IL-18 gene promoter polymorphisms are only moderately associated with periodontal disease in Italian population

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Summary

Objective. **The aim of this study was to determine the impact of the polymorphisms at position -607 (C/A) and -137 (G/C) in the promoter of the IL-18 gene and their haplotypes, on the individual susceptibility of developing Aggressive (AgP) and/or Chronic (CP) periodontitis.**
Materials and methods. **A total of 213 unrelated Italian subjects with periodontitis (AgP=109 and CP=104) and 100 periodontal-health subjects were studied. IL-18 gene promoter polymorphisms were analyzed by TaqMan® SNP Genotyping Assays. Genotype and allele frequencies were analyzed using the chi-square test and multiple logistic regression analysis.**
Results. **χ^2 of comparisons between diseased patients and healthy controls indicated a significant differentiation between the control and AP and CP groups (χ^2=26.359, P<0.02). Interestingly, genotypes AACG, AACC and AACG have a moderate association with AgP and CP. For alleles, multiple logistic regression analysis showed that the polymorphism CG at position -137 is moderately associated with AgP (ExpB=2.880), while the polymorphism AA at position -607 is moderately associated with CP (ExpB=2.076). Finally, a moderate association of CA at position -607 (ExpB=2.099) with the healthy status compared to aggressive periodontitis was found.**
Conclusions. **Results obtained indicated the presence of some potential moderate protective and moderate susceptible alleles and genotypes to both aggressive and chronic periodontitis, demonstrating that IL-18 -607 C/A and -137 G/C gene promoter polymorphisms are not suitable diagnostic features for AgP and CP.**

KEY WORDS: periodontitis; IL-18; inflammation.

Introduction

Periodontal disease afflicts about 60% of Italian population and its increasing prevalence in the world had contributed to insert the development of global policies in oral health promotion and oral disease prevention among the primary prevention programs of the World Health Organization (1). Periodontitis is defined as a multifactorial inflammatory disease of the periodontium caused by predominantly Gram-negative, anaerobic and microaerophilic bacteria that colonize the subgingival area. If the infection continues unchecked, results in a progressive loss of clinical attachment due to destruction of the periodontal ligament with loss of the adjacent supporting bone and eventually tooth loss. In the etiopathogenesis of periodontal disease, the microbial infection is essential in the initiation and progression of periodontitis but bacteria alone are insufficient to cause the disease, the individual level of susceptibility or resistance to periodontitis is influenced also by both environmental factors (e.g. smoking, oral hygiene) and host immune response genetically determined. Several studies support the important role of many candidate genes that may act as disease modifying genes altering the pathogenesis and progression of periodontitis. The host-derived pro-inflammatory cytokines (e.g. IL-1, IL-6, TNFα) produced by periodontal tissues results in destruction of structural components of the periodontum leading to the clinical signs of periodontitis. Among the factors that can modulate the host immune response the cytokine IL-18, also known as IFN γ–inducing factor, is an important regulator of innate and acquired immune responses and has multiple roles in chronic inflammation and autoimmune disorders. IL-18 is a member of IL-1 cytokine family, a pleiotropic cytokine expressed at relatively high levels in different cell types such as macrophages, osteoblasts, monocytes, macrophages, keratinocytes, intestinal epithelial cells, astrocytes, dendritic cells and microglia (2-4). IL-18 is a 18 kDa glycoprotein synthesized as a 23 kDa inactive precursor (pro-IL-18) that needs to be cleaved by caspase-1 in order to become an active cytochine (5). IL-18 has strong pro-inflammatory properties, based on its ability to promote the secretion of other pro-inflammatory cytokines like TNFα, IL-1β, IL-8, and GM-CSF enhancing expansion, migration, and activation of neutrophils during infections (6). Its expression can be induced and up-regulated in response to stimuli, infectious and inflammatory, such as LPS, exotoxins from gram-positive bacteria or other cytokines (e.g. IL-1, TNF, IL-6, IL-10) (7, 8). Recent findings had confirmed as IL-18 might participate in the bone and cartilage destruction favoring the induction of osteoclast formation and bone resorption in rheumatoid arthritis (9, 10). A great number of study about polymorphisms in pro or anti-inflammatory cytokine genes described an association with periodontal disease (11, 12). The aim of this case control study was to determine the impact of the polymorphisms at position -607 (C/A) and -137 (G/C) in the promoter of the IL-18 gene, on the individual susceptibility of developing Aggressive and/or Chronic periodontitis in groups of Italian patients.

Materials and methods

Subject population

Samples of Italian untreated subjects from IRF in Microdentistry

(Florence, Italy) were analysed. The study included 100 healthy subjects, 109 aggressive periodontitis and 104 chronic periodontitis patients. The healthy controls were at least 36 years old, without pocket depth >3mm and they did not show radiographic evidence of bone loss and history of periodontitis. Clinical examination was performed using Florida Probe (Florida Probe Corporation, USA) a computerized periodontal probing that allows tests comparable over time and independent from the operator. The group of 213 patients suffering of periodontitis shows a severe generalized form of the disease with at least 5 sites with PPD≥6mm located in different teeth and distributed amongst the four quadrants, REC, 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. Informed consent was obtained from all subjects.

Exclusion criteria

Exclusion criteria included localized 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.

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 two minutes, on the patient's internal cheek mucosa.

DNA extraction and Genotyping

Genomic DNA was isolated by Biomolecular Diagnostic using QIAxtractor (Qiagen, Germany) according to the manufacture instruction's. Allelic discrimination was performed using TaqMan® SNP Genotyping Assays (rs187238 and rs1946518 functionally tested by Applied Biosystems, USA) on the Rotor-GeneQ instrument (Qiagen, Germany) using 10 ng genomic DNA and QuantiFast Probe PCR Kit (Qiagen, Germany) in PCR tubes. The 2-step cycling conditions were an initial 3' PCR initial heat activation at 95°C, followed by 40 cycles of 95°C for 3s and 60°C for 30s.

Statistical analysis

The significance of the differences in the observed frequencies of IL-18 polymorphisms in the control, CP and AP groups was also computed by Chi-square test (χ^2) and the risk associated with individual alleles or genotypes was as calculated by performing a multiple logistic regression analysis with software SPSS Ver 18.0. The exponentiation of the B coefficient [Exp(B)] indicates the odds ratio.

Results

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.

χ^2 of comparisons between diseased patients and healthy controls considering genotypes indicate a significant differentiation between the control and AgP and CP groups (χ^2=26.359, P<0.02), while single alleles did not allow to show significant difference between the control and AgP and CP groups (Table 2). Calculating the odds ratio (OR), it was revealed that genotypes AACG for AgP (ExpB=2.400) and AACC and AACG for CP (ExpB=2.118) have a moderate association with AgP and CP (Table 3). For alleles (Table 4), multiple logistic regression analysis showed that the polymorphism CG at position -137 is moderately associated with AgP (ExpB=2.880), while the polymorphism AA at position -607 is moderately associated with CP (ExpB=2.076). Concerning a potential protective role of IL-18 promoter polymorphisms (Table 5), we detected a moderate association of CA at position -607 (ExpB=2.099) with the healthy status compared to aggressive periodontitis. No association for chronic periodontitis were found.

Discussion

The present study tests for the first time the possible role of IL-18 gene promoter polymorphisms -607 C>A and -137 G>C in defining the individual susceptibility to develop chronic or aggressive periodontitis in italian untreated periodontal patients. The host's systemic immune status and environmental factors (e.g. smoking and oral hygiene), affect critically on the immune response in the periodontum, influencing the nature of the inflammatory response against periodontopathogens. Population molecular genetic studies had identified numerous genes and gene variations that potentially influenced the individual susceptibility to periodontal disease (IL-1, IL-2, IL-6, IL-10, VDR) (13). The IL-18 is a pleyotropic citokine involved in the amplification of the host inflammatory response and recently the over production of IL18 has been associated in inflammatory disorders and autoimmune diseases (14, 15), such as multiple sclerosis (16), diabetes mellitus, rheumatoid arthritis, Crohn's disease (17). IL-18 acts with IL-12 in a synergistic fashion to stimulate the release of interferon-gamma (IFN-γ) from lymphocytes (18). High serum levels of IL-18 are associated with increased production of the pro-inflammatory cytokine TNF-α (19) and decreased production of the anti-inflammatory cytokine IL-10 (20). Recent studies find also a significant correlation between the main bacteria involved in periodontal disease and IL-18. In particular, *P. gingivalis* specifically stimulates the production and release of the active form of IL-18 (21), while in the monocytes/macrophages, the leukotoxin produced by A. actino-

Table 1 - Demographic characteristics of study subjects*.

	Control (n=100)	Chronic Periodontitis (n=104)	Aggressive Periodontitis (n=109)
Age (mean±SD)	56.07±20.88 a	63.24±8.77 a	27.69±5.14 b
Females (percentage)	55(55%) a	73 (70.19%) a	59(54.21%) a
PPD (mm) (mean±SD)	2.08±0.35 b	7.54±1.30 a	7.38±1.08 a
BOP	2.6% b	99.18% a	100% a
PUS	0.0 (n.a.)	61.43% a	54.60% a
REC(mean±SD)	1.2±0.4mm	2.6±0.9mm	2.2±0.5mm

* Mean value of age, probing pocket depths (PPD) and gingival recession REC (±SD) and the percentage of females, bleeding on probing (BOP) and suppuration (PUS) are reported. SD, standard deviation. Different letters for the same row indicate statistically significant differences (P<0.05) after 1-way ANOVA; n.a., not applicable.

Table 2 - Contingency tables for IL-18 genotypes (A) and IL-18 polymorphisms at position -607 (B), and -137 (C).

A)		IL-18 Genotype								
		AACC	AACG	AAGG	CACC	CACG	CAGG	CCCG	CCGG	Total
Status	**AgP**	5	12	3	0	28	13	3	40	104
	CP	9	9	6	0	31	20	0	34	109
	Control	5	5	5	5	20	20	0	40	100
Total		19	26	14	5	79	53	3	114	313
Pearsons' χ^2						26.359 (P<0.02)				

B)		IL-18 SNP -607			
		AA	CA	CC	Total
Status	**AgP**	20	41	43	104
	CP	24	51	34	109
	Control	15	45	40	100
Total		59	137	117	313
Pearsons' χ^2		3.823 (P<0.4)			

C)		IL-18 SNP -137			
		CC	CG	GG	Total
Status	**AgP**	5	43	56	104
	CP	9	39	61	109
	Control	10	25	65	100
Total		24	107	182	313
Pearsons' χ^2		7.20 (P<0.12)			

Table 3 - Results of multiple logistic regression analysis on IL-18 genotypes with healthy controls as reference category.

Status			CI 95% for Exp(B)	
		Exp(B)	Lower	Upper
AgP	[genotype=AACC]	1.000	0.269	3.724
	[genotype=AACG]	2.400	0.774	7.441
	[genotype=AAGG]	0.600	0.134	2.681
	[genotype=CACG]	1.400	.680	2.882
	[genotype=CAGG]	.650	.285	1.482
CP	[genotype=AACC]	2.118	0.647	6.926
	[genotype=AACG]	2.118	0.647	6.926
	[genotype=AAGG]	1.412	0.396	5.036
	[genotype=CACG]	1.824	0.884	3.764
	[genotype=CAGG]	1.176	0.545	2.541
	[genotype=CCCG]	1.282	1.282	1.282

[a] Odds ratio are expressed as Exp(B) in the cases compared to the healthy control.
[b] Models are not applicable since this genotype is only present in AP group. Some genotypes are not reported since no individuals with these allelic status were detected.

Table 4 - Results of multiple logistic regression analysis on IL-18 polymorphisms at position -607 and -137 with healthy controls as reference category.

Status			CI 95% for Exp(B)	
		Exp(B)	Lower	Upper
AgP	[-607=AA]	0.987	0.346	2.819
	[-607=CA]	0.477	0.220	1.033
	[-137=CC]	0.563	0.143	2.208
	[-137=CG]	2.880	1.311	6.328
CP	[-607=AA]	2.076	0.765	5.634
	[-607=CA]	1.147	0.562	2.343
	[-137=CC]	0.584	0.180	1.895
	[-137=CG]	1.428	0.690	2.954

[a] Odds ratio are expressed as Exp(B) in the cases compared to the healthy control. The GG at -137 and CC at -607allelic status are not reported since no individuals with these allelic status were detected.

mycetemcomitans activates caspase-1, a cysteine proteinase, which causes a proinflammatory response by the activation and secretion of IL-1β and IL-18 (22). Scientific literature evidences some conflicting and limited results about the correlation between the salivary and serum concentration dosage of IL-18 and periodontitis. Two recent studies find IL-18 concentrations higher in saliva and in the gingival crevicular fluid of periodontal patients, suggesting that elevated IL-18 levels could be used as a biomarker for periodontal tissue destruction (23, 24). Conversely, the study of Türkoğlu shows no significant difference in the total amount of gingival crevicular fluid IL-18 among the healthy control group and chronic periodontal patients (25). Moreover, there is no association between periodontitis and plasma levels of IL-18 (26).
Giedraitis et al. postulated that two polymorphisms of the IL-18 gene promoter (-607 C/A, -137 G/C) have been predicted to be nuclear

Table 5 - Results of multiple logistic regression analysis on IL-18 polymorphisms at position -607 and -137 with aggressive periodontitis (a) and chronic periodontitis (b) as reference categories*.

Status			CI 95% for Exp(B)	
		Exp(B)	Lower	Upper
CPa	[-607=AA]	1.013	0.355	2.892
	[-607=CA]	2.099	0.968	4.549
	[-137=CC]	1.777	0.453	6.973
	[-137=CG]	0.347	0.158	0.763
AgPb	[-607=AA]	0.482	0.177	1.307
	[-607=CA]	0.871	0.427	1.779
	[-137=CG]	1.713	.528	5.560

* Odds ratio are expressed as Exp(B) in the cases compared to the aggressive periodontitis (a) and chronic periodontitis (b).

factor binding sites for the cAMP-responsive element-binding protein and the H4TF-1 nuclear factor, respectively. In particular, the carriage of the allele C at position -607 and the G allele at position -137 was associated with an higher transcription and protein production of IL-18 (27), but they also admit that the expression patterns observed for the SNP -137 were in actual fact not fully consistent (28).

Few studies are conducted to assess the putative role of different IL-18 gene polymorphisms in defining the individual susceptibility to the development and progression of periodontal disease. The literature is in accordance in rejecting the hypothesis that IL-18 variants, alone or in combination, could have a correlation with periodontitis. In particular, Folwaczny demonstrated that the distribution of haplotype combination for the IL-18 polymorphisms -607 and -137, showed not significant differences between the healthy control group and the periodontal patients (29). Our findings showed only a moderate association of the polymorphism CG at position -137 with AgP (ExpB=2.880), while the polymorphism AA at position -607 is moderately associated with CP (ExpB=2.076). Concerning a potential protective role of the allele C at position -607, we found indeed a moderate association with the healthy status with respect to aggressive periodontitis (ExpB=2.099). No evidence for protective alleles at -137 were found.

In conclusion, we suggest that IL-18 -607 C/A and -137 G/C gene promoter polymorphisms, though showing some extent of association, may not be a major genetic factor useful for determining individual susceptibility to develop periodontal disease in a sample of Italian population. However, further and larger studies are required to validate our findings.

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