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ORIGINAL ARTICLE

Gene polymorphism of β-defensin-1 is associated with susceptibility to periodontitis in Japanese

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Abstract Periodontitis is a multifactorial disease associated with genetic and environmental factors. Single-nucleotide polymorphisms (SNPs) are associated with susceptibility to common diseases such as diabetes and periodontitis. Although the oral cavity is exposed to various organisms, the conditions are well controlled by innate and acquired immune systems. Antimicrobial peptides (AMPs) play an important role in the innate immune system; however, the association of AMP-SNPs with periodontitis has not been fully elucidated. This study investigated the relationship between AMP-SNPs and periodontitis in Japanese. One hundred and five Japanese subjects were recruited, which included patients with aggressive, severe, moderate and mild periodontitis, and age-matched healthy controls. Genomic DNA was isolated from peripheral blood and genotypes of SNPs of β-defensin-1 and lactoferrin genes (*DEFB1*: rs1799946, rs1800972 and rs11362; and *LTF*: rs1126478) were investigated using the PCR-Invader assay. Protein level of AMPs in gingival crevicular fluid (GCF) was quantified by ELISA. Case–control studies revealed that the −44 CC genotype of *DEFB1* (rs1800972) was associated with periodontitis (OR 2.51), particularly with severe chronic periodontitis (OR 4.15) and with combined severe and moderate chronic periodontitis (OR 4.04). No statistical differences were found in other genotypes. The β-defensin-1 concentrations in GCF were significantly lower in subjects with the −44 CC genotype of *DEFB1* than in those without this genotype. No significant differences between GCF concentrations of AMPs and other genotypes were detected. The −44 CC genotype of the β-defensin-1 gene (*DEFB1* rs1800972) may be associated with susceptibility to chronic periodontitis in Japanese.

Keywords Periodontitis · SNP · β-Defensin-1 · Lactoferrin · Gingival crevicular fluid

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Introduction

Periodontitis is an inflammatory disease primarily caused by the infection of dental plaque microorganisms, followed by periodontal tissue destruction after a continuous excessive immune response. There are many factors that initiate and progress periodontitis, which include not only microbial factors but also other local and systemic factors such as age, gender, ethnicity, smoking, lifestyle, stress, heredity and systemic diseases. Thus, periodontitis is considered to be a multifactorial disease associated with genetic and environmental factors [1]. Genetic factors can convey susceptibility to periodontitis and recent reports have shown that single-nucleotide polymorphisms (SNPs) are important variations among the genetic factors that underlie the host response to diseases [2, 3]. In general, humans have 2–3 million SNPs, which correspond to 0.1 % of genomic DNA. SNPs in non-coding regions can affect the regulation of gene expression, and SNPs in coding regions can change protein sequences and often give rise to different biological functions. Many basic and clinical studies have shown the relationship between SNPs and susceptibility to periodontitis [2–4]. It has been reported

that SNPs of interleukin (IL)-1, IL-6, IL-10, IL-17, tumor necrosis factor (TNF)-α and immunoglobulin G Fc receptors (FcγR) may be associated with the initiation and progression of periodontitis [5–10]. Our collaborating studies reported that vitamin D receptor (VDR) +1056 polymorphisms were related to chronic periodontitis [11] and that α2 integrin +807 polymorphisms were related to drug-induced gingival overgrowth [12].

The oral cavity is continuously exposed to various pathogenic organisms, but oral conditions are well controlled by innate and acquired immune systems. Innate immunity functions in most species, including insects, fungi, plants and mammals, as non-specific host defense at an early stage of microorganism challenge. Antimicrobial peptides (AMPs) play an important role in the innate immune system and more than 800 AMPs have been identified [13, 14]. Many AMPs such as lysozyme, defensin, lactoferrin, histatin, cystatin and calprotectin were found in the human oral cavity, and several reports demonstrated the association between periodontitis and AMP levels in saliva, gingival tissue or gingival crevicular fluid (GCF) [15–17]. We previously reported that the calprotectin level in GCF correlated with gingival index in periodontitis patients [18, 19]. It is conceivable that gene polymorphisms of AMPs cause differences in the innate immune system and confer susceptibility to various infectious diseases. However, there have been few reports concerning the association of AMP-SNPs with periodontitis.

Human β-defensins are small cationic AMPs, and at least four types of β-defensin have been characterized [20]. β-defensin-1 is a prominent molecule of the defensin family that is encoded by the *DEFB1* gene [21]. SNPs of the *DEFB1* gene are located in coding and non-coding regions, including the 5′-untranslated region (5′-UTR) and 3′-UTR [22, 23]. β-Defensin-1 is expressed in keratinocytes and epithelial cells, and is found in the kidney, female reproductive tract, testis, gingival tissue, small intestine, large intestine, cornea and mammary gland [22, 24]. Lactoferrin, also known as lactotransferrin, is a multifunctional metalloprotein, which is a member of the transferrin family [25]. Human lactoferrin is encoded by the *LTF* gene and SNPs of the *LTF* gene have been identified in both coding and non-coding regions [26]. Human lactoferrin is produced by mucosal epithelial cells and widely found in various secretory fluids, such as milk, saliva, tears, sweat and nasal secretions [25]. In addition, human lactoferrin is produced by secondary granules of neutrophils and is released in response to inflammation [27].

In this study, we analyzed the genotype distributions and allele frequencies of three *DEFB1* SNPs and one *LTF*-SNP in Japanese periodontitis patients, and then we quantified the GCF concentrations of these AMPs in the subjects. Since SNPs are generally associated with ethnicity and geography, this study focused on Japanese subjects to determine the relationship between AMP-SNPs and chronic or aggressive periodontitis.

Materials and methods

Subjects and study protocol

The protocol for this study was reviewed and approved by the ethics committee of human genome and gene analysis at the University of Tokushima (Approval No. H23-7). From subjects who agreed to participate in the study, written informed consent was obtained before undergoing periodontal examinations. Periodontal conditions were diagnosed according to the criteria of the 1999 International Workshop for Classification of Periodontal Diseases and Conditions [28]. The subjects were divided into five groups: chronic periodontitis (CP) including severe CP, moderate CP and mild CP, aggressive periodontitis (AgP) and age-matched healthy controls, on the basis of clinical examinations including probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP) and alveolar bone loss (BL). The bone loss was assessed at 2 sites of a tooth on a radiograph [29]. Exclusion criteria were the presence of systemic disease (e.g., diabetes mellitus and kidney disease), drug-induced gingival overgrowth, pregnancy, having fewer than 15 teeth and a history of any periodontal therapy within the previous 6 months. Age-matched healthy controls were subjects more than 40 years old in the case of mild CP and under 35 years old in the case of AgP, both having localized PD $\leq$3 mm and mean BL $\leq$15 %. The severe CP group consisted of subjects more than 40 years old having localized PD $\geq$4 mm and mean BL $\geq$34 %, and the moderate CP group consisted of subjects more than 40 years old having localized PD $\geq$4 mm and 17 % $\leq$ mean BL $\leq$ 28 %. The AgP group consisted of subjects under 35 years old having localized CAL $\geq$5 mm. One hundred and five Japanese subjects were recruited, including 62 periodontitis (28 with severe CP, 13 with moderate CP and 21 with AgP) and 43 controls (22 for CP and 21 for AgP).

Isolation of genomic DNA and genotype determination

Five milliliters of peripheral blood was obtained from the basilic, cephalic or median cubital vein of each subject. Genomic DNA was isolated from the blood sample and the genotypes of the β-defensin-1 gene (*DEFB1*) and the lactoferrin gene (*LTF*) were determined using a PCR-Invader assay by BML Inc. (Saitama, Japan). The PCR-Invader

assay was reported previously [30]. We assayed three known SNPs in the 5′-UTR of *DEFB1*, namely, at positions −52 G/A (rs1799946; a guanine to adenine nucleotide mutation), −44 C/G (rs1800972; a cytosine to guanine nucleotide mutation) and −20 G/A (rs11362; a guanine to adenine nucleotide mutation) from the first AUG codon [31–33]. In addition, we assayed one known SNP of *LTF*, namely, at position 29 in the N-terminal α-helical region of human lactoferrin (rs1126478; an adenine to guanine nucleotide mutation) [34, 35].

Sampling of gingival crevicular fluid and β-defensin-1 quantification

Gingival crevicular fluid (GCF) samples were collected according to a method described previously [36]. Briefly, before the clinical evaluation including PD and BOP, GCF was collected using Periopaper® (Oraflow Inc., New York, NY) from healthy gingival crevices or periodontal pockets with periodontitis. GCF volume was determined using Periotron 8000® (Harco Electronics, Winnipeg, MB, Canada). The GCF perfused in paper strips was extracted using 100 µl of 10 mM Tris–HCl buffer (pH 7.4) containing 200 µM phenylmethylsulfonyl fluoride. The amounts of β-defensin-1 and lactoferrin in GCF samples were determined using enzyme-linked immunosorbent assay (ELISA) kits (Human BD-1 ELISA Kit, pink-ONE, KOMA BOTEC Inc., Seoul, Korea; Human Lactoferrin ELISA Kit; HyCult Biotechnology b.v., Uden, Netherlands). The samples were diluted 2- or 10-fold with the dilution buffer of each kit and used for the determination of β-defensin-1 and lactoferrin according to the manufacturers' instructions. After the total amounts of β-defensin-1 and lactoferrin were measured from the standard curve, the β-defensin-1 and lactoferrin concentrations of GCF samples were obtained as pg or ng/µl by adjusting for the GCF volume.

Statistical analyses

The distribution of each SNP genotype was evaluated for Hardy–Weinberg equilibrium. Statistical analyses were performed using JMP® software (SAS Institute Inc., Cary, NC, USA). The differences in genotype distributions and allele frequencies were tested by Chi-square tests. When an expected value in cells was less than five, Fisher's exact tests were used. Contingency tables (2 × 2) were used for four kinds of comparison: periodontitis vs. controls, severe CP vs. CP control, combined periodontitis (severe and moderate CPs) vs. CP control and AgP vs. AgP control. The strength of the associations was determined using odds ratio (OR) calculation and 95 % confidence intervals (95 % CI). In the ELISA analysis, the relationships between β-defensin-1 concentration in GCF and *DEFB1* genotypes and between lactoferrin concentration in GCF and *LTF* genotype were evaluated using the nonparametric Wilcoxon signed-rank test and Mann–Whitney *U* test. A p value of <0.05 was considered to be statistically significant.

Results

Demographics

Table 1 provides a summary of the demographic and clinical characteristics of the 105 Japanese subjects, 62 periodontitis and 43 controls. The table also provides information of the five groups, consisting of severe CP, moderate CP, AgP, CP control and AgP control. Periodontitis subjects showed significantly higher values of BL, PPD and BOP than the controls ($p < 0.01$). The mean ages of those with severe CP, moderate CP and CP control were similar, whereas those with AgP were significantly older than those in the AgP control group ($p < 0.05$). The severe CP group showed significantly higher values of BL, PPD and BOP than the CP control group. The moderate CP group showed a significantly higher value of BL than the CP control. When the severe and moderate CPs were combined ($n = 41$), higher values of BL, PPD and BOP were observed in the combined group than in the CP control group ($p < 0.01$, data not shown). The AgP group showed significantly higher values of BL, PPD and BOP than the AgP control.

SNP analysis of DEFB1 and LTF

We analyzed three SNPs at positions −52 G/A, −44 C/G and −20 G/A in the 5′-UTR of *DEFB1* and one SNP at position 29, Lys/Arg, in the N-terminal alpha-helical region of human lactoferrin. The genotype distribution of these SNPs did not show any significant difference from Hardy–Weinberg equilibrium. The genotype distributions and allele frequencies of *DEFB1* and *LTF* polymorphisms are summarized in Tables 2, 3 and 4. The frequency of the −44 CC genotype of *DEFB1* was higher in the periodontitis (83.9 %) than in the controls (67.4 %) (Table 2). In the case of the CP groups, the frequency of the −44 CC genotype of *DEFB1* was higher in the severe CP group (85.7 %) and the moderate CP group (84.6 %) than in the CP control (59.1 %) and the frequency of the −44 C allele was higher in the severe CP group (92.9 %) than in the CP control (79.5 %) (Table 3). In the case of the AgP group, no difference of −44 CC genotype or the −44 C allele was observed between AgP and its control (Table 4). These results indicate that not only the frequency of the −44 CC genotype of *DEFB1* was relatively high in the periodontitis, but also the frequencies of the −44 CC genotype and

Table 1 Comparison of the demographic and clinical characteristics of the study groups

	Age (years)	Male/female	Tooth number	Bone loss (%)	PPD (mm)	BOP (%)
Periodontitis ($n = 62$)	48.6 ± 13.9*	22/40	25.2 ± 3.4**	33.8 ± 14.5**	3.5 ± 1.2**	43.7 ± 29.8**
Control ($n = 43$)	41.9 ± 15.6	17/26	26.8 ± 2.3	5.4 ± 5.3	2.3 ± 0.7	13.8 ± 21.2
Severe CP ($n = 28$)	56.2 ± 8.2	11/17	23.8 ± 3.4**	43.1 ± 11.4**	4.0 ± 1.4**	53.8 ± 23.6**
Moderate CP ($n = 13$)	55.3 ± 8.3	4/9	25.8 ± 3.1	22.4 ± 3.1**	2.6 ± 0.6	24.3 ± 17.9
CP control ($n = 22$)	55.3 ± 8.8	8/14	26.2 ± 2.8	8.5 ± 4.8	2.5 ± 0.7	22.2 ± 23.6
AgP ($n = 21$)	33.0 ± 7.9*	7/14	26.6 ± 2.8	28.3 ± 15.1**	3.3 ± 0.8**	42.5 ± 29.6**
AgP control ($n = 21$)	27.8 ± 4.9	9/12	27.8 ± 1.4	2.1 ± 3.7	2.0 ± 0.5	5.0 ± 14.1

* $p < 0.05$, ** $p < 0.01$

Table 2 Genotype and allele frequencies of β-defensin-1 (*DEFB1*) and lactoferrin (*LTF*) gene polymorphisms in subjects with or without periodontitis

Gene	SNP	Periodontitis	Cont	Periodontitis vs. Cont		
		n (%)	*n* (%)	*p* value	OR	95 % CI
DFFB1	rs1799946					
	GG	20 (32.3)	16 (37.2)	0.599	0.804	0.358–1.803
	GA	33 (53.2)	20 (46.5)			
	AA	9 (14.5)	7 (16.3)			
	G	73 (58.9)	52 (60.5)	0.817	0.936	0.535–1.637
	A	51 (41.1)	34 (39.5)			
DFFB1	rs1800972					
	CC	52 (83.9)	29 (67.4)	0.049*	2.510	1.005–6.263
	CG	8 (12.9)	14 (32.6)			
	GG	2 (3.2)	0 (0)			
	C	112 (90.3)	72 (83.7)	0.153	1.815	0.807–4.082
	G	12 (9.7)	14 (16.3)			
DFFB1	rs11362					
	GG	16 (25.8)	14 (32.6)	0.451	0.720	0.309–1.676
	GA	33 (53.2)	20 (46.5)			
	AA	13 (21.0)	9 (20.9)			
	G	65 (52.4)	48 (55.8)	0.628	0.872	0.503–1.513
	A	59 (47.6)	38 (44.2)			
LTF	rs1126478					
	GG	28 (45.2)	20 (46.5)	0.891	0.947	0.436–2.056
	AG	24 (38.7)	16 (37.2)			
	AA	10 (16.1)	7 (16.3)			
	G	80 (64.5)	56 (65.1)	0.929	0.974	0.549–1.729
	A	44 (35.5)	30 (34.9)			

* $p < 0.05$

the −44 C allele of *DEFB1* were relatively high in cases of CP. Next, we performed four kinds of comparison: periodontitis vs. controls, severe CP vs. CP control, combined periodontitis (severe and moderate CPs) vs. CP control and AgP vs. AgP control for genotypic/allelic associations of the *DEFB1* and *LTF* polymorphisms. In the −44 C/G polymorphism of *DEFB1*, statistical significance was observed between the −44 CC genotype and periodontitis (OR 2.510, 95 % CI 1.005–6.263, $p < 0.05$, Table 2). Furthermore, in the −44 C/G polymorphism of *DEFB1*, statistical significance was observed between the −44 CC genotype and severe CP (OR 4.154, 95 % CI 1.113–15.304, $p < 0.05$, Table 3) and between the −44 CC genotype and combined periodontitis (OR 4.038, 95 % CI 1.236–13.186, $p < 0.05$, Table 3). Statistical significance was also identified between the −44 C allele and severe CP

Table 3 Genotype associations of β-defensin-1 (*DEFB1*) and lactoferrin (*LTF*) genes with chronic periodontitis

Gene	SNP	Severe CP	Moderate CP	CP-cont	Severe CP vs. CP-cont			Severe CP + moderate CP vs. CP-cont		
		n (%)	*n* (%)	*n* (%)	*p* value	OR	95 % CI	*p* value	OR	95 % CI
DFFB1	rs1799946									
	GG	9 (32.1)	4 (30.8)	11 (50.0)	0.201	0.474	0.152–1.473	0.154	0.464	0.163–1.323
	GA	15 (53.6)	7 (53.8)	8 (36.4)						
	AA	4 (14.3)	2 (15.4)	3 (13.6)						
	G	33 (58.9)	15 (57.7)	30 (68.2)	0.341	0.670	0.295–1.522	0.288	0.659	0.307–1.416
	A	23 (41.1)	11 (42.3)	14 (31.8)						
DFFB1	rs1800972									
	CC	24 (85.7)	11 (84.6)	13 (59.1)	0.035*	4.154	1.113–15.304	0.020*	4.038	1.236–13.186
	CG	4 (14.3)	1 (7.7)	9 (40.9)						
	GG	0 (0)	1 (7.7)	0 (0)						
	C	52 (92.9)	23 (88.5)	35 (79.5)	0.048*	3.343	1.003–11.041	0.055	2.755	0.978–7.752
	G	4 (7.1)	3 (11.5)	9 (20.5)						
DFFB1	rs11362									
	GG	6 (21.4)	3 (23.1)	6 (27.3)	0.631	0.727	0.206–2.565	0.636	0.750	0.234–2.388
	GA	16 (57.1)	8 (61.5)	11 (50.0)						
	AA	6 (21.4)	2 (15.4)	5 (22.7)						
	G	28 (50.0)	14 (53.8)	23 (52.3)	0.821	0.913	0.417–2.002	0.910	0.959	0.463–1.985
	A	28 (50.0)	12 (46.2)	21 (47.7)						
LTF	rs1126478									
	GG	11 (39.3)	6 (46.2)	10 (45.5)	0.661	0.776	0.254–2.366	0.760	0.850	0.303–2.376
	AG	14 (50.0)	4 (30.8)	9 (40.9)						
	AA	3 (10.7)	3 (23.1)	3 (13.6)						
	G	36 (64.3)	16 (61.5)	29 (65.9)	0.866	0.931	0.410–2.118	0.781	0.897	0.419–1.921
	A	20 (35.7)	10 (38.5)	15 (34.1)						

* $p < 0.05$

(OR 3.343, 95 % CI 1.003–11.041, $p < 0.05$, Table 3). There were no significant differences in the genotypic/allelic associations of other *DEFB1* and *LTF* SNPs for severe CP vs. CP control and combined periodontitis vs. CP control (Table 3). On the other hand, there were no genotypic/allelic associations of the *DEFB1* and *LTF* polymorphisms between AgP and AgP control (Table 4).

GCF analysis of β-defensin-1 and lactoferrin

To evaluate the relationship between the genotype and expressions of AMPs in GCF samples, 67 GCF samples from 17 subjects and 71 GCF samples from 16 subjects were used for the determination of β-defensin-1 and lactoferrin, respectively. As shown in Fig. 1a, ELISA analysis revealed that the β-defensin-1 concentrations were significantly lower in the subjects with the −44 CC genotype than in those without the −44 CC genotype, namely, 0.95 ± 0.36 and 1.55 ± 0.63 pg/μl, respectively ($p < 0.05$). Moreover, the β-defensin-1 concentrations in PD ≤3 mm were significantly lower in the subjects with the −44 CC genotype than in those without it, namely, 1.12 ± 0.43 and 2.20 ± 1.22 pg/μl, respectively ($p < 0.05$, Fig. 1a). There were no significant differences in PD ≥4 mm between the subjects with the −44 CC genotype and those without it, with values of 0.86 ± 0.54 and 0.81 ± 0.13 pg/μl, respectively (Fig. 1a). When the concentrations of lactoferrin in GCF were evaluated, no significant differences were observed between the subjects with the GG genotype of *LTF* and those without it, with values of 7.77 ± 2.09 and 8.14 ± 4.85 ng/μl, respectively (Fig. 1b).

Discussion

In this study, we investigated the association of four AMP-SNPs with the susceptibility to periodontitis in 105 Japanese subjects. We selected three SNPs in the 5′-UTR of the *DEFB1* gene (rs1799946, rs1800972 and rs11362) and one SNP in exon 1 of the *LTF* gene (rs1126478). These SNPs have been considered to be associated with various

Table 4 Genotype and allele frequencies of β-defensin-1 (*DEFB1*) and lactoferrin (*LTF*) gene polymorphisms in subjects with or without aggressive periodontitis

Gene	SNP	AgP	AgP-cont	AgP vs. AgP-cont		
		n (%)	*n* (%)	*p* value	OR	95 % CI
DFFB1	rs1799946					
	GG	7 (33.3)	5 (23.8)	0.367	1.600	0.429–5.927
	GA	11 (52.4)	12 (57.1)			
	AA	3 (14.3)	4 (19.0)			
	G	25 (59.5)	22 (52.4)	0.510	1.337	0.567–3.150
	A	17 (40.5)	20 (47.6)			
DFFB1	rs1800972					
	CC	17 (81.0)	16 (76.2)	0.500	1.328	0.321–5.454
	CG	3 (14.3)	5 (23.8)			
	GG	1 (4.8)	0 (0)			
	C	37 (88.1)	37 (88.1)	0.631	1.000	0.284–3.524
	G	5 (11.9)	5 (11.9)			
DFFB1	rs11362					
	GG	7 (33.3)	8 (38.1)	0.747	0.813	0.236–2.805
	GA	9 (42.9)	9 (42.9)			
	AA	5 (23.8)	4 (19.0)			
	G	23 (54.8)	25 (59.5)	0.659	0.823	0.349–1.942
	A	19 (45.2)	17 (40.5)			
LTF	rs1126478					
	GG	11 (52.4)	10 (47.6)	0.758	1.210	0.367–3.991
	AG	6 (28.6)	7 (33.3)			
	AA	4 (19.0)	4 (19.0)			
	G	28 (66.7)	27 (64.3)	0.818	1.111	0.456–2.706
	A	14 (33.3)	15 (35.7)			

infectious diseases. *DEFB1* gene polymorphisms were reportedly related to *Pseudomonas aeruginosa* airway colonization in cystic fibrosis, oral *Candida* carriage, lepromatous leprosy, HIV infection, *Helicobacter pylori*-induced gastritis, severe acute pancreatitis and dental caries [31, 33, 37–41]. *LTF* gene polymorphism was reportedly related to dental caries [42]. In terms of the SNPs in the 5′-UTR of *DEFB1*, −44 C/G was not associated with early-onset periodontitis [43] and that −20 G/A was not associated with severe CP [32]. In the case of *LTF*-SNP, Lys/Arg polymorphism was shown to be associated with AgP [34, 35]. In this study, we provided the first evidence that the −44 CC genotype of *DEFB1* was associated with periodontitis (OR 2.510), particularly with severe CP and severe/moderate-combined periodontitis (OR 4.154 and 4.038, respectively), whereas there was no association with AgP. This result is similar to a previous report that showed that the −44 C/G polymorphism was not associated with AgP [43].

The present finding, the association of −44 CC genotype with CP, may be linked to the low expression of β-defensin-1 in gingival tissue, because basal levels of β-defensin-1 reflect a protective ability in localized tissues [44, 45]. Our result suggests that the −44 C/G polymorphism may affect β-defensin-1 expression in gingival tissue and enhance the disease susceptibility to CP. It was reported that the constitutive human β-defensin-1 mRNA level was lower in primary gingival keratinocytes associated with the −44 CC genotype than in cells associated with the −44 GG and −44 GC genotypes, and that the −44 G allele was associated with an increase in constitutive antimicrobial activity and expression of β-defensin-1 [46]. Recent study concerning lepromatous leprosy demonstrated that the position −44 was included in the putative NFκB binding site and the variant could change the NFκB binding affinity and thus influenced the regulation of *DEFB1* gene expression at the transcription stage [37]. Furthermore, several reports showed the homology score between the region from positions −57 to −15 of *DEFB1* and the NFκB binding site, and the value for the −44 C allele was shown to be lower than that for the −44 G allele (homology scores of 64.5 and 69.8 %, respectively), indicating that the −44 C/G polymorphism could contribute to susceptibility to infectious disease [37, 47, 48]. Our finding that the subjects with the

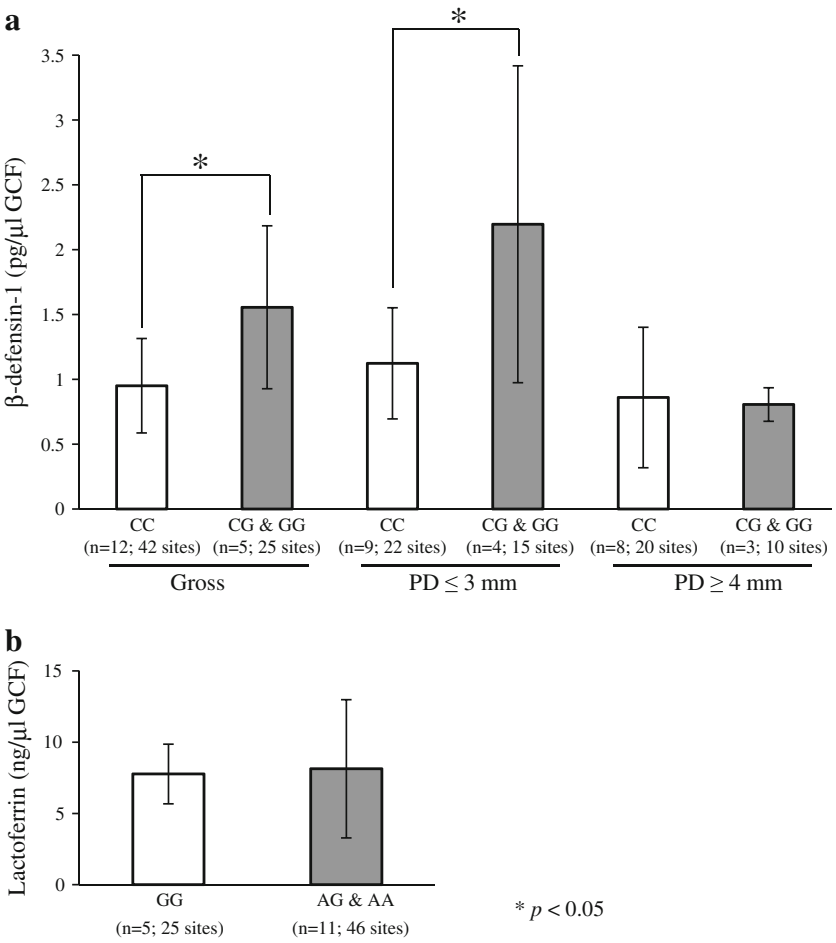

Fig. 1 Associations between the genotypes and concentrations of antimicrobial peptides in GCF samples. GCF samples were collected using paper strips and the volumes were quantified using a calibrated unit. The contents of β-defensin-1 and lactoferrin in GCF were determined using ELISA kits. β-defensin-1 concentrations of subjects with the CC, CG and GG genotypes of rs1800972 (**a**) and lactoferrin concentrations of subjects with the GG, AG and AA genotypes of rs1126478 (**b**) are shown as the mean ± SD. *$p < 0.05$ vs. control

−44 CC genotype exhibited low concentrations of β-defensin-1 in GCF might be attributable to the −44 C allele that being involved in a decrease in NFκB binding affinity. Since the association between −44 C/G polymorphism and NFκB binding affinity is not completely understood, further study including gel mobility shift assay is necessary to clarify the mechanism involved.

The present finding that the β-defensin-1 concentrations in localized PD ≤3 mm, unlike in PD ≥4 mm, were low in the subjects with the −44 CC genotype suggests that the −44 CC genotype may be related to the onset of CP. However, the subjects with the −44 CC genotype did not always suffer from CP, suggesting that periodontitis is a multifactorial disease associated with not only genetic factors but also environmental ones. It is generally known that β-defensins are expressed in mucosal epithelial cells and keratinocytes to protect oral mucosal surfaces. β-defensin-1 was shown to be expressed constitutively in epithelial tissue, but β-defensin-2, -3 and -4 were found to be up-regulated by proinflammatory cytokines and microorganisms [16, 21]. β-defensin-1, -2 and -3 were expressed in both inflamed and non-inflamed gingival tissues, whereas differential expressions were observed among tissues from healthy subjects, and cases of gingivitis and periodontitis [49–51]. The level of β-defensin-1 mRNA expression was low in cases of gingivitis and AgP, but high in CP; conversely, the level of β-defensin-2 mRNA expression was high in AgP but low in CP [52]. From these findings, our result may reflect that β-defensin-1 plays a role in defense of the gingival mucosal surface.

In this study, we demonstrated that the −52 G/A and −20 G/A polymorphisms of β-defensin-1 were not related to periodontitis including CP and AgP. This result is partially consistent with a previous report [32]. Although previous reports showed that the Lys/Arg polymorphism of lactoferrin was associated with AgP [34, 35], our result showed that there were no significant differences in the associations of Lys/Arg polymorphism with periodontitis including CP and AgP. In addition, the concentrations of lactoferrin in GCF did not show any differences between the subjects with and without the GG genotype. It has been reported that Lys/Arg polymorphism of lactoferrin might change the protein sequence and give rise to reduce antimicrobial activity of lactoferrin [34]. However, our results showed that the Lys/Arg polymorphism was not related to CP and AgP in Japanese patients. We assume that these

results might be due to ethnicity and geography because some reports have indicated that a Thr/Ala polymorphism at position 11 resulting from an A/G transition was associated with AgP in African–Americans but not in Caucasians [53].

Taken together, we conclude that the −44 CC genotype of *DEFB1* is associated with the susceptibility to periodontitis particularly CP in Japanese and that −44 C/G polymorphism may be a predictor of CP.

Conclusion

The −44 CC genotype of the β-defensin-1 gene (*DEFB1* rs1800972) may be associated with susceptibility to chronic periodontitis in Japanese.

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