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ORIGINAL ARTICLE

Association among interleukin-6 gene polymorphism, diabetes and periodontitis in a Chinese population

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OBJECTIVES: Diabetics significantly increase risk for periodontitis. Interleukin-6 (IL-6) gene polymorphism may play certain roles in the progression of periodontitis with diabetes. The purpose of this study was to assess the association among IL-6 gene polymorphisms, type 2 diabetes mellitus (T2DM) and chronic periodontitis (CP) in a Chinese population.

MATERIAL AND METHODS: DNA was obtained from 159 patients with CP, 88 patients with T2DM, 110 patients with CP&T2DM and 135 control subjects. The -174/-572/-597 polymorphisms of IL-6 gene were investigated by restriction fragment length polymorphism of polymerase chain reaction products. The results were further confirmed by sequencing. Significance was set at $P < 0.008$ after Bonferroni correction.

RESULTS: Among four groups, CP&T2DM group showed the lowest IL-6-572 CC genotype and C-allele frequencies (54.5% and 74.1%). In this regard, there were significant differences between CP&T2DM group and the control group [$P = 0.006$, odds ratio (OR) = 0.475, 95% CI: 0.279–0.808 and $P = 0.002$, OR = 0.502, 95% CI: 0.319–0.788 respectively]. Logistic regression with adjustment for age, gender, body mass index, smoking and stress showed no significant difference in terms of IL-6-572 genotypes ($P = 0.058$, OR= 0.523, 95% CI: 0.268–1.022).

CONCLUSIONS: The IL-6-572 genotype and allele distributions are unique to subjects with CP&T2DM in a Chinese population.

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Keywords: chronic periodontitis; type 2 diabetes mellitus; polymorphism; interleukin-6; Chinese

Introduction

Periodontitis is a common inflammatory disease caused by oral bacteria, leading to irreversible attachment loss, alveolar bone destruction, and ultimately to tooth loss. Although bacteria are essential for the initiation of periodontitis, the quantity and types of bacteria are not sufficient to explain the differences in disease severity that are routinely seen in adults. In recent years, it has become evident that certain risk factors do not directly cause the disease but rather modify the progression and severity of the disease (Guzman *et al*, 2003). It is suggested that diabetes and genetic factors put certain individuals at relatively high risk for increased severity of periodontitis (Offenbacher, 1996; Kornman *et al*, 1997).

Type 2 diabetes mellitus (T2DM) is a complex disease resulting from resistance to insulin combined with a failure to produce enough additional insulin to compensate for the insulin resistance. This disease usually becomes clinically apparent after 40 years old. Now it has been a well-established consensus that diabetic patients are at significantly increased risk for periodontal complications (Lalla, 2007; Scully, 2007; Skamagas *et al*, 2008). Several pathways have been investigated to explain the relationships between diabetes and periodontal disease, including cellular response, hyperglycemia and microbiological response, but the interaction mechanisms between diabetes and periodontal disease are still not clear (Tan *et al*, 2006; Duarte *et al*, 2007).

The genetic variation in cytokine production may explain some of the differences among individuals in the progression of complex diseases. Therefore the gene polymorphisms of some cytokines have been focused on the population with periodontal disease and diabetes for resent years. Guzman *et al* (2003)evaluated periodontitis in diabetics with different IL-1 genotypes. Perez *et al* (2004) investigated the association between the tumour necrosis factor (TNF)-α (-308) SNP and *in vitro* TNF-α levels in Chilean patients with aggressive periodontitis and/or T1DM (Perez *et al*, 2004). Struch *et al* (2008) recently assessed the association between the IL-1

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genotype and periodontitis in diabetic and non-diabetic subjects ($n = 1515$). However, there is lack of knowledge about IL-6 gene polymorphism effecting the population with periodontitis and diabetes.

Interleukin-6 is a multifunctional cytokine with a central role in host defence. Many cell types produce IL-6 in response to noxious stimuli, including monocytes/macrophages, fibroblasts, endothelial cells, adipocytes, T cells and mast cells. Three single nucleotide polymorphisms (SNPs) at positions -174(rs 1800795), -572(rs 1800796) and -597(rs 1800797) of the IL6 promoter may result in inter-individual variation in IL6 transcription and expression (Terry *et al*, 2000). Several literatures reported that the SNPs of IL-6 are associated with the progression of periodontal disease or diabetes (Holla *et al*, 2004; Illig *et al*, 2004; Brett *et al*, 2005; Hamid *et al*, 2005). So it was hypothesized that these SNPs of IL-6 might play certain roles in the progression of periodontitis aggravated with diabetes. The main purpose of this study was to assess the associations among Interleukin-6 (IL-6) gene polymorphisms, diabetes and periodontitis in a Chinese population.

Material and methods

Subjects

All subjects were recruited from outpatients and inpatients referred to Guangdong Provincial Stomatological Hospital, Zhujiang Hospital of Southern Medical University and the Second Affiliated Hospital of Guangzhou Medical College during August 2007 to September 2008, and a community health check-up in Guangdong, China in April 2008. All participants were 40 years old or greater, Chinese Han descent and genetically unrelated. Exclusion criteria were as follows: known systemic diseases except for diabetes, receiving systemic antibiotic treatment in the preceding 3 months, receiving periodontal therapy in the preceding 1 year, and pregnant or lactating females.

All participants were classified into four groups: chronic periodontitis (CP) group, T2DM group, CP & T2DM group and healthy control group. All patients with T2DM had been diagnosed by diabetes specialists whose diagnosis criteria were in accordance with 1999 WHO criteria (Rathmann *et al*, 2003). T2DM patients had all been accepting diabetes treatment and were categorized as T2DM group or CP&T2DM group by following periodontal examination. Their basic characteristics were: mean age ($\pm$s.d.) 57 $\pm$ 10 years old, body mass index (BMI) 24.14 $\pm$ 4.51 kg m^{-2}, age at clinical diagnosis 52 $\pm$ 11 years old and HbA_1c 7.8 $\pm$ 1.6%.

Chronic periodontitis patients were identified on the clinical criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Disease and Conditions (Armitage, 1999) and the consensus report of the 5th European workshop in periodontology (Tonetti and Claffey, 2005). Periodontal diagnoses were based on clinical measures from six index teeth (teeth numbers 16, 11, 26, 36, 31 and 46) (Wang *et al*, 2007). Periodontal examinations were conducted by a single trained and calibrated examiner. Probing pocket depths (PD), clinical attachment loss (CAL) and bleeding on probing (BOP) were recorded at six sites for each examined tooth with a Williams probe. Missing teeth were recorded, except third molars. In brief, moderate to severe periodontitis cases were defined as those having CAL of $\geq$3 mm in $\geq$30% of the selected tooth sites. Healthy controls and T2DM patients without CP were defined by the absence of CAL, BOP and PD >3 mm on the six index teeth. At last, 159 patients with CP (40–85 years old), 88 patients with T2DM (40–85 years old) and 110 patients with CP&T2DM (40–87 years old) were selected. The control group consisted of 135 healthy individuals (40–82 years old). Characteristics of the subjects were summarized in Table 1.

The study protocol was approved by the Ethics Committee of Southern Medical University. Informed consent was obtained from all participants in accordance with the Helsinki Declaration.

Clinical parameters and sample collection

For this study, each participant was required to complete a questionnaire, containing gender, age, ethnicity, smoking status, body height, weight, medical history

Table 1 Baseline characteristics of study participants ($n = 492$)

Variable	*Control* (n = *135*)	*T2DM* (n = *88*)	*CP* (n = *159*)	*CP&T2DM* (n = *110*)	P-*value*
Gender (male/female, *n*)	42/93	24/64	83/76	46/64	<0.001*
Smoking (no/yes, *n*)	114/21	79/9	108/59	86/24	<0.001*
Stress (no/yes, *n*)	100/35	57/31	75/84	74/36	<0.001*
Age (years, mean $\pm$ s.d.)	51.60 $\pm$ 9.56	59.65 $\pm$ 10.44	51.84 $\pm$ 8.69	62.03 $\pm$ 10.14	<0.001**
BMI (kg m^{-2}, mean $\pm$ s.d.)	22.76 $\pm$ 3.98	24.22 $\pm$ 5.47	22.34 $\pm$ 2.79	24.06 $\pm$ 3.54	<0.001**
Missing teeth (*n*, mean $\pm$ s.d.)	1.01 $\pm$ 2.09	1.42 $\pm$ 2.81	2.09 $\pm$ 2.70	3.83 $\pm$ 3.74	<0.001**
PD (mm, mean $\pm$ s.d.)	2.05 $\pm$ 0.39	1.81 $\pm$ 0.34	3.25 $\pm$ 0.87	2.30 $\pm$ 0.72	<0.001**
CAL (mm, mean $\pm$ s.d.)	0	0	4.86 $\pm$ 1.36	3.4 $\pm$ 0.96	<0.001***
BOP (%, mean $\pm$ s.d.)	0	0	69.38 $\pm$ 32.67	61.27 $\pm$ 36.81	0.078***

Differences in subject numbers are due to missing data.
BMI, body mass index (calculated as body weight divided by body height squared); PD, probing pocket depth; CAL, clinical attachment loss; BOP, bleeding on probing (percentage of sites with bleeding on probing); T2DM, type 2 diabetes mellitus; CP, chronic periodontitis.
**P*-value was obtained using chi-square test; ***P*-value was obtained using one-way ANOVA; ****P*-value was obtained using *t*-test; *P*-values <0.05 were considered significant.

and stress. Participants reported their stress intensity and frequency which were combined into a continuous stress score from 0 to 6. The stress score was categorized into no-stress (points 0–1) and stress (points 2–6) (Nielsen *et al*, 2005). Then a buccal swab sample was obtained by twisting a swab inside each participant's cheek (Duan *et al*, 2001; Dashash *et al*, 2007). In addition, in 10% of the subjects ($n = 50$), a second buccal swab was collected, which was coded with a separate number. These samples were used as a laboratory quality control check to assess the reproducibility of the IL-6 genotyping (Armitage *et al*, 2000).

DNA extraction and genotyping

Genomic DNA was extracted from buccal swabs as described previously (Duan *et al*, 2001), and then stored at −20°C for later analysis. The DNA concentration was estimated at 260 nm using a spectrophotometer.

Genotyping of the -174, -572 and -597 in IL-6 promoter was performed with polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) technique. Details of the PCR–RFLP methods were summarized below:

IL-6-174. Forward primer: 5′-TGA CTT CAG CTT TAC TCT TTG T-3′. Reverse primer: 5′-CTG ATT GGA AAC CTT ATT AAG-3′. Cycling was carried out for one cycle at 94°C for 3 min, 35 cycles each at 94°C for 30 s, 52°C for 30 s, 72°C for 20 s and one cycle at 72°C for 5 min. Digestion of PCR products with *NIa III* (NEB, Ipswich, MA, USA) yielded 153 + 45 bp fragments (CC), a single 198 bp fragment (GG) and 198 + 153 + 45 bp fragments (GC) (Fernandez-Real *et al*, 2000).

IL-6-572. Forward primer: 5′-GAG ACG CCT T-GA AGT AAC TG-3′. Reverse primer: 5′-AAC CAA AGA TGT TCT GAA CTG A-3′. Cycling conditions were similar to the upper, except an annealing temperature of 56°C. Digestion of PCR products with *BsrB I* (NEB, Ipswich, MA, USA) yielded 121 + 61 bp fragments (GG), a single 182 bp fragment (CC) and 182 + 121 + 61 bp fragments (GC) (Seow *et al*, 2005).

IL-6-597. Forward primer: 5′-CTC CTC TAA GTG GGC TGA AG-3′. Reverse primer: 5′-CAA GCC TGG GAT TAT GAA GA-3′. The same cycling conditions of IL-6(-572) were used again. Digestion of PCR products with *Fok I* (NEB, Ipswich, MA, USA) yielded 125 + 87 or 129 + 83 bp fragments (AA), a single 212 bp fragment (GG) and 212 + 125(129) + 87(83) bp fragments (GA) (Komatsu *et al*, 2005).

To confirm the results of the PCR-RFLP, the PCR products of two or more samples in every genotype were randomly selected to sequence directly (Invitrogen, Shanghai, China) (Park *et al*, 2007).

Statistics analysis

Hardy–Weinberg equilibrium was tested in each group by a chi-square test. Haplotypes were constructed on a SHEsis software (Shi and He, 2005). One-way ANOVA was used to calculate means of continuous variables. The *P*-values of age, BMI, missing teeth and PD were calculated using one-way ANOVA among four groups, and the *P*-values of CAL and BOP variables were obtained using *t*-test. *t*-Test was still used to test differences between the numbers of missing teeth according to different IL-6-572 genotypes inside each group. chi-square test was used to test differences in categorical variables (including gender, smoking, stress, alleles and genotypes distributions) among four groups. The multiple comparisons of alleles and genotypes distributions were finished by chi-square test between each two groups. The differences of genotypes were further analyzed through logistic regression models with adjustment for age, gender BMI, smoking and stress. *P*-values < 0.05 were considered significant. To control false-positive errors, the Bonferroni correction was used to assess the results of multiple comparisons, whose significance was set at *P*-values < 0.008 (0.05/6). All analyses were performed using Statistical Package for Social Sciences (SPSS) for Windows, version 13.0.

Results

The genotypes at three loci were in Hardy–Weinberg equilibrium (each *P*-value > 0.05). Only three haplotypes were observed out of four possible haplotypes (Table 2). Two major haplotypes (GGG and GCG) accounted for more than 99% of three haplotypes observed.

The locus, IL-6-174 showed very low allele frequency (Table 3). In 481 subjects (11 failed genotyping), only two GC heterozygotes were found, and others were GG homozygotes. And in the locus IL-6-597, no A-containing variant was detected (Table 3). The two loci were not analyzed statistically because their allele frequencies were too low in the population. IL-6 haplotypes were not further analyzed because they had almost the same distributions as IL-6-572 allele frequencies.

The genotype and allele data of the locus IL-6-572 were shown in Table 3. In terms of its genotypes distribution and allele frequencies, the crud comparison among four groups showed a significant difference ($P = 0.030$ and 0.013 respectively). In detail, the CC genotype frequency in T2DM or CP group (61.4% or 67.9%) was lower than the control group (71.6%), and CP&T2DM group shared the lowest frequency (54.5%). Similar trend could be seen on its allele frequencies.

The multiple comparisons (Table 3) between CP&T2DM group and the control group revealed two significant *P*-values and OR values in CC *vs* GC + GG model, namely CC genotype distribution and C-allele frequency of IL-6-572 ($P = 0.006$, OR = 0.475, 95%

Table 2 Interleukin-6 promoter haplotypes and their frequencies in a Chinese population

Haplotype 1 GGG (%)	*Haplotype 2 GCG (%)*	*Haplotype 3 CGG (%)*
178 (18.8)	768 (81.0)	2 (0.2)

Haplotype refers to the base combination at -174, -572 and -597, and the loci are indicated from left to right in descending order. The values are of a total of 948 chromosomes represented in the 474 subjects except 18 with missing genotyping data in one or more single nucleotide polymorphisms.

Table 3 Genotype and allele data for the -174, -572 and -597 of interleukin-6 promoter in study participants

	Control (n = *135*)	*T2DM* (n= *88*)	*CP* (n= *159*)	*CP&T2DM* (n= *110*)	P-*value*
-174 GC					
GG (%)	132	85	156 (99.4)	106 (99.1)	
GC (%)	0	0	1 (0.6)	1 (0.9)	
CC	0	0	0	0	
-597 GA					
GG	133	88	157	108	
GA	0	0	0	0	
AA	0	0	0	0	
-572 GC					
GG (%)	2 (1.5)	2 (2.3)	3 (1.9)	7 (6.4)	
GC (%)	36 (26.9)	32 (36.4)	48 (30.2)	43 (39.1)	
CC (%)	96 (71.6)	54 (61.4)	108 (67.9)	60 (54.5)	0.030*
C-allele(%)	228 (85.1)	140 (79.5)	264 (83)	164 (74.1)	0.013**

-572 GC	T2DM *vs* control	CP *vs* control	CP&T2DM *vs* T2DM	CP&T2DM *vs* CP	CP&T2DM *vs* Control
CC *vs* GC + GG					
P-value***	0.110	0.491	0.335	0.026	0.006
OR (95% CI)	0.629 (0.355–1.112)	0.838 (0.507–1.385)	0.756 (0.427–1.336)	0.567 (0.343–0.936)	0.475 (0.279–0.808)
C-allele *vs* G-allele					
P-value****	0.130	0.499	0.203	0.784	0.002
OR (95% CI)	0.682 (0.415–1.122)	0.858 (0.549–1.339)	0.735 (0.458–1.182)	0.942 (0.612–1.449)	0.502 (0.319–0.788)

Differences in subject numbers are due to missing genotypes.
OR, odds ratio; CI, confidence interval; T2DM, type 2 diabetes mellitus; CP, chronic periodontitis.
**P*-value for genotype distribution among four groups was obtained using chi-square test in a model (CC *vs* GC + GG); ***P*-value for allele frequency among four groups was obtained using chi-square test; ****P*-values for genotype distribution of multiple comparisons were obtained using chi-square test in a model (CC *vs* GC + GG); *****P*-values for allele frequency of multiple comparisons were obtained using chi-square test.
* and ***P*-values <0.05 were considered significant; *** and **** *P*-values <0.008 were considered significant.

CI: 0.279–0.808 and $P = 0.002$, OR = 0.502, 95% CI: 0.319–0.788 respectively). In other multiple comparisons in the same model, no significant *P*-value was observed (Table 3). In addition, in other two models (GG *vs* GC *vs* CC and GG *vs* GC + CC), the crud comparison among four groups and multiple comparisons between two groups did not observe significant *P*-value (data not shown). The trend demonstrated that CC genotype and C-allele of IL-6-572 might be a protective factor of CP combined with T2DM. However, the further logistic regression analysis with adjustment for age, gender, BMI, smoking and stress did not support the result of the CC genotype completely (Table 4, $P = 0.058$, OR = 0.523, 95% CI: 0.268–1.022).

As shown in Table 4, several significant risk factors were observed in the logistic regression analysis. BMI, age and stress may be risk factors of CP, T2DM or CP combined with T2DM.

Table 4 Logistic regression analyses for associated risk factors of CP&T2DM in a Chinese population

Variables	*T2DM vs* control	*CP vs* control	*CP&T2DM vs* T2DM	*CP&T2DM vs* CP	*CP&T2DM vs* Control
Gender					
P-value	0.791	0.056	0.131	0.937	0.14
OR (95% CI)	1.117 (0.494–2.524)	1.882 (0.983–3.6)	0.577 (0.283–1.177)	1.016 (0.411–2.511)	0.564 (0.264–1.206)
Age					
P-value	<0.001	0.106	0.467	<0.001	<0.001
OR (95% CI)	1.109 (1.07–1.15)	1.026 (0.995–1.058)	0.988 (0.958–1.02)	0.889 (0.849–0.931)	0.886 (0.853–0.919)
BMI					
P-value	0.072	0.181	0.899	<0.001	0.072
OR (95% CI)	1.067 (0.994–1.145)	0.949 (0.879–1.025)	0.996 (0.929–1.067)	0.796 (0.702–0.902)	0.92 (0.839–1.008)
Smoking					
P-value	0.818	0.249	0.201	0.088	0.849
OR (95% CI)	0.878 (0.29–2.658)	1.574 (0.728–3.403)	0.524 (0.195–1.41)	2.404 (0.879–6.574)	0.912 (0.354–2.353)
Stress					
P-value	0.02	<0.001	0.748	0.048	0.006
OR (95% CI)	2.328 (1.14–4.754)	3.048 (1.76–5.281)	1.114 (0.575–2.159)	2.232 (1.005–4.954)	0.361 (0.175–0.745)
IL-6-572					
P-value	0.218	0.998	0.438	0.291	0.058
OR (95% CI)	1.545 (0.773–3.09)	0.999 (0.569–1.754)	0.779 (0.414–1.465)	0.652 (0.295–1.442)	0.523 (0.268–1.022)

OR, odds ratio; CI, confidence interval; T2DM, type 2 diabetes mellitus; CP, chronic periodontitis; BMI, body mass index; IL, interleukin.
Independent variables: gender (dichotomous, male = 1 and female = 0), age and BMI (continuous), smoking (dichotomous, yes = 1 and no = 0), stress (dichotomous, yes = 1 and no = 0), IL-6-572 (dichotomous, CC = 1 and GC + GG = 0).
P-values <0.05 were considered significant.

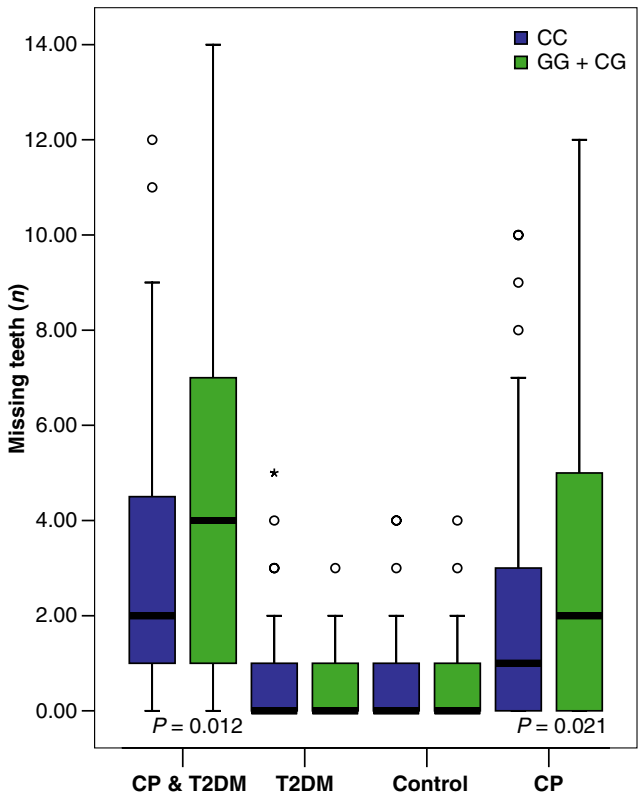

Figure 1 Box plots show different numbers of missing teeth according to different interleukin-6-572 genotypes (CC *vs* GG + CG) inside each group. The box refers to the 25th (bottom) and 75th (top) percentiles and the median is the horizontal bold line inside. The small circles show out-side values and the asterisk shows far out value. *P*-values were obtained using *t*-test with significance of $P < 0.05$

The final outcome of periodontal diseases is the loss of teeth. Despite the large variability of the number of teeth, further indication came from the comparison of the number of missing teeth with respect to the IL-6-572 genotype inside each group (Figure 1). Inside CP&T2DM group or CP group, there was a significant difference ($P = 0.012$ or 0.021) between the number of missing teeth of different genotypes (CC *vs* GG + CG). Additionally, inside T2DM group or control group, no similar significant difference was observed (data not shown). The result indicated that the CP patients carrying G-containing genotype could more lose teeth than the ones carrying CC genotype.

When the code was broken for the 50 duplicate samples used for laboratory quality control purposes, the IL-6 genotyping results matched in all 50 cases. Moreover, all results of sequencing were in accordance with those of the PCR-RFLP.

Discussion

So far, little information is available in the literatures about the association among IL-6 promoter SNPs, diabetes and periodontitis. This study investigated the association among IL-6 promoter SNPs, diabetes and periodontitis in a Chinese population through a case-control model.

A different genetic background in Chinese and Caucasians was found with respect to the allele frequencies of the IL-6 promoter SNPs. The allele frequencies of IL-6-174 and IL-6-597 reported in Caucasian (0.40–0.47) (Terry *et al*, 2000; Holla *et al*, 2004) were much higher than those found in this study (data not shown). On the other hand, the frequencies of the C allele of IL-6-572 in Caucasians (0.03–0.1) were much lower than those of the Chinese population (0.74–0.85). Our result, nevertheless, is similar to a Korean study, a Japanese study and another Chinese study (Zhai *et al*, 2001; Kitamura *et al*, 2002; Park *et al*, 2003). High frequency population-specific alleles are particularly useful for mapping genes responsible for disease susceptibility (Stephens *et al*, 2001). The SNP of IL-6-572 may be a useful marker for the association study in Asians.

In the multiple comparisons, IL-6-572 genotypes distributions and allele frequencies revealed that the CC genotype and C-allele might be a protective factor of CP combined with T2DM. After adjustment for age, gender, BMI, smoking and stress, the result became a borderline trend in terms of IL-6-572 genotypes distribution. A possible explanation is that our subjects were not matched on age, gender and smoking (Table 1; $P < 0.001$). Therefore, subjects matched for age, gender and smoking status should be selected in similar studies hereafter.

The IL-6-572 C-allele as a protective factor of diseases was seldom reported in the previous studies. Holla's research (Holla *et al*, 2004) first observed that IL-6-572 polymorphism may be one of the protective factors in the development of CP. It is interesting that our result of association between the number of missing teeth and IL-6-572 genotype (Figure 1) supports the conclusion of the IL-6-572 C-allele as a protective factor.

The viewpoint above is somewhat in contrast to those published by Ferrari *et al* (2003) who found that the carriage of the C-allele at IL-6-572 was associated with increased IL-6 biological activity in a Caucasian population. The reason for the discrepancy is unclear, but different population and genetic background may be one of reasons (Holla *et al*, 2004). In the previous gene polymorphisms studies, some discrepant results were observed on many occasions in different population, for example IL-1 gene polymorphisms (Laine *et al*, 2002; Rogers *et al*, 2002; Meisel *et al*, 2003). A limitation of this study is that plasma IL-6 levels were not measured in this Chinese population. The further studies are required to interpret the discrepancy.

In this study, the C-allele frequency of IL-6-572 in CP group was lower than that in the control group, but without significant difference. The lowest frequency was observed in CP&T2DM group with a significant difference between CP&T2DM group and the control group. The phenomenon seems to indicate that IL-6-572 polymorphism is a modifying factor which does not directly influence incidence of periodontitis in the population without T2DM but reduce the possibility of periodontitis complication in diabetes patients. To some extent this confirmed the hypothesis that the promoter SNPs of IL-6 might be associated

with the progression of periodontitis aggravated with diabetes.

The study also demonstrated that stress may be a risk factor of CP, T2DM or CP combined with T2DM. The finding is in accordance with that of several previous studies (Johannsen *et al*, 2005; Hilgert *et al*, 2006). Considering the limitation of self-reported stress, more detailed and accurate measurement is required for a definite conclusion.

In this study, six index teeth were used for periodontal examination because these are considered to represent approximately the extent of periodontitis in subjects (Wang *et al*, 2007). However, compared with full-mouth periodontal examination, this protocol might lead to underestimate the true prevalence of periodontal attachment loss and probing depth (Kingman *et al*, 2008). To adjust the possible bias, any subject with gingivitis and periodontitis (PD >3 mm and/or the presence of CAL and BOP) was excluded in the control group and T2DM group. But it needs to be pointed out that it still may result in the misclassification of some controls and T2DM patients without periodontitis. For example, some controls may have had periodontitis on non-index teeth, but not on their index teeth.

In conclusion, there are not enough evidences to confirm that the gene polymorphism at IL-6-572 is one of potential protective factors of CP combined with T2DM for the present, but the IL-6-572 genotype and allele distributions are undoubtedly unique to subjects with CP and T2DM in a Chinese population. The function of the unique distributions needs to be further researched. The study contributes to a genetic understanding of the individual difference in periodontal status of diabetics for clinical practice.

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Author contributions

The corresponding author of this manuscript is Prof. JinCai Zhang, who assumes the overall responsibility for the study and contributes to the research design and to revising the article critically. The first author, Dr LiMin Xiao is primarily responsible for collecting and analyzing data, and writing the manuscript. YuXia Yan takes equal responsibilities of the first author for the research. ChengJie Xie, WeiHua Fan, Dong-Ying Xuan, ChunXian Wang, Lei Chen, ShuYu Sun and BaoYi Xie contribute to acquisition and analysis of data.

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