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Haplotypes of susceptibility to chronic periodontitis in the *Interleukin 8* gene do not influence protein level in the gingival crevicular fluid

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ABSTRACT

Objective: Previously, we identified that the ATC/TTC haplotype formed by polymorphisms in the Interleukin-(*IL*)*8* gene conferred susceptibility to chronic periodontitis (CP). The aim of the study was to investigate whether the *IL8* haplotype ATC/TTC was associated with the volume of gingival crevicular fluid (GCF), the concentration of interleukin IL-8 in the GCF, as well as periodontal conditions in patients with CP in comparison to controls without CP.

Methods: Seventy-nine individuals (CP: $n = 41$, controls: $n = 38$) were grouped according to the presence (susceptible for CP) or absence (not susceptible for CP) of the *IL8* ATC/TTC haplotype. After periodontal clinical evaluation, they were subdivided by the presence or absence of CP. GCF was collected from each patient and the IL-8 levels were determined by ELISA. The GCF volume of each subject was measured by means of a calibrated electronic device. Comparisons of means between carriers and non-carriers of the ATC/TTC haplotype were evaluated using the Mann–Whitney test. Linear regression and stepwise linear regression analysis were used to analyse the association of the GCF volume with potential covariates and their contribution for the phenotype.

Results: We did not find significant differences of both periodontal conditions and IL-8 concentration in the GCF of patients with the presence or absence of the *IL8* ATC/TTC haplotype. However, the GCF volume was significantly higher amongst the patients affected by CP that are absent for the *IL8* ATC/TTC haplotype. In addition, linear regression analysis showed a statistically significant association between GCF volume and CP, IL8 haplotype ATC/TTC and IL-8 concentration.

Conclusions: The *IL8* haplotype of susceptibility to CP was neither associated with IL-8 cytokine levels nor with clinical periodontal parameters. Also, CP, *IL8* haplotype and IL-8 concentration showed a positive association with the GCF volume levels in the studied patients.

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1. Introduction

Periodontal disease (PD) is an infectious disease that can lead to a destructive inflammatory process affecting the supporting tissues of the teeth.[1] Currently, PD is considered a multifactorial disease,[2] and genetic factors were estimated to have approximately 50% heritability, which was unaltered after following adjustments for behavioural variables including smoking.[3]

The immune response of the subjects affected by PD has been widely investigated, and cytokine production and its association with aggressive and/or chronic periodontitis (CP) have been the focus.[4–7] Genetic susceptibility to PD has also been studied with the main focus on genes of the immune system, such as *Interleukin 2* (*IL2*),[8,9] *IL4*,[10–12] *IL6*[13–15] and *IL10*.[16–18]

Interleukin-8 (IL-8) is a chemokine produced primarily by blood mononuclear phagocytes,[19,20] with a potent pro-inflammatory function. IL-8 mediates the migration and activation of neutrophils mainly from the peripheral blood to the injured tissue.[20,21] In the case of PD, such neutrophils are attracted to the lamina propria of the connective tissue. In regard to the importance of neutrophils as the first line of defense against periodontopathic bacteria, the continuous and excessive presence of IL-8 may contribute to the local destruction of periodontal tissues.[22–24]

Inflammatory diseases like CP can be induced and perpetuated by the excessive production of pro-inflammatory cytokines or possibly by a failure in the production of appropriate anti-inflammatory cytokines.[25] Individual differences in the levels of interleukins can be attributed to the polymorphisms in their genes, especially if these polymorphisms are within regions called exons or promoter. The SNP (rs4073) (reference sequence number taken from the NCBI's Entrez system) in the promoter region of the *IL8* gene is thought to be functional because its A allele has been shown to be associated with a higher IL-8 production after the stimulation with lipopolysaccharide.[26] Recent studies from our group investigated polymorphisms in the *IL8* gene in five hundred individuals with and without CP, and demonstrated that the haplotype ATC/TTC, formed by the −251(T/A) (rs4073), +396(T/G) (rs2227307) and +781(C/T) (rs2227306) polymorphisms in the *IL8* gene, conferred two times more susceptibility to CP than the other haplotypes ($OR_{adjusted}$ = 2.24, 95% CI = 1.10–4.55).[27] However, not all polymorphisms in the *IL8* gene that were investigated by our group were associated with susceptibility to CP.[28] Studies involving different ethnic populations showed strong linkage disequilibrium between the SNPs rs4073, rs2227307 and rs2227306, in which TTC was the more frequent haplotype for Italians (61%),[29] English (52%),[30] Chinese (55.4%)[31] and Brazilians (41%).[27]

Because of the multifactorial characteristic of PD, the interrelationship of different factors, like genetic susceptibility, cytokine production and participation of microrganisms in PD need to be better understood. Few studies have investigated the combined influence of more than one factor in the PD, such as genetic polymorphisms and cytokine production[32,33] or analysis of subgingival microflora and genetic polymorphisms.[34,35] Therefore, the aim of this study was to investigate whether the genetic susceptibility to CP given by the ATC/TTC haplotype in the *IL8* gene is associated with the volume of gingival crevicular fluid (GCF), the concentration of interleukin IL-8 in the GCF and with clinical periodontal parameters of the disease.

2. Materials and methods

2.1. Selection of subjects

In a previous study,[27] we found that the AGT/TTC haplotype was more common for both groups (68 subjects without CP and 63 patients with CP), which was significantly different from the ATC/TTC haplotype (22 individuals without CP and 45 patients with CP). Among those 198 genetically pre-screened individuals and after we informed them of their genetic results from the previous study, we selected 79 individuals between January and August, 2009 to participate in the present study. All volunteers were informed about the aims and methods of the current study, and they all gave their written consent to participate. This study was approved by the Committee for Ethical Affairs of the São Paulo State University (Protocol number 52/08).

The inclusion criteria were the following: good general health, age between 30 and 60 years, presence of at least 16 teeth in the mouth and no history of subgingival periodontal debridement or periodontal surgery in the preceding 12 months. The following additional exclusion criteria were applied: oral diseases other than caries and periodontal disease, ongoing orthodontic therapy, smoking (current and former smoker abstinence for less than 5 years); a need for antibiotic prophylaxis, a history of systemic or local disease with influence on the immune system (cancer, cardiovascular and respiratory diseases), diabetes mellitus, hepatitis or HIV infection, immunosuppressive chemotherapy or current pregnancy or lactation.

A single previously calibrated examiner (S.C.T.C./weighted Kappa = 0.80, considering the probing pocket depth clinical outcome) recorded the following clinical parameters: probing pocket depth (PPD), percentage of the periodontal sites with PPD ≥ 4 mm, clinical attachment loss (CAL), bleeding on probing (BOP), visible plaque index (VPI)[29] and gingival bleeding index (GBI).[29] The clinical periodontal parameters were assessed at six sites around each tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual locations) in the whole mouth excluding the third molars. The cemento-enamel junction was accepted as a reference point in the measurement of CAL. The PPD and the CAL measurements were performed using a conventional Williams manual probe (Trinity – Campo Mourão, Brazil). The BOP (deemed positive if occurring within 15 s after the periodontal probing), the visible plaque index (VPI) and the gingival bleeding index (GBI) were recorded dichotomously. Patients were considered to have CP when two or more sites in non-adjacent teeth exhibited probing pocket depths ≥ 5 mm, clinical attachment loss ≥ 3 mm (moderate chronic periodontitis)[30] and bleeding on probing. Subjects without CP exhibited sites with probing pocket depths ≤ 3 mm and absence of clinical attachment loss ≥ 3 mm.