LTF and *DEFB1* polymorphisms are associated with susceptibility toward chronic periodontitis development

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Funding information
Fondo Trieste 2008; Regione FVG, Grant/Award Number: L.26.2008

Objectives: Chronic periodontitis is a common pathological condition that affects the supporting tissue of the teeth, leading to progressive alveolar bone destruction and teeth loss. The disease is caused by bacteria and derives from an altered host immune and inflammatory response, also involving different factors such as the oral hygiene, smoking, and genetic background. The innate immune response, the first line of host defense, could also play an important role in the susceptibility to chronic periodontitis. In this study, we evaluated the possible association between periodontal disease and seven genetic variations within *DEFB1* and *LTF* genes, encoding for β-defensins 1 and lactoferrin (two members of oral innate immune system), in an Italian isolated population.

Subjects and Methods: *DEFB1* 5′UTR g. -52G>A (rs1799946), g. -44C>G (rs1800972), g. -20G>A (rs11362), 3′UTR c*5G>A (rs1047031), c*87A>G (rs1800971), *LTF* p.Ala29Thr (rs1126477), and p.Lys47Arg (rs1126478) single nucleotide polymorphisms (SNPs) were analyzed in 155 healthy individuals and 439 chronic periodontitis patients from North-East Italy.

Results: Significant associations were found between periodontitis and g. -20G>A (rs11362) and g. -44C>G (rs1800972) SNPs in *DEFB1* gene as well as p.Ala29Thr (rs1126477) and p.Lys47Arg (rs1126478) SNPs in *LTF* gene.

Discussion: Our results suggest the involvement of *DEFB1* and *LTF* genetic variations in the susceptibility toward development of periodontitis.

KEYWORDS
DEFB1, genetic polymorphisms, innate immunity, *LTF*, periodontitis

1 | INTRODUCTION

Periodontitis is an inflammatory condition in which the dental supporting tissues are injured and destroyed, leading to pain, loss of connective tissue and bone, until the tooth is lost in the advanced stages (Pihlstrom, Michalowicz, & Johnson, 2005). Periodontitis is caused by pathogenic bacteria that are present in the oral environment and form biofilm or plaque on teeth surface; the presence of bacteria is required to trigger the disease; however, other risk factors such as smoking, diabetes, stress, medication, and nutrition are involved in the onset of gingival inflammation (Van Dyke & Sheilesh, 2005). In addition, the observed variability in periodontitis susceptibility among populations of different ancestral backgrounds suggests a role for the host genome in the disease pathogenesis (Loos, John, & Laine, 2005). Moreover, it has been suggested that periodontitis is also promoted by failure of tissue homeostasis, that is, an altered oral immune response against resident oral microflora (Listgarten, 1976; Moore & Moore, 1994).

The immune inflammatory response plays the major role in the presentation of pathology's clinical signs (Pihlstrom et al., 2005); although the immune response should contribute to protection against periodontitis, either hyporesponsiveness or hyper-responsiveness could be detrimental for the host (Preshaw, Seymour, & Heasman, 2004). In the context of immune molecules possibly involved in periodontitis, the innate immune system, representing the first line of defense against pathogens, could play an important role in the modulation of susceptibility to the diseases as well as to its severity (Darveau, 2010).

Within the molecules of the innate immune system, we focused on the human β-defensin 1 (hBD-1) and lactoferrin (also termed lactotransferrin), two proteins expressed in the oral mucosa with potential action against pathogens involved in periodontitis' susceptibility (Dommisch & Jepsen, 2015; Ebrahem, 2013; Fine, 2015; Wang et al., 2015).

Human β-defensin 1 is a small cationic cysteine-rich peptide possessing antimicrobial activity against viruses, fungi, and gram-negative and gram-positive bacteria (Weinberg, Krisanaprakornkit, & Dale, 1998). hBD-1 is constitutively expressed in the oral mucosa, but it could be also induced by different stimuli, such as microbial infection (Weinberg et al., 1998). Different single nucleotide polymorphisms (SNPs) within the hBD-1 encoding gene, *DEFB1* (8.p22-23), are known to affect peptide expression. Three polymorphisms at *DEFB1* 5′ untranslated region (UTR) at positions g. -52G>A (rs1799946), g. -44C>G (rs1800972), and g. -20G>A (rs11362) have been reported as able to influence hBD-1 levels (Prado-Montes de Oca, 2010), and additionally, two polymorphisms at the 3′ UTR c*5G>A (rs1047031) and c*87A>G (rs1800971) located in microRNA binding sites are potentially able to alter hBD-1 expression (Prado-Montes de Oca, 2010). Furthermore, the above-mentioned SNPs were associated with susceptibility to develop periodontitis among European Caucasian (Schaefer et al., 2010) and Japanese (Ikuta et al., 2015), but not in Americans (Caucasian and African Americans) (Ozturk, Famili, & Vieira, 2010), and in patients from Caucasian and African American cohorts described in the study of Boniotto et al. (Boniotto et al., 2004).

Lactoferrin is another antimicrobial molecule active against bacteria, virus, and fungi (Gonzalez-Chavez, Arevalo-Gallegos, & Rascon-Cruz, 2009). It is produced at mucosal surfaces, and in the oral cavity, it is present in the saliva (Farnaud & Evans, 2003) and in gingival crevicular fluid (Friedman, Mandel, & Herrera, 1983). Lactoferrin is a multifunctional molecule known as "metal iron chelator" with the specific ability to bind iron (Gorr & Abdolhosseini, 2011). Lactoferrin is a host defense protein, with bacteriostatic/bactericidal activities (Arnold, Cole, & McGhee, 1977; Arnold, Russell, Champion, & Gauthier, 1981), and it also works modulating innate and adaptive immunity (Legrand, Elass, Carpentier, & Mazurier, 2005, 2006). Its versatility and importance are proven by its ubiquitously presence in all mucosal tissues of the body, from saliva and tears to gastric secretions and amniotic liquid (Farnaud & Evans, 2003). Lactoferrin is encoded by *LTF* gene located at 3p21.3; two exon 1 missense polymorphisms p.Ala29Thr (AAA>AGA) and p.Lys47Arg (GCC>ACC) (rs1126477 and rs1126478, respectively) are of interest as they cause two amino acid substitutions within the strongly basic N-terminal region of the mature protein possibly affecting its antibacterial properties (Jordan et al., 2005). Previous studies reported associations between these polymorphisms and periodontitis susceptibility in Taiwanese (Wu, Juo et al., 2009), North Americans (Velliyagounder et al., 2003), African Americans but not in Caucasians (Jordan et al., 2005) and in Japanese (Ikuta et al., 2015).

Considering the role hypothesized for hBD1 and lactoferrin in the susceptibility to periodontitis and previous studies with discordant findings in distinct ethnic groups, we analyzed five functional *DEFB1* polymorphisms (rs1799946, rs1800972, rs11362, rs1047031, and rs1800971) and two missense *LTF* polymorphisms (rs1126477, rs1126478) in an isolated population from North-East Italy. We aimed at replicating former results adding more information about the role of *DEFB1* and *LTF* functional genetic variants in the susceptibility toward periodontitis development and disease severity.

2 | MATERIALS AND METHODS

2.1 | Participants

Data from 805 individuals aged 18–88 years were collected between March 2008 and November 2008 thanks to the "Friuli Venezia Giulia Genetic Park" project, aimed at analyzing the genetic background of populations from villages located in the North-East of Italy (San Martino del Carso, Erto e Casso, Clauzetto, Illegio, Sauris and Val di Resia) (Esko et al., 2013).

Participants gave written informed consent, and the ethical committee of IRCCS Burlo Garofolo/the Ethics Committee of the University of Trieste approved the study (Prot. CE/V-78, 06/08/2007). All study experiments and procedures have been performed in accordance with the ethical standards of the 1975 Declaration of Helsinki (7th revision, 2013).

All the patients willing and fully capable to comply with the dental examination and x-ray screening were included.

2.2 | Periodontal status assessment

Medical family and individual history were recorded. All adults were subjected to an accurate oral inspections in which periodontal examinations included measurements of periodontal pocket depth (PD), Plaque Index (PI), Bleeding Index (BoP), clinical attachment loss (CAL), and gingival recession (GR) on all teeth using a periodontal probe PCP12 (Hu-Friedy, Chicago, IL, USA). Four sites on each tooth were assessed: mesial, buccal, distal, and lingual. Moreover, the overall number of teeth present in the mouth was recorded excluding Wisdom teeth. To diagnose the periodontal disease, the periodontal probing and an X-ray examination were used. Although the periodontal status would be more accurate, considering the high number of patients visited in loco, the equipment and the time needed to perform a complete status evaluation, we chose to measure the bone loss by Panoramic x-ray.

Diagnosis was made following the criteria of the American Academy of Periodontology (Armitage, 1999), and subjects were then categorized as summarized in Table 1.

TABLE 1 Criteria of the American Academy of Periodontology used to classify the periodontal status of subjects involved in the study

Code	Condition	Criteria
0	Healthy	Bleeding Index (BoP) <25%
1	Gingivitis	BoP >25%
2	Aggressive pocket depth (PD)	Tooth mobility and pockets of incisors and molars, age <35
3	Chronic localized PD slight or moderate	<30% with support loss <1/3 of the root
4	Chronic generalized PD slight or moderate	>30% with support loss <1/3 of the root
5	Chronic localized PD severe	<30% with support loss >1/3 of the root
6	Chronic generalized PD severe	>30% with support loss >1/3 of the root
7	Edentulous	
8	Not evaluable	No rx, incomplete data

2.3 | Sample collection and phenotype definition

To verify the association between *DEFB1* and *LTF* SNPs with different types of chronic periodontitis, initially a common group of 155 controls was defined composed by people without signs of periodontitis (coded as 0; average teeth number = 26; Tables 1 and 2), and then, the study group was defined including all people (n = 439; average teeth number = 21) affected by chronic periodontitis (coded as 3, 4, 5, and 6, Tables 1 and 2).

Subsequently, cases were regrouped into different categories following the extension and severity of the disease: 271 patients were classified as slight (codes 3 and 4; average teeth number = 22), 168 as severe (codes 5 and 6; average teeth number = 17), 206 as localized (codes 3 and 5; average teeth number = 22), and 233 as generalized (codes 4 and 6; average teeth number = 19) (Tables 1 and 2).

The cases from each category were tested against the common control group.

Patients with aggressive periodontitis, gingivitis, edentulous and patients with incomplete data were excluded from the analysis (n = 211) (Table 2).

2.4 | Single nucleotide polymorphisms selection, genotyping, and imputation

DEFB1 and *LTF* SNPs were selected based on previous literature findings reporting a functional role for these genetic variations (see the Introduction for the references).

For each sample, DNA was extracted from peripheral blood using the EZ1 DNA investigator kit (Qiagen, Milan, Italy) following manufacturer's protocols and then stored at −20°C prior to analysis. Genotyping was carried out using Illumina 370k high-density SNP array (Illumina, Inc., San Diego, CA, USA) on the Illumina HiScan system at CBM (Cluster in Biomedicine, Trieste, Italy).

Genotype calling was performed with the GenomeStudio software (Illumina, Inc.). As quality control (QC) step, individual call rate, excess of heterozygosity, and identity by state (IBS) between each pair of samples were checked. If a pair had IBS >0.95, the sample with lower call rate was excluded.

Single nucleotide polymorphisms which were not present on the genotyping array have been imputed using SHAPEIT2 (Delaneau, Marchini, & Zagury, 2012) for the phasing step and IMPUTE2 (Howie, Donnelly, & Marchini, 2009) for the imputation using the 1000 Genomes phase I v3 reference set (Genomes Project Consortium et al., 2012).

After performing standard filtering (call rate ≥ 97%, Rsq ≥ 0.3, Hardy–Weinberg equilibrium p-value [HWE] ≥ 1×10^{-6}, minor allele frequency [MAF] ≥ 0.05), a total of 8,483,800 SNPs passed the QC (Little et al., 2009).

2.5 | Statistical analysis

For statistical analysis, we treated the logistic trait (0 = controls, 1 = cases) as though it was quantitative which corresponds to the Armitrage Trend Test. We thus used a linear mixed-model regression, assuming an additive model, where the case status was the dependent variant while the SNP dosages were the tested independent variable. Sex, age, smoking status, and number of teeth were included in the model as covariates, according to past evidence that report a relationship between these variables and periodontitis (Martin, Page, Kaye, Hamed, & Loeb, 2009, Office of the Surgeon General & Office on Smoking and Health, 2004; Ragghianti, Greghi, Lauris, Sant'ana, & Passanezi, 2004). All statistical tests were performed with R software 3.1.3 version (R Core Team, 2015).

The genomic kinship matrix was used as random effect to take into account the non-independence of the samples as they are coming from an isolated population. Genomic kinship was estimated using the ibs function from the GenABEL R package (Aulchenko, Ripke, Isaacs, & van Duijn, 2007). Linear regression was conducted using MixABEL (Aulchenko et al., 2007) and the GRAMMAR+ method.

Moreover, we calculated the inflation factor (λ) to check the presence of stratification in FVG population. λ ranges from 1.0061586 to 1.0648490 in the analysis of the different periodontitis subgroups, indicating absence of stratification.

TABLE 2 Demographic features and periodontal disease status of the study population

	All (*n* = 805)	Males (*n* = 357)	Females (*n* = 448)
Age (years) (mean ± *SD*) (range)	51.52 ± 16.19 (18–88)	51.81 ± 15.79 (19–85)	51.29 ± 16.51 (18–88)
Smoking status			
Yes	160 (20%)	71 (20%)	89 (20%)
No	645 (80%)	286 (80%)	359 (80%)
Teeth (mean ± *SD*)	19.73 ± 9.37	19.85 ± 9.11	19.64 ± 9.58
Periodontal status			
Controls (code 0)	155	57	98*
Gingivitis (code 1)	92	41	51
Aggressive (code 2)	10	7	3
Slight (code 3 + 4)	271	130	141
Severe (code 5 + 6)	168	83	85
Localized (code 3 + 5)	206	97	109
Generalized (code 4 + 6)	233	116	117
All (code 3 + 4+5 + 6)	439	213	226
Edentulous (code 7)	93	33	60*
Not evaluable (code 8)	16	6	10

*Indicates significant gender differences (*p*-value < .05).

Finally, as no method can estimate odds ratios when inbreeding is present, we have assessed the odds ratios using standard logistic regression, while standard errors for confidence intervals were derived using the corrected test statistic and the beta from the logistic regression.

3 | RESULTS

Population characteristics are presented in Tables 1 and 2. Briefly, 357 males (mean age 51.81 ± 15.79, range: 19–85) and 448 females (mean age 51.29 ± 16.51; range: 18–88) constituted the study population; 160 patients (20%) were current smokers.

One hundred and fifty-five subjects with no clinical signs of oral pathologies were used to form the control group; the 439 subjects affected by chronic periodontitis were then classified according with the periodontitis status: 271 were categorized as slight (codes 3 and 4), 168 as severe (codes 5 and 6), 206 as localized (codes 3 and 5), and 233 as generalized (codes 4 and 6) Patients with aggressive periodontitis, gingivitis, edentulous, and patients with incomplete data were excluded from the analysis (*n* = 211) (Tables 1 and 2).

The statistically significant associations between *DEFB1* and *LTF* polymorphisms and periodontitis status are summarized in Table 3; the distribution of *DEFB1* and *LTF* polymorphisms frequencies in patients and healthy controls is reported in Table 4.

Initially, the totalities of patients with chronic periodontitis were compared with the controls: No statistically significant different distributions were observed between patients and controls for the five *DEFB1* polymorphisms. When comparing periodontitis subgroups (slight, severe, localized, and generalized) with the control subjects, the genotype distribution of *DEFB1* polymorphisms g. -52G>A (rs1799946), c*5G>A (rs1047031), and c*87A>G (rs1800971) was similar. In contrast, *DEFB1* polymorphisms g. -20 > A (rs11362) and g. -44C>G (rs1800972) were associated with severe periodontitis (*p*-value = .05, OR = 0.64, CI = 0.41–0.99 and *p* = .03, OR = 0.55, CI = 0.31–0.95, respectively). In particular, the g. -20 G/G genotype was more frequent among patients respect to healthy subjects; instead, g. -44 C/C genotype was more represented among controls than in patients (Table 4).

When considering the *LTF* gene, p.Lys47Arg G/G genotype correlated with higher risk of chronic periodontitis (phenotype All, Table 3) (*p* = .04, OR = 1.41; CI = 1.00–2.02), while p.Ala29Thr polymorphism did not. Moreover, in the subgroup of slight periodontitis p.Lys47Arg (rs1126478) and p.Ala29Thr (rs1126477) were correlated with the susceptibility toward disease development (*p*-value = .02; OR = 1.49; CI = 1.05–2.15 and *p* = .02; OR = 1.52; CI = 1.04–2.28, respectively). Specifically, p.Lys47Arg G/G genotype was more frequent among periodontitis patients respect to A/A genotype more represented among controls; moreover, individuals carrying the p.Ala29Thr T allele had an increased risk for the disease. Finally, *LTF* polymorphisms were not correlated with the other subclassification of periodontitis, named severe, localized, and generalized.

4 | DISCUSSION

Human β-defensin 1 and lactoferrin are two members of host innate immunity system previously studied in the context of oral cavity environment. We analyzed five functional polymorphisms in *DEFB1* gene

TABLE 3 Significant results found in the association analysis (analyses adjusted according to the covariates: age, gender, and smoking status)

SNP	Chr	Pos	CA/OA	*N*	Marker Type	*p*	OR	CI	MAF	Gene	Periodontal status
rs11362	8	6735399	A/G	323	G	.05	0.64	0.41–0.99	0.41	*DEFB1*	Severe
rs1800972	8	6735423	C/G	323	I	.03	0.55	0.31–0.95	0.21	*DEFB1*	Severe
rs1126478	3	46501213	A/G	594	G	.04	1.41	1.00–2.02	0.28	*LTF*	All
rs1126478	3	46501213	A/G	426	G	.02	1.49	1.05–2.15	0.28	*LTF*	Slight
rs1126477	3	46501268	T/C	426	I	.02	1.52	1.04–2.28	0.22	*LTF*	Slight

SNP: single nucleotide polymorphism reference number; Chr: chromosome number; Pos: genomic position in base pairs; CA/OA: coded allele/other allele; *N*: total number of samples used for the analysis (patients plus controls); maker type: G = genotyped, I = imputed; *p*: *p*-value; OR, odds ratio; CI, 95% confidence interval values; MAF, minor allele frequency; Gene: gene in which the polymorphism is located; periodontal status: the group of patients with different periodontal condition used for the analysis.

and two in *LTF* gene aimed at disclosing their potential role in the susceptibility to develop periodontitis. In this work, we conducted a replica study in which we considered polymorphisms previously associated with different pathophysiologic conditions, including oral pathologies. So we assumed a priori the correlation of these polymorphisms with periodontal disease, and we did not apply correction for multiple tests.

We found that the *DEFB1* g. -20G>A (rs11362) and g. -44 C>G (rs1800972) functional SNPs were significantly associated with severe periodontitis susceptibility, being the g. -20 G/G genotype more frequent in patients than in healthy controls and g. -44 C/C genotype more represented among healthy individuals than patients.

The g. -20G>A polymorphism was never reported as correlated with periodontitis condition (Ozturk et al., 2010; Schaefer et al., 2010; Wohlfahrt, Wu, Hodges, Hinrichs, & Michalowicz, 2006); however, it was associated with susceptibility to caries among American children (Ozturk et al., 2010) and among Latvian children with cleft lip and/or palate (Krasone et al., 2014). Moreover, the g. -20 A allele was also reported as able to reduce *DEFB1* gene expression (Milanese, Segat, & Crovella, 2007) (Nurjadi, Herrmann, Hinderberger, & Zanger, 2013).

Our results are not in agreement with the study of Boniotto et al. (2004) reporting a lack of association between g. -44C>G polymorphisms and periodontitis susceptibility among patients from Caucasian and African American cohorts and with Ikuta et al. (2015) findings reporting an association between *DEFB1* g. -44 C/C genotype and increased risk toward severe chronic periodontitis development in a Japanese population. Moreover, low hBD-1 levels were reported in subjects carrying g. -44 C/C in vivo in gingival crevicular fluid (Ikuta et al., 2015) and saliva (Polesello et al., 2015).

The exact mechanism of action of hBD-1 in periodontal disease is still unknown. hBD-1, in spite of having been described as antimicrobial peptide, has poor antimicrobial activity, so this peptide feature is not relevant on susceptibility to periodontitis. However, considering its immuno-modulatory action (reviewed in Prado-Montes de Oca, 2010) and the correlation between g- 20 G and g. -44 G alleles with a higher peptide production (Milanese et al., 2007) (Nurjadi et al., 2013), possibly leading to an increased immune response with subsequent inflammation and tissues damage, we can hypothesize that subjects carrying these alleles have an augmented risk toward periodontitis development.

Nevertheless, also a weak innate immune response could be detrimental; in fact, Liu, Chen, Du, Hu, and Chen (2014) observed lower level of *DEFB1* mRNA in chronic periodontitis patients' biopsies when compared to healthy subjects. Instead, other studies failed in measuring altered hBD-1 concentration between periodontitis patients and healthy controls (Bissell et al., 2004; Dommisch, Acil, Dunsche, Winter, & Jepsen, 2005; Kuula et al., 2008), and other authors reported that *DEFB1* mRNA expression was down-regulated in aggressive periodontitis and up-regulated in chronic periodontitis respect to healthy subjects (Vardar-Sengul et al., 2007).

For the other *DEFB1* polymorphisms, no statistically significant associations were observed with periodontitis status in the present study. Our results partially agree with the study of Ikuta et al. (2015) but not with the study of Schaefer et al. (2010) that observed a correlation between *DEFB1* c*5G>A A allele and chronic and aggressive periodontitis among Caucasians.

As regards the role of lactoferrin in periodontal disease (Fine, 2015), higher lactoferrin salivary levels were measured in Australian (Huynh et al., 2015), Indian (Yadav et al., 2014), Swedish (Glimvall, Wickstrom, & Jansson, 2012), Finnish (Suomalainen, Saxen, Vilja, & Tenovuo, 1996), Taiwanese (Tsai, Kao, & Chen, 1998; Wei et al., 2004), American (Fine, Furgang, & Beydouin, 2002), and British (Adonogianaki, Moughal, & Kinane, 1993) periodontitis patients with respect to healthy controls, while low lactoferrin level was found among Brazilian (Rocha Dde et al., 2012) and Chinese (Wu, Shu, Luo, Ge, & Xie, 2009) periodontitis patients. Finally, among Japanese (Ikuta et al., 2015), Swedish (Figueredo & Gustafsson, 2000), and British (Murray, Mooney, & Kinane, 1995) subjects, no differences between patients with periodontitis and controls were detected. Moreover, a study reported that high concentration of lactoferrin in gingival crevicular fluid significantly reduces after periodontal therapy (Yadav et al., 2014). A possible explanation of the differences regarding lactoferrin concentration in patients with periodontitis and healthy controls could be related with the different activity of lactoferrin based on host genetic background not investigated in most studies. In fact, the localization of p.Ala29Thr and p.Lys47Arg nonsynonymous polymorphisms in the N-terminal region of the protein, that mediates its activity, could influence the efficiency of its antibacterial properties (Jordan et al., 2005), independently of protein concentration.

TABLE 4 Frequencies and counts of *DEFB1* and *LTF* polymorphisms that showed a different distribution between patients and healthy controls

	Generalized periodontitis *n* = 233	Severe periodontitis *n* = 168	Slight periodontitis *n* = 271	Localized periodontitis *n* = 206	All periodontitis *n* = 439	Healthy controls *n* = 155
DEFB1 rs1800971						
A/A	225 (0.97)	163 (0.97)	266 (0.98)	204 (0.99)	429 (0.98)	150 (0.97)
A/G	8 (0.03)	5 (0.03)	5 (0.02)	2 (0.01)	10 (0.02)	5 (0.03)
G/G	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
DEFB1 rs1047031						
C/C	182 (0.78)	140 (0.83)	202 (0.74)	160 (0.78)	342 (0.78)	122 (0.79)
C/T	47 (0.20)	26 (0.15)	62 (0.23)	41 (0.20)	88 (0.20)	30 (0.19)
T/T	4 (0.02)	2 (0.01)	7 (0.03)	5 (0.02)	9 (0.02)	3 (0.02)
DEFB1 rs11362						
G/G	88 (0.37)	77 (0.46)*	92 (0.34)	81 (0.39)	169 (0.38)	41 (0.27)
A/G	113 (0.29)	65 (0.39)*	129 (0.48)	81 (0.40)	194 (0.45)	80 (0.52)
A/A	32 (0.14)	26 (0.15)*	50 (0.18)	44 (0.21)	76 (0.17)	34 (0.21)
DEFB1 rs1800972						
C/C	138 (0.61)	94 (0.56)*	172 (0.63)	128 (0.62)	266 (0.61)	110 (0.71)
C/G	81 (0.33)	63 (0.37)*	82 (0.30)	64 (0.31)	145 (0.33)	40 (0.26)
G/G	14 (0.06)	11 (0.06)*	17 (0.06)	14 (0.07)	28 (0.06)	5 (0.03)
DEFB1 rs1799946						
C/C	85 (0.36)	62 (0.37)	113 (0.42)	90 (0.44)	175 (0.40)	64 (0.41)
C/T	108 (0.46)	74 (0.44)	115 (0.42)	81 (0.39)	189 (0.43)	69 (0.45)
T/T	40 (0.17)	32 (0.19)	43 (0.16)	35 (0.17)	75 (0.17)	22 (0.14)
LTF rs1126478						
A/A	117 (0.50)	87 (0.52)	139 (0.51)*	109 (0.53)	226 (0.51)*	92 (0.58)
A/G	94 (0.40)	67 (0.40)	99 (0.37)*	72 (0.35)	166 (0.38)*	54 (0.36)
G/G	22 (0.09)	14 (0.08)	33 (0.12)*	25 (0.12)	47 (0.11)*	9 (0.06)
LTF rs1126477						
C/C	132 (0.57)	102 (0.61)	154 (0.57)*	124 (0.60)	256 (0.58)	107 (0.69)
T/C	87 (0.37)	56 (0.33)	98 (0.36)*	67 (0.33)	154 (0.35)	41 (0.26)
T/T	14 (0.06)	10 (0.06)	19 (0.07)*	15 (0.07)	29 (0.07)	7 (0.05)

*Indicates the significant differences.

In our study, p.Lys47Arg G allele was more frequent in the group of all periodontitis patients respect to healthy controls, correlating with higher risk toward disease development; moreover, p.Ala29Thr T allele as well as p.Lys47Arg G allele was associated with slight periodontitis. Our results regarding p.Lys47Arg polymorphism were in agreement with the study of Wu, Juo et al. (2009) performed in Taiwanese patients, but not with the study of Velliyagounder et al. (2003) on patients from North America with localized juvenile periodontitis and with the study of Ikuta et al. (2015) among Japanese patients.

Furthermore, in our study p.Ala29Thr T allele was associated with increased risk of slight periodontitis, in contrast with the study of Jordan et al. (2005) that observed an association between p.Ala29Thr G allele and aggressive periodontitis in the group of African American patients, but not in Caucasians.

The differences encountered between those studies could be partially explained based on the different ethnic origin of the subjects as well as by the low or medium number of individuals analyzed in each study.

In conclusion, our results report association between *DEFB1* g. -20 A>G, *LTF* p.Ala29Thr, *LTF* p.Lys47Arg polymorphisms and the susceptibility toward the risk of chronic periodontitis development among North-Eastern Italian subjects, possibly indicating a hypothetical role of these molecules in the oral environment and their involvement in the inflammatory immune response against plaque bacteria, the key process for the disease initiation and aggravation. However, the limited number of individuals analyzed together with the heterogeneity of replica comparisons with the literature does indicate the strong need of further studies to be performed on higher numbers of patients and controls, matched for ethnic origin and with biological material (i.e., saliva) to double check the effects of genetic variants in protein expression and functionality.

ACKNOWLEDGEMENTS

We thank all the individuals who participated to the Friuli-Venezia Giulia Genetic Park project.

This study was partially supported by Fondo Trieste (2008) and Regione FVG (L.26.2008). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

AUTHOR CONTRIBUTION

L.Z. performed experiments and drafted the manuscript; R.A. performed experiments, the statistical analyses and participated in writing the manuscript; C.O.N. performed samples collection and manuscript revision; P.N. participated in performing experiments and the statistical analysis; R.D.L. contributed to the supervision of the clinical protocol; P.G. participated in study design and revised the manuscript; SC critically revised the manuscript; L.B. conceived the study and was responsible for the setting of the clinical protocol and management of patients.

CONFLICT OF INTEREST

Authors declare no conflict of interests.

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