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SHORT COMMUNICATION

# Association of − 757T > C polymorphism of C-reactive protein gene with chronic periodontitis of South Indian population

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## Abstract

**Objective** CRP gene polymorphism is common in inflammatory diseases, but such association has not been reported in periodontitis. Our objective was to interrogate SNPs of *crp* in chronic periodontitis in a case–control manner.

**Methods** DNAs were extracted from mouthwash samples of 116 volunteers using salting-out method. Selected 12 5′UTR SNPs of *crp* were genotyped using ARMS–PCR.

**Results** TC genotype of − 757T > C polymorphism (rs3093059) showed protective association (OR— 0.29, 95%CI—0.12–0.68, and *p*—0.004), and wild type − 757TT showed susceptible association with a *p* value of 0.008 (OR—3.09, 95%CI—1.33–7.15).

**Conclusion** The observation of protective and susceptible association of *crp* − 757T > C polymorphism may be useful for better management and prophylaxis of periodontitis.

**Keywords** C-reactive protein (CRP) · Chronic periodontitis (CP) · Amplification refractory mutation system (ARMS)–PCR · Single-nucleotide polymorphism (SNP)

## Introduction

Chronic periodontitis (CP) is more common, and it affects approximately 50% of adults and 60% of people over 65 years old [1, 2]. As reviewed by Cekici et al. [3], the etiology of periodontal diseases is bacterial infection and the host–microbe interactions that mediate inflammatory response. Increased concentration of CRP was observed consistently in periodontitis when compared to healthy controls [4–6]. Very few studies, including the finding of no association of CRP − 717C > T with Indonesian periodontitis cases [7], have been done on understanding CRP gene polymorphisms in periodontitis. Therefore, we decided to interrogate 12 SNPs located at 5′UTR region of CRP gene in periodontitis patients from South India. These 12 SNPs were chosen based on the previous reports on other inflammation-related diseases (Table S1 in Supplementary material-1).

## Materials and methods

### Volunteers' enrolment

Study participants consisting of 65 CP and 51 control cases were enrolled from Sep. 2017 to Sep. 2018 at Sree Balaji Dental College and Hospital, Chennai, Tamil Nadu, India. The age group of CP and control cases was between 30 and 67 years and 27 and 68 years old, respectively. Inclusion criteria used for CP cases were that they must have at least 10 remaining teeth. Clinical parameters of probing depth (PD) and clinical attachment level (CAL) were used and each tooth of patients was examined. Those with PD > 5 mm and CAL > 3 mm were classified as CP. Control subjects had 'healthy periodontium' with no evidence of loss of

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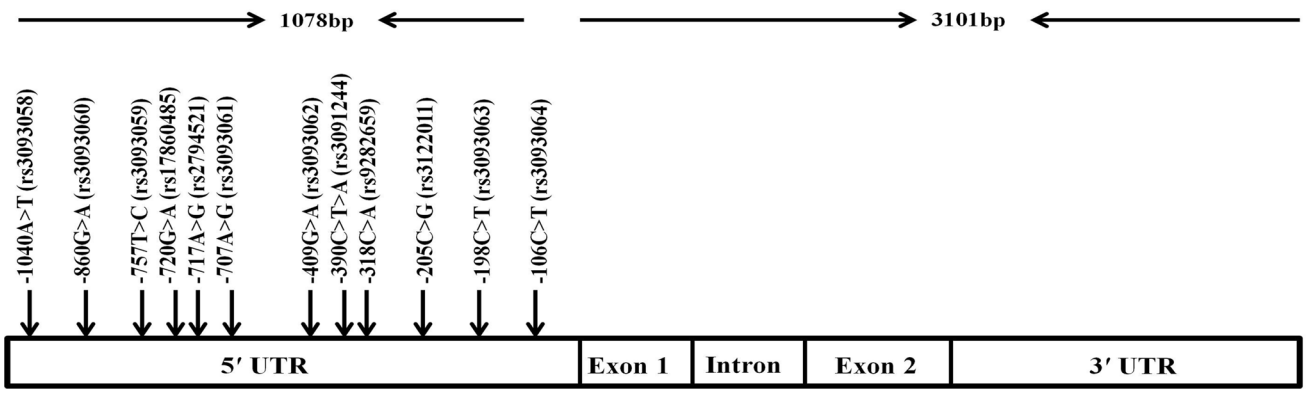


**Fig. 1** Relative positions of 12 SNPs of 5′-UTR of CRP gene analyzed in this study. These SNPs were chosen based on the previous reports in inflammation-related diseases

connective tissue attachment or supporting bone or other signs of disease activity.

### DNA extraction and SNP genotyping

30 ml of mouthwash samples were collected in 50 ml centrifuge tubes using bottled drinking RO-water. DNA was extracted from mouth wash samples by standard salting-out method [8]. The graphical representation of 12 SNPs is shown in Fig. 1. ARMS–PCR method was used for genotyping of SNPs [9, 10]. PCR primers were custom made by Sigma-Aldrich and the list of all primers used is given in Table S2 (Supplementary material-2). The SNPs were genotyped after resolving the PCR products in agarose gel as shown in Figure S1 (Supplementary material-2). To verify the PCR-based genotyping results, 1078 bp 5′UTR region of *crp* (containing 11 SNPs except rs3093064) was amplified for 10 randomly selected samples and Sanger sequencing was performed. An example of chromatogram of − 757T > C obtained is given in Figure S2 (Supplementary material-2).

### Statistical analysis

Sample size was calculated based on prior report of prevalence of 42.3% CP in South Indian population [11]. With 43% incidence of patients group and assuming 5% in controls with odds ratio 1 and a power of 80%, a minimum of 19 cases and 19 controls samples are needed. Other statistics were calculated using standard methods and formulas as reported by Sreekanth et al. [10].

## Results and discussion

### Association of *crp* SNPs in chronic periodontitis

In preliminary screening, alleles of all 12 SNPs were checked on 30 CP samples, and found three SNPs to be interesting to pursue further. Genotype frequencies between CP and controls of the three, viz., − 717A > G, − 757T > C, and − 390C > T > A, obtained with all 116 samples are shown in Table 1. Among these, the TT genotype of SNP − 757T > C showed a high risk in chronic periodontitis (OR 3.09, $p$ 0.008) and TC genotype showed protective association (OR 0.39, $p$ 0.004). The protective association of allele − 757C is previously reported in ischemic stroke patients of the Chinese population [12, 13]. At the protein level, the *crp* promoter SNP − 757T > C was found to increase CRP levels in CAD patients of Caucasian population [14]. The increased CRP levels were correlated with − 757T > C variant in Taiwanese as well as North Indian patients [15, 16].

No significant associations were observed with SNP − 717A > G polymorphism similar to results observed in Indonesian Chronic periodontitis [7]. Similarly the − 390C > T > A polymorphism did not find any significant associations in the present study.

To summarize, genetic association analysis in 5′UTR region of *crp* showed significant association of − 757T > C polymorphism in chronic periodontitis. To our knowledge, this is the first report showing the association of *crp* − 757T > C with South Indian periodontitis.

**Table 1** Association of *crp* polymorphisms in chronic periodontitis patients in South Indian population

| SNP region | Genotype | All (CP) $N=65$ $N+$ (%) | Ctl $N=51$ $N+$ (%) | OR | 95% CI | *p* value |
|---|---|---|---|---|---|---|
| − 717A > G | AA | 30 (46.1) | 21 (41.1) | 1.22 | 0.58–2.56 | 0.592 |
| | AG | 28 (43.0) | 18 (35.2) | 1.38 | 0.65–2.95 | 0.390 |
| | GG | 7 (10.7) | 12 (23.5) | 0.39 | 0.14–1.08 | 0.07 |
| − 757T > C | TT | 53 (81.5) | 30 (58.8) | **3.09** | **1.33–7.15** | **0.008** |
| | TC | 11 (16.9) | 21 (41.1) | **0.29** | **0.12–0.68** | **0.004** |
| | CC | 1 (1.5) | – | 2.39 | 0.09–60.0 | 0.595 |
| − 390C > T > A | CC | 31 (47.6) | 19 (37.2) | 1.05 | 0.48–2.31 | 0.892 |
| | CT | 24 (36.9) | 27 (52.9) | 0.52 | 0.24–1.09 | 0.08 |
| | TT | 3 (4.6) | 1 (1.9) | 2.41 | 0.24–23.9 | 0.450 |
| | CA | 7 (10.7) | 4 (7.8) | 1.41 | 0.39–5.13 | 0.594 |
| | AA | – | | – | – | – |

Bold values indicate significance of association

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## Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

**Ethical approval** Ethical clearance for the study was obtained from the Institutional Ethical Committee of Sree Balaji Dental College and Hospital, Pallikaranai, Chennai, India—600100 (Ethics Committee Registration No: ECR/761/Inst/TN/2015).

**Informed consent** Informed consent was obtained from all individual participants.

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