Association of SOCS1−820 (rs33977706) gene polymorphism with chronic periodontitis: A case–control study in Brazilians

Roger Antoniaci Guedes 1, Aline Cristiane Planello 1, 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

ARTICLE INFO

Article history:
Received 13 May 2015
Accepted 21 June 2015
Available online 9 July 2015

Keywords:
SOCS1
Chronic periodontitis
Genetic polymorphism

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 SOCS1−820 polymorphism in the chronic periodontitis group, especially when severe periodontitis samples were separately analyzed (OR = 0.3933; IC95% 0.2112–0.7324), 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 SOCS1−820/−1478 haplotype revealed that the frequency of A−820/CA−1478 haplotype decreases in ChrP (p = 0.0089). In conclusion, our study found that SOCS1−820 polymorphism is associated with chronic periodontitis.

© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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, 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-1β. 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 et al., 2004).

* Corresponding author at: Department of Morphology, FOP-UNICAMP, Av. Limeira 901, CEP 13414-018, Piracicaba-SP, Brazil.
E-mail address: anapaulapardo@fop.unicamp.br (A.P. de Souza).

1 These authors contributed equally to the study.
et al., 2011). SOCS1 has been shown to play a potential role in modulating periodontitis (Menézes 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 (Mostecki et al., 2011). Assay performed in vitro has demonstrated that A allele increases SOCS1 mRNA levels (Mostecki et al., 2011). The SOCS1−1478 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, Unicamp. 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.

2.3. Isolation of genomic DNA

DNA was collected from a mouthwash of oral cells as described by Trevilatto 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)

PCR was performed in a total volume of 10 μl, containing: 100 ng of gDNA, 5 μl TaqMan Genotyping Master Mix, 0.5 μl TaqMan genotyping assay mix 20× and H2O DNase-free. PCRs were performed in a LightCycler 480 (Roche) under the following conditions: 10′ at 95 °C, 40× (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)

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 μl, containing 1 μl genomic DNA (100 ng), 1.5 μl of each primer and 6 μl of Go Taq Green Master Mix (Promega Corporation, Madison, WI, USA). The solution was incubated under the following conditions:

| Primer | Amplicon | Restriction enzyme |
|--------|----------|--------------------|
| FOW−TGTCGTCACGCTGCACTTC | 250pb | DdeI |
| REV−ACCACAGGCTTCAGAGGAAC | | C/TNAG…3’ |
| | | GANT/C…5’ |

Table 1

| SOCS1−820 locus, sample demographic dataset. |  |
|---|---|
| **SOCS1−820** |  |
| **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 |

Table 2

| SOCS1−1478 locus, sample demographic dataset. |  |
|---|---|
| **SOCS1−1478** |  |
| **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 | 32.83% | 67.17% | 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.

Table 3

| SOCS1−1478 primers set, amplicon size, and restriction endonuclease site. |  |
|---|---|
| **Primer** | **Amplicon** | **Restriction enzyme** |
| FOW−TGTCGTCACGCTGCACTTC | 250pb | DdeI |
| REV−ACCACAGGCTTCAGAGGAAC | | C/TNAG…3’ |
| | | GANT/C…5’ |
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 MgCl₂, 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 H₂O. 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 Mamirauá, Brazil) (M A 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 α = 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 SOCS1−1478 and SOCS1−820 SNPs were successfully performed in all subjects and the distribution of SOCS1−1478/−820 polymorphisms results were found in Hardy–Weinberg equilibrium in the studied groups (χ², p > 0.05). Significant differences were not found in genotypes and allelic distribution between groups for SOCS1−1478 (χ², p = 0.6959 and p = 0.4299, respectively) (Figs. 1 and 2). The same was also observed for SOCS1−820 genotype distribution (χ², p = 0.1551) (Fig. 1). However, SOCS1−820 result was seen bordering significance when allelic distribution was analyzed between controls and periodontitis group (χ², p = 0.080) (Fig. 2), with an increase of C allele in periodontitis group. Then, we performed the next analysis for SOCS1−820 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 distribution between groups (χ², p < 0.05) (Fig. 3). This significance became clearer when control was analyzed versus each ChrP subgroup. We noticed that SOCS1−820 difference was came up from control versus ChrP(S) group, having significant differences for genotypes and alleles (χ², 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; χ², p = 0.0084, Bonferroni correction, [IC95% 0.2112–μ = 0.7324]). In addition, analysis of SOCS1−820/−1478 haplotype revealed that the frequency of A−820/CA−1478 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 SOCS1−820 with severe chronic periodontitis cases, having lower frequency of A−820 allele in the severe chronic periodontitis group, as well as decreased frequency of A−820/CA−1478 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 determine 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. 1. The genotypes frequencies of SOCS1−820/−1478 polymorphisms in Control and ChrP groups. Significant differences were not found for genotype distribution of both polymorphisms in groups.](image1)

![Fig. 2. The alleles frequencies of SOCS1−820/−1478 polymorphisms in Control and ChrP groups. Significant difference was not found for SOCS1−1478 allele frequency in groups.](image2)

![Fig. 3. Genotype frequency of SOCS1−820 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. (χ², p = 0.10, Bonferroni correction.)](image3)
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 (Mostecki 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 (Gylvin et al., 2009). The authors found a higher frequency of SOCS1\(^{-1478}\) 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\(^{+1128}\) polymorphisms (Gylvin et al., 2009). The SOCS1\(^{-820}\) was described as a functional polymorphism since A allele disrupts a promoter inhibitor site, potentiating the gene activity and promoting the increase of mRNA level.

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 (Mostecki 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 (Mostecki 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 it 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-α, 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.

Financial support

CNPq (Conselho Nacional de Pesquisa) supported the study.

Competing interests

The authors have declared that no-competing interests exist.

References

Armitage, G.C., 1999. Development of a classification system for periodontal diseases and conditions. Ann. Periodontol. 4, 1–6.

Borrell, L.N., Papapanou, P.N., 2005. Analytical epidemiology of periodontitis. J. Clin. Periodontol. 32 (Suppl. 6), 132–158.

Chan, H.C., Ke, L.Y., Chang, L.L., et al., 2010. Suppressor of cytokine signaling 1 gene expression and polymorphisms in systemic lupus erythematosus. Lupus 19, 696–702.

Chen, C.Y., Tsay, W., Tang, J.L., et al., 2003. SOCS1 methylation in patients with newly diagnosed acute myeloid leukemia. Genes Chromosomes Cancer 37, 300–305.

Davey, G.M., Heath, W.R., Starr, R. 2006. SOCS1: a potent and multifaceted regulator of cytokines and cell-mediated inflammation. Tissue Antigens 67, 1–9.

Egan, P.J., Lawlor, K.E., Alexander, W.S., Wicks, I.P., 2003. Suppressor of cytokine signaling-1 regulates acute inflammatory arthritis and T cell activation. J. Clin. Invest. 111, 915–924.

Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Eval. Bioinformatics Online 1, 47–50.

Faul, F., Erdfelder, E., Lang, A.G., Buchner, A., 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods 39, 175–191.

Federici, M., Giustizieri, M.L., Scarponi, C., Girolomoni, G., Albani, C., 2002. Impaired IFN-γ gamma-dependent inflammatory responses in human keratinocytes overexpressing the suppressor of cytokine signaling 1. J. Immunol. 169, 434–442.

Flemming, T.F., 1990. Periodontitis. Ann. Periodontol. 4, 32–38.

Galm, O., Yoshikawa, H., Esteller, M., Osieka, R., Herman, J.G., 2003. SOCS1-1, a negative regulator of cytokine signaling, is frequently silenced by methylation in multiple myeloma. Blood 101, 2784–2788.

Garlet, G.P., Cardoso, C.R., Campanelli, A.P., Martinis Jr., W., Silva, J.S., 2006. Expression of suppressors of cytokine signaling in diseased periodontal tissues: a stop signal for disease progression? J. Periodontol. Res. 41, 580–584.

Gylvin, T., Ek, J., Nilose, R., et al., 2009. Functional SOCS1 polymorphisms are associated with variation in obesity in whites. Diabetes Obes. Metab. 11, 190–203.

Harada, M., Nakashima, K., Hirota, T., et al., 2007. Functional polymorphism in the suppressor of cytokine signaling 1 gene associated with adult asthma. Am. J. Respir. Cell Mol. Biol. 36, 491–496.

Lee, T.L., Yeh, J., Van Waes, C., Chen, Z., 2006. Epigenetic modification of SOCS1 differentially regulates STAT3 activation in response to interleukin-6 receptor and epidermal growth factor receptor signaling through JAK and/or MEK in head and neck squamous cell carcinomas. Mol. Cancer Ther. 5, 8–19.
Lin, D.Y., Hu, Y., Huang, B.E., 2008. Simple and efficient analysis of disease association with missing genotype data. Am. J. Hum. Genet. 82, 444–452.

M.A, AJ, M., DL, A., AS dS, 2007. BioEstat 5.0 (364 pp.).

Menezes, R., Garlet, T.P., Trombone, A.P., et al., 2008. The potential role of suppressors of cytokine signaling in the attenuation of inflammatory reaction and alveolar bone loss associated with apical periodontitis. J. Endod. 34, 1480–1484.

Mostecki, J., Cassel, S.L., Klimecki, W.T., et al., 2011. A SOCS-1 promoter variant is associated with total serum IgE levels. J. Immunol. 187, 2794–2802.

O-M, G., M-V, J.L., H-V, P., et al., 2009. Suppressors of cytokine signaling modulate JAK/STAT-mediated cell responses during atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 525–531.

Sachithanandan, N., Graham, K.L., Galic, S., et al., 2011. Macrophage deletion of SOCS1 increases sensitivity to LPS and palmitic acid and results in systemic inflammation and hepatic insulin resistance. Diabetes 60, 2023–2031.

Trevilatto, P.C., Line, S.R., 2000. Use of buccal epithelial cells for PCR amplification of large DNA fragments. J. Forensic Odontostomatol. 18, 6–9.

Yoshida, T., Ogata, H., Kamio, M., et al., 2004. SOCS1 is a suppressor of liver fibrosis and hepatitis-induced carcinogenesis. J. Exp. Med. 190, 1701–1707.

Yoshikawa, H., Matsuura, K., Qian, G.S., et al., 2001. SOCS-1, a negative regulator of the JAK/STAT pathway, is silenced by methylation in human hepatocellular carcinoma and shows growth-suppression activity. Nat. Genet. 28, 29–35.

Yoshimura, A., Naka, T., Kubo, M., 2007. SOCS proteins, cytokine signalling and immune regulation. Nat. Rev. Immunol. 7, 454–465.

Yoshimura, A., Suzuki, M., Sakaguchi, R., Hanada, T., Yasukawa, H., 2012. SOCS, inflammation, and autoimmunity. Front. Immunol. 3, 20.