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CCR5Δ32 (rs333) polymorphism is associated with decreased risk of chronic and aggressive periodontitis: A case-control analysis based in disease resistance and susceptibility phenotypes

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ABSTRACT

Chronic and aggressive periodontitis are infectious diseases characterized by the irreversible destruction of periodontal tissues, which is mediated by the host inflammatory immune response triggered by periodontal infection. The chemokine receptor CCR5 play an important role in disease pathogenesis, contributing to pro-inflammatory response and osteoclastogenesis. CCR5Δ32 (rs333) is a loss-of-function mutation in the CCR5 gene, which can potentially modulate the host response and, consequently periodontitis outcome. Thus, we investigated the effect of the CCR5Δ32 mutation over the risk to suffer periodontitis in a cohort of Brazilian patients (total N = 699), representative of disease susceptibility (chronic periodontitis, N = 197; and aggressive periodontitis, N = 91) or resistance (chronic gingivitis, N = 193) phenotypes, and healthy subjects (N = 218). Additionally, we assayed the influence of CCR5Δ32 in the expression of the biomarkers TNFα, IL-1β, IL-10, IL-6, IFN-γ and T-bet, and key periodontal pathogens P. gingivalis, T. forsythia, and T. denticola. In the association analysis of resistant *versus* susceptible subjects, CCR5Δ32 mutant allele-carriers proved significantly protected against chronic (OR 0.49; 95% CI 0.29–0.83; p-value 0.01) and aggressive (OR 0.46; 95% CI 0.22–0.94; p-value 0.03) periodontitis. Further, heterozygous subjects exhibited significantly decreased expression of TNFα in periodontal tissues, pointing to a functional effect of the mutation in periodontal tissues during the progression of the disease. Conversely, no significant changes were observed in the presence or quantity of the periodontal pathogens P. gingivalis, T. forsythia, and T. denticola in the subgingival biofilm that could be attributable to the mutant genotype.

1. Introduction

Periodontitis is a chronic infectious disease characterized by the irreversible and progressive destruction of teeth-supporting structures. Periodontitis is initiated by the bacteria harbored in the teeth-attached biofilm that activate the host's inflammatory immune response, which provide protection against the infecting agents. However, host response mediators also modulates local proteolytic and osteclastogenic activities, leading to soft and mineralized tissue destruction as collateral damage [1].

In this context, destructive and protective host immune responses have been described to present specific molecular patterns in response to periodontal infection. Pro-inflammatory responses mediated by IL-1, IL-6 and TNFα, along with Th1 and/or Th17 cells, have been associated with the establishment and progression of periodontal destruction, while Th2 and Treg-associated response patterns have been described to attenuate/arrest tissue destruction [2,3]. While the exact mechanisms underlying the host response polarization in periodontium remains to be elucidated, the chemokine system have been described as an important player in the determination of the nature of the host response via the selective recruitment of immune cells subsets, which can amplify the inflammation or suppress it [4].

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The system comprised by the receptor CCR5 and its ligands CCL3, CCL4 and CCL5 has proved important in the inflammatory periodontal destruction associated with periodontitis [5,6]. CCR5 is a 352-amino acid G protein-coupled chemokine receptor which is involved in the selective recruitment of multiple leukocytes subsets, including neutrophils, monocytes, NK cells and lymphocytes [7,8]. Indeed, CCR5 mediate the recruitment of CCR5 + F4/80 + and CCR5 + CD4 + leukocytes, regarded as pre- and pro-osteoclastogenic cells involved in the bone resorption that characterizes periodontitis [9]. The activation of the transduction signals triggered by CCR5 coupling with ligands such as CCL3, CCL4 and CCL5 stimulates not only cell migration, but also influences proliferation, cytokine expression and activation of effector functions [10]. In fact, pharmacological inhibition of CCR5 using metRANTES had proved sufficient to arrest inflammatory cytokine expression, and consequently to suppress alveolar bone loss in mice [8]. Similarly, CCR5 deficient mice (CCR5KO) presents a decreased infiltration of leukocytes and decreased bone loss upon experimental periodontal infection [9]. Importantly, CCR5 + cells also contribute to the protective responses against periodontal infection [8,9].

Interestingly, the human counterpart of the CCR5KO mice strain may be represented by carriers of the CCR5Δ32 mutation. CCR5Δ32 (rs333) is a 32-base pair deletion causing a frameshift variant in the *CCR5* gene leading to a loss-of-function due by the abolition of the receptor's cell-surface expression [11,12]. Indeed, this truncated variant have been demonstrated to be clinically relevant since it has been associated with various phenotypes, including resistance to HIV infection, decreased risk for type 1 diabetes, increased risk for diabetic retinopathy in type 1 diabetes patients and increased risk of multiple sclerosis [13–15]. Thus, based in the relevance of CCR5 to experimental periodontitis pathogenesis, it is reasonable to hypothesize that CCR5Δ32 could be a relevant risk factor for human periodontitis. Two previous studies had found no association between CCR5Δ32 and chronic periodontitis, but methodological shortcomings in their designs warrant a reappraisal of the possible contribution of the mutation to periodontitis' risk profile [16,17].

Indeed, the absence of proper susceptibility and resistance phenotypes definition critically dampen such studies power and odds of identification of genetic factors potentially involved in periodontitis pathogenesis [1,18]. Chronic periodontitis is the most common form of the disease, which is characterized by destruction of teeth-supporting tissues over a long period of time, comprising progressive/active and stable/inactive periods [2,19]. A less common variant is the aggressive form of the disease, characterized by rapid progression of the periodontal destruction [20]. Conversely, chronic gingivitis is a periodontal disease characterized by widespread gingival inflammation without irreversible destruction of the teeth-supporting tissues, even over long periods of time [21]. Despite some differences in the microbial biofilm associated with the different forms of periodontal disease, such conditions are basically initiated by the same stimuli, Gram-negative periodontal bacteria [22,23]. However, the clinical course of each entity reflects inherent differences in the host's capacity to cope with the presence of periodontophatic bacteria [2,3]. Consequently, chronic and aggressive periodontitis can be considered the phenotypic expression of supposed susceptibility genotypes, while chronic gingivitis allegedly represents a resistant phenotype/genotype [18,24]. Indeed, the analysis of opposing resistant and susceptible phenotypes, determined by the observance the outcome of exposure to the microbial challenge, increases the possibilities of finding a true association between genetic markers and disease phenotype [1,18].

Therefore, it is plausible to hypothesize that CCR5Δ32 (rs333) may account for the differential expression of disease susceptibility (such as chronic and aggressive periodontitis) or resistance (such as chronic gingivitis). Also, it is possible that the hypofunctional CCR5 variant could affect the expression of host response factors in periodontal tissues, as well as influence the pattern of bacterial infection in periodontitis.

Therefore, we conducted a case-control study to investigate whether the CCR5Δ32 (rs333) is associated with periodontitis risk; performing for the first time an analysis in groups defined as susceptible and resistant, and also investigated the putative functionality of this SNP in the modulation of CCR5-related host mediators (TNFα, IL-1β, IL-6, IL-10, IFN-γ, and T-bet) *in situ*, as well as its possible impact in the composition of subgingival biofilm, in an attempt to unveil a conceivable mechanism linking the gene variant with the disease phenotype.

2. Materials and methods

2.1. Participants

The sample was recruited at the São Paulo state, south-eastern region of Brazil, from patients scheduled for treatment at the Dentistry School University of Ribeirão Preto. Patients were examined by an experienced periodontist and scored for bleeding on probing (BOP), probing depth (PD) and clinical attachment loss (CAL). Enrolled subjects provided informed consent that was approved by the Institutional Review Board. Subjects were excluded from the study if they were tobacco smokers (including former smokers), had medical history indicating evidence of known systemic modifiers of periodontal disease, and/or had received periodontal therapy in the previous 2 years. No strategy was used to identify subpopulations (population stratification) or population relatedness among the recruited subjects. After the diagnostic phase, patients were subsequently categorized into healthy (H; n = 218) [classic control], chronic gingivitis (CG; n = 193) ['resistant' phenotype control], chronic periodontitis (CP; n = 197) [susceptible subjects] and aggressive periodontitis (AP; n = 91) [highly susceptible subjects], as previously described [1,18].

The control group (H; n = 218) included subjects with clinically healthy gingival tissues scheduled to undergo dental restorative procedures. The chronic gingivitis group (CG; n = 193) corresponded to subjects with history of poor oral hygiene, BOP > 70% of periodontal sites and no CAL or radiographic evidence of alveolar bone loss. Patients in the chronic periodontitis group (CP; n = 197) included subjects diagnosed with moderate to severe periodontitis (at least one teeth per sextant with probing depth > 6 mm and clinical attachment loss > 3 mm plus radiographic evidence of extensive bone loss [> 30% alveolar bone height in at least 50% of teeth]), scheduled to receive periodontal therapy. The aggressive periodontitis group (AP; n = 91) included subject with moderate to severe periodontitis (at least one teeth per sextant with probing depth > 6 mm and clinical attachment loss > 3 mm plus radiographic evidence of extensive bone loss [> 30% alveolar bone height in at least 50% of teeth]) under the age of 35 years. The age of diagnosis was adopted as an operational criterion to define aggressive periodontitis. Table 1 summarize all relevant clinical and demographical information of each sample population.

2.2. DNA sampling and CCR5 genotyping (rs333)

Oral mucosa epithelial cells from each participant (n = 699) were obtained by scrapping the inner cheek after a mouthwash with 3% glucose. DNA was extracted from epithelial cells with sequential phenol/chloroform solution and precipitated with salt/ethanol solution. Extracted DNA was immediately used for genotyping. We genotyped CCR5Δ32 (rs333) by polymerase chain reaction (PCR) with forward primer 5′ – ATGCTGTGTTTGCTTTAAAAGCCAGG – 3′ and reverse primer 5′ – AGGACCAGCCCCAAGATGACTA – 3′ flanking the 32 nucleotide deletion in the CCR5 gene. Amplification was performed in 35 cycles as follows: denaturation step 94 °C for 30 s, annealing 56 °C for 30 s, and extension 72 °C for 30 s. The DNA amplicons were visualized under UV light on 2% agarose gel stained with ethidium bromide. The wild-type amplicon corresponded to a 220-bp band while the CCR5Δ32 allele corresponded to a 188-bp band. Negative and positive controls were included in each reaction.