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Role of familiarity versus interleukin-1 genes cluster polymorphisms in chronic periodontitis

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ABSTRACT

Periodontitis (PO) is a multifactorial disease affecting about 10% to 20% of the general population. Several studies have suggested that part of the clinical variability in PO might be explained by genetic factors. Among the candidate genes for PO, *IL1* gene polymorphisms have been broadly investigated, with variable results, for their relationship with the disease. We studied three *IL1* polymorphisms, IL1A C[-889]T (rs1800587), IL1B C[3953/4]T (rs1143634), and IL1RN VNTR [+2018] (rs419598) in relation to different life styles and familiarities. We did not find correlation between these *IL1* polymorphisms and chronic PO, as well as between chronic PO and life styles (smoking, alcohol, coffee, fizzy drink and fish). We found a strong correlation, also after adjustment for age, between familiarity and PO onset ($P = 0.0062$; OR 5.754, 95% CI 1.644–20.145). In conclusion, we did confirm the previously suggested association between PO and *IL1* gene cluster polymorphisms, and between PO and four common risk factors (coffee, smoking, alcohol and fizzy drinks) and one common protective factor (fish). On the contrary, we found a strong role of familiarity.

1. Introduction

Periodontal (PO) disease is the result of a collection of chronic inflammatory responses activated by the bacterial plaque that accumulates in the gingival margin and induces the inflammatory response. The state of the PO disease can be defined as an imbalance between the quality and quantity of bacterial microflora colonizing periodontal pocket and the immunological potential of the host, which can be modified by several risk factors, among which the genotype is of greatest importance (Kowalski et al., 2006). The presence of specific plaque micro-organisms is necessary for PO occurring, but it is not sufficient. In susceptible individuals, the inflammatory response is greatly exaggerated, leading to progressive destruction of the bony and soft-tissue support for the teeth.

PO is considered to be a complex disease, estimating the involvement of at least 10 and possibly as many as 20 disease-modifying genes, whose effect is also influenced by environmental factors. A large number of studies have been published on the role of genes and their variants (polymorphisms) in host response in PO, many of them focusing on interleukin 1 gene cluster (*IL1A*, *IL1B*, *IL1RN*) (Laine et al., 2012). Since 1997, when Kornman et al. (1997) reported a 19-fold increased risk of bone loss in non-smoking genotype-positive patients when compared to genotype-negative patients, numerous papers analyzed the influence of *IL1* polymorphisms on the outbreak and outcome of PO disease. The mechanism of this linkage seems to be obvious. Rare allele of *IL1* appears to favor a susceptible phenotype in which during inflammatory response macrophages secrete greater amounts of this cytokine, whose effect is an increased inflammatory response. This results in greater damage of periodontal tissues.

IL1 is an inflammatory cytokine involved in the immune response. There are two active forms of IL1, IL1A and IL1B, both binding to type I and type II IL1 receptor. On the contrary, IL1 receptor antagonist (IL1RN) is a protein that binds to IL1 and inhibits the binding of IL1A and IL1B to the receptor. IL1 possesses inflammatory, metabolic, hematopoietic, and immunological properties. It is a potent bone mediator in vitro and in vivo, able to induce bone resorption from osteoclasts. By direct or indirect mechanisms, IL1 induces the proliferation of osteoclast precursors and the differentiation and activation of mature osteoclasts (Assuma et al., 1998). Susceptible individuals carry specific genotypes for *IL1* so that they produce more than normal of this cytokine, increasing their chances of developing PO.

A recent meta-analysis found statistically significant association of *IL1A* C[-889]T (rs1800587) and *IL1B* C[3953/4]T (rs1143634) gene polymorphisms with chronic PO disease (Nikolopoulos et al., 2008). Importantly, no association was found between the aggressive form of

Abbreviations: PO, periodontitis; IL1, interleukin 1; IL1RN, interleukin 1 receptor antagonist; PAG, PO-associated genotype; BMI, body mass index.

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the disease and all the examined cytokines (Nikolopoulos et al., 2008). The *IL1RN* [+2018] (rs419598) gene polymorphism has been investigated in few studies, and only one of them did not find association with chronic PO susceptibility (Laine et al., 2013). Frequently, the so-called "PO-associated genotype" (PAG) fails to associate with the clinical manifestations, probably because many environmental factors play an important and modulating role in the expression of PO (Stellini et al., 2013). These environmental factors include oral hygiene/bacterial plaque, smoking, coffee and alcohol consumption, stress and systemic factors that may exacerbate the inflammatory pathology associated with the disease (Stabholz et al., 2010). Smoking, in particular, has been very frequently queried by a multitude of studies (more than 300) that have shown that not only a relationship between it and PO, but also the action of PAG can be masked by the effects of smoking (Gelskey, 1999; Gustafsson et al., 2000).

Considerable evidence suggested that aggressive PO is equally influenced by genetic and environmental factors (Kinane and Attström, 2005; Loos et al., 2005). Numerous publications have reported familiar aggregation of aggressive PO (in an autosomal dominant inheritance model) in both Caucasian and African-American families, with 73% and 70% penetrance, respectively (Boughman et al., 1992; Marazita et al., 1994; Petit et al., 1994). Other studies in twins showed that approximately half of the variance in aggressive PO disease in the population is attributed to genetic variance (Michalowicz et al., 2000), whereas the magnitude of the genetic effects on chronic PO seems to be less evident (Torres de Heens et al., 2010).

The hypothesis we aimed to test in this study was that *IL1*-PAG, environmental factors (life styles) and familiarity might interplay between each other in PO with relative different weights. To this aim, we analyzed these three components in a cohort of 101 patients affected by chronic PO and 105 controls.

2. Materials and methods

2.1. Subjects

The study has been approved by the Padova Hospital Ethics Committee and was conducted in accordance with the guidelines of the Declaration of Helsinki. The samples have been obtained after advising of the nature of the study and after signing of the appropriate informed consent.

We collected and analyzed 206 cheek swabs of 101 unrelated patients affected by chronic PO and 105 healthy unrelated controls coming from the North-East of Italy (Caucasian). The diagnosis of chronic PO was based on the guidelines of the International Workshop for the Classification of Periodontal Diseases and Conditions (Armitage, 2000). The median (minimum–maximum) age was 53 (25–75) and 25 (22–59) years for patients and controls, respectively. There was no possibility to obtain samples from age-matched control subjects. Nevertheless, the difference in the median age of two cohorts has been normalized by appropriate statistical analysis. Patients and controls were appropriately sex-matched. After an in-depth periodontal visit, each participant filled a form in which the life styles, environmental factors and medical history were investigated in details. In particular, we requested information regarding the family history (positive familiarity was considered when one or more members of the family were affected by PO), body mass index (BMI), smoking status (yes/no), and alcohol (yes/no, more than 1 wine glass/day), coffee (yes/no), fish (yes/no, more than 2 times/week), and fizzy drink (yes/no, more than 1 glass/day) consumption. Data were anonymous and analyzed together with the genetic status.

2.2. Analysis of genetic polymorphisms

Genomic DNA was isolated using QIAamp DNA mini Kit, according to the manufacturer's protocol (Qiagen, Milan, Italy). *IL1A*, *IL1B* and *IL1RN* polymorphisms were analyzed by direct sequencing (ABI PRISM 3730XL DNA Sequencer, Applied Biosystems, Monza, Italy). The oligonucleotide primers used for the analysis were the following: 5′-CATT TGCTAAGAGTCTGGTGTTCTAC-3′ and 5′-TTCTAATAGTAGCTGTAGTTGT GTTCTGG-3′ for rs1800587; 5′-CTAATAGTGTCAGGTCCAGTGTTCTTAG-3′ and 5′-TTTCTAGGACCAAAGTTTGTATATTCCT-3′ for rs1143634; and 5′-CATGCCGTGGGTATACTAAAATACTA-3′ and 5′-GACCTCTGACCTAG ACTCTACCTCTC-3′ for rs419598. Reagents and conditions for the PCR were: 12.5 μl of 2× pre-aliquoted ReddyMix™ PCR Master Mix (Thermo Fisher Scientific, Milan, Italy) 2.5 μl of sterile water, 2 μl of dimethyl sulfoxide, 1 μl of each primer (10 μM; Sigma-Aldrich, Milan, Italy), and 6 μl of 10 ng/μl genomic DNA. The PCR program on a thermal cycler (GeneAMP PCR System 2700, Applied Biosystems, Monza, Italy) was: a first denaturation step at 94 °C for 4 min, followed by 37 cycles of 94 °C for 30 s, 60 °C for 60 s, 72 °C for 60 s, and a final extension step of 4 min at 72 °C. 5 μl of the amplification products was electrophoresed on a 2% agarose gel at 150 V for 30 min. The fragments were visualized by SYBR Safe DNAStain (Invitrogen, Milan, Italy) under ultraviolet (UV) transillumination.

2.3. Statistical methods

The characteristics of patients and controls have been described by count and percentage for categorical variables, and by median (min–max values) for the quantitative variables. The Hardy Weinberg equilibrium in patients and controls has been tested with the chi-square goodness-of-fit test. The comparison between data obtained in this study and previously published studies has been performed by chi-square test. The association of the two groups (cases and controls) with life styles and *IL1* gene cluster genotype has been analyzed with unadjusted, only age-adjusted and age- and BMI-adjusted (Suvan et al., 2011) univariate logistic regression. The results are presented as Odds Ratio (OR), 95% Confidence Interval (95% CI), and P-value. The significance was set at the 5% level. The analysis has been performed by SAS 9.2 for Windows (SAS Institute Inc., Cary, NC, USA).

3. Results

The data regarding *IL1* gene cluster genotype, life styles and familiarity in patients and controls are summarized in Table 1. The distribution of genotypes in patients and controls did not deviate significantly from Hardy–Weinberg equilibrium. The frequency distribution of the *IL1* gene cluster genotypes in our subjects showed some differences, summarized in Table 2, with the frequencies previously reviewed by Laine et al. (2012). In particular, for *IL1A* our data are slightly different from the 3 revised reports (Fiebig et al., 2008; López et al., 2005; Struch et al., 2008), especially for the frequency of this polymorphism in controls from Fiebig et al. (2008) (P = 0.0071). Regarding *IL1B*, our data

Table 1
Results of the *IL1* gene cluster genotype, life styles and familiarity in PO patients and controls.

	Patients (n = 101)	Controls (n = 105)
Sex F/M n (%)	56/45 (55/45)	52/53 (50/50)
Age (years) median (min–max)	53 (25–75)	25 (20–59)
BMI (Kg/m^2) median (min–max)	23.7 (21.3–27.68)	21.7 (19.6–32.0)
IL1A CC/CT/TT n (%)	45/46/10 (45/45/10)	65/34/6 (62/32/6)
IL1B CC/CT/TT n (%)	57/38/6 (56/38/6)	67/30/8 (64/28/8)
IL1RN CC/CT/TT n (%)	7/45/49 (7/45/48)	9/46/50 (8/44/48)
Smoking yes/no n (%)	32/69 (32/68)	28/77 (27/73)
Familiarity for PO yes/no n (%)	61/39 (61/39)	17/69 (20/80)
Alcohol consumption yes/no n (%)	20/81 (20/80)	4/101 (4/96)
Coffee consumption yes/no n (%)	92/9 (91/9)	83/22 (79/21)
Fizzy drink consumption yes/no n (%)	25/76 (25/75)	31/74 (30/70)
Fish consumption yes/no n (%)	38/63 (38/62)	39/66 (37/63)

Results of the *IL1* gene cluster genotype, life styles and familiarity in PO patients and controls.