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# *CSF1* gene associated with aggressive periodontitis in the Japanese population

D. Rabello [a,b,*], N. Soedarsono [a,b], H. Kamei [c], Y. Ishihara [c], T. Noguchi [c], D. Fuma [b,c], M. Suzuki [c], Y. Sakaki [b], A. Yamaguchi [a], T. Kojima [b]

[a] *Oral Pathology, Oral Restitution, Oral Health Science, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan*
[b] *Comparative Systems Biology Team, Computational and Experimental Systems Biology Group, Genomic Sciences Center (GSC), RIKEN Yokohama Institute, Yokohama, Japan*
[c] *Department of Periodontology, School of Dentistry, Aichi-Gakuin University, Nagoya, Japan*

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**Abstract**

Aggressive periodontitis (AgP) is characterized by the early onset of the rapid and progressive destruction of the alveolar bone. We investigated the correlation of single nucleotide polymorphisms (SNPs) in candidate genes with AgP in the Japanese population in order to determine the genetic risk factors for this complex disease. Among 11 genes related to bone formation and resorption, 43 known SNPs were tested in 98 case and 88 control samples for association with AgP by using SNP genotyping techniques. Among these, three polymorphisms located in the colony stimulating factor 1 (*CSF1*) gene showed a positive association with AgP. This is the first case of an association between a *CSF1* polymorphism and a human disease.


*Keywords:* *CSF1* gene; Single nucleotide polymorphism (SNP); Genetic polymorphism; Risk factor; Aggressive periodontitis

Periodontitis is an inflammatory disease of the periodontal tissues that causes tooth loss due to resorption of the alveolar bone. This disease has two main entities: chronic periodontitis (CP) and aggressive periodontitis (AgP). CP is the most common form of periodontitis with a slow clinical course; AgP, however, is relatively rare and is characterized by the early onset of the rapid and progressive destruction of the alveolar bone.

Periodontitis has a complex etiology, including environmental factors (bacterial flora of the oral cavity, stress, lifestyle, etc.) and genetic factors. Although bacterial plaque leads to a host immune response in periodontal tissues and initiates the disease process, no significant relationship has been observed between the amount of bacterial plaque and the progression of periodontitis [1,2]. These findings suggest that the host immune response is a more critical factor in the etiology of periodontitis than the bacterial flora. Many of the host immune responses caused by periodontal pathogens appear to be under genetic control [1]; this influences periodontitis susceptibility and progression [2,3]. Recently, single nucleotide polymorphisms (SNPs) have been efficiently used as a tool for the genetic analysis of such complex traits [4–7].

Although SNPs in several genes associated with both CP and AgP have been reported in the past few years, the variation in the results is still controversial. The majority of these studies concerning CP and/or AgP are focused on genes related to pro-inflammatory cytokines such as interleukins [8–23]. Since periodontitis is modulated by the interaction of polymorphisms of minor effects in multiple genes, the exact profile of the genetic risk factors influencing it has not been determined [24].

The balance between bone formation and bone resorption maintains bone homeostasis. Bone formation is

* Corresponding author. Fax: +81 3 5803 0188.
*E-mail address:* dorarabello@gmail.com (D. Rabello).

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mainly conducted by osteoblasts and bone resorption is mainly performed by osteoclasts, but recent studies have demonstrated that the cross talk between osteoblasts and osteoclasts plays a crucial role in bone remodeling [25]. Since AgP represents rapid bone resorption, the candidate genes for AgP should be selected from various factors involved in bone formation and resorption other than pro-inflammatory cytokines. We therefore selected 11 genes involved in bone remodeling as candidates for AgP in the Japanese population, including bone morphogenetic protein (*BMP2*, *BMP3*, *BMP4*, and *BMP7*), colony stimulating factor 1 (*CSF1*), insulin-like growth factor 1 (*IGF1*), interferon gamma (*IFNG*), IFNG receptor 1 (*IFNGR1*), periostin (*POSTN*), vascular endothelial growth factor (*VEGF*), and nuclear factor of activated T-cells, cytoplasmic, and calcineurin dependent 2 (*NFATC2*/*NFAT1*). Among these genes, we demonstrate here that SNPs found in the *CSF1* gene are significantly associated with AgP.

## Materials and methods

*Subjects.* A total of 98 Japanese patients with AgP (63 females and 35 males; mean age 30.8 ± 6.2 years; range, 17–47 years) were recruited at the Department of Periodontology, Aichi-Gakuin University, from 2002 to 2004. The control group consisted of 88 periodontally healthy Japanese volunteers (33 females and 55 males; mean age, 43.0 ± 7.3 years; range 34–63 years). The diagnostic criteria of AgP based on Tonetti's study [26] were defined as follows: (1) age of onset of periodontitis, less than 35 years and (2) attachment loss, 4 mm or more in at least four permanent teeth, with at least one first molar affected. The alveolar bone loss in all patients with AgP was assessed using full mouth radiographs. An attachment loss of at least 4 mm in at least eight teeth was observed in all the AgP patients. At the time of their initial visit, 27 AgP patients were older than 35 years, but the onset of the periodontitis was confirmed at less than 35 years of age from the oral disease history. The periodontally healthy control subjects were older than 35 years and showed no attachment loss in any teeth; however, some control subjects had gingival pockets in a few teeth. General blood tests indicated that all the AgP patients and control subjects in the present study had no apparent systemic diseases. The degree of periodontitis in all participants was assessed by measuring probing pocket depth (PPD), probing attachment level (PAL), bleeding on probing, and the degree of tooth mobility. The clinical characteristics of the patients with AgP and control subjects are summarized in Table 1. Written informed consent was obtained from all participants. This study was approved by the Ethical Committees of the School of Dentistry, Aichi-Gakuin University, and the RIKEN Yokohama Institute.

*Analysis of genetic polymorphisms.* Genomic DNA was extracted from whole peripheral blood by using the Nucleon Genomic DNA Extraction kit (Tepnel Life Sciences PLC, Manchester, UK).

Eleven candidate genes were selected from genes related to bone destruction and bone formation and included *BMP2*, *BMP3*, *BMP4*, *BMP7*, *IFNG*, *IFNGR1*, *IGF1*, *CSF1*, *NFATC2*, *POSTN*, and *VEGF*. From these genes, a total of 43 SNPs were selected from the JSNP database (http://snp.ims.u-tokyo.ac.jp/) [7], including minor alleles with frequencies greater than 10%. The details of the 43 *SNP*s are listed in Table 2.

For each of the 37 SNPs of the 43, genomic DNA was amplified in a PCR by using an AmpliTaq Gold kit (Applied Biosystems, CA, USA) and a three-primer system [6], including a universal biotinylated primer. Polymerase chain reaction (PCR) products were captured on streptavidin-coated beads to obtain single-stranded DNA and then hybridization of the sequencing primer was accomplished. The polymorphisms were typed by a real-time pyrophosphate non-electrophoretic DNA sequencing method [27] by using a PSQ 96 system (Pyrosequencing AB, Uppsala, Sweden; http://www.pyrosequencing.com). The PCR primers were designed using the DNAsis 3.0 software (Hitachi Software Engineering, Tokyo, Japan) and the sequencing primers and assays by using an assay design software (http://www.biotage.com) [28].

Six SNPs (*BMP2*, *BMP7*, *NFATC2-1*, *NFATC2-5*, *VEGF2*, and *VEGF9*) located in monopolymeric areas (Table 2) were amplified with their respective primers from the JSNP database and the *Taq* polymerase with the best result for each set of primers (LA *Taq*/GC buffer I and Ex *Taq*/Ex *Taq* buffer or LA *Taq*/LA buffer from Takara Biomedicals Inc., Tokyo, Japan). Then, the SNPs were typed using one of the PCR primers (forward or reverse) by the capillary-based sequencing method by using a BigDye Terminator cycle sequencing kit and an ABI Prism 3700 DNA capillary-based sequencer (Applied Biosystems, CA, USA). For these six SNPs, genotype determination was performed using the software Polyphred [29].

After the case and control samples (48 each) were typed for each SNP, the frequency of each allele in every SNP was confirmed by comparison with the JSNP database. Information on the primer sequences that were used for PCR and the sequencing and PCR conditions are available upon request.

*Statistical analysis.* Pairwise linkage disequilibrium (LD) analysis was performed with 48 case and control samples each between all the SNP pairs by using two coefficients: $|D'|$ and $r^2$. A perfect LD is one in which $|D'| = 1$ and $r^2 = 1$. In this study, the SNP pairs were considered to be in LD when $|D'| \geqslant 0.7$ and $r^2 \geqslant 0.7$ [30].

For the association study, the chi-square ($\chi^2$) test was performed between the 98 case and 88 control subjects for each allelic frequency. The haplotypic frequencies for multiple loci between the case and control subjects were obtained by the expectation-maximization method and estimated using the permutation test. The statistical association was considered significant when the $P$ value was lower than 0.05. Calculations were performed using the software SNPalyse v3.2. (Dynacom, Mobara, Japan).

Table 1
Clinical parameters of case and control subjects

| Trait | Case | Control |
|---|---|---|
| Number | 98 | 88 |
| Sex ratio (F/M) | 63/35 | 33/55 |
| Age (year) | 30.8 ± 6.2 | 43.0 ± 7.3 |
| Number of present teeth | 27.7 ± 2.8 | 27.6 ± 2.4 |
| Mean of PPD (mm) | 4.03 ± 1.33 | 1.94 ± 0.30 |
| Mean of PAL (mm) | 4.58 ± 1.56 | 2.14 ± 0.36 |
| Ratio of teeth PPD ⩾ 4 mm | 0.78 ± 0.23 | 0.09 ± 0.13 |
| Ratio of teeth PAL ⩾ 4 mm | 0.83 ± 0.19 | 0.18 ± 0.17 |
| Ratio of teeth bleeding on probing (+) | 0.73 ± 0.28 | 0.14 ± 0.17 |
| Ratio of teeth mobility ⩾ 2 degree | 0.12 ± 0.16 | 0 |

Values are means ± SD. PPD, probing pocket depth; PAL, probing attachment level.

## Results

### *Significant pairwise LD in CSF1*

The strength of LD for each SNP pair within each gene, measured using the values of $|D'| \geqslant 0.7$ and $r^2 \geqslant 0.7$, was significant in four genes (SNPs 2, 4, and 5 in *BMP7*; SNPs 2, 3, and 5 in *CSF1*; SNPs 1 and 4 in *NFATC2*; and SNPs 1, 4, 7, and 9 in *VEGF*). *IGF1* SNPs did not show significant LD. The $|D'|$ and $r^2$ values for the *CSF1* gene are shown in Table 3. The LD values for the other genes are not shown.