^2$ test. First, CKD patients had high serum hs-CRP compared with healthy control group. Moreover, CKD patients accompanied with CP significantly higher hs-CRP serum levels than CKD a single cause. In addition, the association was found between the studied *CRP* polymorphisms (rs1205 and rs2794520) and susceptibility to CKD or CP diseases. CRP levels were the highest in CP patients accompanied with CKD carrying CT genotypes and the lowest in PC subjects with homozygotes (CC or TT) in rs1205 and rs2794520. It was concluded that *CRP* polymorphisms (rs1205 and rs2794520) were associated with CKD and CP diseases in a Uyghur population.

**Keywords:** Chronic kidney disease, periodontitis, hs-CRP, gene polymorphisms

## Introduction

Chronic periodontitis is an inflammatory disease and is a predominantly Gram-negative infection of the oral cavity that seriously endangers the tooth structure, including destruction of periodontal ligament and alveolar bone and eventual tooth loss [1, 2]. Poor oral health outcomes have been observed in national minority, and people with low socioeconomic status (SES) are more likely to have periodontitis compared with people with high SES [3, 4]. Additionally, periodontitis has aroused great concern for its association with diabetes mellitus [3, 5] and hypertension [6]. A role of periodontitis in chronic kidney disease (CKD) has also been reported, the most likely reason is that chronic inflammatory burden has been suggested as a risk factor for CKD [7, 8]. CKD is defined as either proteinuria or an estimated glomerular filtration rate (eGFR) < 60 mL/min/$m^2$ for more than 3 months [9]. In 2005, initial (2,276 individuals) and severe (947 individuals) periodontal diseases are associated with a eGFR less than 60 mL/min/1.73 $m^2$ as compared to healthy controls [10]. These evidences support the contribution of periodontitis to CKD [9, 10].

High-sensitivity C-reactive protein (hs-CRP), which is a robust biomarker of underlying systemic inflammation, is an acute-phase protein and is generated in the liver by IL-6 and TNF-α [11, 12]. Emerging evidence suggests that periodontitis is an independent risk factor for elevated serum hs-CRP levels in CKD patients [13]. Severe chronic periodontitis is associated with increased serum hs-CRP concentration and may increase the risk of death in patients after kidney transplantation [14]. Therefore, understanding periodontitis-induced CRP release is critically important in facilitating the cor-

rect interpretation of elevated CRP concentrations in CKD patients.

Although CRP may have an important role in the pathogenesis and prediction of CKD [15], the gene polymorphisms influencing the CRP concentrations during the development and progression in periodontitis-associated CKD patients are incompletely understood. Previous studies suggest that hs-CRP levels correlate with its gene polymorphisms in various diseases [16-18]. rs3093058_T is associated with higher CRP concentrations, oppositely, rs1205_A and rs2808630_G are associated with lower CRP concentrations [19]. Recently, several large population-based studies show that CRP polymorphism is associated with higher risk of CKD progression [20, 21]. In 55 consecutive patients suffering from periodontitis, individuals homozygous for the +1444T allele show higher CRP concentrations (day 1, 21.10 ± 4.81 mg/L and day 7, 4.89 ± 0.74 mg/L) compared with C-allele carriers (day 1, 12.37 ± 1.61 mg/L and day 7, 3.08 ± 2.00 mg/L) after an intensive course of periodontal treatment [22]. However, hs-CRP gene polymorphisms have not been clearly delineated in periodontitis-associated CKD patients.

The objective of this study was carried out to further investigate the association between periodontitis and CKD in Uyghur adults from Xinjiang Hetian region, a following-up study from 2007 to 2013. Additionally, we examined whether periodontitis-induced up-regulation of serum hs-CRP concentrations was a risk factor for CKD progression. Furthermore, we hypothesized that hs-CRP gene polymorphisms correlated with periodontitis-associated chronic kidney disease patients.

## Materials and methods

### *Study subjects*

The survey is a cross-sectional study belonging to a sampling survey in which dental evaluations were performed in 1650 subjects at 2007 and follow-up survey at 2013 (Supplementary Tables 1 and 2). The study protocol was approved by the Ethics Committee of the Xinjiang Medical University (Urumqi, China), and written informed consent was obtained from all participants. The questionnaire was designed by an experienced research team (Institute of Kidney Diseases, Peking University, Beijing, China). Following professional training, a group of Uyghur medical students fluent in the Chinese and Uyghur languages served as investigators and assisted in filling out the questionnaires. Contents of questionnaire include demographic information (gender, age), medical history (hypertension, diabetes, coronary heart disease, chronic renal disease, chronic respiratory diseases, etc.) behavior and personal habits (e.g., smoking), and family history. Results from the subsequent physical and blood tests were also included. Moreover, 5 mL fasting blood was collected from each participant. The samples were centrifuged, and the serum were placed in sodium fluoride tubes and kept frozen in individual containers. Serum was stored at -80°C in frozen containers in liquid nitrogen tanks.

### *Diagnostic criteria of CP*

Chronic periodontitis was categorized into the following categories. Mild periodontitis: gum inflammation and bleeding on probing, periodontal pocket depth ≤ 4 mm, and attachment loss of 1-2 mm. Moderate periodontitis: gingival inflammation and bleeding on probing, presence of pus, periodontal pocket depth ≤ 6 mm, attachment loss of 3-4 mm, and possible presence of slight loose teeth. Severe periodontitis: obvious inflammation or occurrence of periodontal abscess, periodontal pocket depth > 6 mm, attachment loss ≥ 5 mm, and more than one loose tooth [23].

### *Diagnostic criteria of CKD*

The diagnosis and stage of the CKD were established as recommended by US National Kidney Foundation: kidney damage (such as albuminuria or glomeruar hematuria or structural abnormalities as noted on imaging studies) or a GFR less than 60 mL/min per 1.73 $m^2$ for more than 3 months [24]. Glomerular filtration rate (GFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI): eGFR-CKD-EPI (mL/min·1.73 $m^2)_{female}$ = Scr ≤ 0.7 mg/dl: $144 \times (0.993)^{Age} \times (Scr/0.7)^{-0.329}$; Scr > 0.7 mg/dl: $144 \times (0.993)^{Age} \times (Scr/0.7)^{-1.209}$; eGFR-CKD-EPI (mL/min·1.73 $m^2)_{male}$ = Scr ≤ 0.9 mg/dl: $141 \times (0.993)^{Age} \times (Scr/0.9)^{-0.411}$; Scr > 0.9 mg/dl: $141 \times (0.993)^{Age} \times (Scr/0.9)^{-1.209}$ [25].

**Table 1.** General characteristics of the CKD and non-CKD

| Characteristics | CKD (*n* = 181) | Non-CKD (*n* = 197) | $\chi^2$ | *P*-value |
|---|---|---|---|---|
| Gender (M/F) | 98/83 | 109/88 | 2.338 | 0.126 |
| Age (Years) | 49.89 ± 12.40 | 43.94 ± 12.39 | 4.588 | 0.000 |
| Height (cm) | 162.00 ± 7.69 | 161.00 ± 7.43 | 1.914 | 0.166 |
| Weight (kg) | 68.00 ± 18.66 | 63.00 ± 10.05 | 33.29 | 0.000 |
| Fasting blood glucose (FBG, mmol/L) | 4.60 ± 1.48 | 4.30 ± 0.52 | 4.366 | 0.000 |
| Blood uric acid (BUA, μmol/L) | 259.95 ± 65.09 | 207.76 ± 65.03 | 8.255 | 0.000 |
| Triglyceride (mmol/L) | 1.21 ± 0.88 | 0.95 ± 0.78 | 4.034 | 0.000 |
| Total cholesterol (mmol/L) | 4.90 ± 1.09 | 4.15 ± 0.95 | 5.896 | 0.000 |
| SBP (mmHg) | 120.00 ± 25.10 | 116.67 ± 19.55 | 7.626 | 0.006 |
| DBP (mmHg) | 80.00 ± 13.50 | 78.33 ± 12.79 | 8.266 | 0.004 |
| Chronic periodontitis (CP, %) | 161 (89.0%) | 145 (73.6%) | 14.407 | 0.000 |
| BMI (kg/m$^2$) | 25.96 ± 7.04 | 24.75 ± 3.28 | 4.827 | 0.000 |
| eGFR (mL/min/1.73 m$^2$) | 85.90 ± 18.9 | 131.37 ± 14.78 | 215.775 | 0.000 |
| hs-CRP (mg/L) | 2.00 ± 0.27 | 1.48 ± 0.22 | 14.612 | 0.000 |

**Table 2.** Comparison the inflammatory markers and medical characteristics with periodontitis and non-periodontitis patients in CKD group

| Characteristics | CP (*n* = 161) | Non-CP (*n* = 20) | $\chi^2$ | *P*-value |
|---|---|---|---|---|
| hs-CRP (mg/L) | 2.14 ± 0.48 | 1.95 ± 0.19 | 2.946 | 0.003 |
| IL-6 (pg/mL) | 62.08 ± 9.66 | 60.50 ± 8.03 | 1.036 | 0.300 |
| TNF-α (pg/mL) | 336.35 ± 41.95 | 279.16 ± 34.03 | 3.860 | 0.000 |
| TGF-β (pg/mL) | 2571.67 ± 286.22 | 2488.05 ± 308.37 | 0.876 | 0.381 |
| SBP (mmHg) | 120.45 ± 25.10 | 112.84 ± 19.55 | 1.364 | 0.173 |
| DBP (mmHg) | 80.12 ± 13.50 | 76.95.0 ± 12.79 | 0.811 | 0.417 |
| BMI (kg/m$^2$) | 26.23 ± 7.04 | 25.00 ± 3.28 | 1.693 | 0.090 |

*Cytokines measurement*

Hs-CRP level was measured with immunoturbidimetry. IL-6, TNF-α and TGF-β level were measured with radioimmunoassay (BioSino, Beijing, China).

*Genotyping of CRP polymorphism*

DNA was extracted from whole blood or a buffy coat using the UNIQ-10 Column Clinical Sample DNA Isolation Kit (cat. no: B511341, Sangon Biotech, Shanghai, China) according to the manufacturer's protocol and stored at -80°C until analysis. The SNPs were retrieved from the HapMap database (http://hapmap.ncbi.nlm.nih.gov.) with correlation coefficient $r^2$ > 0.8 and MAF > 15%. We performed genotyping of the SNPs using genomic DNA extracted from 500 μl peripheral blood of the subjects. The genotyping was carried out by SNaPshot multiple single-base extension (SBE) reaction using purified PCR products amplified from genomic regions containing target SNPs. Genotype data were derived from the analysis on SBE products by an ABI PRISM 3130XL DNA sequencer (Applied Biosystems) and GeneMapper 4.0 (Applied Biosystems). The genotyping was performed at least in duplicates to avoid ambiguous genotype data.

*Statistical analysis*

All data input is in duplicate using EpiData3.1 software with the logic and consistency checks. Statistical analysis was performed using the statistical software SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Data are expressed as the mean ± standard deviation (SD). Chi-square ($\chi^2$) test was used to compare the percentage or count data. A logistic regression analysis was performed, using the forward Wald method with $\alpha = 0.05$. $P < 0.05$ was considered to indicate a statistically significant difference.

## Results

*Clinical characteristics of the CKD and non-CKD patients*

The Uyghur nationality in the Karakax County accounts for 98.6% of the total population of 401,000. CKD patients (n = 181) and healthy control (n = 197) were randomly selected from

**Table 3.** Inflammatory markers and medical characteristics by periodontitis and non-periodontitis in non-CKD group

| Characteristics | CP (*n* = 145) | Non-CP (*n* = 52) | $\chi^2$ | *P* |
|---|---|---|---|---|
| hs-CRP (mg/L) | 1.46 ± 3.63 | 1.44 ± 2.07 | 0.747 | 0.455 |
| IL-6 (pg/mL) | 48.58 ± 8.14 | 48.34 ± 11.44 | 0.183 | 0.855 |
| TNF-α (pg/mL) | 257.22 ± 52.57 | 250.74 ± 19.72 | 0.004 | 0.997 |
| TGF-β (pg/mL) | 1907.86 ± 398.74 | 1818.14 ± 406.97 | 1.517 | 0.129 |
| SBP (mmHg) | 119.67 ± 23.21 | 109.61 ± 15.30 | 4.562 | 0.000 |
| DBP (mmHg) | 80.17 ± 12.93 | 69.51 ± 10.67 | 4.812 | 0.000 |
| BMI (kg/m$^2$) | 24.15 ± 3.95 | 24.12 ± 3.77 | 1.853 | 0.064 |

**Table 4.** Inflammatory markers and medical characteristics in the different course of the periodontitis patients

| Groups | Non | Mild/Moderate | Severe |
|---|---|---|---|
| hs-CRP (mg/L) | 1.56 ± 0.21 | 1.74[a] ± 0.36 | 1.86[b,c] ± 0.37 |
| IL-6 (pg/mL) | 65.02 ± 8.14 | 62.14 ± 12.61 | 65.48 ± 10.48 |
| TNF-α (pg/mL) | 269.92 ± 19.72 | 290.93 ± 64.56 | 284.49 ± 43.53 |
| TGF-β (pg/mL) | 2362 ± 406.97 | 2391.35 ± 396.06 | 2471.91 ± 409.18 |
| SBP (mmHg) | 25.42 ± 3.77 | 26.01 ± 3.58 | 25.70 ± 4.28 |
| DBP (mmHg) | 108.6 ± 15.30 | 113.77 ± 19.67 | 121.89 ± 25.85 |
| BMI (kg/m$^2$) | 72.17 ± 10.67 | 76.90 ± 13.00 | 80.27 ± 12.62 |
| eGFR (mL/min/1.73 m$^2$) | 138.71 ± 12.71 | 126.43[a] ± 15.00 | 114.39[b,c] ± 14.83 |

a: non-CP group *vs*. Mild/Moderate PC group, $P < 0.05$; b: non-CP group *vs*. Severe PC group, $P < 0.05$; c: Mild/Moderate PC group *vs*. Severe PC group, $P < 0.05$.

an epidemiological survey with periodontitis research, which contained 1650 subjects. 70, 122 and 22 subjects failed to obtain questionnaires, take the oral examination and blood and urine samples, respectively. 31 cases lacked a full set of teeth. Thus, we received 1415 valid and complete questionnaires. Research participants were all 20 years old and older. The clinical, biochemical and periodontal characteristics of CKD patients and healthy subjects are shown in **Table 1**. The sex ratio and height had no obvious difference in the two groups. Additionally, patients with CKD were more likely to be older, hypertension, periodontitis, hyperlipidemia and higher body weight and BMI. These CKD patients also had higher BUA and eGFR than those of healthy control. Although, the FBG in CKD group was significantly increased compared with healthy control group, the FBG value in the two groups was within the normal range. Intriguingly, CKD patients (2.00 ± 0.27) had high serum hs-CRP compared with healthy control group (1.48 ± 0.22).

*Comparison clinical characteristics and inflammatory markers in CKD patients with or without CP*

Increased values of hs-CRP were found in CKD patients with CP (2.14 ± 0.48) as compared to CKD patients without CP ($P = 0.003$). Moreover, TNF-α levels were statistically different between CKD patients with CP and without CP ($P = 0.000$). IL-6, TGF-β, SBP, DBP and BMI were compared between the groups both showing no significant differences ($P = 0.300$, 0.381, 0.173, 0.417 and 0.090, respectively) (**Table 2**).

*Comparison clinical characteristics and inflammatory markers in non-CKD group with or without CP*

Unexpectedly, no statistically significant of hs-CRP levels was found between CP and non-CP subjects in non-CKD group ($P = 0.455$). The two groups also did not differ significantly in TNF-α, TGF-β, IL-6 and BMI ($P = 0.997$, 0.129, 0.855 and 0.064, respectively). Furthermore, the SDP

**Table 5.** Classification valuation of independent variables

| Variable number | Number |
|---|---|
| Y (CKD) | No = 0; Yes = 1 |
| X1 (CP) | No = 0; Yes = 1 |
| X2 (Hypertension) | No = 0; Yes = 1 |
| X3 (BUA) | Male ≤ 457 umo/L or Female ≤ 357 umo/L = 0; Male > 457 umol/L or Female > 357 umol/L = 1 |
| X4 (Triglyceride) | < 1.7 mmol/L= 0; ≥ 1.7 mmol/L = 1 |
| X5 (Total cholesterol) | < 5.18 mmol/L = 0; ≥ 5.18 mmol/L = 1 |
| X6 (FBG) | < 7 mmol/L = 0; ≥ 7 mmol/L = 1 |
| X7 (BMI) | < 24 Kg/m$^2$ = 0; ≥ 24 Kg/m$^2$ = 1 |

**Table 6.** Univariate logistic regression analysis of CKD risk factors

| | B | S.E. | Wald | *P* | *OR* | 95% CI |
|---|---|---|---|---|---|---|
| CP | 1.658 | 0.339 | 23.963 | 0.000 | 5.250 | 2.703-10.198 |
| Hypertension | 1.728 | 0.246 | 43.398 | 0.000 | 5.632 | 3.478-9.120 |
| Triglyceride | 0.889 | 0.253 | 12.319 | 0.000 | 2.433 | 1.481-3.997 |
| Total cholesterol | 1.030 | 0.254 | 16.491 | 0.000 | 2.800 | 1.703-4.602 |
| BMI | 1.039 | 0.236 | 19.453 | 0.000 | 2.828 | 1.782-4.488 |

**Table 7.** Multivariate logistic regression analysis of CKD risk factors

| | B | S.E | Wald | P | *OR* | 95% C.I |
|---|---|---|---|---|---|---|
| CP | 1.363 | 0.400 | 11.605 | 0.001 | 3.907 | 1.784-8.557 |
| Hypertension | 1.421 | 0.628 | 24.841 | 0.000 | 4.140 | 2.368-7.237 |
| Triglyceride | 0.530 | 0.297 | 3.193 | 0.074 | 1.699 | 0.950-3.040 |
| Total cholesterol | 0.491 | 0.297 | 2.722 | 0.099 | 1.633 | 0.912-2.925 |
| BMI | 0.853 | 0.275 | 9.601 | 0.002 | 2.347 | 1.368-4.025 |

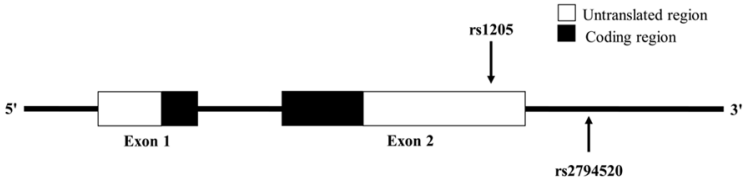


**Figure 1.** Location of the investigated polymorphisms on the CRP gene on chromosome 1.

and DBP in CP patients were significantly higher than in individuals without CP (*P* = 0.000 and 0.000, respectively) (**Table 3**).

*Clinical characteristics and inflammatory markers in the different course of the periodontitis patients*

Clinical characteristics and inflammatory markers, including the IL-6, TNF-α, TGF-β, SBP, DBP and BMI, were no significant difference among the three groups (*P* > 0.05). CRP levels were the highest in severe-CP group and the lowest in non-CP group, and there was significant difference among the three groups respectively (*P* < 0.05). Furthermore, the comparison of eGFR in the three groups respectively was remarkable difference, the highest in non-CP group and the lowest in severe-CP group (**Table 4**).

*Univariate and multivariate logistic regression analysis of CKD-related risk factors*

The further logistic regression analysis was performed. The variables included CP, hypertension, BUA, triglyceride, total cholesterol, FBG and BMI. Classification valuation of independent variables was shown in **Table 5**. These results demonstrated that CP, hypertension, triglyceride, total cholesterol and BMI were the major risk factors to CKD in our study. Without considering the other risk factors, the risk of CP patients with CKD was 5.3-fold higher than non-CP subjects (**Table 6**). The multivariate logistic regression analysis was performed using the forward Wald method with the thresholds of 0.05 for lead into and reject. The findings indicated that CP, hypertension and BMI may serve as independent risk factors for CKD. The risk of CP patients with CKD was 3.9-fold higher than non-CP subjects (**Table 7**).

*Comparison genotype/alleles between CKD and healthy control with or without periodontitis*

The locations of rs1205 and rs2794520 on the CRP gene were shown in **Figure 1**. The frequencies of genotypes/alleles in rs1205 and

**Table 8.** Comparison genotype/alleles between CKD and healthy control with or without periodontitis

| SNP | Genotype/ Alleles | CP | | $\chi^2$ | *p*-Value | Non-CP | | $\chi^2$ | *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| | | CKD (n = 161) | Control (n = 145) | | | CKD (n = 20) | Control (n = 52) | | |
| rs1205 | CC+TT | 56 (34.8%) | 78 (53.8%) | 11.219 | 0.001 | 10 (50%) | 25 (49.2%) | 0.021 | 0.884 |
| | CT | 105 (65.2%) | 67 (46.2%) | | | 10 (50%) | 27 (51.8%) | | |
| | C | 173 (53.7%) | 147 (50.7%) | 0.564 | 0.453 | 28 (70.0%) | 49 (47.1%) | 6.081 | 0.041 |
| | T | 149 (46.3%) | 143 (49.3%) | | | 12 (30.0%) | 55 (52.9%) | | |
| rs2794520 | CC+TT | 56 (34.8%) | 80 (55.2%) | 12.84 | 0.000 | 10 (50.0%) | 25 (49.2%) | 0.021 | 0.884 |
| | CT | 105 (65.2%) | 65 (44.8%) | | | 10 (50.0%) | 27 (51.8%) | | |
| | C | 173 (53.7%) | 145 (50%) | 0.849 | 0.357 | 28 (70.0%) | 49 (47.1%) | 6.081 | 0.014 |
| | T | 149 (46.3%) | 145 (50%) | | | 12 (30.0%) | 55 (52.9%) | | |

rs2794520 could be observed in **Table 8**. The genotype distribution of CC+TT and CT was 56 (34.8%) and 105 (65.2%) in CKD combined with CP group, respectively. In CP group, the following genotype distribution was obtained CC+TT (78, 53.8%) and CT (67, 46.2%). Intriguingly, CC+TT genotypes were signifiantly decreased in CKD combined with CP group as compared to CP group. In contrast to that, CT genotype was signifiantly higher in CKD combined with CP patients than those of patients in CP group. The alleles of C and T were 173 (53.7%) and 149 (46.3%) in CKD combined with CP group. In CP group, the alleles of C and T were 147 (50.7) and 143 (49.3%). Both allele C and T were no obvious difference between CKD combined with CP group and CP group. In non-CP group, both CC+TT and CT had no significant change (P = 0.101) between CKD group and healthy control. The alleles of C and T had obvious difference in CKD group and healthy control from non-CP subjects. Moreover, three genotypes, CC, TT and CT, were checked out in rs2794520 site. Genotypes CC+TT were significantly decreased and CT was significantly increased in CKD combined with CP group as compared to CP group. However, both CC+TT and CT had no obvious change in CKD patients without CP and healthy control. In CP patients, the alleles of C and T were no significantly different with or without CKD. However, the alleles of C and T had decreased in CKD patients without CP compared with healthy control (**Table 8**). As shown in **Figure 2**, the findings demonstrated a significant association between hs-CRP polymorphism and hs-CRP serum levels. CRP levels were the highest in CP patients accompanied with CKD carrying CT genotypes and the lowest in PC subjects with homozygotes (CC or TT) in rs1205 (**Figure 2A**) and rs2794520 (**Figure 2B**). In control group, CT genotypes mirrors higher hs-CRP serum levels than the subjects with homozygotes in rs1205 (**Figure 2C**) and rs2794520 (**Figure 2D**), however, the hs-CRP serum levels in CKD patients had no obvious difference between heterozygotes (CT genotypes) and homozygotes in rs1205 (**Figure 2C**) and rs2794520 (**Figure 2D**). Furthermore, in the CKD patients combined with CP, heterozygotes in rs1205 (**Figure 2E**) and rs2794520 (**Figure 2F**) associated with increased hs-CRP serum levels compared with homozygotes genotypes.

## Discussion

The primary goal of this study was to evaluate the serum hs-CRP levels in CKD patients with CP. The results demonstrated that the serum CRP levels in CKD patients with CP were significantly increased as compared to those of CKD patients without CP. However, the serum hs-CRP levels had no obvious difference between CP patients and non-CP subjects, all of them selected from non-CKD individuals. CP as an independent risk factor associated with CKD progression, and the possible reason for the result was that periodontitis made a significant contribution to the systemic inflammatory burden as measured by up-regulating serum hs-CRP levels. More importantly, we showed an association between the CRP SNP rs1205 and rs2794520 and CP-associated CKD in Uyghur adults. This result is concordant with the previous study in which CRP SNPs were associated with CKD progression [20, 21, 26].

CRP is a biomarker of inflammation with predictive value for cardiac events [27]. Several stud-

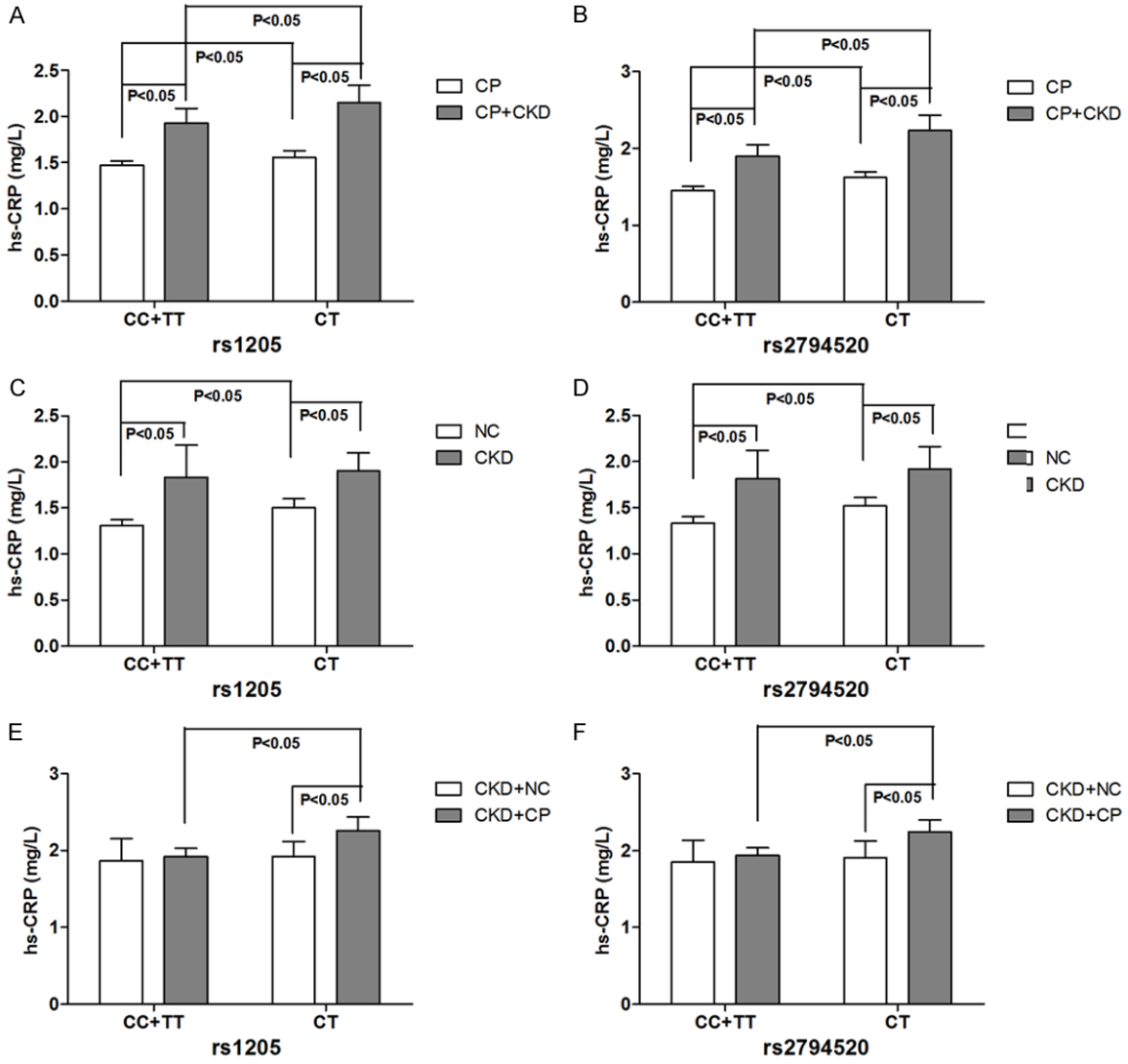


**Figure 2.** The association of hs-CRP serum levels and *CRP* polymorphisms. The association of hs-CRP serum levels and *CRP* polymorphisms rs1205 (A) and rs2794520 (B) in CP patients and CP patients accompanied with CKD. The association of hs-CRP serum levels and *CRP* polymorphisms rs1205 (C) and rs2794520 (D) in healthy controls and CKD patients. The association of hs-CRP serum levels and *CRP* polymorphisms rs1205 (E) and rs2794520 (F) in CKD patients and CKD patients accompanied with CP.

ies indicate an association between plasma hs-CRP levels and CP and/or CKD [13, 28, 29]. Moreover, severe CP is associated with increased serum hs-CRP concentration in patients after kidney transplantation [14]. Patients with high hs-CRP have significantly lower glomerular filtration rates and albumin, and high hs-CRP is independently associated with renal functional decline after partial nephrectomy [30]. Previous study has shown that elevated serum levels of CRP are present in individuals with periodontal disease [31]. There is epidemiological evidence supporting the role of periodontitis as a predictor of acute phase response, as measured by serum hs-CRP levels in the general population [32]. However, there are few research literatures about focusing on the risk relationship with CP in the CKD population by monitoring serum sh-CRP levels. In our study, the results demonstrated that elevated CP prevalence in individuals with CKD in Uyghur adults was confirmed, and the serum hs-CRP levels were significantly increased in CKD patients with CP as compared to CKD patients without CP. These findings suggest that CP may be an independent risk factor associated with

CKD progression. Kamil et al suggest that non-surgical periodontal therapy results in a significant reduction in the serum hs-CRP level [33]. These findings indicate that periodontal problems can affect the hs-CRP level and enhance it. However, the mechanisms underlying the association between plasma CRP and CP and/or CKD, in particular the role of genetic factors, are still unclear.

CRP gene rs1205 (C > T) polymorphism has been associated with lower plasma CRP concentrations in cohorts of healthy and atherosclerotic patients [27]. However, in three hundred consecutive Caucasian patients diagnosed with AS, the rs1205 T allele were characterized by elevated serum CRP levels compared with major homozygotes [27], suggesting existence of a disease-specific molecular regulatory of rs1205. CRP gene variant rs2808630 is associated with higher risk of CKD progression [20, 21]. However, there is no association between CRP gene variant rs1205 and CKD progression in a longitudinal cohort of African American with hypertensive kidney disease [21]. Unexpectedly, participants in the AASK trial has a lower MAF for rs1205 (*A* allele: 18 *versus* 21%, $P = 0.03$) compared with the African Americans from the NHANES III with normal kidney function [20]. In our work, the rs1205 polymorphism heterozygotes/CT frequency of 65.2% was significantly increased in CP combined with CKD patients compared with CP patients without CKD. rs1205 is located in the 3'-untranslated region (3'-UTR) of CRP and may act as a sort of molecular switch of CRP synthesis [27]. Polymorphisms of this region are known for their potential to affect mRNA stability and thus expression of gene harboring them [34], which can potentially provide a functional support for our findings. rs2794520 is located in the downstream of rs1205, which may potentially influence CRP expression in the same way of rs1205 [27]. Carriage of (minor) T allele of rs2794520 is associated with significant increase in CRP levels and is also associated with recurrent pregnancy loss [35]. Similarly, the study of Taichung Community Health Study for Elders (TCHS-E) has consistently shown that rs2794520 is associated with higher levels of CRP and lower handgrip strength in community-dwelling elders in Taiwan [36]. However, in the case-control study, there is no contribution of the CRP rs2794520 polymorphism to the risk of coronary artery disease [37], and rs2794520 genotype contributes limitedly to serum hs-CRP levels in subjects with well-controlled hypertension [38]. Our study demonstrated that the CRP variant rs2794520 was associated with altered hs-CRP secretion and CP-mediated CKD risk. These findings might be supported from the study on a potential role of CRP polymorphisms, including rs1205 and rs2794520, and the risk of CP-related CKD disorders.

Although our study confirms some of the findings in previously published community-based studies, it also offers several important conclusions that rs1205 and rs2794520 variant genotypes predisposed to CP-associated CKD, potentially being a novel genetic risk marker of CP-induced CKD progression.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Jian Liu, Department of Nephrology, The First Affiliated Hospital of Xinjiang Medical University, No. 137 South Liyushan Road, Urumqi 830054, China. Tel: (+86)025-52271000; E-mail: xjjianliu@yeah.net

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**Supplementary Table 1.** General characteristics of the varying degrees periodontitis and non-periodontitis in 2007

| | Non-CP (*n* = 481) | Mild CP (*n* = 373) | Moderate CP (*n* = 192) | Severe CP (n = 369) | *P* |
|---|---|---|---|---|---|
| Age (Years) | 29.5 ± 10.1 | 36.6 ± 13.2 | 41.5 ± 14.4 | 51.7 ± 16.5 | 0.000 |
| Male (%) | 210 (43.7) | 180 (48.3) | 116 (60.4) | 201 (54.5) | 0.000 |
| Smoking (%) | 27 (5.6) | 32 (8.6) | 21 (10.9) | 15 (4.1) | 0.006 |
| Drinking (%) | 0 (0) | 3 (0.8) | 1 (0.5) | 0 (0) | 0.094 |
| History of Myocardial Infarction (%) | 9 (1.9) | 12 (3.2) | 9 (4.7) | 19 (5.1) | 0.052 |
| History of stroke (%) | 1 (0.2) | 7 (1.9) | 4 (2.1) | 4 (1.1) | 0.069 |
| History of hepatitis B (%) | 10 (2.1) | 8 (2.1) | 6 (3.1) | 5 (1.4) | 0.57 |
| History of hepatitis C (%) | 10 (2.1) | 8 (2.1) | 2 (1.0) | 4 (1.1) | 0.53 |
| BMI (kg/m$^2$) | 23.8 ± 3.4 | 24.9 ± 11.06 | 24.4 ± 3.5 | 24.1 ± 5.0 | 0.117 |
| 25.0-29.9 kg/m$^2$ | 114 (24.1) | 98 (26.6) | 60 (31.7) | 93 (25.4) | 0.157 |
| ≥ 30 kg/m$^2$ | 29 (6.1) | 28 (7.6) | 17 (9.0) | 28 (7.7) | |
| SBP (mmHg) | 119.7 ± 19.0 | 127.7 ± 25.6 | 127.8 ± 20.6 | 138.7 ± 26.8 | 0.000 |
| DBP (mmHg) | 77.6 ± 11.9 | 90.0 ± 14.1 | 80.6 ± 10.7 | 84.6 ± 14.3 | 0.000 |
| Hypertension (%) | 90 (18.7) | 131 (35.1) | 62 (32.3) | 174 (52.8) | 0.000 |
| Diabetes mellitus (%) | 9 (1.9) | 13 (3.5) | 7 (3.6) | 27 (7.3) | 0.000 |
| Fasting blood-glucose (mmol/L) | 5.2 ± 0.8 | 5.3 ± 0.8 | 5.4 ± 0.9 | 5.5 ± 1.3 | 0.000 |
| Cholesterol (mmol/L) | 4.1 ± 0.9 | 4.4 ± 0.9 | 4.5 ± 1.0 | 4.7 ± 1.0 | 0.000 |
| Triglyceride (mmol/L) | 1.5 ± 0.9 | 1.6 ± 0.9 | 1.8 ± 0.9 | 1.8 ± 1.3 | < 0.05 |
| Hyperlipemia (%) | 154 (32.0) | 130 (34.9) | 86 (44.8) | 168 (45.5) | 0.000 |
| BUA (μmol/L) | 200.4 ± 65.4 | 207.4 ± 63.3 | 213.3 ± 60.5 | 216.6 ± 59.9 | < 0.05 |
| Serum creatinine (μmol/L) | 81.8 ± 14.1 | 83.0 ± 17.3 | 86.9 ± 13.2 | 86.3 ± 31.2 | < 0.05 |
| Microalbuminuria (mg/g Cr) | 6.6 ± 9.9 | 6.7 ± 11.0 | 6.9 ± 7.1 | 9.5 ± 14.2 | 0.000 |
| Metabolic syndrome (%) | 55 (11.4) | 70 (18.8) | 37 (19.3) | 97 (26.3) | 0.000 |
| eGFR (mL/min/1.73 m$^2$) | 97.2 ± 23.8 | 94.7 ± 33.9 | 87.4 ± 16.9 | 86.6 ± 23.8 | 0.000 |
| Decreased eGFR (%) | 4 (0.8) | 5 (1.3) | 7 (3.6) | 35 (9.5) | 0.000 |
| Proteinuria (%) | 12 (2.5) | 15 (4.0) | 4 (2.1) | 29 (7.9) | 0.000 |
| CKD (%) | 13 (2.7) | 16 (4.3) | 6 (3.1) | 37 (10.03) | 0.000 |

**Supplementary Table 2.** General characteristics of the periodontitis subjects in 2007 and 2013

| | CP in 2007 year (*n* = 934) | CP in 2013 year (*n* = 757) | $\chi^2$ | *P* |
|---|---|---|---|---|
| Age (Years) | 43.6 ± 16.3 | 48.2 ± 19.9 | 190.4 | 0.000 |
| Smoking (%) | 68 (7.3) | 48 (6.3) | 1.02 | 0.796 |
| Drinking (%) | 4 (0.4) | 4 (0.5) | 0.013 | 0.909 |
| BMI ($Kg/m^2$) | 24.5 ± 7.8 | 25.4 ± 11.2 | 4.4 | 0.037 |
| Waistline (cm) | 84.4 ± 9.7 | 91.4 ± 11.4 | 177.9 | 0.000 |
| SBP (mmHg) | 132.1 ± 25.7 | 130.2 ± 25.5 | 0.109 | 0.742 |
| DBP (mmHg) | 82.3 ± 13.6 | 84.0 ± 14.4 | 11.4 | 0.001 |
| BUA (μmol/L) | 212.3 ± 61.5 | 228.3 ± 138.3 | 23.8 | 0.000 |
| Cholesterol (mmol/L) | 4.5 ± 1.0 | 4.2 ± 1.0 | 27.2 | 0.000 |
| Triglyceride (mmol/L) | 1.7 ± 1.1 | 1.4 ± 1.1 | 45.6 | 0.000 |
| Fasting blood-glucose (mmol/L) | 5.4 ± 1.0 | 5.1 ± 1.7 | 40.2 | 0.000 |
| Diabetes mellitus (%) | 28 (3.3) | 44 (5.8) | 2242.9 | 0.000 |
| Hypertension (%) | 410 (43.9) | 430 (56.8) | 158.3 | 0.000 |
| Metabolic syndrome (%) | 204 (21.8) | 147 (19.4) | 12.5 | 0.000 |
| Hyperlipemia (%) | 397 (42.5) | 315 (41.6) | 11.3 | 0.024 |
| History of hepatitis B (%) | 19 (2.0) | 16 (2.1) | 1.02 | 0.796 |
| History of hepatitis C (%) | 14 (1.5) | 11 (1.5) | 2.35 | 0.503 |
| History of Myocardial Infarction (%) | 40 (4.3) | 31 (4.7) | 11.29 | 0.010 |
| History of stroke (%) | 15 (1.6) | 15 (2.0) | 9.26 | 0.026 |
| Microalbuminuria (mg/g Cr) | 7.6 ± 11.9 | 16.5 ± 39.9 | 21.4 | 0.000 |
| Albuminuria (%) | 48 (5.1) | 59 (7.8) | 24.7 | 0.000 |
| reduced renal function (%) | 47 (5.0) | 35 (4.6) | 23.9 | 0.000 |
| CKD (%) | 85 (9.1) | 87 (11.5) | 12.7 | 0.000 |