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VDR TaqI polymorphism is associated with chronic periodontitis in Italian population

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ABSTRACT

Aim: The aim of this study is to investigate the relationship between a vitamin D receptor polymorphism and the diagnosis of periodontal disease in non-smoker Italian patients with aggressive and chronic periodontitis.

Materials and methods: DNA was obtained from the internal cheek mucosa of 115 patients with chronic periodontitis, 58 with aggressive periodontitis and 65 healthy controls. Allelic discrimination was performed using TaqMan® SNP Genotyping Assays. Genotype and allele frequencies were calculated.

Results: Comparisons between diseased patients and healthy controls showed significant differences. Moreover, calculating the odds ratio, individuals with the TT genotype, was more susceptible than individuals with tt to chronic periodontitis and individuals with Tt to aggressive periodontitis. Interestingly, the dominant model (TT + Tt vs. tt) was applicable to chronic periodontitis, whilst for aggressive periodontitis the recessive model (TT vs. Tt + tt) gave the highest odds ratio.

Conclusions: These data indicated that VDR TaqI polymorphism is differentially associated with development of chronic periodontitis and aggressive periodontitis in Italian population. The study of VDR polymorphisms may therefore be essential for the prevention of periodontitis and for a pre-treatment periodontal and/or for implant assessment. Moreover VDR TaqI polymorphism could be useful to discriminate between aggressive and chronic forms of periodontal disease.

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1. Introduction

Periodontitis is a common multifactorial disease characterised by inflammatory response to altered levels of subgingival pathogens, resulting in destruction of periodontal ligament, cement and bone, if left untreated can lead to tooth loss. The principal feature of bone-loss in periodontitis is enhanced osteoclast activity without a corresponding bone formation. Periodontal disease is traditionally classified as chronic (CP) or aggressive (AP) in relation to some clinical parameters such as the age of onset or detection, rate of progression, patterns of destruction, signs of inflammations and amount of plaque and calculus. However, clinical distinction between chronic and aggressive periodontitis is still not clear cut.3

The key point of periodontitis is the interaction of periodontal pathogens and host immune response is modulated by genetic and environmental factors involved in the inflammatory disease aetiology. Most genetic research in periodontitis has focused on gene polymorphisms that play roles in immunoregulation or metabolism, such as interleukin-1 family (IL-1), interleukin-6 (IL-6), interleukin-10 (IL-10) or tumour necrosis factor (TNF). Currently, the presence of interleukin genetic variations appears to identify individuals...
who are increased risk for more severe form of periodontitis and for a more difficult response to therapy.

Vitamin D receptor (VDR) gene has also been suggested as one of the candidate genes for genetic control of bone mass. The VDR gene is polymorphic at several sites; the BsmI, Apal, and TaqI polymorphisms are in strong linkage disequilibrium in Caucasians. These common polymorphisms in the gene may alter transcriptional activity and mRNA stability, and may be associated with circulating levels of 1,25(OH)2D3.5–7 The active form of vitamin D, 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] (calcitriol), is a key regulator of bone and calcium metabolism. In addition, 1,25(OH)2D3 is involved in cellular differentiation and immunity with a role in the induction of monocytes differentiation to macrophages and modulating macrophage responses preventing them from releasing proinflammatory cytokines and chemokines.5 Vitamin D exerting also direct modulatory effects on T-cells and B-cells functions.5–8

There are numerous studies confirming the association of VDR polymorphisms with several diseases such as osteoporosis, rheumatoid arthritis, cancer, diabetes and periodontitis.9–11 Several authors have looked for association between VDR polymorphisms and periodontitis at TaqI, BsmI, FokI and Apal restriction sites.3,12–17 However, the mode by which VDR gene polymorphism may influence susceptibility to periodontitis is not clear yet. In fact, the most studied VDR polymorphisms such as those shown by restriction enzymes TaqI, BsmI, Apal involve synonymous substitutions, leaving unchanged the protein sequence, whereas the FokI polymorphism may affect VDR functions by creating an alternative start codon.18 The VDR TaqI (−1056) is characterised by a single base transition (T < C) at codon 352 in exon 9 of the VDR gene that creates a TaqI restriction site. The resulting alleles are called “t” (TaqI site present) and “T” (TaqI site absent). The allele “t” correlates with increased transcriptional activity, mRNA stability, and high serum level of 1,25-D3.3 Several studies on VDR gene polymorphisms have found an association with periodontitis, however not always unconditionally. The carriage of VDR TaqI TT genotype and T allele was associated with periodontitis in Caucasian and Japanese patients,12–14 whilst the tt genotype and t allele are associated with aggressive periodontitis in Chinese subjects. VDR BsmI, FokI, and Apal polymorphisms showed no association with periodontitis as a single SNP but in different haplotype combination with the other VDR polymorphisms.15–17 Conversely, the study of VDR TaqI SNP may be associated with periodontal disease as a single polymorphism or in combination with other VDR polymorphisms which shows a significant linkage disequilibrium.3

The discrepancies between studies addressing genetic risks may be attributed to genetic heterogeneity population and to the small size of sample. For these reasons the aim of this study is to investigate the relationship between VDR TaqI polymorphism and the diagnosis and progression of periodontal disease in Italian patients with AP and CP. In particular, thanks to the large dataset presented we address the question if doubtful clinical cases of AP and CP patients could be associated to VDR TaqI polymorphism.

2. Materials and methods

2.1. Subject population

Samples of Italian untreated subjects from IRF in Microdentistry (Florence) were analysed. The study included 65 healthy subjects, 58 aggressive periodontitis and 115 chronic periodontitis patients. The healthy controls were at least 36 years old, without pocket depth >3 mm and they did not show radiographic evidence of bone loss and history of periodontitis. Clinical examination was performed using Florida Probe (www.florida-probe.com) a computerised periodontal probing that allows tests comparable over time and independent from the operator.

The group of 173 patients suffering from periodontitis shows a severe generalised form of the disease with at least 5 sites with PPD ≥ 6 mm located in different teeth and distributed amongst the four quadrant, BOP and PUS. The differential diagnosis of CP and AP was made according to the age of onset: subjects with CP were >50 years old; patients with AP were <36 years old and exhibited highly destructive forms of periodontitis (Table 1). Informed consent was obtained from all subjects.

2.2. Exclusion criteria

Exclusion criteria included localised periodontitis, history or current manifestation of systemic diseases that could affect the progression of periodontitis, chronic usage of anti-inflammatory drugs, smoke, diabetes, hepatitis, HIV infection, current pregnancy and lactation.

2.3. Sample collection

Sampling was carried out following the procedures reported in the kit (Genetic Periodontal Screening GPS Biomolecular Diagnostics, Italy) with a sterile foam tipped applicator that must be firmly rubbed, for about 2 min, on the patient’s internal cheek mucosa.

Table 1 – Demographic characteristics of study subjects.*

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 65)</th>
<th>Chronic periodontitis (n = 115)</th>
<th>Aggressive periodontitis (n = 58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>56.07 ± 20.88a</td>
<td>59.59 ± 8.74a</td>
<td>29.07 ± 5.77b</td>
</tr>
<tr>
<td>Females (%)</td>
<td>45 (69.23%)a</td>
<td>97 (84.35%)a</td>
<td>40 (68.96%)a</td>
</tr>
<tr>
<td>PPD (mm) (mean ± SD)</td>
<td>2.08 ± 0.35b</td>
<td>7.26 ± 1.13a</td>
<td>7.14 ± 1.02a</td>
</tr>
<tr>
<td>BOP</td>
<td>25.6%b</td>
<td>99.14%a</td>
<td>97.10%a</td>
</tr>
<tr>
<td>PUS</td>
<td>0.0% (n.a.)</td>
<td>57.76%a</td>
<td>62.32%a</td>
</tr>
</tbody>
</table>

* Mean value of age and PPD (±SD) and the percentage of females and smokers of Caucasian subjects are reported. SD, standard deviation. Different letters for the same row indicate statistically significant differences (P < 0.05) after one-way ANOVA; n.a., not applicable.
2.4. DNA extraction and genotyping

Genomic DNA was isolated by Biomolecular Diagnostic using commercial kit (QIAGEN). Allelic discrimination was performed using TaqMan® SNP Genotyping Assays (rs731236 functionally tested by Applied Biosystems) on the StepOne instrument (Applied Biosystems, Forster City, CA, USA) with 10 ng genomic DNA in 48 well plates. Thermocycler conditions were an initial 35 s denaturation at 95 °C, followed by 40 cycles of 95 °C for 10 s and 60 °C for 45 s.

2.5. Statistical analysis

One-way ANOVA was computed with Analyse-It 2.20 (Analyse-it Software, Ltd., http://www.analyse-it.com/). Tukey post hoc protection was used for inferring statistical significance of differences and of proportions. Genetic association analyses were conducted following already reported guidelines.20 The population genetic parameters of control, AP and CP groups were assessed by testing the Hardy–Weinberg equilibrium and genetic differentiation amongst groups by Fixation Index and pairwise $F_{ST}$ analysis with the software Arlequin ver. 3.5.1.21 Statistical significant for Hardy–Weinberg equilibrium was assessed by running exact tests using a Markov chain with length 1,000,000 and dememorisation steps 100,000, whilst for pairwise $F_{ST}$ by a permutation test with 1000 random permutation. The significance of the differences in the observed frequencies of Taq polymorphism in the control, CP and AP groups was also computed by Chi-square test ($\chi^2$) with Analyse-It 2.20 (Analyse-it Software, Ltd., http://www.analyse-it.com/). The risk associated with individual alleles or genotypes was calculated as the Conditional Maximum Likelihood Estimate (CMLE) of odds ratio22 with 95% confidence intervals (CI) by using OpenEpi 2.3 software suite23 and SPSS Ver 18.0. The dominant (TT + Tt vs. tt), recessive (TT vs. Tt + tt) and additive models (linear relation) were tested.

3. Results

3.1. Clinical data

The demographic and clinical characteristics of the population are presented in Table 1. The population recruited for our study is composed of non-smoker Italian individuals only, with a prevalence of females in all three groups. The frequencies of the alleles and genotypes are reported in Tables 2 and 3. Hardy–Weinberg equilibrium was assessed by running exact tests using a Markov chain with length 1,000,000 and dememorisation steps 100,000, whilst for pairwise $F_{ST}$ by a permutation test with 1000 random permutation. The significance of the differences in the observed frequencies of Taq polymorphism in the control, CP and AP groups was also computed by Chi-square test ($\chi^2$) with Analyse-It 2.20 (Analyse-it Software, Ltd., http://www.analyse-it.com/). The risk associated with individual alleles or genotypes was calculated as the Conditional Maximum Likelihood Estimate (CMLE) of odds ratio22 with 95% confidence intervals (CI) by using OpenEpi 2.3 software suite23 and SPSS Ver 18.0. The dominant (TT + Tt vs. tt), recessive (TT vs. Tt + tt) and additive models (linear relation) were tested.

### Table 2 – Conditional maximum likelihood estimates of odds ratio (OR) and 95% confidence interval (CI) of comparison between patients with chronic periodontitis and healthy controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases (chronic periodontitis) n = 115</th>
<th>Controls n = 65</th>
<th>OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dominant model TT + Tt vs. tt</td>
<td>Additive model Linear relation</td>
<td>Recessive model TT vs. Tt</td>
</tr>
<tr>
<td>t allele</td>
<td>88 (38.3%)</td>
<td>70 (53.9%)</td>
<td></td>
</tr>
<tr>
<td>T allele</td>
<td>142 (61.7%)</td>
<td>60 (46.1%)</td>
<td>1.78 (1.02–3.11)</td>
</tr>
<tr>
<td>tt genotype</td>
<td>14 (12.2%)</td>
<td>20 (30.8%)</td>
<td>3.18 (1.45–7.01)</td>
</tr>
<tr>
<td>Tt genotype</td>
<td>60 (52.2%)</td>
<td>30 (41.6%)</td>
<td>3.59 (1.62–7.55)</td>
</tr>
<tr>
<td>TT genotype</td>
<td>41 (35.6%)</td>
<td>15 (20.0%)</td>
<td>1.84 (0.93–3.76)</td>
</tr>
</tbody>
</table>

* OR: odds ratio in the cases compared to the control. $\chi^2$ of comparisons between patients with CP and healthy controls were 9.35 (P < 0.002) for alleles and 10.01 (P < 0.007) for genotypes.

b Reference category.

c Models are not applicable.

### Table 3 – Conditional maximum likelihood estimates of odds ratio (OR) and 95% confidence interval (CI) of comparison between patients with aggressive periodontitis and healthy controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases (aggressive periodontitis) n = 43</th>
<th>Controls n = 65</th>
<th>OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dominant model TT + Tt vs. tt</td>
<td>Additive model Linear relation</td>
<td>Recessive model TT vs. Tt</td>
</tr>
<tr>
<td>t allele</td>
<td>34 (39.5%)</td>
<td>70 (53.9%)</td>
<td></td>
</tr>
<tr>
<td>T allele</td>
<td>52 (60.5%)</td>
<td>60 (46.1%)</td>
<td>1.97 (1.27–3.06)</td>
</tr>
<tr>
<td>tt genotype</td>
<td>12 (27.9%)</td>
<td>20 (30.8%)</td>
<td>1.15 (0.49–2.75)</td>
</tr>
<tr>
<td>Tt genotype</td>
<td>10 (23.2%)</td>
<td>30 (41.6%)</td>
<td>2.71 (1.15–5.24)</td>
</tr>
<tr>
<td>TT genotype</td>
<td>21 (48.8%)</td>
<td>15 (20.0%)</td>
<td>3.15 (1.37–7.38)</td>
</tr>
</tbody>
</table>

* OR: odds ratio in the cases compared to the control. $\chi^2$ of comparisons between patients with AP and healthy controls were 4.25 (P < 0.04) for alleles and 8.89 (P < 0.01) for genotypes.

b Reference category.

c Models are not applicable.
healthy control groups as theoretical populations in genetic equilibrium, whilst for AP an excess of homozygosity was observed (Supplemental Material S1). Whilst analysing genetic differentiation on the basis of allele frequencies a significant Fixation Index was obtained ($F_{ST} = 0.025, P < 0.02$) and pairwise $F_{ST}$ comparisons indicated healthy control group as statistically differentiated from CP group. No statistical significant differences were on the contrary obtained for pairwise $F_{ST}$ comparisons between AP and CP and between healthy control and AP (Supplemental Material S2). $\chi^2$ of comparisons between diseased patients and healthy controls considering both alleles and genotypes gave similar results, showing a significant difference between the control and AP and CP groups. Calculating the odds ratio (OR), it was revealed that dominant and additive models produced higher values for CP (OR = 3.18 and 3.59, respectively) (Table 2), whilst for AP the highest value was shown under the recessive model (OR = 3.15) (Table 3). However possible biases cannot be completely excluded due to the excess of homozygosis within the AP group. For the T vs. t alleles, slightly higher OR was scored for CP than for AP.

4. Discussion

Periodontitis is a complex multifactorial disease. Periodontopathogens are essential for the initiation of disease but the amount of plaque and the species of bacteria do not necessarily correlate with periodontitis severity. Each person has an individual dose-dependent response to the bacterial load that determines the susceptibility to periodontitis. This is the reason of the increasing scientific interest on the role of genes and of their polymorphism in: (i) host immune responses to periodontitis, (ii) progression of the disease, and (iii) tissue destructive processes.

Amongst the large number of candidate gene polymorphisms investigated in relation to susceptibility to periodontitis, the vitamin D receptor (VDR) gene polymorphism is one of the most studied because it affects both bone metabolism and immune functions.⁵ Hennig and co-authors found an association between the carriage of the less-frequent VDR t allele (detected as polymorphism for TaqI restriction enzyme site) with an increased risk of developing localised, but not generalised, disease in Caucasian subjects.²⁴ Others studies associated the TT genotype and T allele with CP in Caucasian and Japanese patients.¹²,¹³ According to these results our study confirmed that VDR TaqI polymorphism is significantly associated with CP but also with AP. In particular, for CP dominant and additive models support the association of T allele (TT and Tt genotypes vs. tt) whilst for AP the recessive model (TT vs. Tt + tt) better explain the association. However, due to excess of homozygosis in AP group this result should be carefully considered. More studies are needed to better elucidate these findings. Our study also has some potential limitations. In particular we did not perform any sample size calculation of subject population, and there are missing data in clinical parameters such as plaque index and clinical attachment loss (CAL).

From the molecular aspect, the VDR TT genotype has been correlated with lower serum levels of vitamin D3.⁵ Low serum levels of vitamin D3 due to reduced activation of the provitamin D by UV radiation, to VDR polymorphism or to nutritional factors, are associated with increased circulating-reactive protein (CRP),¹⁶ with cardiovascular disease⁶ and with autoimmune diseases such as inflammatory bowel disease (IBD), rheumatoid arthritis (RA) and multiple sclerosis (MS).²⁷ Furthermore, vitamin D deficiency induces a decreased bone mineral density at skeletal level (osteoporosis) including maxilla and mandible with an increased alveolar porosity and more rapid alveolar bone resorption following invasion by periodontal pathogens. These evidence may explain a higher susceptibility to periodontitis of patients showing VDR TT genotype, hypothesising a more difficult host response to periodontopathogenic bacteria and to a marked bone loss. Unlike the effects of other gene polymorphisms associated with the development and progression of periodontal disease, such as IL-1, IL1RN, IL-6, that produce immediate consequences in the immune response, the metabolic effects produced by low serum levels of vitamin D are only noticeable over the years and therefore likely to be more associated with CP rather than AP, suggesting gene-dosage effect as a possible hypothesis for the differences between CP and AP in genotype’s susceptibility. For both forms the homozygous T condition is the most associated, but whilst for AP homozygous and heterozygous (Tt) individuals differentiate, for CP they behave similarly (dominant model). Gene dosage effect has previously been shown to be related to Down syndrome as well as several cancer diseases,²⁸,²⁹ but no association with periodontitis have been reported.

The determination of VDR polymorphisms may therefore be essential for the prevention of periodontitis through a mass screening from a very early age and for a pre-treatment periodontal and/or for implant assessment. In case of individual susceptibility (TT genotype), patients treated with vitamin D before surgery may reduce the risk of implant failure, whilst the administration during treatment may promote the response to periodontal treatment.³⁰

In conclusion, this is the first study in which the VDR TT/Tt/ tt genotypes show strong differential correlation with the development of CP and AP. This result could be useful to discriminate between aggressive and chronic forms of periodontitis, especially in the range 36–50 years in which clinical distinction between these two forms is still dubious.²

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Competing interest: None declared.
Ethical approval: Not required.

Appendix A

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.archoralbio.2011.06.012.

References


