Evaluation of IL6, IL10 and VDR alleles distribution in an Italian large sample of subjects affected by chronic periodontal disease

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Abstract
In recent decades, the role played by the immune response to bacteria in the pathogenesis of chronic periodontal disease (PD) has long been studied. Although from the clinical point of view, adequate oral hygiene is essential to ensure a satisfactory response of the host to infections, modulation of the reaction of immune system could be influenced by genetic factors. The aim of the present study was to investigate the distribution of alleles of polymorphisms relevant for chronic periodontitis in a sample of adult subjects affected by chronic PD. The present study was conducted with sample collected in Italian private practice offices from January 2013 to December 2017. The sample included 744 adult patients diagnosed with chronic periodontitis. The inclusion criteria were as follows: age > 18 years, diagnosis of chronic PD. The diagnosis of chronic periodontitis was based on the criteria established by the American Academy of Periodontology. No significant difference in allele distribution among patients from different Italian regions was found. Results, supporting absence of population heterogeneity for the investigated polymorphisms in Italy, suggest similar effect in periodontitis etiology.

Keywords
chronic periodontal disease (PD), genetic susceptibility, Italian population

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Introduction

Background
Periodontitis is represented by the loss of the supporting tissues of the teeth, characterized by a change in the composition of the bacterial biofilm (changing aerobic to anaerobic) that trigger the immune response.¹

In recent decades, the role played by the immune response to bacteria in the pathogenesis of chronic periodontal disease (PD) has long been studied. Antibodies are essential to avoid bacterial adherence and tissue colonization, improving bacterial phagocytosis and detoxifying bacterial toxins.²

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Although from the clinical point of view, adequate oral hygiene is essential to ensure a satisfactory response of the host to infections, especially for PD in immunosuppressed subjects, the exact role and the exact serum antibody concentration for the pathogenesis of periodontal tissue loss has not yet been fully understood.

Some genetic polymorphisms have been found associated with PD, although PD is considered multifactorial, therefore due to a combination of genetic factors and environmental factors (oral hygiene, cigarette smoking, diet, stress, etc.).

Research of genetic factors of PD have mainly focused on genes that modulate immune system reaction, such as genes coding for cytokines, cell-surface receptors, chemokines, enzymes and proteins related to antigen recognition. Cytokines, such as IL1A, IL1B, IL10 and IL6, were intensely investigated because were considered key factors that mediate the inflammatory process during periodontal disease. Because alveolar bone resorption is a key factor in PD, vitamin D receptor (VDR) has been considered as a possible periodontitis susceptibility factor.

Conflicting results have been obtained by different studies investigating genetic association between gene polymorphisms and PD. This could be related with different study design, sampling criteria or phenotype definition, but more likely could be due to low statistical power due to moderate sample size, low effect of risk allele or population heterogeneity. Indeed, allele frequency and allele risk may vary considerably among different populations and geographic areas.

The present study investigated the three gene polymorphisms that were found most consistently associated with chronic periodontitis in different populations and particularly in Italians. The incidence of risk genotypes of IL6, IL10 and VDR genes was evaluated among periodontitis patients from Northern, Central and Southern Italy. Considering that no evidence of genetic heterogeneity was found, data supported that the investigated polymorphisms could have similar diagnostic relevance in different Italian regions.

**Materials and methods**

The present study was conducted in different Italian private practice offices between January 2013 and December 2017. The sample included 744 patients all diagnosed with chronic PD.

The diagnosis in based on the same criteria that the American Academy and Periodontology has stabilized, for instance that the patient must have one site with probing depth and clinical attachment loss \( \geq 4 \) mm. The inclusion criteria were as follows: age \( \geq 18 \) years and chronic PD.

The exclusion criteria were medically compromised patients, patients who have been administered antibiotic or antimicrobial in the past 6 months, and pregnant and lactating mothers. According to the regional boundaries adopted by the Italian Institute of Statistics (Istat) (www.Istat.it/it/Archivio/regioni), the sample that included a total of 744 patients can be stratified in 195 patients from northern Italy (26.2%), 497 from central Italy (66.8%) and 52 of the South (6.9%) of Italy.

The tips of paper, left inside the periodontal pocket for 30 s, were then inserted into a sterile container and were transmitted for subsequent DNA extraction and analysis by polymerase chain reaction (PCR). Specimens included periodontal microflora but also a sufficient number of host cells that enabled genetic profiling of patients. Genotyping was performed as previously described. The following polymorphisms were investigated: at IL6, the XR_108749.1:n.50-321G>C (rs1800795); at IL10, the NG_012088.1:g.4433A>C (rs1800872); at VDR, the NM_000376.2:c.1056T>C (rs731236).

Genotyping were performed by an ABI PRISM 7500 Sequence Detection System and TaqMan chemistry according to manufacturer protocols (Applied Biosystems, Foster City, CA).

SPSS program was used for statistical analysis to evaluate the geographic distribution of variant allele carriers in \( 2 \times 2 \) and \( 2 \times 3 \) contingency tables. A level of significance of 5% was used in the study.

This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee no. 29579 University Study of L’Aquila.

**Results**

The sample study included 744 subjects; genotypes of IL6, IL10 and VDR polymorphisms were obtained from each one of the samples.

The number of patients stratified by risk genotypes observed in the three different geographic
areas, Northern, Central and Southern of Italy, were shown in Table 1. No differences among the groups from different geographic areas were observed for IL6 \((P=0.79)\), IL10 \((P=0.54)\) and VDR \((P=0.07)\). The high risk TT genotype at VDR gene appeared rarer in samples collected in Southern Italy, although this deviation was not significant.

### Discussion

PD, over the years, has been considered as an inflammation of the chronic oral cavity, with gingival inflammation, destruction of periodontal tissues, loss of alveolar bone and with it early loss of the dental elements.\(^6\) Several cytokines of the immune system are implicated in the pathogenesis of the disease: interleukin-1a and b (IL-1a and IL-1b) and the transforming growth factor beta.\(^6\) These cytokines have been shown to have a function in the digestion of collagen and in the remodelling of periodontal tissues. Considering the functional role of these protein and their possible role in periodontitis etiology, several polymorphisms of candidate genes have been studied.

Evidence of association with periodontitis was obtained in Italian population for alleles of IL6, IL10 and VDR genes.\(^7\) These polymorphisms assumed a potential relevance as diagnostic and prognostic factors for PD. In the present investigation, an independent sample of 744 Italian PD patients was investigated for the same polymorphisms. Considering the high level of genetic heterogeneity observed among different population detected so far, the aim of the study was to check whether genetic heterogeneity was evident among different Italian regions. The comparison of variant allele carrier prevalence between patients from Northern, Central and Southern Italy showed no significant differences. This suggest that the diagnostic relevance of the polymorphisms IL6, IL10 and VDR genes is comparable in the different Italian regions.

Recently, additional evidence of association between genetic polymorphisms and PD have been obtained. Indeed, alleles of IL6 increased the risk of PD in Brazilian and Turkey population.\(^{15,16}\) The role of IL6 was supported also by meta-analysis of previous publications.\(^{17,18}\) Four recent meta-analysis reached similar conclusion supporting IL10 polymorphisms as possible biomarkers for PD.\(^{19–22}\)

The effect of VDR appeared less consistent among different populations.\(^{23,24}\)

Although increasing number of papers reported association between genetic polymorphisms and PD, the way for their use for diagnostic or prognostic purposes appeared full of obstacles. Indeed, PD can be considered a complex, multifactorial disease, with a strong environment influence, as well as polygenic component. Indeed, different factors, such as oral hygiene level, diet, smoke and alcohol consumption, age, gender, among others, make it difficult to evaluate the level of risk provided by different genotypes. A limitation of the present study was the absence of a control population of unaffected patients. This hampered genetic association testing with PD. On the

### Table 1. Observed variant allele distribution in different Italian regions.

| Polymorphism | Risk | Genotypes | Amount | Italy | Total | P |
|--------------|------|-----------|--------|-------|-------|---|
|              |      |           |        | Northern | Central | Southern | |
| IL6 rs1800795 | Higher | GG | n | 233 | 97 | 25 | 583 | 0.79 |
|              | Lower | GC/CC | n | 264 | 98 | 27 | 161 | |
| IL10 rs1800872 | Higher | CA/AA | n | 234 | 101 | 25 | 360 | 0.54 |
|              | Lower | CC | n | 263 | 94 | 27 | 384 | |
| VDR rs731236 | Higher | TT | n | 163 | 70 | 10 | 242 | 0.07 |
|              | Lower | TC/CC | n | 334 | 125 | 42 | 502 | |
contrary, the main goal of the investigation was to test for genetic heterogeneity of potential PD polymorphisms in Italy. The present study support that no difference in the allelic distribution of IL6, IL10 and VDR is detected in three Italian geographical areas.

Declaration of conflicting interests
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References
1. Marchetti E, Monaco A, Procaccini L, et al. (2012) Periodontal disease: The influence of metabolic syndrome. Nutrition & Metabolism (London) 9(1): 88
2. Ebersole JL (1990) Systemic humoral immune responses in periodontal disease. Critical Reviews in Oral Biology & Medicine 1(4): 283–331.
3. Gherlone EF, Cappare P, Tecco S, et al. (2016) Implant prosthetic rehabilitation in controlled HIV-Positive patients: A prospective longitudinal study with 1-year follow-up. Clinical Implant Dentistry and Related Research 18(4): 725–734.
4. Lauritano D, Candotto V, Bignozzi CA, et al. (2018) Zinc plus octenidine: A new formulation for treating periodontal pathogens. A single blind study. Journal of Biological Regulators and Homeostatic Agents 32(2 Suppl. 1): 231–236.
5. Laine ML, Loos BG and Crielaard W (2010) Gene polymorphisms in chronic periodontitis. International Journal of Implant Dentistry 2010: 324719.
6. Ranney RR (1992) Differential diagnosis in clinical trials of therapy for periodontitis. Journal of Clinical Periodontology 63(Suppl 12S): 1052–1057.
7. Scapoli L, Girardi A, Palmieri A, et al. (2012) IL6 and IL10 are genetic susceptibility factors of periodontal disease. Journal of Dental Research (Isfahan) 9: S197–S201.
8. Brett PM, Zyngianni P, Griffiths GS, et al. (2005) Functional gene polymorphisms in aggressive and chronic periodontitis. Journal of Dental Research 84: 1149–1153.
9. Mashhadiaabas F, Neamatdezeh H, Nasiri RF, et al. (2018) Association of vitamin D receptor Bsml, TaqI, FokI, and Apal polymorphisms with susceptibility of chronic periodontitis: A systematic review and meta-analysis based on 38 case-control studies. Journal of Dental Research (Isfahan) 15: 155–65.
10. Scapoli L, Girardi A, Palmieri A, et al. (2015) Interleukin-6 gene polymorphism modulates the risk of periodontal diseases. Journal of Biological Regulators & Homeostatic Agents 29: 111–116.
11. Lauritano D, Scapoli L, Mucchi D, et al. (2016) Infectogenomics: Lack of association between VDR, IL6, IL10 polymorphisms and red complex bacterial load in a group of Italian adults with chronic periodontal disease. Journal of Biological Regulators & Homeostatic Agents 30: 155–160.
12. Tecco S, Grusovin MG, Sciara S, et al. (2018) The association between three attitude-related indexes of oral hygiene and secondary implant failures: A retrospective longitudinal study. International Journal of Dental Hygiene 16(3): 372–379.
13. Giuca MR, Pasini M, Tecco S, et al. (2014) Levels of salivary immunoglobulins and periodontal evaluation in smoking patients. BMC Immunology 15: 5.
14. van der Zee E, Everts V and Beertsen W (1997) Cytokines modulate routes of collagen breakdown: Review with special emphasis on mechanisms of collagen degradation in the periodontium and the burst hypothesis of periodontal disease progression. Journal of Clinical Periodontology 24(5): 297–305.
15. Farhat SB, de Souza CM, Braosi AP, et al. (2017) Complete physical mapping of IL6 reveals a new marker associated with chronic periodontitis. Journal of Periodontal Research 52: 255–261.
16. Toker H, Gorgun EP and Korkmaz EM (2017) Analysis of IL-6, IL-10 and NF-kB gene polymorphisms in aggressive and chronic periodontitis. Central European Journal of Public Health 25: 157–162.
17. Zhu J, Guo B, Fu M, et al. (2016) Interleukin-6-174G/C polymorphism contributes to periodontitis susceptibility: An updated meta-analysis of 21 case-control studies. Disease Markers 2016: 9612421.
18. Zhao B and Li R (2018) The association between periodontitis and interleukin-6 genetic polymorphism -174 G/C: A meta-analysis. Archives of Oral Biology 96: 13–20.
19. Zhang Z, Zheng Y and Li X (2018) Interleukin-10 gene polymorphisms and chronic periodontitis susceptibility: Evidence based on 33 studies. Journal of Periodontal Research.
20. Li Y, Feng G, Deng Y, et al. (2018) Contribution of Interleukin-10-592 (-590, -597) C>A polymorphisms to periodontitis susceptibility: An updated meta-analysis Based on 18 case-control studies. Disease Markers 2018: 2645963.
21. Yang SL and Huang SJ (2019) Interleukin-10 polymorphisms (rs1800871, rs1800872 and rs1800896) and periodontitis risk: A meta-analysis. Archives of Oral Biology 97: 59–66.
22. Wong HC, Ooi Y, Pulikkotil SJ, et al. (2018) The role of three interleukin 10 gene polymorphisms
(-1082 A > G, -819 C > T, -592 A > C) in the risk of chronic and aggressive periodontitis: A meta-analysis and trial sequential analysis. BMC Oral Health 18: 171.

23. Ji XW, Wang Y, Cao C, et al. (2016) Assessment of the link between Vitamin D receptor TaqI gene polymorphism and periodontitis: A meta-analysis in a Chinese population. Genetics and Molecular Research 15.

24. Cai T, Yang ZY, Nie L, et al. (2017) Association between vitamin D receptor BsmI gene polymorphism and periodontitis: A meta-analysis in a single ethnic group. Cellular and Molecular Biology (Noisy-le-grand) 63: 1–4.