



# Association of *SOCS1*<sup>-820</sup> (rs33977706) gene polymorphism with chronic periodontitis: A case–control study in Brazilians



Roger Antoniacci Guedes<sup>1</sup>, Aline Cristiane Planello<sup>1</sup>, Denise Carleto Andia, Naila F.P. De Oliveira, Ana Paula de Souza\*

Department of Morphology, School of Dentistry of Piracicaba, University of Campinas, Piracicaba, SP, Brazil

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## ABSTRACT

It is evident that the accumulation of periodontal pathogens over the teeth surface triggers periodontitis; however, its aggravation and severity depend on other elements such as environmental factors, systemic health and the host genetic and/or epigenetic background. To address this issue, we investigated the association of two genetic polymorphisms placed on promoter region of *SOCS1* gene with chronic periodontal disease. *SOCS1* regulates Jak/Kinase signaling pathway and changes in its mRNA expression have been related to different types of cancer and chronic inflammation, including chronic periodontitis. The frequency of alleles and genotypes of two polymorphisms in *SOCS1* gene promoter (position –820 (rs33977706) and position –1478 (rs33989964)) were analyzed by performing RFLP and TaqMan system in a total of 257 non-smoking subjects. We found a low frequency of A allele and A/A genotype of *SOCS1*<sup>-820</sup> polymorphism in the chronic periodontitis group, especially when severe periodontitis samples were separately analyzed (OR = 0.3933; *p* = 0.0084 (IC95% 0.2112 <  $\mu$  < 0.7324)), suggesting that A allele plays protective effect against chronic periodontitis. We did not find association between *SOCS1*-1478 polymorphism and periodontitis. In addition, analysis of *SOCS1*<sup>-820/-1478</sup> haplotype revealed that the frequency of A<sup>-820</sup>/CA<sup>-1478</sup> haplotype decreases in ChrP (*p* = 0.0089). In conclusion, our study found that *SOCS1*<sup>-820</sup> polymorphism is associated with chronic periodontitis.

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## 1. Introduction

As the second most frequent infection disease worldwide, chronic periodontitis represents an infection-mediated inflammation whose hallmark is the destruction of the connective tissue that attaches the surface of teeth roots to alveolar bone. In addition, chronic periodontitis has also been related to lethal diseases, such as endocarditis, stroke, myocardial infarction and atherosclerosis (Borrell and Papapanou, 2005; Chan et al., 2010; Chen et al., 2003; Davey et al., 2006; Egan et al., 2003).

The association between the presence of bacterial biofilm on teeth and oral mucosa surface (Federici et al., 2002) and periodontitis is clear. Besides, it is well known that environment factors, social habits, some drugs, systemic health and smoke influence the periodontitis prognosis. Likewise, a vast number of studies published in the last two decades have shown the importance of host genetic background as a key modulator of the response against the infection (Flemmig,

1999; Galm et al., 2003; Garlet et al., 2006; Gylvin et al., 2009; O-M et al., 2009).

Suppressor Of Cytokine Signaling (SOCS) represents a family of signaling intracellular proteins that take part in the immune-system regulation, modulating the signal transduction of several inflammatory cytokines, including IL-1 $\beta$ . At least eight members of SOCS family have been described: *SOCS1*, *SOCS2*, *SOCS3*, *SOCS4*, *SOCS5*, *SOCS6*, *SOCS7* and *CIS* (Yoshimura et al., 2007). Addressing the role of *SOCS1* to inflammation, its N-terminal domain contains inhibitory kinase-associated activity that strongly inhibits the JAK/STAT pathway (JAK (Janus kinase)/STAT (Signal Transducer and Activator of Transcription)) (Yoshimura et al., 2007, 2012). Recently, authors have reported that the TOLL-LIKE signaling may be modulated by *SOCS1*, affecting cell functions, cell differentiation and cell maturation Yoshimura et al., 2012.

*SOCS1* gene is a short sequence placed within chromosome 16. The coding sequence is composed by two exons that are regulated by a promoter region characterized by an extensive CpG island that covers the gene from its promoter until the end of the second exon. Downregulation of *SOCS1* has been observed in different types of cancer (Galm et al., 2003; Lee et al., 2006; Yoshikawa et al., 2001) and chronic inflammation (Yoshida et al., 2004). The macrophage deletion of *SOCS1* increased sensitivity to LPS in systemic inflammation (Sachithanandan

\* Corresponding author at: Department of Morphology, FOP-UNICAMP, Av. Limeira 901, CEP 13414-018, Piracicaba-SP, Brazil.

E-mail address: [anapaulapardo@fop.unicamp.br](mailto:anapaulapardo@fop.unicamp.br) (A.P. de Souza).

<sup>1</sup> These authors contributed equally to the study.

et al., 2011). SOCS1 has been shown to play a potential role in modulating periodontitis (Menezes et al., 2008) and it is down-regulated in chronic periodontitis when compared to chronic gingivitis (Garlet et al., 2006). A number of genetic polymorphisms have been described within SOCS1 gene. Two single nucleotide polymorphisms (SNP) placed at  $-820$  (rs33977706) and  $-1478$  (rs33989964) in the promoter region were shown to possess functional activity on gene transcription level. The SNP placed at  $-820$  creates the alleles C > A and the presence of A allele disrupts a cis-consensus sequence to YY1 transcription factor binding, a negative regulator of SOCS1 (Mostecky et al., 2011). Assay performed *in vitro* has demonstrated that A allele increases SOCS1 mRNA levels (Mostecky et al., 2011). The SOCS1<sup>-1478</sup> SNP represents a dinucleotide CA insertion/deletion that has been associated with respiratory immune-inflammatory diseases (Chan et al., 2010; Harada et al., 2007). There are no studies relating the functional role of SOCS1 polymorphisms with the susceptibility or severity of periodontitis. Thus, the aim of the study was to investigate the relationship between these SOCS1 polymorphisms with chronic periodontitis.

## 2. Material and methods

### 2.1. Ethics statement

The study was performed in accordance with the current recommendations of the National Health Council—Ministry of Health of Brazil for research in human subjects and with the approval of the Ethics Committee in Research of the School of Dentistry of Piracicaba—State University of Campinas, Unicamp. Written informed consent was obtained from all volunteers.

### 2.2. Study population

Subjects of both genders from the Southeastern region of Brazil were recruited from the patient pool of the Dental Clinics of the School of Dentistry of Piracicaba, State University of Campinas. Two hundred and fifty-seven (257) non-smoking subjects were included in this cross-sectional study and the demographic characteristics of the study population are presented in Tables 1 and 2. All subjects were in good general health and had at least 20 teeth in their mouth. Subjects did not have any of the following exclusion criteria: smoking, diseases of the oral hard or soft tissues (except caries and periodontitis), use of orthodontic appliances, need for pre-medication for dental treatment, chronic usage of anti-inflammatory drugs, a history of diabetes, hepatitis or HIV infection, immunosuppressive chemotherapy, history of any disease known to severely compromise immune function, presence of acute necrotizing ulcerative gingivitis, or current pregnancy or lactation.

Diagnosis and classification of generalized chronic periodontitis were performed based on the 1999 Consensus Classification of Periodontal Diseases (Armitage, 1999). The diagnostic examination considered the clinical parameters and consisted of physical examination, full medical and dental history, probing pocket depth (PPD), assessment of clinical attachment loss (CAL), tooth mobility and observation of gingival bleeding on probing and radiographic evaluation. Measurements of probing depth and attachment level were recorded

**Table 1**  
SOCS1<sup>-820</sup> locus: sample demographic dataset.

SOCS1 <sup>-820</sup>	Age		Gender		Ancestry (%)			
	Average	Men	Women	C	J	M	AA	
Control (N = 105)	37.28	38.78%	61.22%	82	1	9	8	
ChrP(M) (N = 86)	45.06	35.94%	64.06%	72	0	19	8	
ChrP(S) (N = 51)	44.78	28.21%	71.79%	67	0	28	5	

C—Caucasian; J—Japanese; M—Mulatto; AA—Afro-American.

Control—Healthy; ChrP(M)—Moderate Chronic Periodontitis; ChrP(S)—Severe Chronic Periodontitis.

**Table 2**  
SOCS1<sup>-1478</sup> locus: sample demographic dataset.

SOCS1 <sup>-1478</sup>	Age		Gender		Ancestry (%)			
	Average	Men	Women	C	J	M	AA	
Control (N = 111)	37.27	37.86%	61.17%	82	1	9	8	
ChrP(M) (N = 90)	45.95	33.85%	66.15%	76	0	16	8	
ChrP(S) (N = 56)	44.95	26.83%	73.17%	68	0	27	5	

C—Caucasian; J—Japanese; M—Mulatto; AA—Afro-American.

Control—Healthy; ChrP(M)—Moderate Chronic Periodontitis; ChrP(S)—Severe Chronic Periodontitis.

at six points around each tooth. The individuals of the control group were examined attending the same clinical parameters as used in the chronic periodontitis group. Clinical parameters were obtained from one calibrated examiner (intra-class correlation = 0.94):

Control: Healthy subjects did not show any sites with CAL and PPD  $\geq 3$  mm and bleeding in any quadrant; tooth mobility was not found; age over 25 years.

Generalized Chronic Periodontitis (ChrP): Patients with at least 30% of the sites are affected with CAL  $\geq 5$  mm; age over 35 years.

Chronic Periodontitis subgroups:

Moderate Chronic Periodontitis (ChrP(M)): Patients with at least 30% of the sites are affected with CAL  $\geq 5$  mm and  $\leq 7$  mm; age over 35 years.

Severe Chronic Periodontitis ChrP(S): Patients with at least 30% of the sites are affected with CAL  $> 7$  mm; age over 35 years.

### 2.3. Isolation of genomic DNA

DNA was collected from a mouthwash of oral cells as described by Trevisatto and Line, (2000). DNA was purified by the sequence of phenol/chloroform extraction and salt/ethanol precipitation. Following, DNA was dissolved in nuclease-free water and its concentration and quality were estimated by measuring absorbance at 280/260 nm and 280/230 nm, respectively (Nanodrop, Thermo).

### 2.4. Polymerase chain reaction (PCR) and genotyping (SNP $-820$ (rs33977706))

TaqMan System (Applied Biosystems by Life Technologies) was applied to investigate SOCS1<sup>-820</sup> polymorphism. PCRs were carried out in a total of 10  $\mu$ l, containing: 100 ng of gDNA, 5  $\mu$ l TaqMan Genotyping Master Mix, 0.5  $\mu$ l TaqMan genotyping assay mix 20 $\times$  and H<sub>2</sub>O DNase-free. PCRs were performed in a LightCycler 480 (Roche) under the following conditions: 10' at 95 °C, 40 $\times$  (15" at 95°, 1' at 60 °C). The VIC (HEX) (580 nm) discriminated the A allele while FAM (510 nm) discriminated the C allele.

### 2.5. Polymerase chain reaction (PCR) and genotyping (SNP $-1478$ (rs33989964))

A fragment of 250 bp of the SOCS1 gene promoter was PCR amplified with specific primer set (Table 3). PCR was carried out in a total volume of 12  $\mu$ l, containing 1  $\mu$ l genomic DNA (100 ng), 1.5  $\mu$ l of each primer and 6  $\mu$ l of Go Taq Green Master Mix (Promega Corporation, Madison, WI, USA). The solution was incubated under the following conditions:

**Table 3**  
SOCS1<sup>-1478</sup> primers set, amplicon size, and restriction endonuclease site.

Primer	Amplicon	Restriction enzyme
FOR—TGTCGTCCAGCTGCACCTC	250pb	<b>DdeI</b> C↓TNAAG...3' GANT↓C...5'
REV—ACCACAGGCTTCAGAGGAAC		

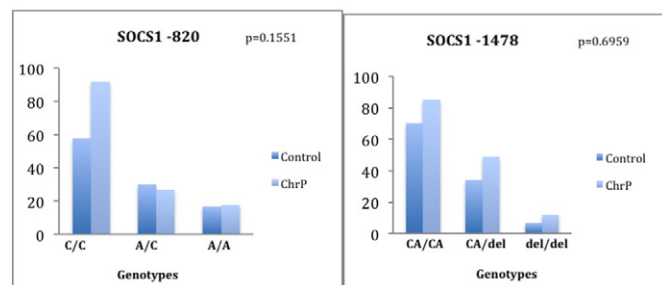
5 min. at 95 °C, 28× (1' at 95 °C, 1' at 60 °C, 1' at 72 °C), 7' at 72 °C. The restriction endonuclease digestion was prepared using 3 µl aliquot of *SOCS1* PCR products mixed with solution containing 1 µl 10 × NE Buffer (50 mM NaCl, 10 mM Tris–HCl, 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, pH 7.9), 0.20 µl *DdeI* (10,000 U/ml) (New England Biolabs, Inc., Beverly, MA, USA) and 5.8 µl sterile deionized H<sub>2</sub>O. The solution was incubated at 37 °C overnight. The *DdeI* restriction endonuclease cuts the CA(+) allele, while the CA(−) allele does not. The length difference of the RFLP fragments was 145 bp and 105 bp (CA(−) 250 bp; CA(−)/CA(+) 250 bp, 145 bp and 105 bp; CA(+) 145 bp and 105 bp). Aliquots of 5 µl of the RFLP products were electrophoresed on 10% vertical non-denaturing polyacrylamide gel at 20 mA. Gels were stained using nucleic acid gel stain (SYBR Gold, Eugene, Oregon, USA).

## 2.6. Statistical analysis

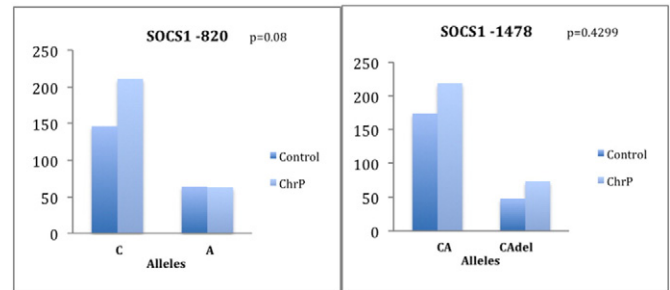
We evaluated Hardy–Weinberg equilibrium (HWE), linkage disequilibrium, and haplotype frequency estimation using ARLEQUIN 3.0 software and HAPSTAT 3.0 software (The statistical analysis of haplotype–disease association, University of North Carolina at Chapel Hill) (Excoffier et al., 2005; Lin et al., 2008). We also performed Chi-squared analysis in order to evaluate the genotype distribution and allele frequency in the groups using BIOESTAT 5.0 software (Instituto Mamiraua, Brazil) (M A et al., 2007). A p-value of less than 5% was taken to be statistically significant for all analyses. We verified the statistical power of our sample using G\*POWER 3.0.5 software (Faul et al., 2007). The input parameters were: moderate effect size = 0.3, based on genotype distribution data; statistical significance level  $\alpha = 0.05$ ; and one and two degrees of freedom for allelic and genotype analysis, respectively. The statistical power for samples was higher than 80% for association detection.

## 3. Results

Genotype distribution and allele frequency of *SOCS1*<sup>−1478</sup> and *SOCS1*<sup>−820</sup> SNPs were successfully performed in all subjects and the distribution of *SOCS1*<sup>−1478/−820</sup> polymorphisms results were found in Hardy–Weinberg equilibrium in the studied groups ( $\chi^2$ ,  $p > 0.05$ ). Significant differences were not found in genotypes and alleles distribution between groups for *SOCS1*<sup>−1478</sup> ( $\chi^2$ ,  $p = 0.6959$  and  $p = 0.4299$ , respectively) (Figs. 1 and 2). The same was also observed for *SOCS1*<sup>−820</sup> genotype distribution ( $\chi^2$ ,  $p = 0.1551$ ) (Fig. 1). However, *SOCS1*<sup>−820</sup> result was seen bordering significance when alleles distribution was analyzed between controls and periodontitis group ( $\chi^2$ ,  $p = 0.080$ ) (Fig. 2), with an increase of C allele in periodontitis group. Then, we performed the next analysis for *SOCS1*<sup>−820</sup> separating the moderate chronic periodontitis samples (CAL ≤ 7 mm) from the severe chronic periodontitis samples (CAL > 7 mm). Chronic periodontitis group was sub-classified into: moderate (ChrP(M)) and severe (ChrP(S)). Performing analysis in control versus ChrP(M) versus ChrP(S), we observed a statistical significant difference in the genotype



**Fig. 1.** The genotypes frequencies of *SOCS1*<sup>−820/−1478</sup> polymorphisms in Control and ChrP groups. Significant differences were not found for genotype distribution of both polymorphisms in groups.



**Fig. 2.** The alleles frequencies of *SOCS1*<sup>−820/−1478</sup> polymorphisms in Control and ChrP groups. Significant difference was not found for *SOCS1*<sup>−1478</sup> allele frequency in groups.

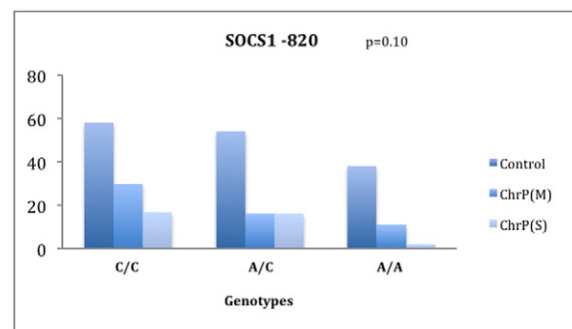
distribution between groups ( $\chi^2$ ,  $p < 0.05$ ) (Fig. 3). This significance became clearer when control was analyzed versus each ChrP subgroup. We noticed that *SOCS1*<sup>−820</sup> difference was came up from control versus ChrP(S) group, having significant differences for genotypes and alleles ( $\chi^2$ ,  $p = 0.0309$  and  $p = 0.0042$ , respectively) (Fig. 4). We clearly observed an increment of C allele within ChrP(S) followed a frequency decrease of A allele in the same group, probably due to the A allele protective effect against severe chronic periodontitis (OR = 0.3933;  $\chi^2$ ,  $p = 0.0084$ , Bonferroni Correction, (IC95% 0.2112 <  $\mu$  < 0.7324). In addition, analysis of *SOCS1*<sup>−820/−1478</sup> haplotype revealed that the frequency of A<sup>−820</sup>/CA<sup>−1478</sup> haplotype decreases in ChrP, having a statistically significant effect of this haplotype on ChrP ( $p = 0.0089$ ) (Fig. 5).

## 4. Discussion

The aim of this case–control study was to investigate the association between *SOCS1* genetic polymorphisms and chronic periodontitis. We observed association of *SOCS1*<sup>−820</sup> with severe chronic periodontitis cases, having lower frequency of A<sup>−820</sup> allele in the severe chronic periodontitis group, as well as decreased frequency of A<sup>−820</sup>/CA<sup>−1478</sup> haplotype in chronic periodontitis group.

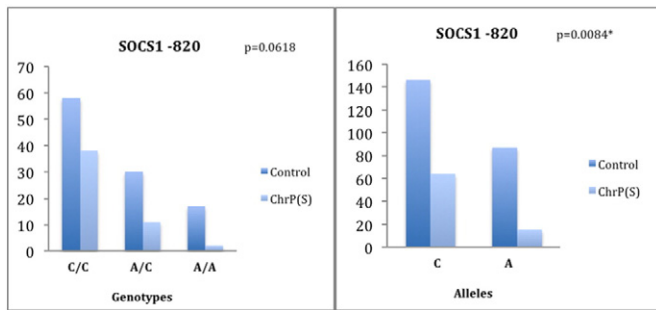
Chronic periodontitis is triggered by bacterial biofilm that covers teeth and soft tissues surfaces. Periodontal disease, once established, leads to a turnover imbalance between matrix extracellular components synthesis versus its destruction followed by periodontal tissue loss, including the Sharpey's fibers of collagen type I. The periodontitis prognosis will depend on the genetic background of host that will determinate the intensity of immune-inflammatory response against the infection.

The *SOCS1* protein, also known as JAK binding protein-1 or STAT-induced STAT inhibitor-1, takes part in this environment. *SOCS1* modulates the JAK-STAT intracellular signaling that represents a pathway used by a wide-range of pro-inflammatory cytokines. The target of *SOCS1* is JAK kinase. The *SOCS1*–JAK association recruits ubiquitination



**Fig. 3.** Genotype frequency of *SOCS1*<sup>−820</sup> in Control, ChrP(M) and ChrP(S) groups. Significant difference was found when ChrP samples were sub-classified into ChrP(M) and ChrP(S) groups. ( $\chi^2$ ,  $p = 0.10$ , Bonferroni correction.)





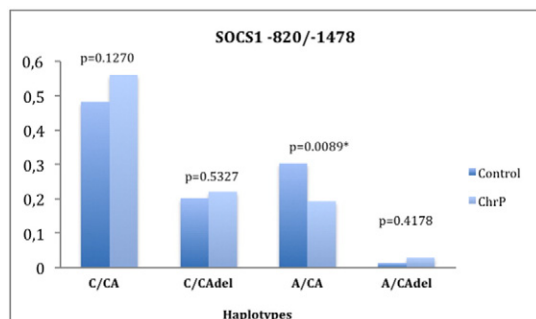
**Fig. 4.** Genotype and allele frequency of  $SOCS1^{-820}$  in Control and ChrP(S) groups. The significant differences are greater when control allele result was plotted against ChrP(S) results. ( $\chi^2$ ,  $p = 0.0618$  and  $p = 0.0084$ , Bonferroni correction.)

enzymes that recognize and mark  $SOCS1$ -JAK complexes, activating the proteosomal system and leading to degradation of the complex; consequently, the STAT phosphorylation is inhibited as well its activation (Mostecky et al., 2011). Additionally, increasing evidences suggest that  $SOCS1$  can also bind straightly to the cytokine receptor to trigger its degradation and/or mask the connection sites for adapting molecules.  $SOCS1$  level is essential to regulate the action of some cytokines. Changes in the  $SOCS1$  transcriptional activity may have important implication on the severity of inflammation.

$SOCS1$  gene is found within chromosome 16. The transcription begins on the first exon; however, the coding sequence initiates on the second exon, where an Open Reading Frame (ORF) is found. Several genetic polymorphisms have been described all over  $SOCS1$  gene; the majority, apparently, is not functional.

Although we have not observed association between  $SOCS1^{-1478}$  polymorphism and chronic periodontitis, Harada et al. (2007) did show association between CA/CAdel heterozygous genotype and asthma in adults. The same authors observed an increased  $SOCS1$  promoter activity after they had performed luciferase report assay carrying on CA sequence. Another study investigated the association of  $SOCS1^{-1478}$  polymorphism with Systemic Lupus Erythematosus (SLE) in DNA samples from subjects with serious clinical manifestations of SLE, such as thrombocytopenia and central nerve system (CNS) involvement. Authors found a higher frequency of  $SOCS1^{-1478del/del}$  genotype in subjects with SLE and CNS involvement than those without CNS involvement (Chan et al., 2010).

For the best of our knowledge, this is the first study that reports an association of  $SOCS1^{-820}$  polymorphism with chronic periodontitis. This polymorphism has also been described associated to obesity and insulin sensibility in Caucasians (Gylvin et al., 2009). Linkage disequilibrium is observed between  $SOCS1^{-820}$  and  $SOCS1^{+1125}$  polymorphisms (Gylvin et al., 2009). The  $SOCS1^{-820}$  was described as a functional polymorphism since A allele disrupts a promoter inhibitory site, potentiating the gene activity and promoting the increase of mRNA level.



**Fig. 5.** Haplotype frequency of  $SOCS1^{-820/-1478}$  in Control and ChrP(S) groups. The decrease frequency of  $A^{-820}/CA^{-1478}$  polymorphisms in ChrP group impacts significantly the disease.

Performing  $pGL3$  basic vector construction containing different inserts, some of which containing the  $-820$  region, an increased Luciferases activity was seen at the occurrence of the A allele (Mostecky et al., 2011). The same study demonstrated a higher expression of  $SOCS1$  associated to A/A and A/C genotypes when it was compared to C/C genotype (Mostecky et al., 2011). The increased promoter activity is thought to have occurred due to the disruption of a conserved DNA consensus sequence that binds to YY1 inhibitory transcription factor at the occurrence of A allele. Therefore, we suggest that the lower A allele frequency found in the severe chronic periodontitis group is due to the fact that A allele promotes protection against chronic periodontitis since A allele increases  $SOCS1$  mRNA level and consequently  $SOCS1$  protein level what promotes down-regulation of JAK/STAT pro-inflammatory pathway. It also explains the higher frequency of C/C genotype associated to chronic periodontitis group.

Although the  $A^{-820}/CA^{-1478}$  haplotype was associated with control groups, the result must be a consequence of the protective effect of A allele on this haplotype, as previously the  $CAdel^{-1478}$  allele was shown to increase  $SOCS1$  promoter activity.

$SOCS1$  regulates the signaling of several pro-inflammatory cytokines. Among them, we highlight the signaling promoted by IL-6, IFN-1 and TNF- $\alpha$ , all of them highly transcribed during chronic periodontal inflammation (Garlet et al., 2006). Therefore, the gene down regulation promoted by C allele created by  $SOCS1^{-820}$  polymorphism can represent one of the risk factors addressed to periodontitis severity while A allele plays a protective role during periodontitis.

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## Competing interests

The authors have declared that no-competing interests exist.

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