

Association between periodontal disease and Interleukin-1 β +3953 and vitamin D receptor Taq1 genetic polymorphisms in an Italian caucasian population

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Summary

Aim. Periodontal diseases entail a variety of conditions affecting the periodontium. The pathogenesis results from a complex interaction of genetic and environmental factors. Although there are evidences to confirm a role of genetic determinants, the outcome of the available studies is controversial and the largest part of the research has been carried out in Asian populations.

Methods. We investigated two polymorphisms in the genes encoding Interleukin-1 β (IL-1 β +3953 C>T; rs1143634) and vitamin D receptor (VDR Taq1; rs731236) in 42 Caucasian patients with chronic periodontal disease and 39 Caucasian subjects, matched for age and gender.

Results. The IL-1 β C allele was present in 100% of cases and 92% of controls ($p=0.07$), the T allele was present in 19% of cases and in 44% controls ($p=0.017$). The prevalence of the VDR Taq1 tt genotype was lower in patients as compared with controls (i.e., 10 versus 59%; $p<0.01$), whereas the tT and TT genotypes were disproportionally higher in patients than in cases (i.e., 62 versus 33% for tT and 29% versus 8% for TT; $p<0.01$).

The t allele was present in 71% of cases and 92% of controls ($p=0.016$), whereas the T allele was present in 90% of patients with periodontal disease and in

41% controls ($p<0.01$).

Conclusion. The results of this case control study attest that the T allele of VDR Taq1 is strongly associated with periodontal disease, whereas the t allele of the IL-1 β +3953 confers a slightly protection against the risk of periodontitis.

Key words: periodontal diseases, periodontitis, genetics, interleukins, gene expression.

Introduction

Periodontal diseases encompass a variety of conditions affecting the periodontium. A simple classification of this condition has been agreed upon in 1999, during the International Workshop for a Classification of Periodontal Diseases and Conditions, and is now widely used by clinicians and research scientists worldwide (1).

Basically, this classification ranks periodontal disease and conditions into eight leading categories, that are gingival diseases (including dental plaque-induced gingival diseases, non-plaque-induced gingival lesions), chronic periodontitis (either localized or generalized), aggressive periodontitis (either localized or generalized), periodontitis as a manifestation of systemic diseases (i.e., associated with hematological disorders, genetic disorders, or not otherwise specified), necrotizing periodontal diseases (which can be either necrotizing ulcerative gingivitis or necrotizing ulcerative periodontitis), abscesses of the periodontium, periodontitis associated with endodontic lesions, as well as developmental or acquired deformities and conditions (1, 2).

According to the Research, Science and Therapy Committee of the American Academy of Periodontology, the prevalence of severe generalized periodontitis is comprised between 5% and 15% of the general population, although moderate disease may affect a majority of adults (2). Accordingly, the prevalence of gingivitis among school children in the US ranges from 40% to 60%, whereas 47% of males and 39% of females aged 18 to 64 exhibit at least one site which bled on probing (BOP). Although reliable data attests that relatively few sites with gingivitis progress to develop manifest periodontitis, a genetically determined response has been hypothesized, with non-smoking or former smoking, interleukin (IL)-1 genotype-positive individuals having greater risk of BOP than non-smoking and former smoking IL-1 negatives (3).

As specifically regards the pathogenesis, the old view of periodontitis as the outcome of infection is now overcome by the new concept that the disease would emerge from a complex interaction of several genetic

and environmental factors (4-6), including bacterial infection, host response and inflammation, with tobacco smoking, ageing, male gender and low socioeconomic status being considered other important risk factors (7, 8). The host response mediated by several cytokines is now regarded as a leading aspect in the clinical expression of periodontitis, and several inflammatory biomarkers have been consistently associated with periodontal disease, especially prostaglandin E2 (PGE2), tumor necrosis factor-alpha (TNF- α), Interleukin-1 alpha (IL-1 α), and Interleukin-1 beta (IL-1 β) (9, 10). Interestingly, only 20% of periodontal diseases is now attributed to bacterial variance, 20% to tobacco use, whereas 50% of disease expression has been attributed to genetic variance at several loci, including specific polymorphisms at the loci encoding for some cytokines (i.e., IL-1 α , IL-1 β , IL-6, TNF- α) (9), as well as for the vitamin D receptor (VDR) gene (10).

Although some studies (11) and meta-analyses (12, 13) are now available to attribute a certain role to these genetic determinants in the pathogenesis of periodontal disease, the outcome is often controversial. As such, the aim of this article was to investigate whether two polymorphisms in the genes encoding IL-1 β (i.e., IL-1 β +3953 C>T; rs1143634) and VDR (i.e., VDR Taq1; rs 731236) are associated with periodontal disease in a Caucasian population.

Materials and methods

The study population consisted in 42 Caucasian patients (27 females and 12 males, mean age of 60 \pm 9 years) with chronic periodontal disease diagnosed according to the well-established criteria of the International Workshop for a Classification of Periodontal Diseases and Conditions (1).

Accordingly, each of the 42 patients with chronic periodontal disease had severe and generalized form of periodontal disease, showing at least 5 sites with probing pocket depth (PPD) >6 mm located in different teeth and distributed among the four quadrants, BOP and pus. The control group consisted in 39 Caucasian subjects matched for age and gender (30 females and 9 males, mean age 56 \pm 15 years) not affected by periodontal disease, i.e., with no history of periodontal disease, without periodontal pockets >3 mm and radiographic evidence of bone loss (Tab. 1).

All the subjects were enrolled in the study after signing an informed consent form, and the experimental protocol was accepted by the ethical committee.

No case or control subject was affected by systemic diseases that are known to influence development or progression of periodontal disease (e.g., diabetes), or was pregnant, currently smoking or using anti-inflammatory drugs. Each patient signed the informed consent to be included in the study, which was carried out in accordance with the Declaration of Helsinki, and under the terms of all relevant local legislation. Genomic testing was carried out after collecting material with a sterile foam tipped applicator. The DNA was extracted using a commercial kit purchased from Qiagen (Qiagen GmbH,

Table 1. Leading characteristics of the study population. Values are shown as mean \pm standard deviation.

PPD, probing pocket depth; BOP, bleeding on probing.

	Chronic periodontal disease	Healthy controls
n	42	39
Age (yrs)	60 \pm 9	55 \pm 15
Gender, females (%)	27 (64%)	30 (77%)
PPD (mm)	4,17	1,34
Presence of PUS (%)	58	0
BOP (%)	99	26

Hilden, Germany). Allelic analysis was performed using Taq Man1 SNP Genotyping Assays rs731236 and rs1143634 (Applied Biosystems, Forster City, CA, USA), which are based on a predesigned mix of unlabeled polymerase chain reaction (PCR) primers and the Taq Man[®] minor groove binding group (MGB) probe (FAM[™] and VIC[®] dye-labeled), on the instrument Step One (Applied Biosystems). Thermocycler conditions were an initial 35 s denaturation at 95 $^{\circ}$ C, followed by 40 cycles of 95 $^{\circ}$ C for 10 s and 60 $^{\circ}$ C for 45 s. The significance of differences between cases and controls was evaluated with Chi-Square test using Analyse-it for Microsoft Excel (Analyse-it Software Ltd, Leeds, UK).

Results

The results of IL-1 β +3953 are shown in Figure 1. A significant difference was observed in the overall distribution of C/T polymorphisms (Pearson Chi-Square = 7.106; DF=2; p=0.029), in that patients with periodontal disease exhibited a higher prevalence of the CC genotype (81 versus 56%), whereas the TT genotype was present in 8% of controls but in none of the cases (Fig. 1). The heterozygous allele CT was present in 19% of cases versus 36% of controls. Globally, the C allele was present in 100% of cases and 92% of controls (Pearson Chi-Square = 3.36; DF=1; p=0.07), whereas the T allele was present in 19% of patients with periodontal disease and in 44% without (Pearson Chi-Square = 5.71; DF=1; p=0.017).

An even more statistically significant associations was however found for the VDR Taq1 polymorphism (Fig. 2) (Pearson Chi-Square = 23.024; DF = 2; p<0.01), in that the prevalence of the tt genotype very low in patients with periodontal disease as compared with controls (i.e., 10 versus 59%), whereas the tT and TT genotypes were disproportionately higher in patient than in cases (i.e., 62 versus 33% for tT and 29% versus 8% for TT). Overall, the prevalence of the t allele was present in 71% of cases and 92% of controls (Pearson Chi-Square = 5.84; DF=1; p=0.016), whereas the T allele was present in 90% of patients with periodontal disease and in 41% controls (Pearson Chi-Square = 22.25; DF=1; p<0.01).

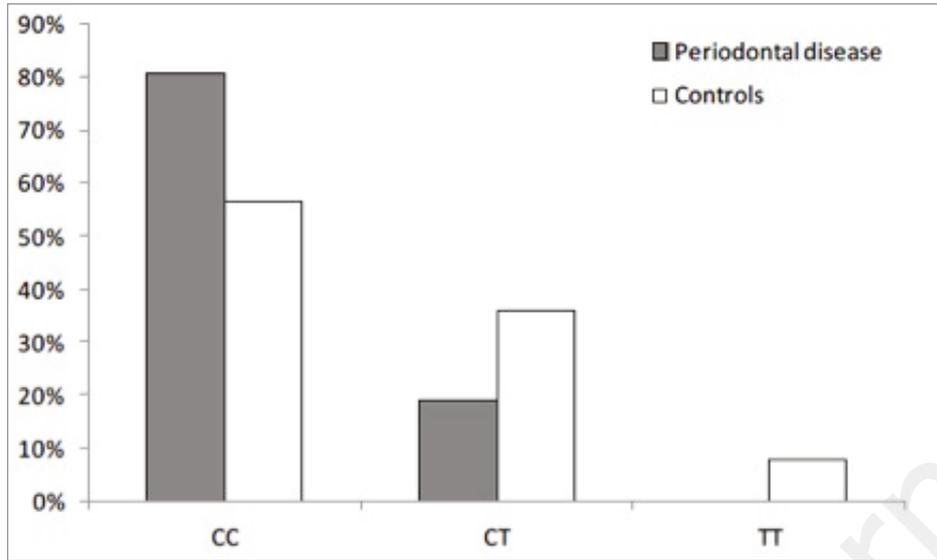


Figure 1. Results for IL-1 β +3953.

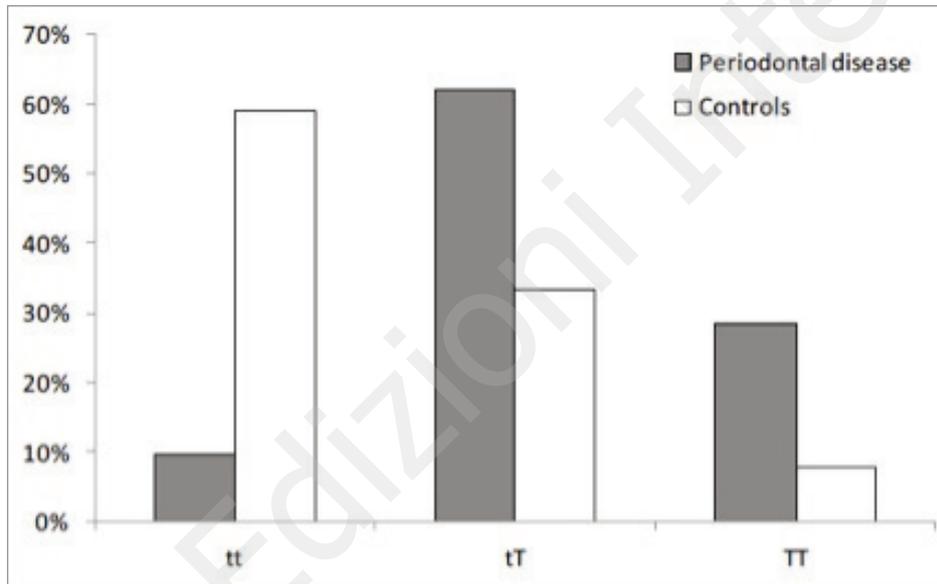


Figure 2. Results for VDR Taq1.

Discussion and conclusion

Periodontal disease is a public health problem afflicting the vast majority of adults in mild to moderate forms and being associated with important clinical implications not only for dental medicine, but also for its causal relationship with a variety of other important disorders, namely cardiovascular disease (13, 14). Recent and remarkable advances in our understanding of the pathogenesis of periodontal disease have led to a radical approach for prevention and treatment, which now deeply involve the assessment of personal susceptibility. The outcome of several epidemiological investigations have allowed to identify a variety of genetic polymorphisms that may be associated with periodontal disease (7, 15), but the heterogeneity of the studies, the different diagnostic criteria as well as the prevalent enrolment of

Asian populations did not allow to draw definitive conclusions on this topic.

The results of our investigation, which were limited to the two genetic polymorphisms for which more solid evidence are available (i.e., IL-1 β +3953 and VDR Taq1), attest that patients who are more prone to develop chronic periodontitis may have an important genetic predisposition.

This finding may have important clinical implications in terms of genotype-based risk assessment, early prevention of predisposed individuals (e.g., smoking and genetic interactions are important contributory factor in severity of periodontitis), and more aggressive treatment of patients at greater risk.

The two genotypes that have been assessed in this study exert different but synergic role in the pathogenesis of periodontal disease. The pivotal role of the IL-1

family of interleukins as mediators of inflammatory reactions has been well established. Both IL-1 α and IL-1 β exert similar proinflammatory activities, whereas IL-1 receptor antagonist (IL-1Ra) is an anti-inflammatory cytokine that counteract the former interleukins by binding to the IL-1 receptor (16).

Although some similarities exist between IL-1 α and IL-1 β , the latter is a different molecular form, which is also known as catabolin. IL-1 β exerts a kaleidoscope of biological functions; it is an important mediator of the inflammatory response and actively involved in several cellular activities such as cell proliferation, differentiation, and apoptosis. In a seminal article by Liu et al., the severity of periodontal disease (in terms of gingival index, probing depth and gingival crevicular fluid flow) was first associated with IL-1 β activity (17). Hou et al. also reported that the tissue concentration of IL-1 β determined from diseased gingivae were remarkably higher than those of controls from both healthy sites of periodontitis and non-periodontitis subjects (18), thereby confirming that IL-1 β plays a significant role in the pathogenic mechanisms of periodontal tissue destruction and that its assessment in periodontal tissue might be a valuable aid and useful diagnostic marker of disease.

The important role played by IL-1 β on periodontal disease has been attributed to the potency of inducing bone resorption and connective tissue destruction, paving the way to further studies assessing whether genetic polymorphisms in the IL-1 β gene might induce variations of cytokine levels in the periodontal tissue and thereby predispose to periodontitis. Nevertheless, conflicting results recently reviewed by Laine et al. were published, so that this polymorphism cannot be considered as yet a definitive risk factor for susceptibility to chronic periodontitis for the worldwide population (15). In a recent meta-analysis of 53 studies including 4178 cases and 4590 controls, Nikolopoulos et al. found a significant association of IL-1 β +3953 C>T polymorphism and chronic periodontal disease (9). In particular, the carriage of the T allele conferred a 45% relative increase in the risk for chronic periodontitis (Odds Ratio [OR]: 1.45, 95% CI: 1.13, 1.85) and more than doubled the hazard in populations of Asian origin (OR: 2.18, 95% CI: 1.22, 3.92). Both TT versus CT+CC and TT+CT versus CC contrasts showed evidence of an association between the IL-1 β 3953 C>T polymorphism and chronic periodontal disease (OR: 1.60, 95% CI: 1.11, 2.311, and OR: 1.50, 95% CI: 1.16, 1.93, respectively). An even stronger association was observed comparing carriers of TT or CT genotype with the CC homozygotes in Asian populations (OR: 2.42, 95% CI: 1.49, 3.94). A statistically significant heterogeneity was however found in these analyses, which hence decreased the overall statistical significance of the associations.

Overall the data of Nikolopoulos et al. in Asian populations is in disagreement with our findings in Caucasians, whereby the T allele was two times more prevalent in controls than in patients with periodontal disease (i.e., 19 versus 44%; $P = 0.017$).

Vitamin D plays a key role in bone metabolism. Alveolar bone is now considered a major characteristic of peri-

odontal disease, so that mediators of bone metabolism like the VDR and its genetic polymorphisms may play a role in the pathogenesis (19). Unfortunately, the potential mechanism/s linking VDR and its gene polymorphisms with periodontal disease are still largely obscure. Some hypothesis were postulated, including negative influences on bone mineral density, turnover, and bone loss (10, 20). In 2011 Deng et al. carried out a database search retrieving 15 studies involving 1338 cases and 1302 controls, and found that that chronic periodontitis cases had a weak significantly higher frequency of TT genotype of VDR Taq1 (OR = 1.86, 95% CI, 1.00, 3.46; $p = 0.049$) in Asians (10). These results are globally in agreement with those obtained in our case control study in a Caucasian population, since we observed that the prevalence of the T allele was more than twice in with patients with periodontal disease as compared with healthy controls (i.e., 90 versus 41% $P < 0.01$).

Taken together, the results of this case control study attest that the T allele of VDR Taq1 are strongly associated with periodontal disease, whereas the t allele of the IL-1 β +3953 confers a slightly protection against the risk of periodontitis.

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