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ORIGINAL RESEARCH

Interleukin-16 rs4072111 Polymorphism is Associated with the Risk of Peri-Implantitis in the Chinese Population

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Purpose: Peri-implantitis (PI) is a major contributor to dental implant failure. Genetic predisposition plays an essential role in the development of PI. The purpose of this study was to investigate the correlation of *IL-16* gene single nucleotide polymorphisms (SNPs), rs11556218 and rs4072111, with PI at the gene level.

Patients and Methods: A total of 162 patients with PI and 162 cases with healthy implants were recruited as the case and control groups, respectively. The genotypes were analysed using direct sequencing. The genotype and allele proportion between the case and control groups were compared using the chi-square test. The periodontal status of patients carrying different genotypes was analysed, including gingival index, plaque index, calculus index, peri-implant pocket depth (PPD), and clinical attachment level (CAL).

Results: The case and control groups were age- and gender-matched. In the case group, the rs4072111 CT genotype was majorly observed, and the T allele carriers showed a high PI risk. Patients with the rs4072111 CT genotype had worse periodontal status, which was reflected by the higher levels of the gingival index, plaque index, calculus index, PPD and CAL. The distribution of the rs11556218 genotype and T allele showed no significant difference between the case and control groups ($P > 0.05$).

Conclusion: The CT genotype of *IL-16* gene rs4072111 SNP can be used as a factor assessing PI risk. Therefore, *IL-16* genetic variation may be related to PI susceptibility in the Chinese Han population.

Keywords: peri-implantitis, IL-16, rs4072111, rs11556218, susceptibility

Introduction

Peri-implantitis (PI) is the reversible inflammation of the soft tissue surrounding oral implants.[1] Bacterial growth on oral implants is the root cause PI, which causes the accumulation of plaque around the implant and stimulates the inflammatory response of the body.[2] The clinical manifestations of PI are gingival redness, swelling, bleeding on probing, and radiographic signs of bone loss.[3] In severe cases, a fistula around the dental implant may sometimes overflow with pus, causing alveolar bone absorption around the implant.[4] The occurrence of PI negatively affects the success of dental implantation. Various factors have been reported to influence the occurrence of PI, such as microbial growth, dental plaque and oral hygiene.[5] In addition, recent studies have demonstrated that genetic polymorphism is a major contributor to PI.[6]

Single nucleotide polymorphisms (SNPs) are DNA sequence polymorphisms caused by single nucleotide variation at the genome level. They are common

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heritable variants in humans and are widely present in the human genome, accounting for more than 90% of all known polymorphisms.[7] Regarding PI, multiple SNPs in different genes have been identified to be associated with the genetic predisposition for PI. In the Chinese Han population, osteoprotegerin gene (*OPG*) rs2073618 SNP has been reported to be associated with the risk of PI. In addition, the rs2073618 CC genotype carriers are susceptible to PI.[8] Recently, the genetic association of TNF-α, IL-1A, and IL-1B polymorphisms with PI has been studied in the Chinese non-smoking population.[9] Activated T cells primarily produce IL-16 that can bind to CD^{4+} molecules and can activate monocytes to produce inflammatory factors, such as IL-6, TNF-α, IL-1β and IL-15.[10] *IL-16* is located on chromosome 15q26.3, comprises seven exons and six introns. Rs11556218 and rs4072111 are two common SNPs located in the exon region of *IL-16* gene. As previously reported, rs11556218 and rs4072111 are associated with the susceptibility to several human diseases, such as osteoporosis and coronary artery disease.[11,12] The two SNPs have been reported to be related to the genetic susceptibility of periodontitis.[13] In light of the important role of periodontitis in PI, the genetic correlation between *IL-16* gene and PI should be investigated. Therefore, this study was designed to explore the genetic association of *IL-16* gene rs11556218 and rs4072111 SNPs with PI at the gene level.

Materials and Methods

Study Objects

A total of 162 patients with PI who visited the School and Hospital of Stomatology, Fujian Medical University from August 2019 to March 2021 were selected as the case group. In addition, 162 cases with healthy implants were included as the control group. All the participants were Chinese Han population. The inclusion criteria were as follows: (1) Patients have signed the informed consent form; (2) Patients have at least one implant in the mouth; (3) The insertion of the superstructure has been completed for at least 1 year. The exclusion criteria were as follows: (1) Patients who have systemic diseases; (2) Patients who have been administered NSaids and adrenocorticosteroids within the past 3 months; (3) Patients with other oral infections, jawbone cysts and other local lesions; (4) Women who are pregnant or breastfeeding; (5) Patients caused by iatrogenic factors such as unreasonable design, type of restoration, use of adhesive and removal of residual adhesive, etc. The diagnostic criteria for healthy implants were as follows: (1) No soft tissue redness and swelling; (2) No probing bleeding; (3) No excessive concentration; (4) Unexplored depth increased; (5) Bone resorption at the top level of the alveolar ridge after bone remodeling. The diagnostic criteria for PI were as follows: (1) Excessive concentration or probing bleeding; (2) The probing depth increased compared with the baseline; (3) Bone resorption at the top level of alveolar ridge was observed after bone remodeling. In the absence of baseline data, the following criteria were used for diagnosis: (1) Probing depth ≥6 mm; (2) Excessive concentration or probing bleeding; (3) A distance of ≥3 mm from the crest of the bone around the implant to the crown of the bone within the implant.[14] The clinical information of the patients was collected subsequently after the completion of the dental implant. This study was conducted in accordance with the Declaration of Helsinki. The current study design was approved by the ethics committee of School and Hospital of Stomatology, Fujian Medical University (ethical code: 2019–028-02; Approval date: 10th January, 2019).

Template DNA Extraction

For DNA extraction, the patients were asked to abstain from food and water for 30 minutes before the extraction, followed by water gargling. Using sterile cotton swabs, the buccal mucosa of patients was taken and placed in sterile 15×10^3 μL Eppendorf tubes. The genomic DNA was extracted using phenol/chloroform/isoamyl alcohol followed by isopropanol precipitation and cleaned using an oral swab genomic DNA extraction kit (Tian Gen, DP322-03, Beijing, China). DNA concentration was determined by NanoDrop 1000 (Thermo Fisher Scientific, Waltham, MA USA) via measuring the A260/A280 ratios.

PCR Assay

Polymerase chain reaction (PCR) was performed using the genomic DNA as the template. The primer sequences were as follows: rs11556218, forward primer: 5′-GCTCAGGTTCACAGAGTGTTTCCATA-3′, reverse primer: 5′-TGTGACAATCACAGCTTGCCTG-3′; rs4072111, forward primer: 5′- CACTGTGATCCCGGTCCAGTC-3′, reverse primer: 5′- TTCAGGTACAAACCCAGCCAGC −3′. 25 μL PCR reaction system was prepared, including 5 μL DNA template, 12.5 μL Go Tag mixed enzyme (including Taq DNA polymerase, dNTP, 10 × PCR buffer), 0.25 μL forward and 0.25 μL reverse primes, and 7 μL sterile deionized water. The PCR cycle conditions were as follows:

94°C for 5 min, denaturation for 1 min, renaturation for 30 s at 72°C, extension for 1 min at 61°C, and 30 cycles were run. Finally, the temperature was maintained at 72°C for 5 min.

Genotyping

After purification, the PCR products were outsourced to Sangon Biotech Company (Beijing, China) for sequencing using an ABI 3130xL Genetic Analyzer (Applied Biosystems, Foster City, California, USA) according to the manufacturer's recommendations.[15]

Statistical Approach

SPSS 17.0 software (SPSS, Chicago IL, USA) was applied for the data analysis. The genotype and allele frequency was directly counted. The hardy-Weinberg equilibrium was calculated for the control group. Differences between two groups were calculated using the Mann–Whitney *U*-test for non-normally distributed continuous variables, Student's *t* test for normally distributed continuous variables, chi-squared test for categorical variables. Differences between multiple groups were compared using one-way analysis of variance (ANOVA) analysis. A *P* value less than 0.05 was defined as statistically significant.

Results

Demographic and Clinical Information of the Study Population

Chi-square test results revealed no significant difference between the case and control groups regarding age and gender distribution, demonstrating that the two groups were age ($P = 0.249$) and gender ($P = 0.578$) matched (Table 1). The comparison of clinical information including periodontitis history ($P = 0.059$), tooth loss reason ($P = 0.369$), platform type ($P = 0.317$), position ($P = 0.055$) and peri-implant phenotype ($P = 0.149$) showed no significant difference between the two groups (Table 1). In addition, no significant difference was observed in the lifestyle information of the study population, including history of alcohol and smoking, frequencies of brushing, dental floss and mouth washing ($P > 0.05$, Table 1). Therefore, the two groups were comparable.

Table 1 Clinical Information of the Subjects

Indicators	HC (n=162)	PI (n=162)	*P*
Age (years, mean±SD)	42.44±5.92	43.30±7.36	0.249
Gender (n/%)			0.578
Male	84/51.85	89/54.94	
Female	78/48.15	73/45.06	
Alcohol consumption(n/%)			0.910
Yes	65/40.12	66/40.74	
No	97/59.88	96/59.26	
Smoking (n/%)			0.823
Yes	75/46.30	73/45.06	
No	87/53.70	89/54.94	
Periodontitis (n/%)			0.059
Yes	73/45.06	90/55.56	
No	89/54.94	72/44.44	
Tooth loss reason (n/%)			0.369
Periodontitis	71/43.83	83/51.23	
Deep caries	2/1.23	1/0.62	
Trauma	89/54.94	78/48.15	
Platform type (n/%)			0.317
External hex	76/46.91	68/41.94	
Internal hex	24/14.81	37/22.84	
Morse cone	52/32.11	49/30.24	
Others	10/6.17	8/4.94	
Position (n/%)			0.055
Anterior region	104/64.20	87/53.70	
Posterior region	58/35.80	75/46.30	
Per-implant phenotype (n/%)			0.149
Thin	89/54.94	76/46.91	
Thick	73/43.45	86/53.09	
Brushing daily (n/%)			0.754
1–3times	137/84.57	139/85.80	
More than 3 times	25/15.43	23/14.20	
Dental floss daily (n/%)			0.231
Yes	63/38.89	67/41.36	
No	30/18.52	19/11.73	
Infrequent	69/42.59	76/46.91	
Mouth washing daily (n/%)			0.363
Yes	48/29.63	60/37.04	
No	37/22.84	32/19.75	
Infrequent	77/47.53	70/43.21	

Abbreviations: HC, healthy controls; PI, peri-implantitis.

Patient's Periodontal Condition

Patient's periodontal condition was assessed by detecting gingival index, plaque index, calculus index, peri-implant pocket depth (PPD), clinical attachment level (CAL), and the periodontal condition was compared between the case and control groups. As shown in Table 2, patients who suffer from PI had higher levels of gingival index, plaque index, calculus index, PPD and CAL ($P < 0.001$) than those in the control group. The findings indicated that patients with poor periodontal conditions were at high risk of PI.

Table 2 Periodontal Status of Subjects

Indicators	HC (n=162)	PI (n=162)	*P*
Gingival index	0.58 ± 0.55	2.41 ± 0.60	< 0.001
Plaque index	0.87 ± 0.62	2.23 ± 0.66	< 0.001
Calculus index	0.20 ± 0.40	0.56 ± 0.58	< 0.001
PPD (mm)	1.84 ± 0.56	5.42 ± 0.78	< 0.001
CAL (mm)	1.35 ± 0.56	4.52 ± 0.80	< 0.001

Notes: The data are represented as mean±standard deviation.
Abbreviations: HC, healthy controls; PI, peri-implantitis; PPD, peri-implant pocket depth; CAL, clinical attachment level.

Genotype Distribution Frequencies of IL-16 Gene rs11556218 and Rs4072111 Polymorphisms

As shown in Table 3, the three genotypes of *IL-16* gene rs11556218 SNP were detected, including TT, TG and GG. The three genotypes showed minor differences in distribution frequency between the two groups (P = 0.795; P = 0.378, respectively). In addition, the T and G allele frequency between the two groups was not statistically significant (P = 0.521). For rs4072111 polymorphism, the three genotypes of CC, CT and TT were observed in the study subjects. The comparison of differences between groups suggested that the frequency of CT genotypes was significantly increased in the PI group compared with CC genotypes (P = 0.007, Table 3). Moreover, the frequency of T allele in the PI group was significantly higher than that in the control group (P = 0.030, Table 3). These results suggested that rs4072111 CT genotype and T allele carriers were at higher risk of PI.

The Periodontal Status of Patients Carrying Different Genotypes

According to the rs4072111 genotype information, all the patients were divided into three groups, and the periodontal status index of the three groups was compared. ANOVA analysis results revealed that patients with the CC genotype had lower scores of the gingival index, plaque index, calculus index, PPD and CAL, whereas patients with the CT genotype had significantly higher scores (Table 4). The periodontal status of individuals with different rs11556218 genotypes was also analysed. As shown in Table 5, the levels of gingival index (P = 0.578), plaque index (P = 0.407), calculus index (P = 0.551), PPD (P = 0.482) and CAL (P = 0.784) showed no significant difference among the three groups (P > 0.05). These findings indicated that individuals carrying rs4072111 CT genotype had poor periodontal status.

Discussion

IL-16 is a pro-inflammatory cytokine that was originally designated as a lymphocyte chemoattractant factor. IL-16 can inhibit the growth of periodontal membrane cells such as fibroblasts, collagen cells and stromal cells, and reduce the adhesion of fibroblasts. This affects the repair and metabolic functions of periodontal membrane cells and leads to the damage of periodontal tissue, which is not

Table 3 Frequency Distribution of *IL-16* Gene Rs11556218 and Rs4072111 Genotype and Allele in Healthy Controls and Peri-Implantitis Groups

Genotype/Allele	HC (n=162)/%	PI (n=162) /%	χ^2	OR (95% CI)	*P*
rs11556218					
TT	88/54.32	92/56.79	–	1	–
TG	66/40.74	65/40.12	0.068	0.942 (0.601–1.478)	0.795
GG	8/4.94	5/3.09	0.776	0.598 (0.188–1.897)	0.378
T	242/74.69	249/76.85	–	1	–
G	82/25.31	75/23.15	0.412	0.889 (0.620–1.274)	0.521
P^{HWE}	0.323				
rs4072111					
CC	130/80.25	110/67.90	–	1	–
CT	29/17.90	50/30.87	7.246	2.038 (1.208–3.438)	0. 007
TT	3/1.85	2/1.23	0.067	0.788 (0.129–4.800)	0.796
C	289/89.20	270/83.33	–	1	–
T	35/10.80	54/16.67	4.702	1.651 (1.046–2.607)	0.030
P^{HWE}	0.365				

Abbreviations: HC, healthy controls; PI, peri-implantitis; OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.

Table 4 Periodontal Status in Patients with Rs4072111 Gene Polymorphism

Indicators	Genotype			*P*
	CC	CT	TT	
PPD (mm)	3.46±1.89	4.14±1.93	3.60±1.82	0.024
CAL (mm)	2.74±1.77	3.53±1.35	2.80±3.03	0.002
Gingival index	1.23±0.98	2.29±0.98	1.40±0.89	<0.001
Plaque index	1.43±0.87	1.89±1.05	1.80±0.84	0.001
Calculus index	0.25±0.45	0.76±0.56	0.50±0.55	<0.001

Note: The data are represented as mean±standard deviation.
Abbreviations: PPD, peri-implant pocket depth; CAL clinical attachment level.

Table 5 Periodontal Status in Patients with Rs11556218 Gene Polymorphism

Indicators	Genotype			*P*
	TT	TG	GG	
PPD (mm)	3.65±1.90	3.66±1.93	3.00±2.04	0.482
CAL (mm)	2.96±1.75	2.92±1.73	2.62±1.61	0.784
Gingival index	1.53±1.11	1.47±1.07	1.23±0.73	0.578
Plaque index	1.52±0.96	1.62±0.90	1.31±0.95	0.407
Calculus index	0.37±0.53	0.40±0.54	0.23±0.44	0.551

Note: The data are represented as mean±standard deviation.
Abbreviations: PPD, peri-implant pocket depth; CAL clinical attachment level.

conducive to the success of implants.[16] Early studies have reported that patients with periodontal disease secrete more TNF-α, IL-6 and IL-16 factors than healthy people.[17] In addition, various studies have reported the association between *IL-16* gene polymorphism and inflammatory diseases susceptibility. Gu et al have studied the effect of *IL-16* polymorphism in Graves' disease and identified that the rs4072111 locus was associated with Graves' disease and corresponding eye diseases. Moreover, individuals carrying the TT genotype and T allele had a high risk to suffer from Graves' disease.[18] In addition, in the Chinese Han population, the G allele of rs11556218, C allele of rs4778889, and T allele of rs4072111 were frequently detected in patients with systemic lupus erythematosus (SLE), indicating the genetic association of IL-16 gene polymorphism with systemic lupus erythematosus (SLE) susceptibility.[19] Notably, *IL-16* gene SNPs have been identified to be related to the development of periodontitis in several study populations.[13,20,21] In the present study, two common SNPs in *IL-16* gene, including rs11556218 and rs4072111, were selected, and genotype and allele distribution in PI cases were analyzed in 162 healthy implants and 162 PI cases. The case and control groups were age and gender matched. The distribution of gene frequency of the two SNPs in the two groups conforms to Hardy–Weinberg equilibrium, indicating that the selected samples are representative and comparable. Moreover, *IL-16* gene rs4072111 polymorphism showed a close association with the PI onset.

Rs4072111 polymorphism is located in exon 6 of *IL-16* gene and carries the C/T mutation, resulting in a serine to alanine substitution.[12] In the present study, the genotype and allele distributions of rs4072111 polymorphism showed statistical significance between the case and control groups. It implied that *IL-16* gene rs4072111 polymorphism plays an important role in the genetic predisposition of PI. It was found that more PI cases carried rs4072111 CT genotype, whereas the CC genotype carriers showed higher frequencies in the control group. The allele distribution analysis results demonstrated that there were more T-allele carriers in PI patients than in the control group, indicating that the T allele might be a risk factor for the hereditary susceptibility of PI. Moreover, individuals carrying rs4072111 CT genotype had worse periodontal status. It was concluded that the CT genotype and T allele of rs4072111 might be a pathogenic site of PI, which may lead to altered cytokine expression or biological activity and further modulate the individual's risk for PI. Consistently, *IL-16* gene rs4072111 SNP exists in a variety of inflammatory diseases. In SLE cases, the rs4072111 T allele was predominant.[19] In the Brazilians population, more rs4072111 CT genotype carriers are detected in individuals with periodontitis than that in the healthy control group.[13] These results are consistent with our findings in patients with PI. It was concluded that the PI susceptibility in patients with rs4072111 T allele may be related to the relationship between this SNP and inflammation.

Rs11556218 is also located in exon 6 of *the IL-16* gene, and brings the change of alanine to lysine. The SNP has been reported to be associated with the genetic susceptibility of several cancers, including nasopharyngeal carcinoma and colorectal cancer.[22] In China, it is also suggested to have a regulatory effect on the pathogenesis of coronary artery disease, osteoporosis, type 2 diabetes mellitus and so on.[11,23,24] In the current study, the three genotypes of rs11556218 were detected in patients with PI. However, the genotype and allele distributions showed no difference between the PI and control groups. It was concluded that *IL-16* gene rs11556218 might lack a genetic

association with PI susceptibility. As genetic association studies are susceptible to false positive results owing to genetic population diversity, population structure and multiple testing, this study requires further validation in a larger study population. Moreover, other clinical samples from the gingival sulcus or the gingival tissue around implants are also necessary for further validation.

Conclusion

In the Chinese Han population, *IL-16* rs4072111 genetic variation can be used as a genetic marker for PI susceptibility. The findings may be useful in public health genomics and future advanced clinical practice. Because detection of gene polymorphism can be completed in advance, it is of great benefit for early intervention and prognosis in the early disease stage.

Ethics Statement

The current study design was approved by the ethics committee of School and Hospital of Stomatology, Fujian Medical University and written informed content was obtained from each participant.

Disclosure

The authors report no conflicts of interest in this work.

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