

# 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.<sup>1</sup>

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.<sup>2</sup>

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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,<sup>3</sup> 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.).<sup>4</sup>

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.<sup>5-7</sup> Because alveolar bone resorption is a key factor in PD, vitamin D receptor (VDR) has been considered as a possible periodontitis susceptibility factor.<sup>8,9</sup>

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.<sup>7</sup> 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 is 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  $> 18$  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](http://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 30s, 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.<sup>10,11</sup> 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

**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
			% in region	47%	50%	47%	48%	
	Lower	GC/CC	n	264	98	27	161	
			% in region	53%	50%	53%	52%	
IL10 rs1800872	Higher	CA/AA	n	234	101	25	360	0.54
			% in region	47%	52%	48%	48%	
	Lower	CC	n	263	94	27	384	
			% in region	53%	48%	52%	52%	
VDR rs731236	Higher	TT	n	163	70	10	242	0.07
			% in region	33%	36%	18%	33%	
	Lower	TC/CC	n	334	125	42	502	
			% in region	67%	64%	82%	67%	

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.<sup>12</sup> 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.<sup>6,13,14</sup> 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.<sup>7</sup> 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.<sup>15,16</sup> The role of IL6 was supported also by meta-analysis of previous publications.<sup>17,18</sup> Four recent meta-analysis reached similar conclusion supporting IL10 polymorphisms as possible biomarkers for PD.<sup>19–22</sup>

The effect of VDR appeared less consistent among different populations.<sup>23,24</sup>

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

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

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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