Th2 cell regulatory and effector molecules single nucleotide polymorphisms and periodontitis

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Suggested summary sentence

This candidate gene study explored the genetic association between Th2 regulatory genes polymorphism and periodontitis. A haplotype (GTT) in a block of 3 *CTLA4* SNPs was identified. Meta-analysis of disease risk SNPs detected from the current cohort indicated allele T of *CTLA4* rs5740909 and allele G of *IL6* rs1800796 appeared associated with human periodontitis.

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Abstract

Aim: To investigate the association between T helper 2 (Th2) cell regulatory and effector molecules' genetic polymorphisms and periodontitis.

Materials and methods: Single nucleotide polymorphisms (SNPs) of eleven Th2 cell regulatory or effector molecules genes (*CD28*, *CTLA4*, *IL4*, *IL5*, *IL6*, *IL9*, *IL10*, *IL13*, *IL4R*, *GATA3*, *STAT6* and rs1537415; total 130 SNPs) were studied in Chinese non-smokers (163 periodontitis-free controls, 141 periodontitis patients) using Sequenom iPlex assays. SNPs potentially associated with periodontitis (adjusted allelic $P < 0.1$) in this cross-sectional study were further investigated via meta-analysis.

Results: Allele G of rs4553808 in promoter of *CTLA4* was more frequently detected in periodontitis than controls ($P < 0.005$), but did not remain significant after age and gender adjustment. Haplotype (GTT) in a block of three *CTLA4* SNPs (rs4553808, rs16840252, rs5742909) was significantly associated with periodontitis. Meta-analysis of SNPs identified indicated allele T of *CTLA4* rs5742909 (3 studies; 461 control, 369 periodontitis) and allele G of *IL6* rs1800796 (18 studies; 2,760 control, 2,442 periodontitis) were significantly associated with periodontitis (OR = 1.44 and OR = 1.30, respectively).

Conclusions: Within limitations of this study, a haplotype of *CTLA4* concerning Th2 cell regulation, may be associated with periodontitis in Chinese non-smokers followed. Meta-analysis indicated rs5742909 of *CTLA4* and rs1800796 of *IL6* appeared significantly associated with periodontitis.

Word count: 210

KEYWORDS

CTLA-4 antigen; interleukin-6; periodontitis; polymorphisms, single nucleotide; Th2 cells

Abbreviations

ANNOVAR, annotate variation; AP, aggressive periodontitis; BOP, bleeding on probing; CAL, clinical attachment level; CD, cluster of differentiation; CHB, Han Chinese population in Beijing, China; CI, confidence interval; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; GATA-3, globin transcription factor binding protein 3; GLIDE, Gene-Lifestyle Interactions and Dental Endpoints; GLT6D1, glycosyltransferase 6 domain containing 1; GWAS, genome-wide association study; HWE, Hardy-Weinberg equilibrium; IL-4R, interleukin 4 receptor; LD, linkage disequilibrium; MAF, minor allele frequency; OPG, orthopantomogram; OR, odds ratio; PAL, probing attachment level; PPD, probing pocket depth; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; SHP, Src homology region 2 domain-containing tyrosine phosphatase; SIGLEC5, sialic acid binding Ig-like lectin 5; SNP, single nucleotide polymorphism; STAT6, signal transducer and activator of transcription 6; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology; TCR, T-cell receptor; Th1, T helper 1 cell; Th2, T helper 2 cell; TNF-α, tumor necrosis factor alpha; Treg, regulatory T cell; UTR, untranslated region; VNTR, variable nucleotide tandem repeat polymorphisms.

1. INTRODUCTION

T helper 2 (Th2) cells have been suggested to play role in the development of periodontitis.[1,2] They influence human humoral immunity via modulation of B cell IgG and IgE secretion. T helper 1 (Th1) cells, on the other hand, promote macrophage activation and delayed hypersensitivity, hence influencing cell-mediated immune responses. There are some hypotheses suggested that the imbalance between Th1 and Th2 cells and their related cytokines production may be associated with periodontitis.[1,2] If the subgingival biofilm continuously irritate the periodontal tissue, B cells and plasma cells would be the major immune cell types recruited in the advanced lesions, implied a more significant role for Th2 cells in periodontal defense.[3] Th2 cells were regarded as agents to ameliorate periodontal disease symptoms, because less Th2 type cytokines (interleukin 4 or IL-4 and interleukin 6 or IL-6) were detected in the disease tissue.[4,5] The controversy remained for decades, but many studies supported or implied Th2 cells may play an important role in periodontitis.

Caused by mixed opportunistic bacterial infection, periodontal disease expression/progression could be modified by smoking, diabetes, stress and genetic factors.[6,7] Interests in genetic predisposition of periodontitis grew over last couple of decades.[7] An important gene reported in the first genome-wide association study (GWAS) on previously named aggressive periodontitis (AP), or periodontitis in young adults,[8,9] was related to Th2 cells. It was reported that a single nucleotide polymorphism (SNP) rs1537415 in glycosyltransferase 6 domain containing 1 gene (*GLT6D1*) would encode a transcription factor binding site with reduced globin transcription factor binding protein 3 (GATA-3) attachment affinity and the SNP is

significantly associated with AP.[10] GATA-3 is considered the master switch required for the development of Th2 cell[11] and its mRNA expression appeared significantly higher in healthy gingiva than advanced periodontitis lesions.[12] Although the GWAS study was on young adults with periodontitis, it is possible that periodontitis of all ages in general may share similar/same genetic risk indicator. However, a recent meta-analysis on periodontitis GWAS, including the aforementioned report, with a sample size of 45,651 stated an insignificant association between rs1537415 and periodontitis.[13] The same paper also reported low heritability of periodontitis, a common limitation for GWAS study on complex traits.[14]

In brief, Th1 cells exhibit pro-inflammatory responses and can enhance the destructive pathway in periodontal tissues. Th2 cells and T regulatory (Treg) cells, on the other hand, associate with anti-inflammatory responses and immune suppressive properties which can control or attenuate periodontal disease development. Treg is a protective T cell subset to prevent tissue damage in the periodontal environment.[15] We hypothesized that hereditary acquired inadequate Th2 responses may associate with increased risk for periodontitis and such genetic predisposition could be detectable from periodontitis patients via a candidate gene approach.

The differentiation of Th2 cells involved many regulatory molecules, including cluster of differentiation 28 (CD28), cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), IL-4, IL-4 receptor (IL-4R), GATA-3 and signal transducer and activator of transcription 6 (STAT6) (Supplementary Fig. 1). Th2 cells-produced effector molecules mainly include IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13.[16-18]

A few studies investigated the association between periodontitis and genetic polymorphisms of genes coding for the aforementioned molecules, including *CD28*, *CTLA4*, *IL-4*, *IL-6*, *IL-4R*, *IL-10* and *IL-13*.[7,19,20] However, only limited SNPs

concerning these gene regions were covered in the previous investigations. Here we carried out a candidate gene study on periodontitis, based upon the aforementioned hypothesis, to investigate the association between periodontal disease and SNPs of genes known to control or regulate Th2 cell differentiation (*CD28*, *CTLA4*, *IL-4R*, *GATA3*, *STAT6*) and Th2 cell effector molecules production (*IL-4*, *IL-5*, *IL-6*, *IL-9*, *IL-10*, *IL-13*) in Chinese non-smokers. We postulated that the same SNPs reported by Song et al.[21] and Zhao et al.[22] would be significantly associated with periodontitis in the cohorts followed.

Based upon the results of this study, we also undertook a meta-analysis of any relevant Th2 regulatory SNPs, tentatively identified in the current study to be potentially associated with periodontitis, in an attempt to improve the strength of findings observed in the current study.

2. MATERIALS AND METHODS

2.1 Participants

We computed the study sample size using a web browser program, Genetic Association Study Power Calculator[23] which was derived from CaTS power calculator.[24] Taking rs1537415 as a reference SNP, to reach a power of 95%, with a disease prevalence of 40%,[25] a disease allele frequency of 38.8% and a genotype relative risk of 1.59,[10] the anticipated sample size should be 130 cases and 130 controls.

Patients' records, orthopantomogram (OPG) plus other available radiographs were screened within one month of first attendance to a dental hospital. Potential

166 eligible subjects were invited to attend a clinical examination. Roughly one control
167 case (gender- and age-matched) was recruited for each test case included.[26,27]

168 Chinese, non-smokers, > 35 year-old, who might be periodontitis-free or have
169 periodontitis (based on OPG on first attendance[26,27]) but otherwise healthy were
170 invited for periodontal examination. Demographic information and medical and
171 dental histories were obtained from patients' records, supplemented by information
172 obtained during the day of the clinical examination. Race and ethnicity were self-
173 reported, with a participant being considered Chinese if his or her biological parents,
174 grandparents, and great grandparents were all reported to be ethnic Chinese.[26,27]
175 Smoking history was self-reported; patients who currently smoked or who had quit
176 within 12 months were considered to be smokers and those who had never smoked or
177 who had quit for more than 12 months were considered to be non-smokers. Smokers
178 were excluded. For periodontitis subjects, scans of their OPG had to show > 50%
179 alveolar bone loss at > 30% of proximal sites measured by a trained examiner using
180 Schei ruler, with each tooth contributing two sites: mesial and distal.[28] After OPG
181 screening, clinical periodontal examination by another designated examiner had to
182 confirm the presence of at least two teeth in each quadrant with probing pocket depth
183 (PPD) ≥ 5mm and bleeding on probing (BOP). Periodontitis-free participants or
184 controls were recruited as someone with no PPD > 4mm and no gum recession >
185 2mm in any site, and had no history of tooth loss due to periodontal diseases.[26,27] Over
186 the three years study period (2005-2008), 304 Chinese participants, including 163
187 periodontitis-free controls and 141 periodontitis patients fulfilling all selection
188 criteria, consented and with acceptable DNA quality/quantity from blood sample
189 (section 2.2) were recruited in Prince Philip Dental Hospital, Hong Kong (Fig. 1). The
190 Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)

guidelines were followed. All participants were systemic healthy and were non-smokers (Table 1). Written informed consent was obtained from all participants. The study was approved by Ethics Committee, Faculty of Dentistry, the University of Hong Kong (1/8/12d).

2.2 DNA isolation and genotyping

Ten milliliters of venous blood were collected from each participant and stored in tubes containing ethylene-diamine-tetra-acetic acid at -70°C before DNA extraction. Genomic DNA was extracted using a QIAamp DNA mini-kit according to the manufacture's instruction and were stored at -70°C before genotyping. All DNA samples were checked before genetic analysis, and those with insufficient quantity and/or quality for genotyping (< 10ng/μl, or A/280 ratio not within the range of 1.7-2.0, or showing DNA degradation upon electrophoresis) were discarded.

In total, 135 SNPs of *CD28, CTLA4, GATA3, STAT6, IL4, IL4R, IL5, IL6, IL9, IL10* and *IL13* were selected for genotyping (Supplementary Table 1), including rs1537415, the GATA-3 binding site associated with AP.[10] All the samples were genotyped by MassARRAY iPlex Gold assay (Sequenom, San Diego, CA).

2.3 Single nucleotide polymorphisms (SNPs) selection

By using the HapMap Genome Browser release #27[29] (Phase 1, 2 and 3 - merged genotypes and frequencies), SNPbrowser™ software version 4.0 (Applied Biosystems, Foster City, CA, USA) and the dbSNP database in the US National Center for Biotechnology Information website,[30] the SNPs of *CD28, CTLA4, GATA3, STAT6, IL4, IL4R, IL5, IL6, IL9, IL10* and *IL13* to be analyzed in this study were selected according to the following criteria: i) tagging SNPs were selected by the SNP Tagging

Wizard of SNPbrowser™ [haplotype $r^2 \geq 80\%$ and minor allele frequency (MAF) $\geq$ 0.05 in Han Chinese population in Beijing, China (CHB)] and tag SNP picker of HapMap Genome Browser (tagger multimarker $r^2 \geq 80\%$ and MAF ≥ 0.05 in CHB population); ii) SNPs in coding regions were selected directly on SNPbrowser™ (in CHB population); iii) SNPs in regulatory regions were selected if their published MAFs were more than 0.1; iv) SNPs in introns were selected only if they were adjacent to exons within 100 bp and if their published MAFs were also more than 0.1; and v) SNPs of the eleven genes of interest previously reported to be associated with periodontitis.

2.4 Genotyping

For genotyping, an online assay design software (MassARRAY Assay Design Suite, Sequenom) was used to design PCR and single-base extension primers.[31] rs1537415 was designed as priority. Within the 135 SNPs, five SNPs were failed to design because of the technical limitation of iPlex platform (Supplementary Tables 1 and 2).

For each 96-well plate of assay, there were 89 wells for samples, five for random duplicates, one water control well and one blank well. Four of the duplicate check samples and six randomly selected samples for each sample plate were ran on 1% agarose gel and the bands were required to be intact after the electrophoresis. PCR amplification, the shrimp alkaline phosphatase treatment and the primer extension reactions were performed according to the manufacture's instruction of iPLEX Gold assay (Sequenom, San Diego, CA). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was used to read the signals and detect the genotypes. Online annotation tool, Annotate Variation (ANNOVAR), was used to annotate the SNPs.

241

242 **2.5 Quality control**

243 Before being considered suitable for further statistical analysis, the 130 SNPs were
244 screened according to the following three criteria: i) SNP call rate > 0.8; ii) MAF >
245 0.1; iii) Hardy-Weinberg equilibrium (HWE) test showing $P \geq 0.01$ in the
246 periodontitis-free group.

247 Twenty samples were duplicated for each SNP during genotyping and the
248 concordance rate was 99.8%. One SNP (rs13306436, a downstream variant) in *IL6*
249 has no call on one of two alleles in any individual and was excluded (Supplementary
250 Table 1). Eleven SNPs with call rate less than 0.8. Out of the remaining 118 SNPs, 29
251 were with MAF less than 0.1 or with only one genotype detected hence were excluded
252 from further analysis. After filtering by MAF and call rate, three SNPs with HWE *P*-
253 value <0.01 in the periodontitis-free group was detected and one was excluded except
254 rs1537415 and rs231775, which were reported to be associated with periodontitis in
255 previous studies,[10,32,33] were included for further analysis. Together with 70 of the 130
256 SNPs, which passed the subsequent quality control tests, 72 SNPs were analyzed
257 (Supplementary Tables 2 and 3).

258

259 **2.6 Meta-analysis**

260 As SNPs nominally implicated ($P < 0.1$) may represent true disease risk loci,[34] SNPs
261 with adjusted *P* value (allelic) < 0.1 in the current periodontitis association analysis
262 were identified. A systematic search was performed by searching electronic
263 biomedical databases, including PubMed, Web of Science, Wanfang Data and China
264 National Knowledge Infrastructure. All articles published on or before 31 December
265 2019 were searched in these databases. The name of the molecules encoded by the

genes potentially associated was used in the search. The key words used for search were: “Cytotoxic T-Lymphocyte Associated Protein 4” or “CTLA-4” and “periodontitis” and “polymorphism”; “interlukin-4” or “IL-4” and “periodontitis” and “polymorphism”; “interlukin-4 receptor” or “IL-4R” and “periodontitis” and “polymorphism”; “interlukin-6” or “IL-6” and “periodontitis” and “polymorphism.” The eligibility criteria were cross-sectional human case-control studies with periodontal healthy individuals as controls. Reports analyzing the association between the SNPs of interest (adjusted allelic $P < 0.1$) identified in the current cross-sectional study and periodontitis were selected. No language restriction was set. The following data were extracted from the studies directly: the authors, funding source, the year of publication, the country, the ethnicity of the population, the sample size of the cases and controls with different genotypes and allele types. The related references in the articles or relevant publications citing the selected articles were also read and included if relevant.

Possibilities of data avaible from GWAS on periodontitis was also explored. The related literature search followed the same protocol described above with key words as “periodontitis” and “GWAS” or “genome wide association study”. The final results were verified in GWAS Catalog,[35] an open GWAS database summaries published GWAS. One recent GWAS meta-analysis on periodontitis[13] included and deposited most if not all GWAS on periodontitis data available in the Gene-Lifestyle Interactions in Dental Endpoints (GLIDE) consortium and the corresponding data were extracted accordingly.[36]

The screening and selection of the finally included studies followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[37] We also conducted a risk of bias assessment on the included studies, which is a common

procedure for ordinary meta-analyses but is seldom performed in genetic risk meta-analyses.

2.7 Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) Statistics 23.0 (Chicago, IL, USA) together with PLINK 1.90 when appropriate. Differences between the distributions of sex among periodontitis-free and periodontitis individuals were tested with chi-square statistic. Differences between cases and controls in number of standing teeth and age were assessed with *t* test.

The allele types and genotypes of each suitable SNP single marker were screened initially by Chi-square test. To avoid false-negative findings on the SNPs with intermediate frequency or effects, a less-stringent significance level was used at beginning ($P < 0.005$).[38] To eliminate random SNP association, haplotype analysis was carried out on the potentially associated region. For the logistic regression, an adaptive permutation test was performed by PLINK 1.90 to correct against multiple comparisons.[39] Full mode of PLINK was performed, including Cochran-Armitage trend test assuming co-dominant mode (2 degree of freedom), genotypic test (2 degree of freedom), dominant gene action test (1 degree of freedom), recessive gene action test (1 degree of freedom) and allelic test. Fisher exact test was used when there was any cell less than five. Odds ratios and 95% confidence intervals were determined from the result of allelic test.

The association of disease and haplotype in the block of these SNPs were analyzed. The linkage disequilibrium analysis was performed by Haploview 4.2.

Review Manager 5.3 was used for meta-analysis. Random effect model was used when the number of studies was more than 4 or I square was more than 50% in

case the *P* value of heterogeneity was less than 0.05, otherwise fixed effect model was chosen as default. Subgroup analysis was performed in different populations. Funnel plots were drawn under fixed effect model to identify publication bias. The evaluation of bias of the studies included were following Cochrane collaboration's tool.[40]

3. RESULTS

The demography and clinical profile of the participants were shown in Table 1 and there was no significant difference between cases and controls concerning age and gender.

3.1 Association study of Th2 related gene polymorphisms

DNA samples from all 304 non-smoking participants were genotyped and analyzed. After filtering by minor allele frequency (< 0.1), call rate (< 0.8) and Hardy-Weinberg equilibrium (HWE) *P*-value (< 0.01). Seventy out of the 130 candidate SNPs passed the quality control tests. Adding rs231775 (HWE with $P < 0.01$ but found significantly associated with periodontitis in other populations[32,33]) and rs1537415 (AP-associated SNP[10] but with *P* value of HWE < 0.01), a total of 72 SNPs were analyzed (Supplementary Table 1). The distribution of genotypes and alleles of the 72 SNPs analyzed were elaborated in Supplementary Table 2.

Cochran-Armitage trend test under the heredity mode of co-dominant showed rs4553808 appeared significantly associated with periodontitis ($P = 0.0042$), but such observation did not remain significant after age and gender adjustment.

Four SNPs (rs4553808, rs16840252, rs5742909 and rs3087243) in *CTLA4* with $P < 0.05$ in the Cochran-Armitage trend test were analyzed on linkage disequilibrium. rs4553808, rs16840252 and rs5742909 from the region of *CTLA4* were closely linked ($r^2 = 0.98$) and located in the regulatory regions (Fig. 2). The haplotype (GTT) in a block of the three *CTLA4* SNPs above was significantly associated with periodontitis, after adjustment against age and gender (Table 2).

The AP-associated SNP, rs1537415,[10] did not appear to be associated with periodontitis in the current case-control investigation.

3.2 Meta-analysis

Nine SNPs presented adjusted allelic *P* value < 0.1, including rs4553808, rs16840252, rs5742909 and rs3087243 of *CTLA4*; rs2243290 and rs2243291 of *IL4*, rs1800796, rs2066992 and rs2069852 of *IL6* (Supplementary Table 3). No candidate gene study was available regarding association between rs4553808, rs16840252 or rs3087243 of *CTLA4*; rs2243290 or rs2243291 of *IL4*; rs2066992 or rs2069852 of *IL6* with periodontitis. The downloaded data files regarding periodontitis GWAS studies[36] from GLIDE consortium were consulted. Unfortunately, the data set only contain summary statistics of the SNPs analyzed. It did not include allelic/genetic data for individual cohort followed. Such data are needed for the present meta-analysis hence incorporating the related GWAS data for further investigation is considered impossible. At the end, meta-analysis of the aforementioned seven SNPs or incorporation of GWAS data into the meta-analyses of rs5742909 or rs1800796 was not carried out.

Four papers[32,33,41,42] were identified reporting *CTLA*4 candidate genes and periodontitis by key words search, but only two papers[32,41] involved rs5742909, hence

the flow of the selection on rs5742909 relevant articles was not illustrated in graphic presentation. Reports citing and cited by the included studies were looked up and no additional relevant study was detected.

For rs5742909 of *CTLA4*, the included Iranian study surveyed 218 controls and 126 cases[32] and the later Chinese study included 80 controls and 103 periodontitis patients.[41] The bias assessment of the included studies was detailed in Supplementary Figures 2 and 3. Data meta-analysis on rs5742909 and periodontitis with current report and the two studies available (461 controls and 369 cases) showed significant association between allele T of the former and periodontitis (Table 3 and Fig. 3).

Twenty articles were found reporting rs1800796 of *IL6* and periodontitis.[20,43-61] Special attention was paid upon the full-text and reported absolute values. Regarding articles on rs1800796 of *IL6* and periodontitis, two of the twenty studies were suspected reporting data from almost same cohort,[49,52] only the recent report with a bigger sample size was included in the final analysis.[52] Two groups of studies on rs1800796 were using exactly the same cohort,[20,55;46,51] i.e. same control/case counts, so only data from the later reports was used.[20,51] Reports citing and cited by the included studies were looked up and no additional relevant study was detected. As a result, a total of seventeen studies[20,43-45,47,48,50-54,56-61] together with data of the current study were finally included in the meta-analysis (Fig. 4, Supplementary Table 4; controls = 2,760, periodontitis = 2,442). The bias assessment was detailed in Appendix (Supplementary Fig. 4 and 5).

The seventeen included papers plus the current study on rs1800796 of *IL6* and periodontitis comprised thirteen studies on east Asians (including eleven on Chinese), one on central Asians (Iranians), one on south Asians (Indians), one on Caucasians and one study investigated a multicultural population, including Asians, Caucasians

and Africans (Supplementary Table 4). When analyzing data from all included studies (Supplementary Table 4, Fig. 5), G allele of rs1800796 appeared to be associated with increased risk of periodontitis (P = 0.0009, OR = 1.30). Meta-analysis stratified by ethnicity showed the G allele remained significant both in Caucasians (P = 0.003, OR = 2.15) and in East Asians with periodontitis (P < 0.0001, OR = 1.35). When considering only the eleven studies on Chinese, significantly more rs1800796 G allele were reported in the periodontitis group than controls (P = 0.0002, OR = 1.33) (Fig. 5).

In general, studies included in the meta-analyses presented different levels of bias. Unknown risk of selection bias and performance bias exist as the criteria for the control or non-periodontitis group were not clearly defined and the concordance test on the genotyping was not mentioned (Supplementary Fig. 2 and 4). Smokers or participants with diabetes might be included in the data in three studies on rs1800796 while exact details were lacking, which presented as unknown or high risk of selection bias (Supplementary Fig. 4). Although smoking as a confounding factor was adjusted in one of the studies,[52] the data of the non-smokers was not defined, so both smokers and non-smokers were included in the meta-analysis, which indicate a high risk of bias (Supplementary Fig. 4).

4. DISCUSSION

Th2 cell has been reported to be associated with periodontitis,[3] but the genetic risk indicators of Th2 cell-related genes have not been thoroughly investigated. This study investigated a selection of SNPs from eleven genes, which are closely related with Th2 cells or their function regulation. The current candidate gene study indicated G

allele of rs4553808 appeared significantly associated with periodontitis (P = 0.0042) but not after age and gender adjustment (Supplementary Table 3; allelic P = 0.0073, genotypic P = 0.0280 compare to predetermined P < 0.005). The observation indicated further study with a bigger sample size is needed to confirm the association between rs4553808 polymorphism and periodontitis.

Four *CTLA4* SNPs, i.e. rs4553808, rs16840252, rs5742909 (upstream) and rs3087243 (CT60, downstream) were found to qualify as periodontitis risk loci (P = 0.0073, 0.0106, 0.0099 or 0.0213, respectively; Supplementary Table 3). Further investigations with bigger sample size are needed to confirm the above observations. It was however noteworthy that rs3087243 was previously reported in GWAS to be associated with several autoimmune diseases.[62]

The haplotype GTT in a block of these SNPs from *CTLA4* promoter region (rs4553808, rs16840252, rs5742909) was significantly enriched in Chinese periodontitis patients (15.0%) compare to the periodontitis-free controls (8.3%). The association between rs5742909 and periodontitis was supported by the meta-analysis of candidate gene data that follows (Table 3). CTLA-4 is encoded by a gene located on chromosome 2q33 and it is a key T cell downregulation molecule. The closely linked three SNPs located in the same promoter region of *CTLA4*, which indicated a lower possibility of false positive result or positive result by chance. The current observation implied fine mapping and functional study of the *CTLA4* promoter especially at the rs4553808, rs16840252, rs5742909 haplotype GTT region might potentially shed light on the related Th2 regulation biology and hence the corresponding periodontitis risks in human.

In attempt to control periodontal inflammation hence the possibility of reverting the former to health, a series of chemical signals need to be activated to downregulate

441 inflammatory cytokines, attenuate immune cell trafficking, induce immune cell
442 apoptosis and clearance.[63] The current meta-analysis based on candidate gene studies
443 data indicated rs5742909 T allele at promoter region of *CTLA4* was associated with
444 periodontitis (Table 3). CTLA-4 (CD152) is an important negative regulator of T cell
445 activation which transiently express on the surface of T cells. Within periodontal
446 tissue, CTLA-4 can suppress proliferation of T-cells in response to
447 periodontopathogens.[64] Therefore Tregs expressing inhibitory molecule CTLA-4 are
448 supposed to attenuate inflammatory responses against periodontopathogens or
449 antigens.[32,42]

450 Despite the available GWAS data did not provide genetic information of each
451 cohort hence prevented further meta-analysis in relation to SNPs of interests
452 detectable in the current investigation, a Supplementary Table 5 is prepared to
453 summarize the stated *P* concerning the nine SNPs with adjusted allelic $P < 0.1$
454 reported in the present study. It is noteworthy that Shungin et al.[13] reported none of
455 the nine SNPs, including rs5742909 or rs1800796, was associated with periodontitis.

456 Some previous studies tested the association between periodontal disease and
457 *CTLA4* SNPs. A missense variant in a coding region, rs231775, was tested in studies
458 on periodontitis but the results were controversial.[32,33,65] Result from the current
459 investigation showed the SNP was not significantly associated with periodontitis ($P =$
460 0.464; Supplementary Table 3). The current rs231775 HWE test indicated $P < 0.01$,
461 demonstrating the SNP may be influenced by selection, genetic drift, mutation or
462 other factors in the Chinese cohort followed. G allele of rs231775 was the minor
463 allele in Iranian (28%)[32] and in Brazilian controls (27%),[33] but was major allele in
464 Chinese control of the current study (72.9%). The latter, was consistent with the data
465 reported on the Chinese population of HapMap. The present observation perhaps

indicated genetic variance of the rs231775 among different populations. Similar to report by Houshmand and coworkers,[32] *CTLA4* upstream SNP rs733618 was not associated with periodontitis (P = 0.658). A recent study reported the G allele of rs56102377, a 3 prime untranslated region variant, might be regulated by miR-105, which caused a down-regulation of *CTLA4*.[41] rs56102377 was not included in the current study, because the published MAF in the 1000 Genome and HapMap was < 0.05 at the time of study design. The implication of rs56102377 in periodontitis therefore warrant further investigation.

Limited published reports could be included in our candidate gene data-based meta-analysis on *CTLA4* as few studies investigated periodontitis and rs4553808, rs16840252, rs5742909 or rs3087243. At the end, only a SNPs in the *CTLA4* upstream region, rs5742909, could be considered. The SNP of interest was studied in Iranian and Chinese.[32,41] Interestingly, both earlier studies independently reported no association between the SNP and periodontitis. However, after combining their data with the current investigation (461 control, 369 periodontitis), allele T of rs5742909 appeared significantly associated with periodontitis (Fig. 3, Table 3). Putting the observation together, more investigations regarding association and/or functional studies related to rs5742909 polymorphism need to be carried out.

Only two of nine potential periodontitis risk loci identified from this candidate gene study, i.e. rs5742909 of *CTLA4* discussed above or rs1800796 of *IL6*, published reports with usable data. Seventeen reports (Supplementary Table 4) were available related to *IL6* rs1800796 and periodontitis, together with the present study consists of a candidate gene database of 2,760 control and 2,442 periodontitis patients. In line with previous reports, the current meta-analysis indicated significant association between allele G of *IL6* rs1800796 and periodontitis (Fig. 5A). The observation

remained significant even if the data was segregated in subgroups of East Asians, Caucasians or Chinese (Fig. 5B-D).

Having dual roles, IL-6 acts as either pro- or anti-inflammatory signalling agents.[66,67] At the initial phase of periodontitis, the gram-negative pathogens and their lipopolysaccharides activates transcription of proinflammatory cytokines in affected host cells via the NFκB pathway,[68-70] resulting in IL-1, IL-6 and TNF-α, production and related matrix metalloproteinase activation leading to degradation of periodontal extracellular matrix and bone resorption through increased RANKL secretion.[71,72] IL-6 activates transcription mediated by nuclear factor of activated T cells leading to production of IL-4 by naïve CD4+ T cells and their differentiation into effector Th2 cells.[18] Since IL-6 is abundantly produced by antigen-presenting cells, it is also a likely source of early Th1/Th2 control during CD4+ T cell activation.[18,73] On the other hand, being an anti-inflammatory cytokine, at the later stage of an inflammatory process, IL-6 can inhibit the synthesis of proinflammatory cytokines such as IL-1, TNF-α, and increase the production of anti-inflammatory signals by Th2 cells.[74] Periodontitis patients appeared to express higher serum and salivary IL-6 level than controls[75,76] might indicate perhaps a modulatory role of the cytokine at situation when chronic periodontal inflammation is established. In fact, rs1800796 located in the promoter region of *IL6*, which contributes to the functional regulation of IL-6 expression.[77]

The effect of G allele of rs1800796 on *IL6* transcriptional activity and expression level unfortunately remained controversial.[78-82] The current meta-analysis reported association between G allele of rs1800796 with periodontitis (Table 3). Provided rs1800796 G allele *in vivo* would inference *IL6* expression in human periodontal tissue, which in theory it could, the importance of such polymorphism as

516 one possible genetic periodontitis risk is reinforced. More studies are however,
517 warranted to elucidate the role of rs1800796 in periodontal inflammation/defense.

518 Indeed, a meta-analysis published in 2013 analyzed eight reports on rs1800796
519 and periodontitis indicated that the SNP might be associated with increased risk of
520 periodontitis in Europeans.[21] Interestingly, frequencies of G allele at rs1800796
521 appeared very different among the two Caucasian studies, being 5%[48] or 94%.[43] Four
522 Chinese studies were included in the Song et al.[21] meta-analysis, however two were
523 suspected to report cohorts from the same or almost same source and we excluded the
524 duplication in the current investigation (Please refer to Results: Meta-analysis
525 section).

526 A recent meta-analysis included eighteen studies as well as the suspected
527 duplicated ones from two groups of studies[20,55;46,51] exploring relationship between
528 rs1800796 and periodontitis reported significant association between the two, be that
529 Asians only or all subjects included.[22] The present report, excluding the suspected
530 duplicated data, adding data from two latest studies[20,51] and current report confirmed
531 such observation. However, because of putting central Asian participants' data under
532 Caucasians, Zhao et al.[22] reported rs1800796 did not associate with periodontitis in
533 the latter.

534 The meta-analysis contained studies with different levels of bias, so the result
535 should be considered with caution. Like a recent report,[83] we adopted in part the
536 PRISMA checklist to qualitatively assess the possibility of bias in the current meta-
537 analysis in order to assist readers' appreciation of the results observed.

538 One of the limitations of the current study is that some common risk indicators
539 especially that of socioeconomic status, education etc. were not recorded. These data

were known to associate with periodontitis[84] and hence perhaps best be adjusted during the data analysis.

Genes of T-cell receptor (TCR) were not investigated in this study because of the high complexity. There is no candidate gene study to date on associations between TCR genetic polymorphisms and periodontitis. However, SNPs in the genes of TCR complex were reported to be associated with other diseases, such as type 1 diabetes.[85] The latest periodontitis GWAS meta-analysis by Shungin et al.,[13] (n = 45,651) together with an earlier report by Munz and coworkers[86] (n = 25,003) in the same year, revealed that a SNPs rs11084095 of *SIGLEC5* (sialic acid binding Ig-like lectin 5, Siglec-5 or CD170) remained the only one SNP significantly associated with periodontitis ($P = 1.3 \times 10^{-9}$ or 5.0×10^{-8}, respectively). Siglec-5 is an inhibitory receptor with expression in various myeloid immune cells and may mediate Src homology region 2 domain-containing tyrosine phosphatase (SHP)-1/-2 dependent signaling. SHP-1/-2, two cytoplasmic protein tyrosine phosphatases, are critical regulators of T-cell by inhibiting TCR signalling, including not only Th1/2 cells, but also other T cells, such as Treg and thymic T cells.[87] The association between periodontitis and SNPs in the region coding for different TCR segments, *i.e.* constant segment or variable segment, could be studied in the future.

From the available thirteen GWASs[10,88-99] and two GWAS meta-analyses,[13,86] none except one[13] provided a full set of association study result summary (n = 45,651) online.[36] Only one GWAS[88] reported a SNP, i.e. rs11084095 of *SIGLEC5*, which was found to be associated with periodontitis after GWAS meta-analyses.[13,86] The authors, nonetheless, cautioned against heterogeneity introduced by the different approaches to periodontitis classification, different patterns of periodontal treatment and varying distributions of age in some of the cohorts followed or gene–environment interactions

not accounted for in the study design.[13] Regarding the nine SNPs identified to be of interest in the current candidate gene study, none was associated with periodontitis from the Shungin et al. meta-analysis (Supplementary Table 5).[13] The low number of significant locus (n = 1) identified so far from both GWAS meta-analyses is in fact is in agreement theoretically, with the total GWAS sample size available.[100]

In general, GWAS screened the whole genome for the disease associated genetic polymorphisms without a hypothesis, while candidate gene studies focus on some specific genes based on the potential biological function/mechanism, such as Th2 regulation upon periodontitis in the current study. Theoretically, as a hypothesis-free approach, GWAS should be able to identify more disease associated genetic polymorphisms and reveal the heritability of the disease. However, compared to twins/family studies, GWAS on periodontitis detected a lower heritability estimate of 0.07 for combined definitions of periodontitis, increasing with disease severity.[101] The atheoretical GWAS approach, until present moment, not able to capture all variation due to variable nucleotide tandem repeat polymorphisms (VNTRs), nor does it capture all variation due to copy number variations. Being atheoretical, the nature of GWAS and large number of potential main effects/interactions forced substantial corrections are needed for GWAS suggest that even genuine effects will be "corrected" away or overlooked.[102] In contrast to GWAS, candidate gene approah study specific alleles of genes that are hypothesized to be related to a phenotype. Candidate genes may be VNTRs with well-known functional impact, SNPs that have emerged from prior GWAS studies, etc. It enables theory driven investigation between SNPs and particular polymorphisms taking into considerations of of potential environmental interactions, i.e. a broader network of physiological and contextual findings, allows for stronger hypotheses based research. [101]

A systematic review compared the SNPs on cancer-associated genes identified via GWAS versus candidate gene approach.[103] The study analysed a database with research since 2000 and found GWAS reported 269 significant associations, while candidate gene methods reported 349. Only 7.1% (41 of total 577) of associations were found significantly associated with cancer in both methods and with similar effect sizes. Therefore, this study group was not overtly surprised regarding the lack of concordance between the current candidate-gene-based *CTLA4* and *IL6* results versus the corresponding data reported from recent GWAS meta-analyses.

There appears yet insufficient evidence to support the exclusive role of Th2 cells in pathogenesis of periodontitis, because neither CTLA-4 nor IL-6 is produced or works exclusively in Th2 cells. CTLA-4 in general is a negative immune modulator expressed by many T cells, including Treg and T helper cells, while IL-6 does not only work on Th2 cells through IL-4 but also has inhibitory effect on IL-1 and TNF-α. A recent study focused on risk indicators related to recurrent pregnancy loss, postulated that CTLA-4 upregulates transforming growth factor-β1 production from endometrial epithelial cells which in turn modulate IL-6 expression thereby positively influencing maturation of naïve CD4+ T cells to Tregs then reduces pregnancy failure.[104] However, further functional studies should be carried out on the expression and effect of *CTLA4* and *IL6* on Th2 cells from subjects with or without periodontitis. More genetic studies, including candidate gene approach, on *CTLA4* should be carried out to identify possible periodontitis associating SNPs, to clarify the limited observations made so far.

5. CONCLUSION

Among the eleven Th2 related genes, a haplotype in a block of three SNPs (rs4553808, rs16840252 and rs5742909) from *CTLA4*, the most important inhibitory receptor on Th2 cell which modulates cell activation and development of the latter, appeared significantly associated with chronic periodontitis in a non-smoking southern Chinese cohort. The present candidate gene study results did not indicate *IL6* rs1800796 associate with periodontitis in the Chinese cohort followed. Nevertheless, the following candidate gene-based meta-analysis on rs5742909 of *CTLA4* and rs1800796 of *IL6*, provided another opportunity to reassess all relevant studies involved and demonstrated the potential association between *CTLA4* rs5742909 or *IL-6* rs1800796 and the susceptibility of periodontitis. Functional studies should be performed to validate the effect of disease-associated SNPs and haplotype reported in this study and periodontitis in the future. As CTLA-4 and IL-6 are not exclusively expressed in Th2 cells, the current study is not sufficient to clarify the role of the genetic polymorphisms on Th2 cells of affected individuals on the pathogenesis of periodontitis.

AUTHORSHIP

The authors contributed in the following manner: study conception and design: WKL, Y-QS, YZ; institutional review board submission: LC, K-YZ, WKL, Y-QS, YZ; blood sampling and analysis: LC, YZ; patient recruitment: LC, K-YZ, LJJ, WKL, YZ; acquisition of data: LC, WWT, YHF, YZ,; statistical analysis: WKL, YHF, Y-QS, YZ; interpretation of data: WKL, WWT, YHF, Y-QS, YZ; manuscript preparation:

WKL, WWT, Y-QS, YZ; and critical manuscript revision: WKL, Y-QS, YZ. All authors approved the final version of the manuscript.

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DISCLOSURES

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional information may be found online in the Supporting Information section at the end of the article.

LEGEND

FIGURE 1 Flow diagram showing participants selection process in the candidate genes study.

FIGURE 2 Pairwise linkage disequilibrium (LD) of the four single nucleotide polymorphisms (SNP) associated with periodontitis in the cytotoxic T-lymphocyte-associated antigen 4 (*CTLA4*) gene (rs4553808, rs16840252, rs5742909 and rs3087243). The enlarged region at middle of the figure highlighted the relative location of the four SNPs along chromosome 2 at 2q33. The numbers above the bar are the corresponding chromosome positions. rs4553808, rs16840252, rs5742909 located in a promoter region upstream of, while rs3087243 located downstream of *CTLA4*. Diamonds in the haplotype blocks represent pairwise LD (r^2) between all SNPs assessed; as shown in the figure key, the darker the diamond, the stronger the LD between the SNPs.

FIGURE 3 Odds ratios (OR) and 95 % confidence intervals (CI) of individual studies and pooled data for the association between the T allele of the *CTLA4* rs5742909 and periodontitis. Data were synthesized with fixed effects meta-analysis with OR and 95% CIs being calculated.

FIGURE 4 Flow diagram showing the selection process of the published *IL6* studies included in the meta-analysis. The number of publications (n) in each stage is labeled. Seventeen studies plus results of current study were included in the meta-analysis.

FIGURE 5 Forest plot on eighteen studies for the association between the G allele of the *IL6* rs1800796 and periodontitis in A) all studies; B) subgroup of East Asians; C) subgroup of Caucasians; D) subgroup of Chinese. Data were synthesized with fixed effects meta-analysis with odds ratios (OR) and 95% confidence intervals (CI) being calculated. Pooled effect estimates are indicated by diamonds, with I^2 and significance of the overall effect being given. When the number of studies was more than four or the I^2 was more than 50% in case *P*-value of heterogeneity less than 0.05, odds ratio and *P*-value were calculated using random effect model.

TABLE 1 Demographics and clinical characteristics of study participants

	Periodontitis-free n = 163	Periodontitis n = 141	Test	*P*-value
Age in years	44.2 ± 6.4 (35-72)	45.2 ± 5.6 (35-59)	t	0.16
Gender				
Male	64	50	χ^2	0.38
Female	99	91		
Standing teeth	27.3 ± 2.0 (22-32)	25.1 ± 2.9 (16-28)	t	< 0.05
% Sites with radiographic Bone loss ≥ 50%[a]	0.0 ± 0.0	43.7 ± 14.2	-	-
% Sites PAL ≥ 5mm	0.0 ± 0.0	51.7 ± 28.8	-	-
BOP%	ND	74.4 ± 25.5	-	-

Data presented as mean ± SD (range in parenthesis) or as absolute numbers; ND, not determined.

[a]Panoramic proximal alveolar bone loss measured using Schei ruler.[28]

TABLE 2 Haplotype association results of *CTLA4* (rs4553808, rs16840252, rs5742909)

Haplotype	Periodontitis-free[a] n = 162[b]	Periodontitis[a] n = 140[b]	χ^2 *P*	Adjusted *P*[c]
CTLA4				
-GTT	27 (8.3)	42 (15.0)	0.006	0.007
-ACC	297 (91.7)	238 (85.0)		

Data presented as absolute numbers; percentage in parenthesis

[a]Haplotype count: numbers of alleles with haplotypes.

[b]One case failed genotyping on SNP rs5742909 and one periodontitis-free participant with haplotype ATC were excluded.

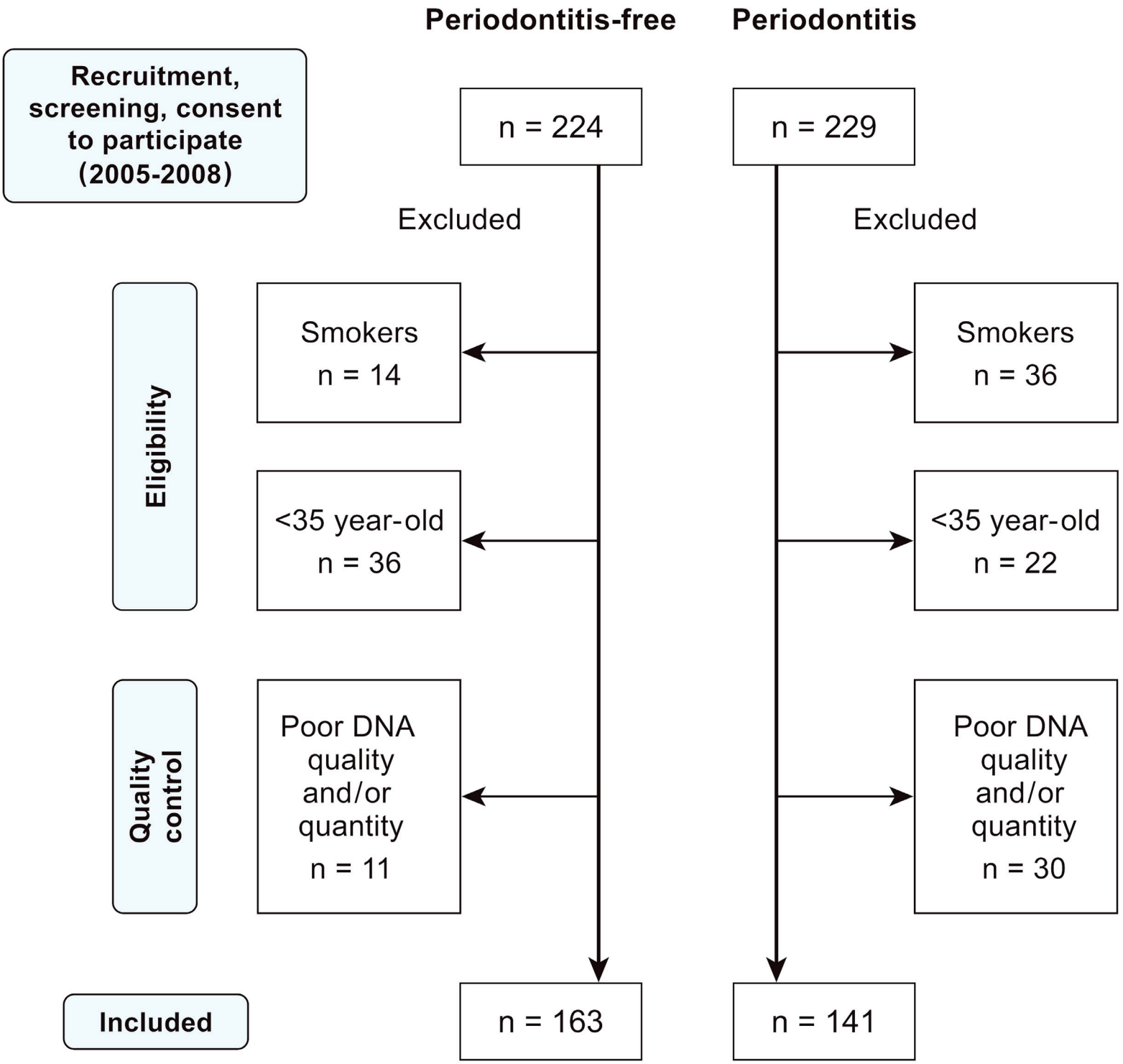
[c]Logistic regression with the adjustment for age and gender. The significant level was set at $P < 0.01$.

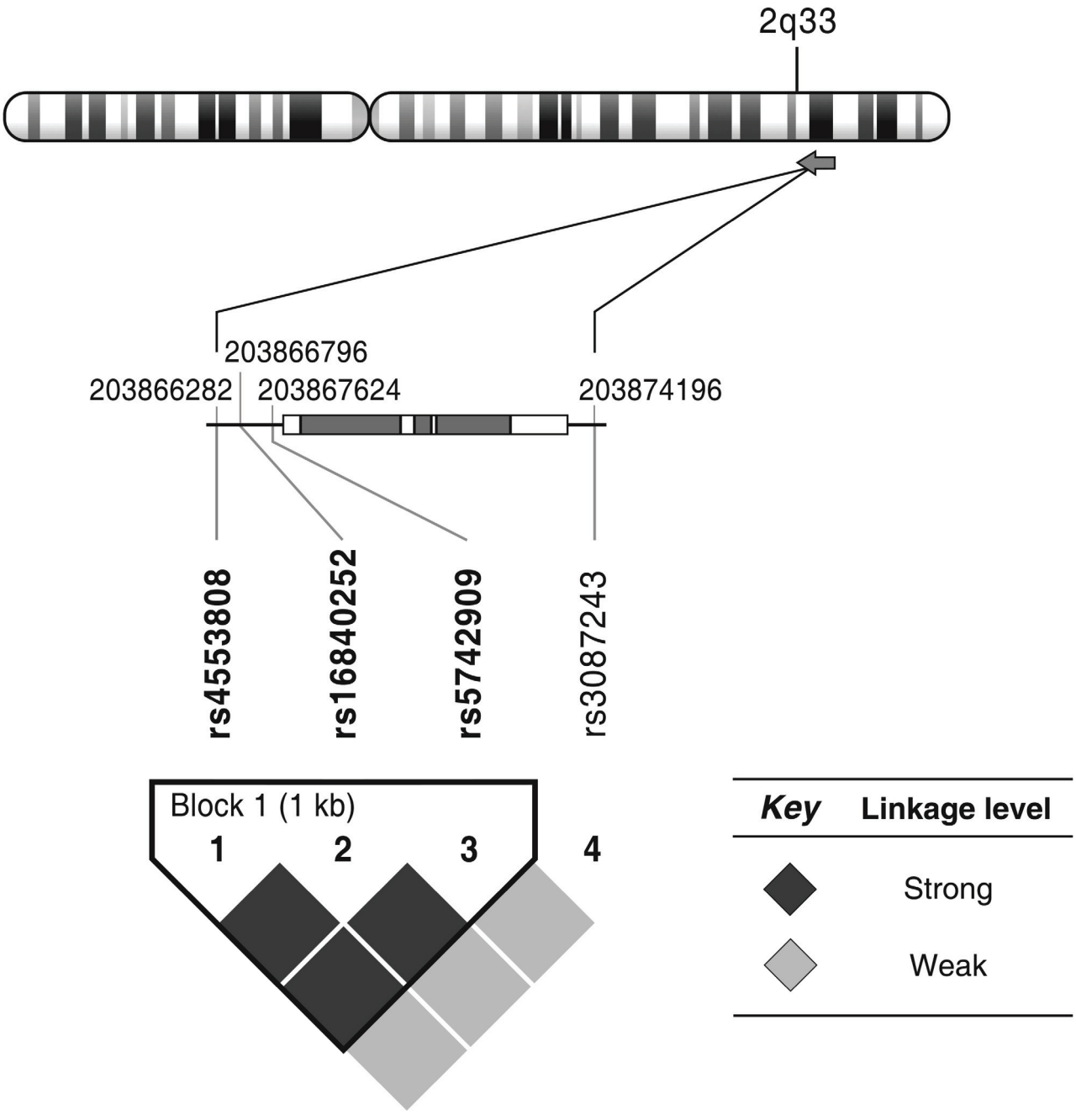
TABLE 3 Meta-analysis of association between *CTLA4* rs5742909, *IL6* rs1800796 and periodontitis[a]

SNPs/Ethnicity	OR	CI (95%)	*P*-value	Study groups	Sample size (n)
***CTLA4* rs5742909**	1.44	1.07-1.94	0.02	3	830
***IL6* rs1800796**					
All	1.30	1.11-1.52	0.0009	18	5202
Asian	1.25	1.08-1.45	0.003	15	3974
Chinese	1.35	1.17-1.56	< 0.0001	11	3365
Caucasian	2.12	1.25-3.58	0.005	2	717

CI: confidence interval; OR: Odds ratio; SNP: single-nucleotide polymorphism

[a]*CTLA4* rs5742909: T *vs.* C allele; C: reference allele; *IL6* rs1800796: G *vs.* C allele; C: reference allele.

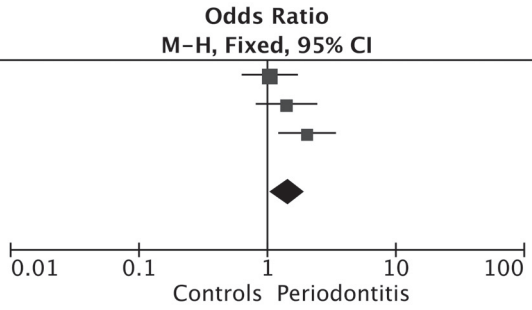

Periodontitis-free
Periodontitis
Recruitment, screening, consent to participate (2005-2008)
n = 224
n = 229
Excluded
Excluded
Eligibility
Smokers n = 14
Smokers n = 36
<35 year-old n = 36
<35 year-old n = 22
Quality control
Poor DNA quality and/or quantity n = 11
Poor DNA quality and/or quantity n = 30
Included
n = 163
n = 141

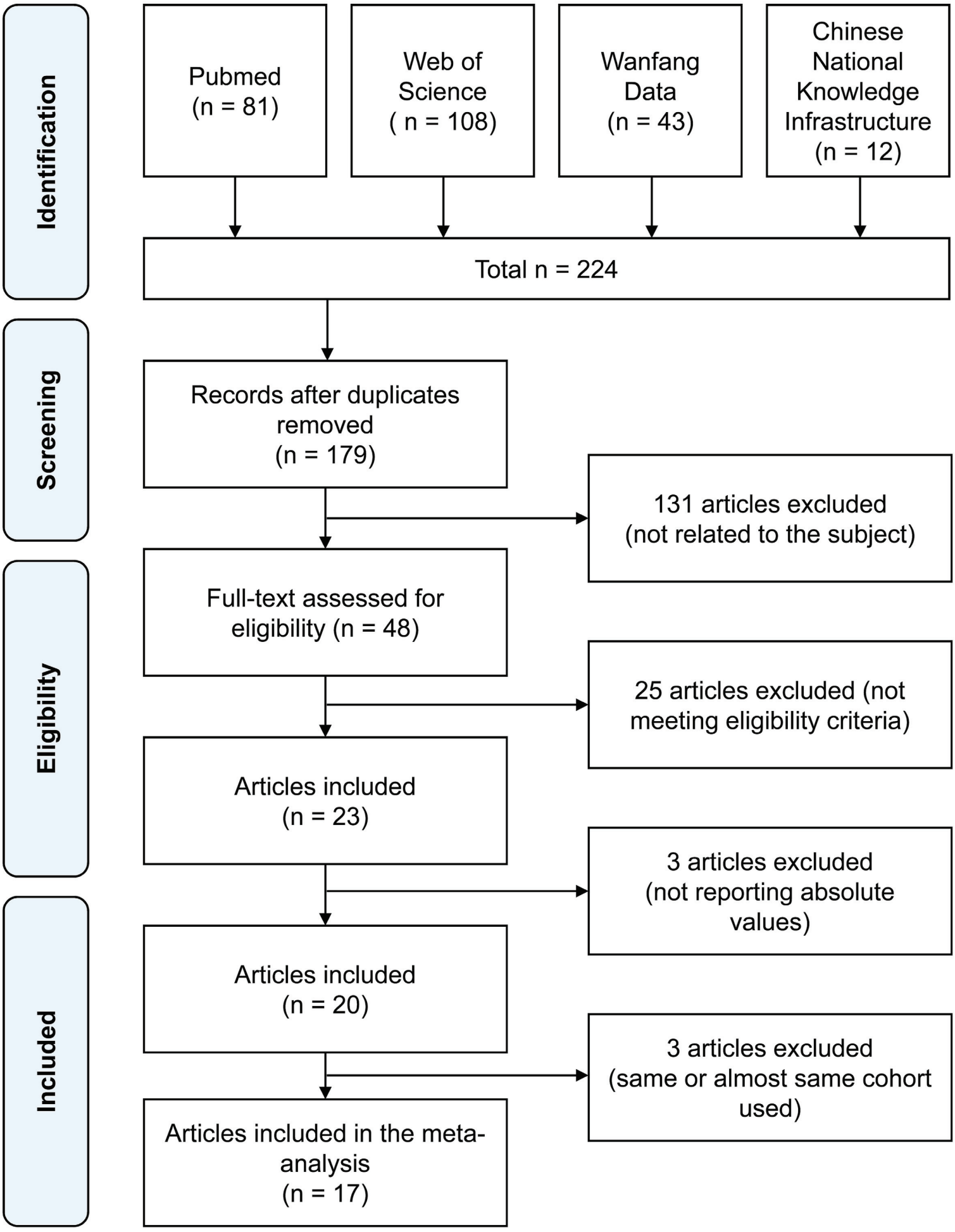

2q33
203866796
203866282
203867624
203874196
rs4553808
rs16840252
rs5742909
rs3087243
Block 1 (1 kb)
1
2
3
4
Key
Linkage level
Strong
Weak

Study	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Houshmand et al.2012	27	252	45	436	41.2%	1.04 [0.63, 1.73]
Song et al.2014	41	206	24	160	30.3%	1.41 [0.81, 2.45]
Current study	42	280	26	326	28.6%	2.04 [1.21, 3.42]
Total (95% CI)		**738**		**922**	**100.0%**	**1.44 [1.07, 1.94]**
Total events	110		95			

Heterogeneity: $Chi^2 = 3.30$, df = 2 (P = 0.19); $I^2 = 39\%$

Test for overall effect: Z = 2.38 (P = 0.02)

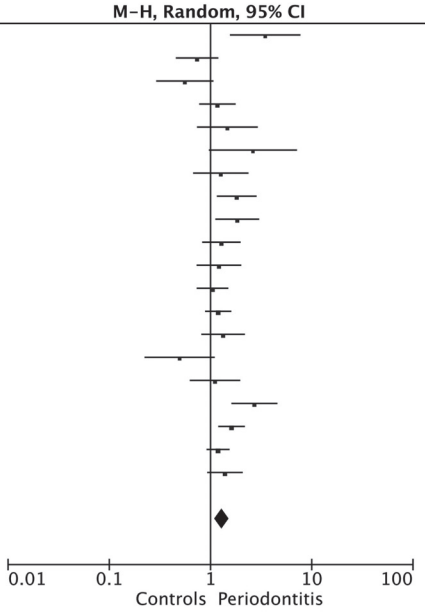



Identification
Screening
Eligibility
Included
Pubmed (n = 81)
Web of Science (n = 108)
Wanfang Data (n = 43)
Chinese National Knowledge Infrastructure (n = 12)
Total n = 224
Records after duplicates removed (n = 179)
131 articles excluded (not related to the subject)
Full-text assessed for eligibility (n = 48)
25 articles excluded (not meeting eligibility criteria)
Articles included (n = 23)
3 articles excluded (not reporting absolute values)
Articles included (n = 20)
3 articles excluded (same or almost same cohort used)
Articles included in the meta-analysis (n = 17)

A

Study or Subgroup	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Holla et al. 2004	287	296	193	214	2.8%	3.47 [1.56, 7.74]
Komatsu et al. 2005	46	224	40	154	5.1%	0.74 [0.45, 1.20]
Bang et al. 2008	51	104	38	60	3.7%	0.56 [0.29, 1.07]
Kobayashi et al. 2009	68	234	56	216	5.9%	1.17 [0.77, 1.77]
Nibali et al. 2009-1	35	636	11	288	3.4%	1.47 [0.73, 2.93]
Nibali et al. 2009-2	23	172	5	90	2.0%	2.62 [0.96, 7.16]
Nibali et al. 2009-3	64	168	19	58	3.8%	1.26 [0.67, 2.37]
Franch-Chillida et al. 2010	47	94	145	408	5.5%	1.81 [1.15, 2.85]
Liu et al. 2010	50	186	32	192	5.0%	1.84 [1.12, 3.03]
Fan et al. 2011	64	356	38	260	5.7%	1.28 [0.83, 1.98]
Huang et al. 2011	54	318	26	180	4.9%	1.21 [0.73, 2.01]
Chen et al. 2012	104	396	65	256	6.6%	1.05 [0.73, 1.50]
Zhang et al. 2014	126	398	121	432	7.4%	1.19 [0.88, 1.60]
Hu et al. 2016	42	240	33	240	5.0%	1.33 [0.81, 2.18]
Salman et al. 2016	134	162	87	96	2.8%	0.50 [0.22, 1.10]
Li C et al. 2017	24	80	53	190	4.3%	1.11 [0.62, 1.97]
Li J et al. 2017	66	166	27	138	4.8%	2.71 [1.61, 4.58]
Shi 2017	124	462	96	518	7.4%	1.61 [1.19, 2.18]
Dong et al. 2018	163	546	150	568	7.9%	1.19 [0.91, 1.54]
Current study	62	282	55	326	6.1%	1.39 [0.93, 2.08]
Total (95% CI)		**5520**		**4884**	**100.0%**	**1.30 [1.11, 1.52]**
Total events	1634		1290			

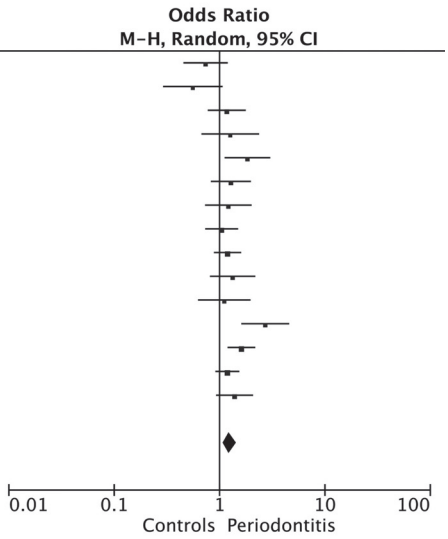
Heterogeneity: $Tau^2 = 0.06$; $Chi^2 = 41.60$, df = 19 (P = 0.002); $I^2 = 54\%$
Test for overall effect: Z = 3.31 (P = 0.0009)



B

Study or Subgroup	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Komatsu et al. 2005	46	224	40	154	5.9%	0.74 [0.45, 1.20]
Bang et al. 2008	51	104	38	60	3.9%	0.56 [0.29, 1.07]
Kobayashi et al. 2009	68	234	56	216	7.1%	1.17 [0.77, 1.77]
Nibali et al. 2009-3	64	168	19	58	4.1%	1.26 [0.67, 2.37]
Liu et al. 2010	50	186	32	192	5.7%	1.84 [1.12, 3.03]
Fan et al. 2011	64	356	38	260	6.7%	1.28 [0.83, 1.98]
Huang et al. 2011	54	318	26	180	5.6%	1.21 [0.73, 2.01]
Chen et al. 2012	104	396	65	256	8.2%	1.05 [0.73, 1.50]
Zhang et al. 2014	126	398	121	432	9.6%	1.19 [0.88, 1.60]
Hu et al. 2016	42	240	33	240	5.7%	1.33 [0.81, 2.18]
Li C et al. 2017	24	80	53	190	4.7%	1.11 [0.62, 1.97]
Li J et al. 2017	66	166	27	138	5.4%	2.71 [1.61, 4.58]
Shi 2017	124	462	96	518	9.5%	1.61 [1.19, 2.18]
Dong et al. 2018	163	546	150	568	10.5%	1.19 [0.91, 1.54]
Current study	62	282	55	326	7.3%	1.39 [0.93, 2.08]
Total (95% CI)		**4160**		**3788**	**100.0%**	**1.25 [1.08, 1.45]**
Total events	1108		849			

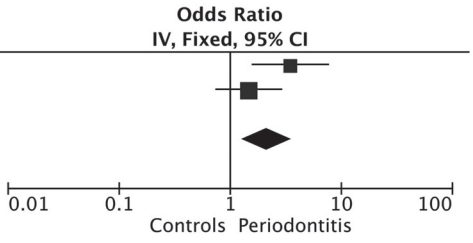
Heterogeneity: $Tau^2 = 0.04$; $Chi^2 = 25.76$, df = 14 (P = 0.03); $I^2 = 46\%$
Test for overall effect: Z = 2.93 (P = 0.003)



C

Study or Subgroup	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio IV, Fixed, 95% CI
Holla et al. 2004	287	296	193	214	42.7%	3.47 [1.56, 7.74]
Nibali et al. 2009-1	35	636	11	288	57.3%	1.47 [0.73, 2.93]
Total (95% CI)		**932**		**502**	**100.0%**	**2.12 [1.25, 3.58]**
Total events	322		204			

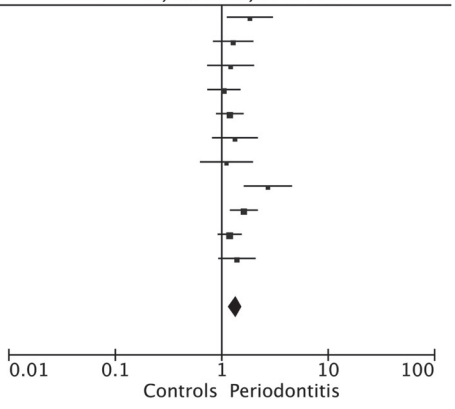
Heterogeneity: $Chi^2 = 2.54$, df = 1 (P = 0.11); $I^2 = 61\%$
Test for overall effect: Z = 2.81 (P = 0.005)



D

Study	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Liu et al. 2010	50	186	32	192	6.4%	1.84 [1.12, 3.03]
Fan et al. 2011	64	356	38	260	7.9%	1.28 [0.83, 1.98]
Huang et al. 2011	54	318	26	180	6.3%	1.21 [0.73, 2.01]
Chen et al. 2012	104	396	65	256	10.6%	1.05 [0.73, 1.50]
Zhang et al. 2014	126	398	121	432	13.5%	1.19 [0.88, 1.60]
Hu et al. 2016	42	240	33	240	6.5%	1.33 [0.81, 2.18]
Li C et al. 2017	24	80	53	190	5.1%	1.11 [0.62, 1.97]
Li J et al. 2017	66	166	27	138	6.0%	2.71 [1.61, 4.58]
Shi 2017	124	462	96	518	13.2%	1.61 [1.19, 2.18]
Dong et al. 2018	163	546	150	568	15.6%	1.19 [0.91, 1.54]
Current study	62	282	55	326	8.9%	1.39 [0.93, 2.08]
Total (95% CI)		**3430**		**3300**	**100.0%**	**1.35 [1.17, 1.56]**
Total events	879		696			

Heterogeneity: $Tau^2 = 0.02$; $Chi^2 = 13.88$, df = 10 (P = 0.18); $I^2 = 28\%$
Test for overall effect: Z = 4.20 (P < 0.0001)



SUPPLEMENTARY TABLE 1 Candidate single-nucleotide polymorphisms investigated

SNP ID	Type	Variation	Call Rate	MAF	HWE *P*-value[a]
IL4[b]					
Regulatory					
rs2243248	upstream	G/T	1.00	< 0.1	
rs17772853	upstream	T/C	1.00	< 0.1	
rs2070874	upstream	C/T	1.00	0.20	0.35
rs2243291	downstream	G/C	0.99	0.19	0.36
Other[c]					
rs734244	intronic	G/A	1.00	0.20	0.35
rs2227284	intronic	C/A	1.00	0.12	0.19
rs2227282	intronic	C/G	0.97	0.12	0.17
rs2243283	intronic	G/C	1.00	0.30	0.26
rs2243290	intronic	C/A	1.00	0.18	0.35
IL5					
Coding					
rs2069818	synonymous	0/C	0.99	one genotype	
Regulatory					
rs2069812	upstream	C/T	1.00	0.28	0.24
IL6[b]					
Coding					
rs2069830	nonsynonymous	0/C	1.00	one genotype	
rs2069860	nonsynonymous	0/A	1.00	one genotype	
rs2069849	synonymous	0/C	1.00	one genotype	
Regulatory[d]					
rs2069852	downstream	G/A	0.97	0.28	1.00
Other					
rs2066992	intronic	G/T	0.99	0.19	0.26
rs2069837	intronic	G/A	0.97	< 0.1	
rs2069840	intronic	G/C	1.00	< 0.1	
rs1800796	ncRNA exonic	G/C	1.00	0.19	0.57
rs2069827	ncRNA exonic	0/G	1.00	one genotype	
rs1800795	ncRNA intronic	C/G	1.00	< 0.1	
IL9[b]					
Regulatory					
rs2069868	downstream	T/C	1.00	0.13	0.72
Other					
rs31564	intronic	0/A	< 0.80	one genotype	
IL10					
Regulatory					
rs1800871	upstream	C/T	1.00	0.31	1.00
rs1800872	upstream	C/A	1.00	0.31	1.00
rs3024496	downstream	C/T	1.00	< 0.1	
rs3024497	downstream	0/A	1.00	one genotype	

rs3024498	downstream	G/A	0.99	< 0.1	
rs1800896	downstream	G/A	1.00	< 0.1	
Other					
rs1518111	intronic	G/A	1.00	0.31	1.00
rs1554286	intronic	T/C	< 0.80	0.40	
rs3021094	intronic	A/C	1.00	0.46	1.00
rs3790622	intronic	T/C	1.00	< 0.1	
IL13					
Coding					
rs20541	nonsynonymous	T/C	0.92	0.35	0.48
Regulatory					
rs1800925	upstream	T/C	0.99	0.16	0.77
rs1295685	downstream	T/C	0.99	0.33	0.28
rs2069750	downstream	C/G	< 0.80	< 0.1	
rs847	downstream	A/G	1.00	0.33	0.28
rs848	downstream	T/G	0.85	0.30	0.20
Other					
rs2069744	intronic	T/C	1.00	< 0.1	
rs1295686	intronic	A/G	1.00	0.34	0.60
rs1295687	intronic	G/C	1.00	0.10	0.67
CTLA4					
Coding					
rs231775	synonymous	A/G	0.86	0.26	<0.01
Regulatory					
rs16840252	upstream	T/C	1.00	0.11	0.61
rs11571315	upstream	G/A	1.00	0.33	0.22
rs11571316	upstream	T/C	< 0.80	< 0.1	
rs4553808	upstream	G/A	1.00	0.11	0.60
rs5742909	upstream	T/C	1.00	0.11	0.60
rs733618	upstream	G/A	1.00	0.40	0.20
rs231725	downstream	G/A	1.00	0.40	0.33
rs3087243	downstream	A/G	1.00	0.22	0.29
Other					
rs231779	intronic	C/T	1.00	0.33	0.22
CD28					
Regulatory					
rs1879877	upstream	C/A	1.00	0.39	0.62
rs3181094	upstream	G/T	1.00	0.39	0.51
rs3181096	upstream	T/C	1.00	0.25	0.84
rs3181097	upstream	A/G	1.00	0.48	0.75
rs3181098	upstream	A/G	0.99	0.25	0.84
rs3181113	downstream	G/T	1.00	0.49	0.64
Other					
rs3181100	intronic	G/C	0.95	0.12	1.00
rs3769686	intronic	G/A	1.00	< 0.1	
rs3769687	intronic	C/T	0.84	< 0.1	

rs4673259	intronic	T/C	< 0.80	0.37	
rs4675360	intronic	T/A	1.00	0.50	0.76
rs4675363	intronic	C/T	1.00	< 0.1	
rs10932017	intronic	T/C	0.99	0.32	0.48
rs1181388	intronic	C/T	1.00	0.46	0.28
rs2140148	intronic	G/T	1.00	0.13	0.72
rs3116486	intronic	C/T	1.00	< 0.1	
rs3116494	intronic	G/A	1.00	< 0.1	
rs3116496	intronic	C/T	1.00	0.11	1.00
rs12693993	intronic	A/G	1.00	0.13	0.31
***IL4R*[b]**					
Coding					
rs1801275	nonsynonymous	G/A	1.00	0.19	1.00
rs1805010	synonymous	A/G	< 0.80	0.49	
rs1805011	nonsynonymous	C/A	1.00	< 0.1	
rs1805013	nonsynonymous	0/C	1.00	one genotype	
rs1805015	nonsynonymous	C/T	1.00	< 0.1	
rs1805016	nonsynonymous	G/T	0.98	0.49	< 0.01
rs2234897	synonymous	0/T	1.00	one genotype	
rs2234898	synonymous	T/G	1.00	< 0.1	
rs3024677	nonsynonymous	0/G	1.00	one genotype	
rs3024679	synonymous	0/T	1.00	one genotype	
Regulatory					
rs3024685	downstream	C/T	1.00	0.49	1.00
rs12102586	downstream	T/C	0.97	< 0.1	
rs4787956	downstream	A/G	1.00	0.49	0.64
rs3024682	downstream	G/A	0.97	< 0.1	
rs8832	downstream	A/G	0.99	0.49	0.88
rs1049631	downstream	A/G	1.00	0.50	1.00
Other					
rs3024638	Non-coding transcript exon	0/C	1.00	one genotype	
rs12925861	intronic	A/T	1.00	0.49	0.09
rs2301807	intronic	T/G	1.00	< 0.1	
rs3024530	intronic	G/A	< 0.80	0.47	
rs3024570	intronic	0/G	1.00	one genotype	
rs3024585	intronic	G/A	1.00	0.37	0.14
rs3024608	intronic	G/C	1.00	< 0.1	
rs3024613	intronic	T/C	1.00	0.43	1.00
rs3024614	intronic	G/A	1.00	< 0.1	
rs3024619	intronic	A/G	< 0.80	0.40	
rs3024622	intronic	G/C	1.00	0.47	0.53
rs3024633	intronic	0/A	1.00	one genotype	
rs3024658	intronic	A/G	< 0.80	0.18	
rs3024660	intronic	C/T	1.00	< 0.1	
rs3024668	intronic	A/G	1.00	< 0.1	

rs3024691	intronic	0/G	1.00	one genotype	
rs4787423	intronic	C/T	0.99	< 0.1	
rs6498012	intronic	G/C	< 0.80	0.21	
GATA3					
Coding					
rs575091	synonymous	0/G	0.97	one genotype	
Regulatory					
rs1058240	downstream	G/A	1.00	< 0.1	
rs11255509	downstream	0/C	1.00	one genotype	
rs2229360	downstream	T/C	0.98	0.27	0.33
rs263419	downstream	T/A	0.99	0.26	0.44
rs263418	downstream	G/A	0.99	0.27	0.44
Other					
rs10905284	intronic	C/A	1.00	0.43	0.27
rs12572421	intronic	T/G	1.00	0.10	0.22
rs2280015	intronic	A/G	0.99	0.27	0.34
rs3802604	intronic	C/T	1.00	0.32	0.73
rs3824662	intronic	T/G	1.00	0.27	0.85
rs406103	intronic	T/C	0.99	0.27	0.34
rs422628	intronic	C/T	1.00	< 0.1	
rs570613	intronic	G/A	1.00	0.27	0.69
rs2275806	ncRNA exonic	G/A	< 0.80	0.10	
STAT6					
Regulatory					
rs1059513	downstream	G/A	0.91	0.10	0.13
rs324015	downstream	A/G	0.95	0.49	0.01
rs703817	downstream	A/G	1.00	0.23	0.30
Other					
rs841718	intronic	T/C	1.00	0.32	0.73
rs10783813	intronic	G/T	1.00	0.40	0.25
rs3024971	intronic	C/A	0.99	< 0.1	
rs3024974	intronic	T/C	0.99	0.19	0.18
rs324011	intronic	T/C	1.00	0.22	1.00
GLT6D1					
Other					
rs1537415	intronic	G/C	1.00	0.26	< 0.01

HWE: Hardy-Weinberg equilibrium; MAF: minor allele frequency; SNP: single-nucleotide polymorphism.

[a]HWE *P*-value for control group; the *P*-value was not calculated if SNPs with i) call rate < 80%, ii) MAF < 0.1 or iii) only one genotype detected.

[b]Five SNPs were rejected by assay design and not included: rs2243250 in *IL4*, rs3024535 and rs2074570 in *IL4R* were with high dimer potential for reverse extend primer; rs1524107 in *IL6* for hybridization Tm minimum not satisfied for any forward extend primer within length bounds; rs2069828 in *IL9* for proximal SNP blocks reverse extend primer design.

[c]Other: Tag SNPs, which were non-coding SNPs and not located in regulatory region.

[d]rs13306436 in the downstream of *IL6* has no call on one of two alleles in any individual and was excluded.

SUPPLEMENTARY TABLE 2 Allelotype and genotype of the included candidate single-nucleotide polymorphisms after quality control[a]

				Periodontitis-free						Periodontitis					
				Allelotype		Genotype				Allelotype		Genotype			
SNPs	Position	A1	A2	1 (%)	2 (%)	11 (%)	12 (%)	22 (%)	n	1 (%)	2 (%)	11 (%)	12 (%)	22 (%)	n
IL4															
rs2070874	5:132037609	C	T	71 (21.8)	255 (78.2)	10 (6.1)	51 (31.3)	102 (62.6)	163	48 (17.0)	234 (83.0)	6 (4.3)	36 (25.5)	99 (70.2)	141
rs734244	5:132038625	G	A	71 (21.8)	255 (78.2)	5 (3.1)	33 (20.8)	121 (76.1)	159	26 (9.3)	254 (90.7)	3 (2.1)	20 (14.3)	117 (83.6)	140
rs2227284	5:132040624	C	A	45 (13.8)	281 (86.2)	5 (3.1)	35 (21.5)	123 (75.4)	163	28 (9.9)	254 (90.1)	3 (2.1)	22 (15.6)	116 (82.3)	141
rs2227282	5:132041078	C	G	43 (13.5)	275 (86.5)	11 (6.7)	74 (45.4)	78 (47.9)	163	88 (31.2)	194 (68.8)	14 (9.9)	60 (42.6)	67 (47.5)	141
rs2243283	5:132044492	G	C	96 (29.4)	230 (70.6)	10 (6.1)	51 (31.3)	102 (62.6)	163	40 (14.2)	242 (85.8)	4 (2.8)	32 (22.7)	105 (74.5)	141
rs2243290	5:132046068	C	A	71 (21.8)	255 (78.2)	10 (6.2)	51 (31.7)	100 (62.1)	161	40 (14.4)	238 (85.6)	4 (2.9)	32 (23.0)	103 (74.1)	139
rs2243291	5:132046882	G	C	71 (22.0)	251 (78.0)	10 (6.1)	51 (31.3)	102 (62.6)	163	47 (16.7)	235 (83.3)	6 (4.3)	35 (24.8)	100 (70.9)	141
IL5															
rs2069812	5:131907815	C	T	91 (27.9)	235 (72.1)	9 (5.5)	73 (44.8)	81 (49.7)	163	80 (28.4)	202 (71.6)	7 (5.0)	66 (46.8)	68 (48.2)	141
IL6															
rs1800796	7:22732771	G	C	55 (16.9)	271 (83.1)	3 (1.8)	49 (30.1)	111 (68.1)	163	62 (22.0)	220 (78.0)	7 (5.0)	48 (34.0)	86 (61.0)	141
rs2066992	7:22734774	G	T	53 (16.4)	271 (83.6)	2 (1.2)	49 (30.2)	111 (68.6)	162	59 (21.2)	219 (78.8)	6 (4.3)	47 (33.8)	86 (61.9)	139
rs2069852	7:22738785	G	A	78 (24.5)	240 (75.5)	9 (5.7)	60 (37.7)	90 (56.6)	159	85 (31.5)	185 (68.5)	13 (9.6)	59 (43.7)	63 (46.7)	135
IL9															
rs2069868	5:135260865	T	C	41 (12.6)	285 (87.4)	3 (1.8)	35 (21.5)	125 (76.7)	163	36 (12.8)	246 (87.2)	2 (1.4)	32 (22.7)	107 (75.9)	141
IL10															
rs1518111	1:205011268	G	A	101 (31.0)	225 (69.0)	16 (9.8)	69 (42.3)	78 (47.9)	163	86 (30.5)	196 (69.5)	17 (12.0)	52 (36.9)	72 (51.1)	141
rs1800872	1:205013030	C	A	101 (31.0)	225 (69.0)	16 (9.8)	69 (42.3)	78 (47.9)	163	86 (30.5)	196 (69.5)	17 (12.0)	52 (36.9)	72 (51.1)	141
rs1800871	1:205013257	C	T	101 (31.0)	225 (69.0)	16 (9.8)	69 (42.3)	78 (47.9)	163	86 (30.5)	196 (69.5)	17 (12.0)	52 (36.9)	72 (51.1)	141
rs3021094	1:205011575	A	C	146 (44.8)	180 (55.2)	33 (20.2)	80 (49.1)	50 (30.7)	163	134 (47.5)	148 (52.5)	38 (27.0)	58 (41.1)	45 (31.9)	141
IL13															
rs1800925	5:132020708	T	C	53 (16.4)	271 (83.6)	13 (7.9)	78 (47.9)	72 (44.2)	163	93 (33.2)	187 (66.8)	9 (6.4)	75 (53.6)	56 (40.0)	140

rs1295687	5:132022361	G	C	33 (10.1)	293 (89.9)	16 (9.8)	76 (46.6)	71 (43.6)	163	93 (33.0)	189 (67.0)	9 (6.4)	75 (53.2)	57 (40.4)	141
rs1295686	5:132023742	A	G	108 (33.1)	218 (66.9)	2 (1.2)	29 (17.8)	132 (81.0)	163	28 (9.9)	254 (90.1)	0 (0.0)	28 (19.9)	113 (80.1)	141
rs20541	5:132023863	T	C	105 (34.8)	197 (65.2)	5 (3.1)	43 (26.5)	114 (70.4)	162	41 (14.7)	237 (85.3)	2 (1.4)	37 (26.6)	100 (72.0)	139
rs1295685	5:132024344	T	C	104 (31.9)	222 (68.1)	16 (10.6)	73 (48.3)	62 (41.1)	151	90 (34.6)	170 (65.4)	9 (6.9)	72 (55.4)	49 (37.7)	130
rs848	5:132024399	T	G	73 (27.7)	191 (72.3)	13 (7.9)	78 (47.9)	72 (44.2)	163	93 (33.0)	189 (67.0)	9 (6.4)	75 (53.2)	57 (40.4)	141
rs847	5:132024568	A	G	104 (31.9)	222 (68.1)	13 (9.8)	47 (35.6)	72 (54.6)	132	78 (31.0)	174 (69.0)	9 (7.1)	60 (47.6)	57 (45.3)	126
STAT6															
rs1059513	12:55775976	G	A	36 (11.9)	266 (88.1)	0 (0.0)	36 (23.8)	115 (76.2)	151	22 (8.7)	232 (91.3)	1 (0.8)	20 (15.7)	106 (83.5)	127
rs703817	12:55776095	A	G	80 (24.5)	246 (75.5)	27 (16.6)	70 (42.9)	66 (40.5)	163	121 (42.9)	161 (57.1)	29 (20.6)	63 (44.7)	49 (34.7)	141
rs324015	12:55776367	A	G	144 (47.7)	158 (52.3)	8 (5.0)	41 (25.5)	112 (69.5)	161	57 (20.4)	223 (79.6)	3 (2.1)	51 (36.4)	86 (61.5)	140
rs3024974	12:55779012	T	C	57 (17.7)	265 (82.3)	7 (4.3)	54 (33.1)	102 (62.6)	163	66 (23.4)	216 (76.6)	3 (2.1)	60 (42.6)	78 (55.3)	141
rs841718	12:55779263	T	C	110 (33.7)	216 (66.3)	42 (27.8)	60 (39.7)	49 (32.5)	151	135 (49.6)	137 (50.4)	35 (25.7)	65 (47.8)	36 (26.5)	136
rs10783813	12:55780627	G	T	124 (38.0)	202 (6.02)	7 (4.3)	66 (40.5)	90 (55.2)	163	62 (22.0)	220 (78.0)	2 (1.4)	58 (41.1)	81 (57.5)	141
rs324011	12:55788449	T	C	68 (20.9)	258 (79.1)	17 (10.4)	76 (46.6)	70 (43.0)	163	83 (29.4)	199 (70.6)	8 (5.7)	67 (47.5)	66 (46.8)	141
CD28															
rs3181094	2:204277962	G	T	129 (39.6)	197 (60.4)	15 (9.3)	77 (47.5)	70 (43.2)	162	87 (30.9)	195 (69.1)	13 (9.2)	61 (43.3)	67 (47.5)	141
rs1879877	2:204278245	C	A	128 (39.3)	198 (60.7)	32 (19.6)	89 (54.6)	42 (25.8)	163	123 (43.6)	159 (56.4)	23 (16.3)	77 (54.6)	41 (29.1)	141
rs3181096	2:204278337	T	C	83 (25.5)	243 (74.5)	1 (0.6)	41 (25.2)	121 (74.2)	163	37 (13.1)	245 (86.9)	3 (2.1)	31 (22.0)	107 (75.9)	141
rs3181097	2:204278384	A	G	155 (47.5)	171 (52.5)	23 (14.1)	82 (50.3)	58 (35.6)	163	106 (37.6)	176 (62.4)	17 (12.0)	72 (51.1)	52 (36.9)	141
rs3181098	2:204278623	A	G	83 (25.5)	243 (74.5)	3 (1.8)	35 (21.5)	125 (76.7)	163	37 (13.1)	245 (86.9)	3 (2.1)	31 (22.0)	107 (75.9)	141
rs3181100	2:204280251	G	C	36 (11.9)	266 (88.1)	1 (0.6)	31 (19.0)	131 (80.4)	163	37 (13.1)	245 (86.9)	1 (0.7)	35 (24.8)	105 (74.5)	141
rs2140148	2:204280385	G	T	41 (12.6)	285 (87.4)	23 (14.1)	83 (50.9)	57 (35.0)	163	106 (37.6)	176 (62.4)	17 (12.0)	72 (51.1)	52 (36.9)	141
rs1181388	2:204284196	C	T	153 (46.9)	173 (53.1)	11 (6.7)	61 (37.4)	91 (55.9)	163	67 (23.8)	215 (76.2)	8 (5.6)	51 (36.2)	82 (58.2)	141
rs10932017	2:204286150	T	C	107 (33.0)	217 (67.0)	35 (21.5)	85 (52.1)	43 (26.4)	163	136 (48.6)	144 (51.4)	31 (22.1)	74 (52.9)	35 (25.0)	140
rs4675360	2:204292701	A	T	167 (51.2)	159 (48.8)	11 (6.7)	61 (37.4)	91 (55.9)	163	67 (23.8)	215 (76.2)	8 (5.6)	51 (36.2)	82 (58.2)	141
rs3116496	2:204302757	C	T	33 (10.1)	293 (89.9)	2 (1.3)	32 (21.2)	117 (77.5)	151	35 (12.9)	237 (87.1)	3 (2.2)	29 (21.3)	104 (76.5)	136
rs12693993	2:204303842	A	G	43 (13.2)	283 (86.8)	36 (22.0)	86 (52.8)	41 (25.2)	163	138 (48.9)	144 (51.1)	28 (19.8)	82 (58.2)	31 (22.0)	141

rs3181113	2:204310155	G	T	158 (48.5)	168 (51.5)	41 (25.2)	85 (52.1)	37 (22.7)	163	136 (48.2)	146 (51.8)	29 (20.6)	78 (55.3)	34 (24.1)	141
CTLA4															
rs231775	2:204405682	A	G	78 (27.1)	210 (72.9)	0 (0.0)	26 (16.0)	137 (84.0)	163	43 (15.2)	239 (84.8)	3 (2.1)	37 (26.2)	101 (71.7)	141
rs11571315	2:204439146	G	A	107 (32.8)	219 (67.2)	0 (0.0)	27 (16.6)	136 (83.4)	163	43 (15.2)	239 (84.8)	3 (2.1)	37 (26.2)	101 (71.7)	141
rs733618	2:204439189	G	A	132 (40.5)	194 (59.5)	0 (0.0)	26 (16.0)	137 (84.0)	163	42 (15.0)	238 (85.0)	3 (2.1)	36 (25.7)	101 (72.2)	140
rs4553808	2:204439250	G	A	26 (8.0)	300 (92.0)	14 (8.6)	79 (48.5)	70 (42.9)	163	93 (33.0)	189 (67.0)	13 (9.2)	67 (47.5)	61 (43.3)	141
rs16840252	2:204439764	T	C	27 (8.3)	299 (91.7)	23 (14.1)	85 (52.1)	55 (33.8)	163	108 (38.3)	174 (61.7)	19 (13.5)	70 (49.6)	52 (36.9)	141
rs5742909	2:204440592	T	C	26 (8.0)	300 (92.0)	4 (2.8)	70 (48.6)	70 (48.6)	144	59 (24.8)	179 (75.2)	2 (1.7)	55 (46.2)	62 (52.0)	119
rs231779	2:204442732	C	T	107 (32.8)	219 (67.2)	14 (8.6)	79 (48.5)	70 (42.9)	163	93 (33.0)	189 (67.0)	13 (9.2)	67 (47.5)	61 (43.3)	141
rs3087243	2:204447164	A	G	81 (24.8)	245 (75.2)	31 (19.0)	70 (42.9)	62 (38.1)	163	110 (39.0)	172 (61.0)	18 (12.7)	74 (52.5)	49 (34.8)	141
rs231725	2:204448920	G	A	131 (40.2)	195 (59.8)	7 (4.3)	67 (41.1)	89 (54.6)	163	48 (17.0)	234 (83.0)	5 (3.5)	38 (27.0)	98 (69.5)	141
IL4R															
rs12925861	16:27250097	A	T	156 (47.9)	170 (52.1)	41 (25.2)	81 (49.6)	41 (25.2)	163	140 (50.0)	140 (50.0)	37 (26.4)	66 (47.1)	37 (26.5)	140
rs3024585	16:27267345	G	A	125 (38.3)	201 (61.7)	43 (26.4)	70 (42.9)	50 (30.7)	163	141 (50.0)	141 (50.0)	40 (28.4)	61 (43.3)	40 (28.3)	141
rs3024613	16:27271754	T	C	149 (45.7)	177 (54.3)	5 (3.1)	49 (30.1)	109 (66.8)	163	57 (20.2)	225 (79.8)	9 (6.4)	39 (27.7)	93 (65.9)	141
rs3024622	16:27272954	G	C	146 (44.8)	180 (55.2)	29 (17.8)	67 (41.1)	67 (41.1)	163	102 (36.2)	180 (63.8)	19 (13.5)	64 (45.4)	58 (41.1)	141
rs1801275	16:27281901	G	A	59 (18.1)	267 (81.9)	34 (20.9)	81 (49.7)	48 (29.4)	163	111 (39.4)	171 (60.6)	17 (12.1)	77 (54.6)	47 (33.3)	141
rs1049631	16:27283043	G	A	163 (50.0)	163 (50.0)	35 (21.5)	76 (46.6)	52 (31.9)	163	142 (50.4)	140 (49.6)	31 (22.0)	80 (56.7)	30 (21.3)	141
rs8832	16:27283288	A	G	158 (48.5)	168 (51.5)	38 (23.3)	81 (49.7)	44 (27.0)	163	137 (48.6)	145 (51.4)	37 (26.2)	63 (44.7)	41 (29.1)	141
rs3024685	16:27284411	C	T	157 (48.2)	169 (51.8)	42 (25.8)	78 (47.8)	43 (26.4)	163	137 (48.6)	145 (51.4)	35 (24.8)	67 (47.5)	39 (27.7)	141
rs4787956	16:27285750	A	G	162 (49.7)	164 (50.3)	39 (23.9)	80 (49.1)	44 (27.0)	163	135 (48.2)	145 (51.8)	36 (25.7)	63 (45.0)	41 (29.3)	140
GATA3															
rs3802604	10:8142278	C	T	107 (32.8)	219 (67.2)	31 (19.0)	88 (54.0)	44 (27.0)	163	112 (39.7)	170 (60.3)	21 (14.9)	70 (49.6)	50 (35.5)	141
rs3824662	10:8144214	T	G	92 (28.2)	234 (71.8)	0 (0.0)	35 (21.5)	128 (78.5)	163	27 (9.6)	255 (90.4)	1 (0.7)	25 (17.7)	115 (81.6)	141
rs12572421	10:8144573	T	G	35 (10.7)	291 (89.3)	10 (6.1)	71 (43.6)	82 (50.3)	163	71 (26.3)	199 (73.7)	5 (3.7)	61 (45.2)	69 (51.1)	135
rs570613	10:8146508	G	A	86 (26.4)	240 (73.6)	10 (6.2)	70 (43.2)	82 (50.6)	162	73 (25.9)	209 (74.1)	5 (3.5)	63 (44.7)	73 (51.8)	141
rs2280015	10:8150944	A	G	90 (27.8)	234 (72.2)	10 (6.1)	69 (42.3)	84 (51.6)	163	70 (25.2)	208 (74.8)	5 (3.6)	60 (43.2)	74 (53.2)	139

rs406103	10:8151627	T	C	90 (28.0)	232 (72.0)	10 (6.1)	69 (42.3)	84 (51.6)	163	69 (24.6)	211 (75.4)	5 (3.6)	59 (42.1)	76 (54.3)	140
rs10905284	10:8155368	C	A	150 (46.0)	176 (54.0)	18 (11.0)	71 (43.6)	74 (45.4)	163	86 (30.5)	196 (69.5)	14 (9.9)	58 (41.1)	69 (49.0)	141
rs2229360	10:8156085	T	C	91 (27.9)	235 (72.1)	12 (7.4)	68 (41.7)	83 (50.9)	163	73 (25.9)	209 (74.1)	10 (7.1)	53 (37.6)	78 (55.3)	141
rs263419	10:8158099	T	A	89 (27.3)	237 (72.7)	10 (6.2)	70 (43.5)	81 (50.3)	161	71 (25.5)	207 (74.5)	5 (3.6)	61 (43.9)	73 (52.5)	139
rs263418	10:8158197	G	A	89 (27.3)	237 (72.7)	10 (6.1)	66 (40.5)	87 (53.4)	163	74 (26.2)	208 (73.8)	8 (5.7)	58 (41.1)	75 (53.2)	141
GLT6D1															
rs1537415	9:137669543	G	C	83 (25.5)	243 (75.5)	18 (11.0)	47 (28.8)	98 (60.2)	163	75 (26.6)	207 (73.4)	11 (7.8)	53 (37.6)	77 (54.6)	141

A1: minor allele; A2: major allele; n: number of cases/controls; SNP: single-nucleotide polymorphism.
[a]Included rs231775 and rs1537415; two SNPs reported to be associated with periodontitis.[10,32,33]

SUPPLEMENTARY TABLE 3 Association statistics for the 72 candidate single-nucleotide polymorphisms listed under Supplementary Table 2

				Heredity Mode[a]							
SNPs	**A1**	***P* (allelic)[a]**	***P* (geno)[a]**	***P* (co-dom)**	***P* (dom)**	***p* (rec)**	**adjust *P* (allelic)[b]**	**adjust OR (allelic, 95% CI)[b]**	**adjust *P* (geno)[b]**	**adjust OR (het) (95% CI)[b]**	**adjust OR (hom, 95% CI)[b]**
IL4											
rs2070874	C	0.1520	0.3681	0.1580	0.1820	0.6084	0.1649	0.76 (0.51-1.12)	0.3768	0.74 (0.44-1.24)	0.60 (0.21-1.72)
rs734244	G	0.1234	0.2955	0.1289	0.1442	0.6084	0.1319	0.74 (0.50-1.1)	0.3107	0.71 (0.42-1.19)	0.59 (0.21-1.70)
rs2227284	C	0.1688	0.3449	0.1646	0.1625	0.7288	0.1988	0.73 (0.45-1.18)	0.4014	0.68 (0.38-1.23)	0.67 (0.16-2.90)
rs2227282	C	0.1238	0.3021	0.1283	0.1161	0.7274	0.1481	0.70 (0.42-1.14)	0.3046	0.63 (0.34-1.17)	0.65 (0.15-2.81)
rs2243283	G	0.6587	0.6030	0.6303	1.0000	0.4031	0.4994	1.13 (0.79-1.63)	0.6113	1.01 (0.63-1.63)	1.55 (0.64-3.79)
rs2243290	C	0.0158	0.0667	0.0203	0.0357	0.2722	*0.0231*	0.62 (0.41-0.94)	0.0746	0.61 (0.36-1.03)	0.39 (0.12-1.30)
rs2243291	G	0.0201	0.0661	0.0205	0.0351	0.2720	*0.0235*	0.62 (0.40-0.94)	0.0760	0.61 (0.36-1.03)	0.39 (0.12-1.30)
IL5											
rs2069812	C	0.9281	0.9269	0.8939	0.8188	1.0000	0.9194	1.02 (0.69-1.51)	0.7537	1.13 (0.70-1.81)	0.78 (0.26-2.33)
IL6											
rs1800796	G	0.1221	0.1987	0.1059	0.2287	0.1965	*0.0922*	1.44 (0.94-2.19)	0.2102	1.31 (0.80-2.16)	2.95 (0.73-11.86)
rs2066992	G	0.1416	0.1884	0.1161	0.2739	0.1500	*0.0980*	1.44 (0.93-2.23)	0.1892	1.28 (0.78-2.11)	3.94 (0.77-20.20)
rs2069852	G	0.0649	0.1603	0.0592	0.1014	0.2661	*0.0312*	1.52 (1.04-2.21)	0.0972	1.54 (0.94-2.54)	2.23 (0.89-5.58)
IL9											
rs2069868	T	1.0000	0.9631	0.9443	0.8930	1.0000	0.7926	1.07 (0.66-1.73)	0.8622	1.14 (0.66-1.98)	0.79 (0.13-4.85)
IL10											
rs1518111	G	0.9299	0.5811	0.9003	0.6456	0.5817	0.7546	0.95 (0.68-1.33)	0.4278	0.75 (0.46-1.23)	1.13 (0.53-2.41)
rs3021094	A	0.5148	0.2917	0.5178	0.9013	0.1767	0.7168	1.06 (0.78-1.45)	0.2294	0.73 (0.43-1.25)	1.18 (0.63-2.20)
rs1800872	C	0.9299	0.5811	0.9003	0.6456	0.5817	0.7546	0.95 (0.68-1.33)	0.4278	0.75 (0.46-1.23)	1.13 (0.53-2.41)
rs1800871	C	0.9299	0.5811	0.9003	0.6456	0.5817	0.7546	0.95 (0.68-1.33)	0.4278	0.75 (0.46-1.23)	1.13 (0.53-2.41)
IL13											
rs1800925	T	0.6527	0.6901	0.5859	0.7995	0.4573	0.6787	0.91 (0.58-1.43)	0.5783	1.04 (0.62-1.76)	0.42 (0.08-2.24)
rs1295687	G	1.0000	0.5534	0.9356	0.8851	0.5010	0.7978	0.93 (0.54-1.61)	0.9737	0.99 (0.56-1.77)	ND[c]
rs1295686	A	1.0000	0.4032	0.9666	0.6416	0.3028	0.8514	0.97 (0.67-1.39)	0.3695	1.21 (0.75-1.95)	0.66 (0.27-1.62)
rs20541	T	1.0000	0.4102	0.9674	0.6248	0.3020	0.8707	0.97 (0.66-1.42)	0.3594	1.23 (0.74-2.04)	0.67 (0.27-1.67)
rs1295685	T	0.7942	0.5947	0.7090	0.4857	0.6620	0.8046	1.05 (0.72-1.53)	0.6081	1.21 (0.75-1.96)	0.85 (0.33-2.14)

rs848	T	0.4393	0.1436	0.4096	0.1706	0.5073	0.4885	1.15 (0.78-1.69)	0.1436	1.61 (0.95-2.74)	0.82 (0.32-2.10)
rs847	A	0.7948	0.6268	0.7591	0.5611	0.6613	0.8538	1.04 (0.71-1.51)	0.6374	1.19 (0.74-1.92)	0.83 (0.33-2.11)
STAT6											
rs1059513	G	0.2651	0.0994	0.1922	0.1393	0.4568	0.2302	0.7 (0.39-1.26)	0.1603	0.65 (0.35-1.19)	ND[c]
rs703817	A	0.5014	0.3681	0.4236	0.7288	0.1836	0.3695	0.83 (0.55-1.25)	0.3883	0.94 (0.59-1.51)	0.32 (0.06-1.62)
rs324015	A	0.6761	0.3725	0.6603	0.3012	0.7897	0.6074	1.09 (0.79-1.49)	0.4491	1.43 (0.81-2.50)	1.16 (0.61-2.19)
rs3024974	T	0.4655	0.0725	0.4079	0.1457	0.2307	0.4014	1.19 (0.79-1.8)	0.0682	1.64 (0.99-2.72)	0.48 (0.12-1.89)
rs841718	T	0.2578	0.3195	0.2338	0.5632	0.1476	0.2116	0.79 (0.55-1.14)	0.3368	0.90 (0.56-1.45)	0.5 (0.20-1.25)
rs10783813	G	0.2459	0.5234	0.2423	0.3432	0.3779	0.2401	1.21 (0.88-1.67)	0.4999	1.23 (0.74-2.05)	1.46 (0.75-2.81)
rs324011	T	0.4925	0.1712	0.4283	0.2419	0.3487	0.3691	1.21 (0.80-1.81)	0.1864	1.47 (0.92-2.37)	0.62 (0.15-2.48)
CD28											
rs3181094	G	0.6763	0.8629	0.6028	0.8106	0.6148	0.7207	0.94 (0.67-1.32)	0.8626	1.01 (0.62-1.67)	0.84 (0.40-1.75)
rs1879877	C	0.6771	0.8747	0.6607	0.9048	0.6148	0.7783	0.95 (0.68-1.34)	0.8525	1.04 (0.63-1.71)	0.85 (0.41-1.77)
rs3181096	T	0.6385	0.8972	0.6290	0.7281	0.8139	0.6431	0.92 (0.63-1.33)	0.8938	0.93 (0.57-1.51)	0.81 (0.31-2.13)
rs3181097	A	0.8073	0.9640	0.7959	0.7938	0.8900	0.9125	1.02 (0.73-1.42)	0.9320	1.10 (0.64-1.91)	1.03 (0.53-2.01)
rs3181098	A	0.6385	0.8972	0.6290	0.7281	0.8139	0.6431	0.92 (0.63-1.33)	0.8938	0.93 (0.57-1.51)	0.81 (0.31-2.13)
rs3181100	G	0.7998	0.8893	0.7332	0.8887	0.6704	0.6706	1.11 (0.68-1.83)	0.8584	1.06 (0.60-1.87)	1.63 (0.27-10.02)
rs2140148	G	0.9034	1.0000	0.8437	0.8930	1.0000	0.8248	1.06 (0.66-1.69)	0.9714	1.04 (0.60-1.81)	1.19 (0.23-6.04)
rs1181388	C	0.4156	0.6912	0.3877	0.5220	0.5504	0.4490	0.88 (0.62-1.23)	0.7172	0.93 (0.55-1.60)	0.76 (0.38-1.51)
rs10932017	T	0.6008	0.7619	0.5580	0.4883	1.0000	0.5470	0.9 (0.63-1.28)	0.6957	0.81 (0.5-1.31)	0.91 (0.40-2.08)
rs4675360	A	0.4655	0.6419	0.4436	0.7872	0.4126	0.5321	0.90 (0.64-1.26)	0.6066	1.08 (0.61-1.91)	0.81 (0.41-1.58)
rs3116496	C	0.2546	0.4855	0.2322	0.2695	1.0000	0.2367	1.37 (0.81-2.31)	0.4547	1.42 (0.82-2.47)	1.08 (0.07-17.69)
rs12693993	A	1.0000	0.4354	0.9794	0.7912	0.3401	0.8228	1.06 (0.65-1.72)	0.5355	0.92 (0.54-1.58)	3.42 (0.35-33.44)
rs3181113	G	0.9352	0.6522	0.9027	0.5889	0.6738	0.8063	1.04 (0.74-1.47)	0.5002	1.36 (0.77-2.40)	1.08 (0.54-2.14)
CTLA4											
rs4553808	G	0.0068	0.0079	**0.0042**	0.0117	0.0986	*0.0073*	2.10 (1.22-3.62)	0.0280	1.91 (1.07-3.40)	ND[c]
rs231775	A	0.6179	0.7501	0.4956	0.6209	0.6925	0.4641	0.84 (0.54-1.33)	0.7285	0.87 (0.53-1.44)	0.56 (0.10-3.15)
rs11571315	G	1.0000	0.9850	0.9658	1.0000	0.8433	0.9851	1.00 (0.70-1.44)	0.9533	0.95 (0.59-1.54)	1.08 (0.47-2.48)
rs733618	G	0.7400	0.1724	0.7109	0.6329	0.1604	0.6578	0.93 (0.67-1.29)	0.1430	1.35 (0.81-2.24)	0.70 (0.34-1.43)
rs16840252	T	0.0104	0.0112	0.0063	0.0180	0.0986	*0.0106*	2.01 (1.18-3.45)	0.0400	1.82 (1.03-3.23)	ND[c]
rs5742909	T	0.0068	0.0103	0.0056	0.0166	0.0975	*0.0099*	2.05 (1.19-3.52)	0.0370	1.85 (1.04-3.30)	ND[c]
rs231779	C	1.0000	0.9850	0.9658	1.0000	0.8433	0.9851	1.00 (0.70-1.44)	0.9533	0.95 (0.59-1.54)	1.08 (0.47-2.48)
rs3087243	A	0.0220	0.0277	0.0167	0.0093	0.7772	*0.0213*	0.61 (0.41-0.93)	0.0329	0.52 (0.32-0.85)	0.67 (0.20-2.18)

rs231725	G	0.6774	0.842	0.6227	0.6303	1.0000	0.5943	0.91 (0.65-1.28)	0.7797	0.84 (0.51-1.38)	0.88 (0.43-1.80)
IL4R											
rs12925861	A	0.6258	0.8840	0.6204	0.7063	0.7009	0.5217	1.10 (0.82-1.50)	0.7722	1.19 (0.69-2.06)	1.22 (0.66-2.24)
rs3024585	G	0.6143	0.5486	0.5948	1.0000	0.3457	0.6801	0.93 (0.68-1.29)	0.4241	1.19 (0.72-1.97)	0.76 (0.39-1.51)
rs3024613	T	0.1191	0.1214	0.1036	0.5352	0.0459	0.1309	0.77 (0.55-1.08)	0.1011	1.05 (0.63-1.76)	0.51 (0.25-1.05)
rs3024622	G	0.1926	0.0955	0.1639	0.0392	1.0000	0.2202	1.23 (0.88-1.72)	0.0646	1.94 (1.11-3.40)	1.45 (0.74-2.85)
rs1801275	G	0.5355	0.3981	0.5209	0.9034	0.1830	0.5340	1.13 (0.76-1.69)	0.3436	0.91 (0.55-1.52)	2.19 (0.70-6.83)
rs1049631	G	1.0000	0.9211	1.0000	0.8952	0.8952	0.9637	1.01 (0.73-1.39)	0.9423	0.93 (0.53-1.63)	1.02 (0.54-1.92)
rs8832	A	1.0000	0.7753	0.952	0.7012	0.7897	0.9824	1.00 (0.73-1.37)	0.8343	0.87 (0.50-1.50)	1.00 (0.53-1.88)
rs3024685	C	0.9353	0.6829	0.9193	0.7024	0.5948	0.8945	1.02 (0.75-1.40)	0.7314	0.86 (0.50-1.48)	1.06 (0.56-1.98)
rs4787956	A	0.8075	0.9659	0.7892	0.8970	0.8952	0.7577	0.95 (0.69-1.30)	0.9472	0.98 (0.56-1.69)	0.90 (0.48-1.70)
GATA3											
rs3802604	C	0.5424	0.8293	0.5431	0.5657	0.852	0.5052	0.89 (0.63-1.26)	0.7880	0.86 (0.53-1.40)	0.82 (0.38-1.78)
rs3824662	T	0.5238	0.7512	0.5172	0.4898	1.0000	0.4636	0.87 (0.60-1.26)	0.6871	0.81 (0.50-1.31)	0.86 (0.35-2.12)
rs12572421	T	0.6879	0.3860	0.6231	0.5668	0.4638	0.6558	0.88 (0.51-1.54)	0.5408	0.84 (0.47-1.48)	ND[c]
rs570613	G	1.0000	1.0000	0.9682	1.0000	1.0000	0.9173	0.98 (0.67-1.43)	0.9740	1.01 (0.63-1.63)	0.90 (0.33-2.41)
rs2280015	A	0.6463	0.6119	0.5775	0.9083	0.4270	0.6737	0.92 (0.62-1.36)	0.6040	1.04 (0.65-1.67)	0.58 (0.19-1.80)
rs406103	T	0.5191	0.6336	0.4807	0.7292	0.4270	0.5607	0.89 (0.60-1.32)	0.6218	0.99 (0.62-1.60)	0.58 (0.19-1.78)
rs10905284	C	0.1198	0.2460	0.1069	0.1353	0.3632	0.1658	0.79 (0.56-1.11)	0.3665	0.74 (0.44-1.25)	0.63 (0.32-1.27)
rs2229360	T	0.7116	0.6709	0.6378	0.9078	0.4293	0.7542	0.94 (0.63-1.39)	0.6476	1.06 (0.66-1.71)	0.62 (0.20-1.91)
rs263419	T	0.516	0.6104	0.4346	0.6460	0.4272	0.4945	0.87 (0.59-1.29)	0.6238	0.96 (0.60-1.55)	0.57 (0.19-1.76)
rs263418	G	0.5789	0.6461	0.5338	0.8175	0.4274	0.6262	0.91 (0.62-1.34)	0.6351	1.02 (0.63-1.63)	0.59 (0.19-1.82)
GLT6D1											
rs1537415	G	0.7812	0.2386	0.7660	0.3534	0.4343	0.7572	1.06 (0.75-1.50)	0.2474	1.43 (0.87-2.36)	0.79 (0.35-1.78)

A1: minor allele; CI: confidence interval; co-dom: codominant mode by Cochran-Armitage Trend test; dom: dominant mode; geno: genotype; het: heterozygous of A1; hom: homozygous of A; ND: not determined; OR: Odds ratio; rec: recessive mode; SNP: single-nucleotide polymorphism

Significant level set at P <0.005; statistic significant result was bolded; adjustment against age and gender; SNPs with adjusted allelic P value <0.1 (italic) were selected for meta-analysis

[a]Fisher exact test.

[b]Logistic regression with the adjustment of age and gender

[c]no individual with the genotype of homozygous minor(A1) or major allele (A2).

SUPPLEMENTARY TABLE 4 Characteristics of candidate gene studies included in meta-analyses

SNPs	Author year	Country	Ethnicity	Sample size		Genotypes/Allelotypes		
				Control	Case		Control	Case
CTLA4 **rs5742909**	Houshmand et al. 2012	Iran	Iranian	218	126	CC	177	101
						CT	37	23
						TT	4	2
						C	391	225
						T	45	27
	Song et al. 2014	China	Chinese	80	103	CC	57	64
						CT	22	37
						TT	1	2
						C	136	165
						T	24	41
	Current study	China	Chinese	163	140	CC	137	101
						CT	26	36
						TT	0	3
						C	300	238
						T	26	42
IL6 **rs1800796**	Holla et al. 2004	Czechia	Caucasian	107	148	CC	0	0
						GC	21	9
						GG	86	139
						C	21	9
						G	193	287
	Komatsu et al. 2005	Japan	Japanese	77	112	CC	41	71
						GC	32	36
						GG	4	5
						C	114	178
						G	40	46
	Bang et al. 2008	Korea	Korean	30	52	CC	6	12
						GC	10	29
						GG	14	11
						C	22	53
						G	38	51
	Kobayashi et al. 2009	Japan	Japanese	108	117	CC	60	58
						GC	40	50
						GG	8	9
						C	160	166
						G	56	68
	Nibali et al. 2009-1	UK	Caucasian	144	318	CC	133	285
						GC	11	31
						GG	0	2

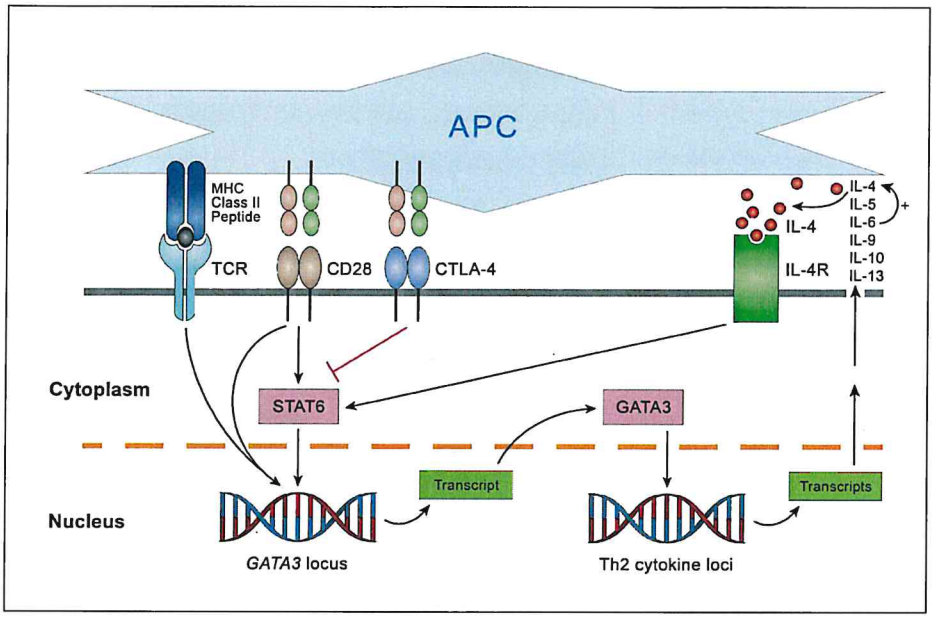
					C	277	601
					G	11	35
Nibali et al. 2009-2	UK	African	45	86	CC	39	68
					GC	5	13
					GG	0	5
					C	83	149
					G	5	23
Nibali et al. 2009-3	UK	Asian	29	84	CC	13	33
					GC	13	38
					GG	3	13
					C	39	104
					G	19	64
Franch-Chillida et al. 2010	UK	Indian	204	47	CC	72	14
					GC	87	19
					GG	29	14
					C	231	47
					G	145	47
Liu et al. 2010	China	Chinese	96	93	CC	65	49
					GC	30	38
					GG	1	6
					C	160	136
					G	32	50
Fan et al. 2011	China	Chinese	130	178	CC	95	120
					GC	32	52
					GG	3	6
					C	222	292
					G	38	64
Huang et al. 2011	China	Chinese	90	159	CC	66	108
					GC	22	48
					GG	2	3
					C	154	264
					G	26	54
Chen et al. 2012	China	Chinese	128	198	CC	71	118
					GC	49	56
					GG	8	24
					C	191	292
					G	65	104
Zhang et al. 2014	China	Chinese	216	199	CC	117	94
					GC	77	84
					GG	22	21
					C	311	272

					G	121	126
Hu et al. 2016	China	Chinese	120	120	CC	89	82
					GC	29	34
					GG	2	4
					C	207	198
					G	33	42
Salman et al. 2016	Iranian	Iranian	48	81	CC	0	0
					GC	9	28
					GG	39	53
					C	9	28
					G	87	134
Li C et al. 2017	China	Chinese	95	40	CC	47	21
					GC	43	14
					GG	5	5
					C	137	56
					G	53	24
Li J et al. 2017	China	Chinese	69	83	CC	43	31
					GC	25	39
					GG	1	13
					C	111	100
					G	27	66
Shi 2017	China	Chinese	259	231	CC	167	120
					GC	88	98
					GG	4	13
					C	422	338
					G	96	124
Dong et al. 2018	China	Chinese	284	273	CC	157	139
					GC	104	105
					GG	23	29
					C	418	383
					G	150	163
Current study	China	Chinese	163	141	CC	111	86
					GC	49	48
					GG	3	7
					C	271	220
					G	55	62

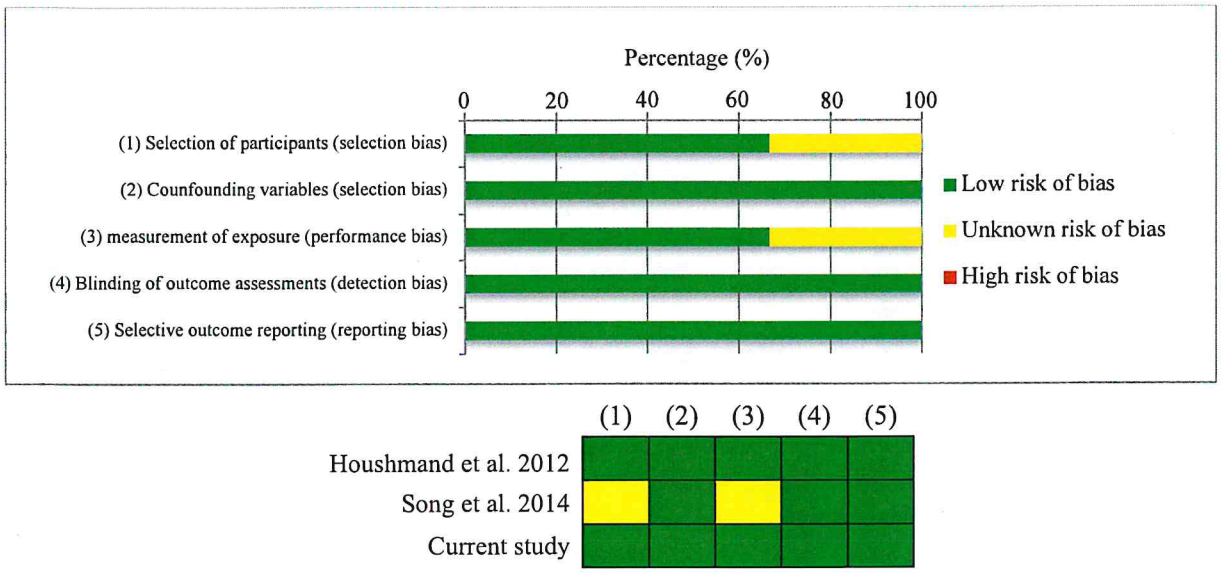
SUPPLEMENTARY TABLE 5 Association statistics extracted from Shungin et al. 2019[13] (n = 45,651) for candidate single-nucleotide polymorphisms identified with adjusted allelic $P < 0.1$ in current study

	Position	Allele1	Allele2	Effect	SE	*P*-value	Controls (n)	Cases (n)	MAC
CTLA4									
rs4553808	2:204731005	A	G	-0.0256	0.0196	0.192	43817	19033	18388.0
rs16840252	2:204731519	T	C	0.0257	0.0196	0.190	43817	19033	18621.4
rs5742909	2:204732347	T	C	0.0101	0.0250	0.687	43817	19033	11538.7
rs3087243	2:204738919	A	G	0.0263	0.0150	0.079	35402	18363	39904.1
IL4									
rs2243290	5:132018169	A	C	-0.0158	0.0179	0.376	43817	19033	26643.2
rs2243291	5:132018983	C	G	-0.0219	0.0176	0.213	43817	19033	27514.2
IL6									
rs1800796	7:22766246	C	G	0.0227	0.0257	0.379	37054	18108	11806.5
rs2066992	7:22768249	T	G	0.0164	0.0259	0.526	37054	18108	11650.0
rs2069852	7:22772260	A	G	0.0055	0.0262	0.833	43817	19033	16213.4

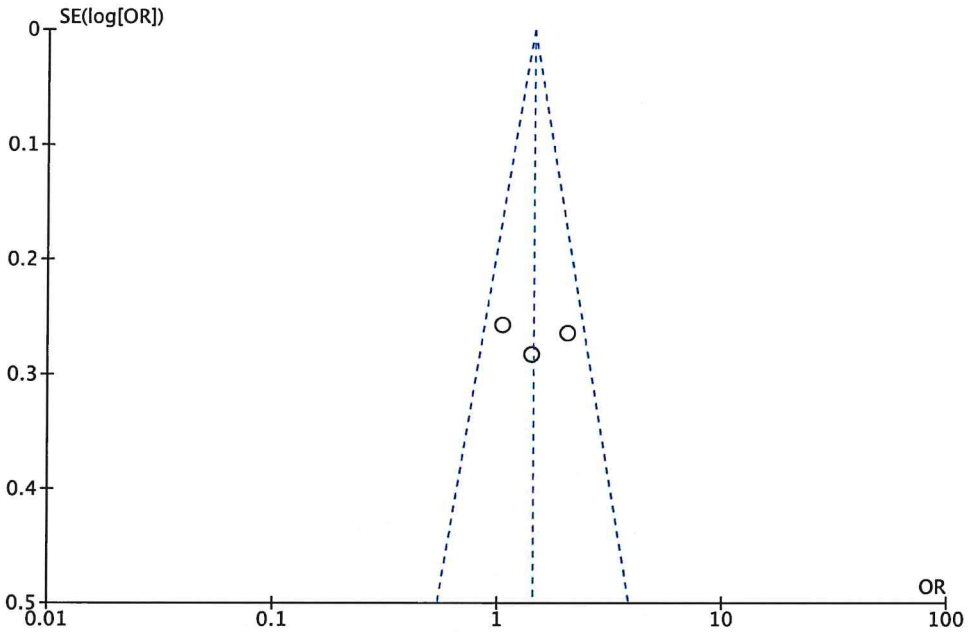
Effect, estimated effect of allele 1 on a log-odds scale from fixed-effects meta-analysis; MAC, minor allele count; SE, standard error for effect. SNPs includable in candidate gene meta-analysis are in italics.



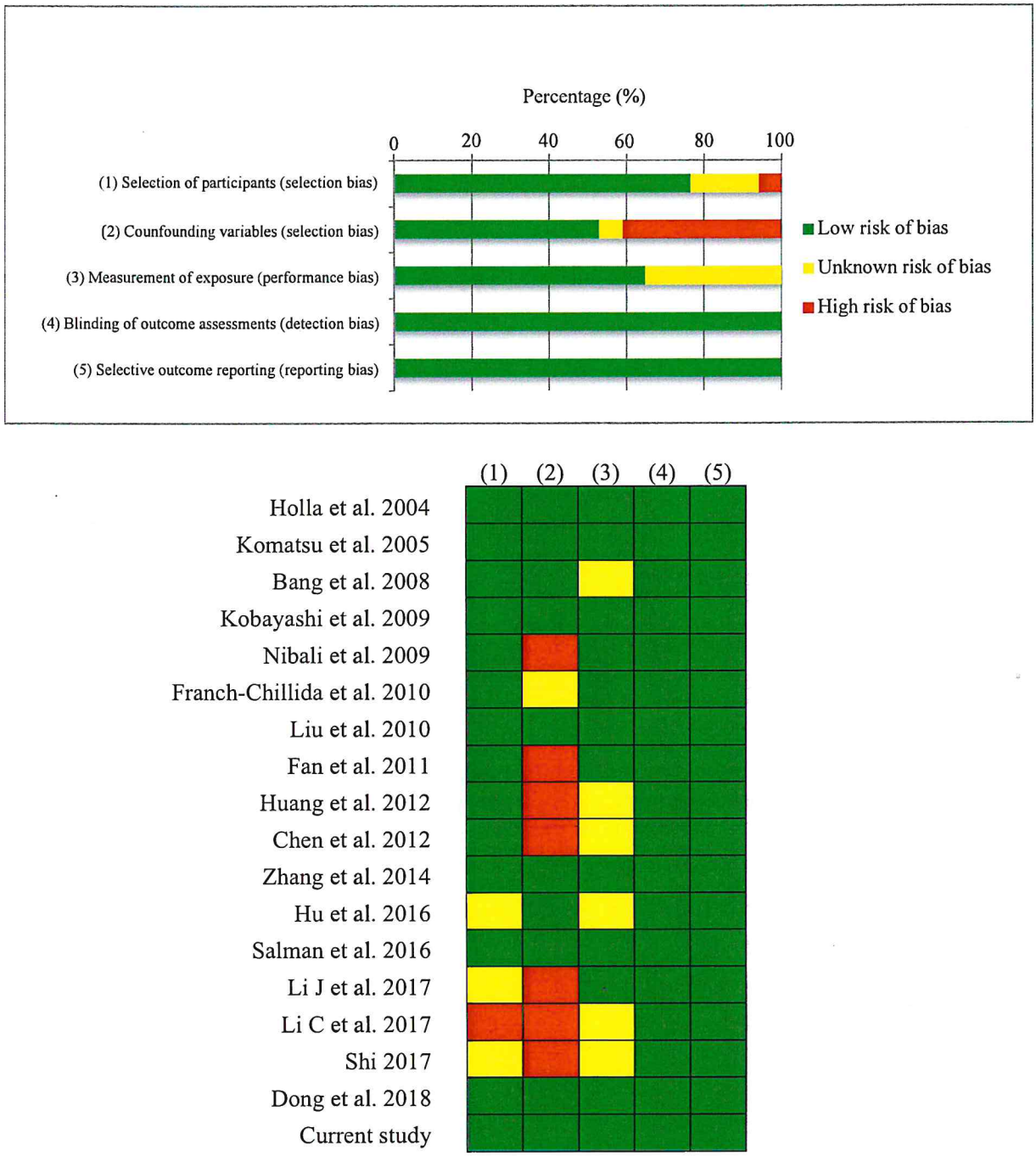
SUPPLEMENTARY FIGURE 1 Regulation of T helper 2 (Th2) cell differentiation. The differentiation into Th2 cell is activated by two essential pathways: i) cell-cell interaction pathway consisting of T-cell receptor (TCR) and the costimulatory molecules such as cluster of differentiation 28 (CD28); ii) the cytokine-receptor pathway with interleukin 4 (IL-4) and IL-4 receptor (IL-4R). Cytotoxic T-lymphocyte antigen 4 (CTLA-4), an inhibitory regulator sharing the same ligand (peripheral membrane protein B7-1/B7-2) with CD28 and of higher affinity, can competitively occupy B7-1/B7-2 and block the activation via CD28. Both of the TCR/CD28 and IL-4/IL-4R pathways can induce the transcription of GATA-binding protein gene (*GATA3*) with or without activating signal transducer and activator of transcription 6 (STAT6). GATA3, as the master switch to develop Th2 cell from naïve T (Th0) cell, later promotes the production of the Th2 cytokines, such as IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13. IL-6 activates transcription mediated by nuclear factor of activated T cells leading to production of IL-4 by naïve CD4+ T cells and their differentiation into effector Th2 cells.[18]



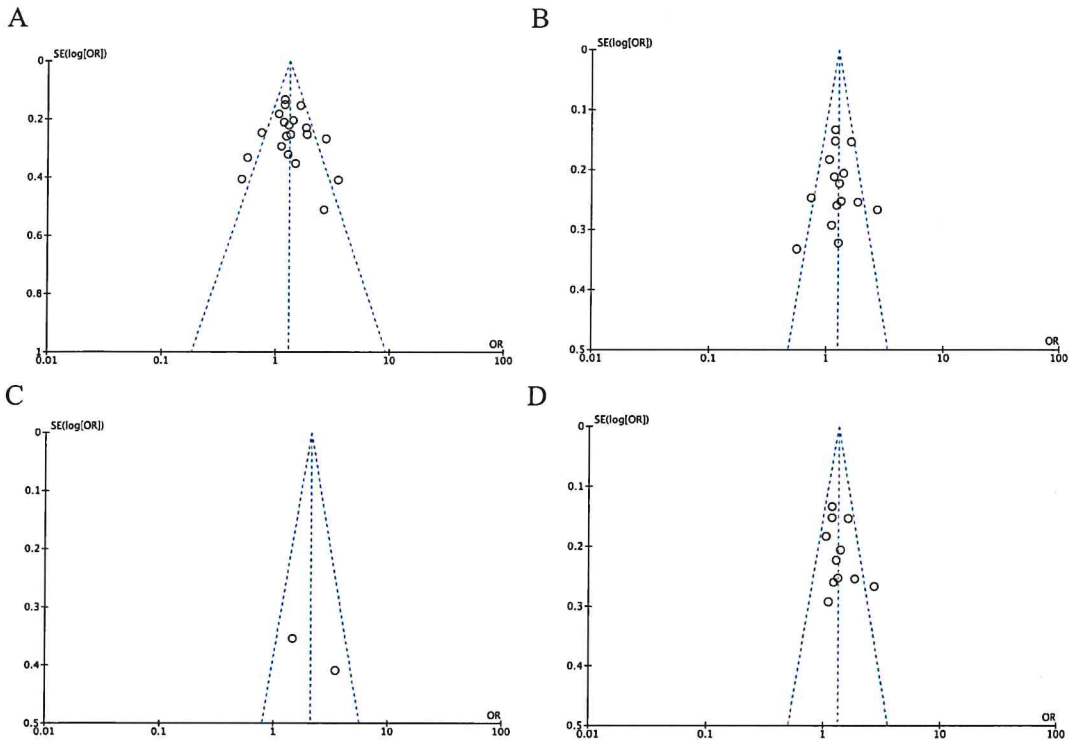
SUPPLEMENTARY FIGURE 2 Risk of bias assessment of included studies regarding *CTLA4* rs5742909 and periodontitis. The upper panel displays a risk of bias graph that indicates the ratio of studies with bias for each assessed item. The figure in the lower panel is a risk of bias summary showing the details of each included study.



SUPPLEMENTARY FIGURE 3 Funnel plot analyzing the included studies concerning *CTLA4* rs5742909 and periodontitis. The symmetric funnel plots indicated absence of publication bias in the meta-analysis.



SUPPLEMENTARY FIGURE 4 Risk of bias assessment of included studies regarding *IL6* rs1800796 and periodontitis. The upper panel displays a risk of bias graph that indicates the ratio of studies with bias for each assessed item. The figure in the lower panel is a risk of bias summary showing the details of each included study.



SUPPLEMENTARY FIGURE 5 Funnel plots analyzing the included studies concerning *IL6* rs1800796 and periodontitis. A) all studies; **B)** East Asians; **C)** Caucasians; **D)** Chinese. The symmetric funnel plots indicated absence of publication bias in the meta-analysis.