A meta-analysis of MMP-9 promoter −1562 C/T polymorphism on susceptibility of chronic periodontitis

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Abstract

Background: Although many studies have focused on the association of the MMP-9 promoter −1562 C/T polymorphism with the susceptibility and/or severity of chronic periodontitis (CP), results have been inconsistent. Therefore, a meta-analysis of all eligible studies was performed to derive a more precise estimation of the association between this polymorphism and CP risk.

Methods: All relevant studies were identified through a database search in PubMed, Medline, and Web of Science. All the full-text studies with appropriate analytical design, published in English, which evaluated the association of MMP-9 promoter −1562 C/T polymorphism with CP risk using validated genotyping methods, and with non-duplicated data were selected for this study. A fixed-effect model was used to calculate pooled ORs in the absence of heterogeneity across included trials (P > 0.1 and I² < 50 %), otherwise the random-effect model was applied.

Results: In an overall meta-analysis, pooled ORs revealed that T variant in the MMP-9 promoter −1562 C/T polymorphism was associated with a significantly decreased risk for CP under all comparison models. In subgroup analyses by ethnicity, pooled ORs showed that a significant association of the MMP-9 promoter −1562 C/T polymorphism with CP risk was only detected in Caucasians and Asians but not in mixed population. In the subgroup analysis by severity of CP, pooled ORs indicated that T allele of the MMP-9 promoter −1562 C/T polymorphism was associated with decreased susceptibility to severe CP while there was no significant association between this polymorphism and moderate CP.

Conclusions: Our meta-analysis showed that T allele in the MMP-9 promoter −1562 C/T polymorphism might be a protective factor for CP, especially in Caucasians and Asians. Moreover, there was a significant association of the MMP-9 promoter −1562 C/T polymorphism with decreased susceptibility to severe CP, while the allelic and/or genotype distributions of this polymorphism were not associated with moderate CP.

Keywords: Matrix metalloproteinase-9, Polymorphism, Chronic periodontitis, Meta-analysis

Background

Chronic periodontitis (CP), the most commonly occurring and slowly progressive form of periodontal disease, can lead to continual inflammatory host response, which may finally result in periodontal attachment loss and bone resorption (Deo and Bhongade 2010; Pihlstrom et al. 2005). CP is a highly prevalent disease and has shown to affect 90 % of the worldwide individuals (Tatakis and Kumar 2005). Even though the key etiological factor that results in progression of CP is the formation of complex biofilm on the surfaces of teeth adjacent to their periodontal tissues, determinants like demographic, social, environmental, behavioral, systemic and genetic factors have also been coupled with the epidemiology of this disease (Stabholz et al. 2010; Yoshie et al. 2007).
Matrix metalloproteinases (MMPs) belong to a family of zinc-dependent endopeptidases, which are served as the vital enzymes. They have the combined capacity to degrade extracellular matrix (ECM) components such as several kinds of collagen, that are essential for the tissue repair and remodeling involved with the development of inflammation (Hannas et al. 2007; Sorsa et al. 2006). MMPs are secreted in an inactive form as pro-MMPs, which can be activated by other MMPs or serine proteinases (Malemud 2006; Sternlicht and Werb 2001). The imbalance between MMPs, identified in the inflamed periodontal tissues and their host inhibitors is thought to be responsible for the process of destruction in structural proteins during CP (Emingil et al. 2006; Ingman et al. 1996; Víctor et al. 2014).

Matrix metalloproteinase-9 (MMP-9) or gelatinase B, involved in the breakdown of various connective tissue proteins, including types IV, V and XI collagen, proteoglycans as well as elastin, is abundantly expressed in CP (Bildt et al. 2008; Rai et al. 2008). Various cell lines such as polymorphonuclear leukocytes, macrophages, keratinocytes, fibroblasts, osteoclasts, eosinophils and neutrophils have been connected with the expression of MMP-9 gene, located on chromosome 20q11.2-13.1 (Seguier et al. 2001). Genetic variations in the promoter region of MMP-9 gene may have an effect on its transcription and protein synthesis (Chang et al. 2002; Soder et al. 2009), which may influence connective tissue degradation of the protein and thus contributing to genetic susceptibility to CP.

The fact that a functional C-to-T single nucleotide polymorphism (SNP) exists in MMP-9 gene at position −1562, which affects transcription, and also decreased transcriptional activity shown by CC genotype is well documented (Haberbosch and Gardemann 2005; Zhang et al. 1999). Despite comprehensive studies focusing on the association of this polymorphisms with the susceptibility and/or severity of CP using the similar methodology (de Souza et al. 2005; Gurkan et al. 2008; Holla et al. 2006; Keles et al. 2006), the results show high degree of variation. Therefore a meta-analysis of all eligible studies was carried out to draw an accurate assessment of the association of this polymorphism with CP risk.

Methods

Selection of studies

The following criteria were designed and used for including the identified studies into the present meta-analysis and system review: (1) Studies that evaluated the association of MMP-9 promoter −1562 C/T polymorphism with CP risk among CP affected and unaffected individuals; (2) Studies applied validated genotyping methods such as PCR–RFLP; (3) Studies with appropriate analytical design, for example case–control, cohort, or nested case control; (4) Studies published in English, and available full-text; (5) Study data not duplicated or overlapped with those of any other article. Besides, we barred those studies, of which the pertinent data were not available to figure up the odds ratios (ORs) and its variance.

Data extraction

The data extraction was performed by two independent reviewers (ADH and PS) under a pre-defined strategy. Any disagreements between investigators were settled through consensus decision with the third evaluator (LWY). The following items were collected from each included trial: first author’s surname, publication year, nation, race, sample size, severity of CP, matching criteria, genotyping method, as well as the results of HWE in controls were calculated using an online software (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl).

Heterogeneity

Heterogeneity across the included studies was evaluated by the Cochrane-Q test. $I^2$ directly calculated from the Cochrane-Q test was also used to describe the presence of inconsistency among the included studies caused by heterogeneity. The range of $I^2$ varies from 0 to 100 %, with 0 % suggesting that there is no heterogeneity. Moreover, low heterogeneity was assumed if $I^2$ was below 50 %,
while, if $I^2$ exceeded 50%, a significant heterogeneity was believed to exist.

**Statistical analysis**

The association of the MMP-9 promoter $-1562$ C/T polymorphism with CP risk was assessed by ORs with their 95% confidence intervals (CIs). The Z-test was applied to find out statistical significance of pooled ORs. Initially, the allele-frequency comparison model (T vs. C) was used to evaluate the potential relationship between the allele T of MMP-9 promoter $-1562$ C/T polymorphism and CP risk. After that, the association of this polymorphism with CP risk was also examined by four other comparison models, including TT versus CC, CT versus CC, dominant (TT + CT vs. CC), as well as recessive (TT vs. CT + CC) genetic models. In addition, subgroup analyses concerning the study characteristics of ethnicity and severity of CP were respectively carried out.

To calculate pooled ORs, a fixed-effects model was planned to be used with the absence of heterogeneity across included trials ($P > 0.1$ and $I^2 < 50%$), otherwise, the random-effects model was applied. Furthermore, the potential source of heterogeneity was evaluated via a sensitivity analysis. Besides, we used funnel plots to examine publication bias, in which a standard error of log (OR) of each included trial was plotted against its log (OR), and Egger’s linear regression test was applied to assess the asymmetry. Meta-analyses was executed by using software Stata (version 11.0, Stata Corp., College Station, TX), with a statistical significance defined as two-sided $P$ value <0.05. If the results of trials could not be pooled through the meta-analysis, we assessed them using descriptive statistics.

**Results**

The workflow and results of literature search are summarized in Fig. 1. The comprehensive search of literature under defined terms retrieved 197 articles. Of those 185 articles were barred due to the irrelevance to this topic, after a thorough screening of titles and abstracts. After the detailed screening of 12 potentially relevant references, 9 articles were taken for a further full-text review. Following full-text retrieval of the articles, two studies were eliminated as they investigated the association of the MMP-9 promoter $-1562$ C/T polymorphism with aggressive periodontitis (Chen et al. 2007; Gurkan et al. 2007). Finally, seven case–control studies involving a total of 859 CP cases and 1186 controls were included in this meta-analysis (de Souza et al. 2005; Gurkan et al. 2008; Holla et al. 2006; Isaza-Guzman et al. 2011; Keles et al. 2006; Li et al. 2012; Loo et al. 2011). The baseline characteristics of all the included studies are summarized in Table 1. As far as stratification by ethnicity is concerned, 3 studies reported on Caucasians, 2 on Asians, and 2 on subjects with mixed populations were included in the ultimate analysis. Also, concerning the severity of CP, the final analysis included two studies (1 reported on Caucasians and 1 on mixed populations) that investigated the association of MMP-9 promoter $-1562$ C/T polymorphism with risk for both moderate CP and severe CP. Moreover, the genotype distributions of all the included studies were in accordance with HWE except for the study of Gurkan et al. (2008), Loo et al. (2011) and Li et al. (2012).

**Meta-analysis results**

As shown in Table 2 and Fig. 2, the allele-frequency comparison model produced a significant relation between the MMP-9 promoter $-1562$ C/T polymorphism and CP risk (T vs. C: OR 0.49, 95% CI 0.31–0.77, $P = 0.002$), but with a considerable heterogeneity among the included studies ($P_h = 0.000, I^2 = 85.8\%$) (Table 2; Fig. 2). Besides, statistically significant relation of this polymorphism with CP risk was also identified under all other different genetic models (Table 2).

**Subgroup analysis**

We performed subgroup analysis according to the ethnicity and severity of CP. We found that the MMP-9 promoter $-1562$ C/T polymorphism was associated with a significantly decreased CP risk under all comparison models except for the recessive genetic model in Caucasians (Table 2; Fig. 3). Besides, pooled analysis using the fixed-effects model for subgroup analysis by ethnicity revealed a significant association between this
polymorphism and CP susceptibility in Asians under all comparison models (Table 2; Fig. 3). However, under all comparison models, we failed to detect any significant association between this polymorphism and CP risk in a mixed population (Table 2; Fig. 3). As long as the severity of CP was concerned, significant differences were detected in the allelic and/or genotype distributions of the MMP-9 promoter –1562 C/T polymorphism between a subgroup of patients with severe CP versus controls (Table 2; Fig. 4), while under all comparison models the overall OR with its 95% CI demonstrated that the difference between this polymorphism in patients

### Table 1 Baseline characteristics of studies included for investigating the association of the MMP-9 promoter –1562 C/T polymorphism with periodontitis risk

| References          | Country | Ethnicity | Sample size (case/control) | Type of periodontitis | Matching criteria | Genotype method | HWE in controls |
|---------------------|---------|-----------|----------------------------|-----------------------|-------------------|----------------|-----------------|
| de Souza et al. (2005) | Brazil  | Mixed     | 62/38                      | Moderate or severe CP | –                 | PCR–RFLP       | 0.623           |
| Holla et al. (2006) | Czech   | Caucasian | 169/135                    | Moderate or severe CP | Age, gender, smoker ratios | PCR–RFLP       | 0.586           |
| Keles et al. (2006) | Turkey  | Caucasian | 70/70                      | Severe CP             | Age, gender       | PCR–RFLP       | 0.816           |
| Gurkan et al. (2008) | Turkey  | Caucasian | 87/107                     | Severe CP             | Age, gender, smoker ratios | PCR–RFLP       | 0.17            |
| Loo et al. (2011)   | China   | Asian     | 280/250                    | Severe CP             | Age, gender       | PCR–RFLP       | 0.001           |
| Isaza-Guzman et al. (2011) | Colombia | Mixed  | 69/54                      | Slight to moderate to severe CP | Gender | PCR–RFLP | 0.163 |
| Li et al. (2012)    | China   | Asian     | 122/532                    | Severe CP             | –                 | PCR–RFLP       | 0.001           |

**MMP-9** matrix metalloproteinase-9; **CP** chronic periodontitis; **GAgP** generalized aggressive periodontitis; **HWE** Hardy–Weinberg equilibrium

Slight periodontitis: patients with teeth exhibiting <3 mm attachment loss; moderate periodontitis: patients with teeth exhibiting ≥3 and <7 mm attachment loss; severe periodontitis: patients with teeth exhibiting ≥7 mm attachment loss

A P value less than 0.05 of HWE was considered significant

### Table 2 Meta-analysis results of the association of the MMP-9 promoter –1562 C/T polymorphism with CP risk

| MMP-9 (−1562 C/T) | Studies (cases/controls) | T versus C OR (95% CI), P P<sub>h</sub> (I<sup>2</sup> %) | TT versus CC OR (95% CI), P P<sub>h</sub> (I<sup>2</sup> %) | CT versus CC OR (95% CI), P P<sub>h</sub> (I<sup>2</sup> %) | Dominant genetic model OR (95% CI), P P<sub>h</sub> (I<sup>2</sup> %) | Recessive genetic model OR (95% CI), P P<sub>h</sub> (I<sup>2</sup> %) |
|--------------------|--------------------------|------------------------------------------------------|------------------------------------------------------|------------------------------------------------------|------------------------------------------------------|------------------------------------------------------|
| Total              | 7 (859/1186)             | 0.49 (0.31–0.77), 0.02 (0.001, 85.8)                 | 0.16 (0.12–0.22), 0.001 (0.564, 0.0)                  | 0.53 (0.33–0.85), 0.008 (0.001, 73.4)                 | 0.46 (0.26–0.82), 0.009 (0.001, 85.8)                | 0.27 (0.20–0.35), 0.001 (0.897, 0.0)                |
| Ethnicity          |                          |                                                      |                                                      |                                                      |                                                      |                                                      |
| Caucasian          | 3 (326/312)              | 0.61 (0.39–0.97), 0.037 (0.101, 56.3)               | 0.35 (0.13–0.97), 0.044 (0.452, 0.0)                 | 0.66 (0.47–0.92), 0.016 (0.227, 32.5)                | 0.62 (0.45–0.87), 0.005 (0.139, 49.3)                | 0.40 (0.14–1.10), 0.076 (0.535, 0.0)                |
| Asian              | 2 (402/782)              | 0.26 (0.21–0.31), 0.001 (0.902, 0.0)                | 0.14 (0.10–0.20), 0.001 (0.926, 0.0)                 | 0.27 (0.19–0.39), 0.001 (0.522, 0.0)                 | 0.19 (0.14–0.26), 0.001 (0.756, 0.0)                 | 0.26 (0.20–0.35), 0.001 (0.782, 0.0)                |
| Mixed              | 2 (131/92)               | 0.89 (0.49–1.59), 0.684 (0.596, 0.0)                | 0.23 (0.02–2.25), 0.206 (0.983, 0.0)                 | 1.09 (0.56–2.11), 0.808 (0.453, 0.0)                 | 0.98 (0.51–1.88), 0.093 (0.512, 0.0)                 | 0.23 (0.02–2.22), 0.202 (0.915, 0.0)                |
| Severity of chronic periodontitis |                          |                                                      |                                                      |                                                      |                                                      |                                                      |
| Severe             | 6 (687/1132)             | 0.46 (0.28–0.75), 0.002 (0.001, 87.6)               | 0.16 (0.12–0.22), 0.001 (0.260, 23.2)                | 0.48 (0.29–0.81), 0.006 (0.001, 75.9)                | 0.41 (0.21–0.78), 0.006 (0.001, 87.2)                | 0.27 (0.21–0.36), 0.001 (0.680, 0.0)                |
| Moderate           | 2 (103/173)              | 0.65 (0.40–1.07), 0.090 (0.358, 0.0)                | 0.34 (0.06–2.08), 0.245 (0.831, 0.0)                 | 0.70 (0.39–1.24), 0.218 (0.319, 0.0)                 | 0.65 (0.37–1.14), 0.132 (0.323, 0.0)                 | 0.37 (0.06–2.26), 0.284 (0.891, 0.0)                |

**MMP-9**: matrix metalloproteinase-9; **CP**: chronic periodontitis; dominant genetic model: TT + CT versus CC; recessive genetic model: TT versus CT + CC; P<sub>h</sub>, the P value of heterogeneity; OR: odds ratio; CI: confidence interval

When P<sub>h</sub> is < 0.1 and I<sup>2</sup> exceeded 50%, the random-effects model was used. Conversely, the fixed-effects model was used

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It appears that the text contains tables and figures (Table 1 and Table 2) which are essential for understanding the data and conclusions presented in the text. The tables provide detailed information about studies included in the meta-analysis, their characteristics, and the results of the analysis. The figures are likely visual representations of the data, which can help in understanding the relationships and trends described in the text. The text itself discusses the association of the MMP-9 promoter –1562 C/T polymorphism with CP risk, with a focus on detecting differences in susceptibility among different ethnic populations and severity levels of periodontitis.
with moderate CP and control population was not significant (Table 2; Fig. 4).

**Sensitivity analysis**

Sensitivity analyses indicated that the major contributor of heterogeneity in different comparison models was the study by Li et al. (2012). After exclusion of this study, pooled ORs of the remaining trials revealed similar results except under the recessive genetic model (OR 0.54, 95% CI 0.29–1.01, P = 0.054) (Table 3). Moreover, when we performed population-specific and severity-specific sensitivity analyses, we found that studies conducted by Keles et al. (2006) and Holla et al. (2006) were the main contributor of heterogeneity in subgroups of Caucasians and severity CP, respectively. The exclusion of the study by Keles et al. (2006) could eliminate the heterogeneity, and using a fixed-effects model, pooled ORs showed that there was no significant association between the MMP-9 promoter −1562 C/T polymorphism and CP risk in Caucasian population under the allele-frequency comparison model (OR 0.75, 95% CI 0.54–1.04, P = 0.080) (Table 3). Besides, with the exclusion of study by Holla et al. (2006), pooled ORs of the remaining trials in the subgroup of severe CP still showed similar results (Table 3).

**Publication bias**

In order to assess the publication bias of the included trials, Begg’s funnel plot and Egger’s test were performed. Funnel plot shapes were found to be symmetrical in all the comparison models of the MMP-9 promoter −1562 C/T polymorphism (Fig. 5). In addition, for the purpose of providing statistical evidence to funnel plot symmetry, Egger’s test was used in this study. In the end, the results of the tests once again suggested that no publication bias existed for the association of this polymorphism (P = 0.057 for allele-frequency model, P = 0.295 for TT versus CC, P = 0.295 for CT versus CC, P = 0.213 for the dominant genetic model, P = 0.103 for the recessive genetic model) with CP risk.

**Discussion**

Current understanding of the etiology of CP indicates the bacterial infection by a group of predominantly gram-negative and anaerobic organisms as the essential cause for disease initiation (Flemmig 1999), however, different evolution and the poor prognosis of severe CP cannot be justified by mere existence, type or quantity of infection. And the severity of periodontal destruction is rather dependent on a dynamic equilibrium of bacteria–host interactions that can be modified by multiple...
environmental and genetic factors (Page et al. 1997). Therefore, the effect of host genetic factors on disease susceptibility, progression and treatment outcome of CP has been suggested by many authors (Michalowicz et al. 2000; Offenbacher 1996). Evidence from these studies has implicated that about half of the variations found in the expression of CP can be attributed to genetic factors (Michalowicz et al. 2000). As a result, the need for searching some specific genetic markers for CP is on the rise.

A single nucleotide polymorphism (rs3918242) in the promoter region of MMP-9 gene has been reported at position −1562 relative to the transcription start site, where a conversion between C and T (C−1562-T) occurs (Zhang et al. 1999). This functional polymorphism causes loss of binding of a nuclear repressor protein, thereby resulting in an increase of mRNA, protein level and MMP-9 activity in T allele carriers while the CC genotype has been shown to decrease transcriptional activity (Zhang et al. 1999).

Moreover, it has been proved that inappropriate activation of MMP-9 at a site of infection can have a profound effect in the tissue destruction of CP (Rai et al. 2008; Soder et al. 2009). However, studies about the association between this polymorphism and CP risk have not yielded consistent results. Several studies have supported that risk for CP is associated with this polymorphism (Keles et al. 2006; Li et al. 2012; Loo et al. 2011), whereas others have failed to find an association (de Souza et al. 2005; Gurkan et al. 2008; Holla et al. 2006; Isaza-Guzman et al. 2011).

Therefore, we performed the present meta-analysis to clarify the relationship between this polymorphism and CP risk. In this study, we first summarized the data about the association between the MMP-9 promoter −1562 C/T polymorphism and CP risk in overall population. Surprisingly, pooled ORs revealed that T variant in the MMP-9 promoter −1562 C/T polymorphism was associated with a significantly decreased risk for CP under all comparison models. The possible explanation might be

![Fig. 3](image)

**Fig. 3** Forest plots demonstrating the association of MMP-9 promoter −1562 C/T polymorphism to CP risk in regards to the ethnicity under the allele contrast.
Fig. 4 Forest plot illustrating the association between MMP-9 promoter −1562 C/T polymorphism and CP risk with regards to the severity of CP under the allele contrast

### Table 3 Sensitivity analysis for heterogeneity

| MMP-9 (−1562 C/T) | Study majorly contributed to the heterogeneity | Heterogeneity after excluding the study P_{h} | I^{2}, % | Pooled ORs of the remaining studies OR 95% CI, P |
|-------------------|-----------------------------------------------|---------------------------------------------|--------|-----------------------------------------------|
| Total             |                                              |                                             |        |                                              |
| T versus C        | Li et al’s study                             | 0.000                                       | 85.2   | 0.56 (0.33, 0.97), 0.040                      |
| CT versus CC      | Li et al’s study                             | 0.013                                       | 65.4   | 0.62 (0.39, 0.98), 0.040                      |
| Dominant genetic model | Li et al’s study                         | 0.000                                       | 82.9   | 0.54 (0.29, 1.01), 0.054                      |
| Ethnicity         |                                              |                                             |        |                                              |
| Caucasian         |                                              |                                             |        |                                              |
| T versus C        | Keles et al’s study                          | 0.440                                       | 0.0    | 0.75 (0.54, 1.04), 0.080                      |
| Severity of chronic periodontitis |                                              |                                             |        |                                              |
| Severe            |                                              |                                             |        |                                              |
| T versus C        | Holla et al’s study                          | 0.003                                       | 75.2   | 0.37 (0.25, 0.54), 0.000                      |
| CT versus CC      | Holla et al’s study                          | 0.108                                       | 47.3   | 0.36 (0.28, 0.47), 0.000                      |
| Dominant genetic mode | Holla et al’s study                      | 0.003                                       | 75.2   | 0.32 (0.19, 0.53), 0.000                      |

MMP-9: matrix metalloproteinase-9; dominant genetic model: TT + CT versus CC; P_{h}: the P value of heterogeneity; OR: odds ratio; CI: confidence interval
that the MMP-9 expression is primarily controlled at the transcriptional level, where the promoter of MMP-9 gene responds to stimuli of various cytokines and growth factors (Kondapaka et al. 1997). Furthermore, the T allele of this variant can abolish a binding site for a transcription repressor and thus change the promoter activity of MMP-9, leading to increased MMP-9 expression. Besides, an exchange of C-to-T at position −1562 can also alter the binding of a nuclear protein to this region, resulting in increased transcriptional activity in macrophages (Zhang et al. 1999). Therefore, it can be suggested that carriage of T allele may down-regulate the transcription activity and thus decreases the expression level of MMP-9 protein, which may offer a lower susceptibility of CP. In conclusion, MMP-9 promoter −1562 C/T polymorphism may be a good biomarker for diagnosis and prognosis of CP.

In the subgroup analysis by ethnicity, pooled ORs revealed that a significant association of the MMP-9 promoter −1562 C/T polymorphism with CP risk was only detected in Caucasian and Asian populations but not in mixed population. The reasonable explanation for this would be, firstly, the mixed population included not only Caucasians and Asians but also a large ethnic mixture of mestizos (white-Amerindian mix), Latin-European whites, mulattoes (black-white mix), zambos (black-Amerindian mix), blacks (Afro-Americans), and pure indigenous Amerindians (de Souza et al. 2005; Isaza-Guzman et al. 2011). Therefore, the distribution of alleles and/or genotypes is often different in different races. Secondly, distinct races having different risk alleles for a different phenotype which can exhibit different genotypic frequencies which may be influenced by sampling methods, racial and regional factors that may complicate interpretation of the results of genetic studies (Gurkan et al. 2008; Loos et al. 2005). It can also be speculated that there might be other transcription and/or activity, such as other regulatory elements, or promoter methylation (Cotignola et al. 2007) responsible for these contrasting results. Also, functionality of this polymorphism may depend on interaction with other factors such as genetics, environmental, tissue remodeling, and bacterial pathogens, to modulate susceptibility to disease causing increased MMP-9 expression (Demacq et al. 2008; Holla et al. 2006). Therefore, possible positive associations between a genetic marker and disease within one population may not necessarily be extrapolated to other populations. Based on these results, we can make a conclusion that the etiology of CP is genetically heterogeneous.

In the subgroup analysis by severity of CP, the results indicated that T allele of the MMP-9 promoter −1562 C/T polymorphism was associated with decreased susceptibility to severe CP while there was no significant association between this polymorphism and moderate CP. Our results were in accordance with the previous studies (Gurkan et al. 2008; Keles et al. 2006); however, more convincing evidence, such as larger sample size and number of studies, is still required to draw a more substantial conclusion. In combination with our results of the overall analysis, this subgroup analysis may lead to a
conclusion that the MMP-9 promoter −1562 C/T polymorphism not only contributes to the disease susceptibility but also the severity of CP.

Most of the evidence from our study should be considered to be stable and convincing albeit substantial heterogeneity since the heterogeneity detected in many pooled analyses did not have a significant impact on the results of our study. However, some potential limitations still exist in our study that needs to be addressed before interpreting these results. Firstly, a relatively large heterogeneity was evident in this meta-analysis. Nevertheless, through stratified analysis by ethnicity and severity of CP, heterogeneity reduced significantly. As a result, we presumed that relatively large heterogeneity mainly results from differences in ethnicity and severity of CP. Secondly, our overall outcomes were based on individual unadjusted ORs. A more precise analysis would have been conducted by adjustment of other covariates, such as age, gender, smoking status, environmental factors, and etc. thereby avoiding serious confounding bias. Accordingly, control group individuals might develop CP later within the age range, if unmatched by age and gender, as seen in some studies (de Souza et al. 2005; Li et al. 2012) hence underestimating the OR association with the genotype. Moreover, further evaluation of potential interactions between gene-to-environment, different polymorphic loci of the same gene, and even gene-to-gene, was limited due to unavailability of original data of the included studies, all of which may modulate CP risk. Finally, in our meta-analysis, a relatively small number of studies and sample size was used. Also, our meta-analysis included only those studies which were published in English. Therefore, it is possible that some relevant studies published in other languages were not included, which might introduce publication bias. The results for publication bias were statistically insignificant in our study though.

In conclusion, our meta-analysis showed that T allele in the MMP-9 promoter −1562 C/T polymorphism might be a protective factor for CP, especially in Caucasian and Asian populations. Moreover, there was a significant association of the MMP-9 promoter −1562 C/T polymorphism with decreased susceptibility to severe CP, while there was no association seen between the allelic and/or genotype distributions of this polymorphism and moderate CP. However, more studies of high quality with larger sample sizes and also other ethnic populations are still needed to draw a more solid inference on the relation between this polymorphism and CP risk.

Authors’ contributions
DHA: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, critical revision of the article. PS: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, critical revision of the article. YZ: construction of 3D model, drafting the article, critical revision of the article, final approval of the version to be published. SJ: critical revision of the article, final approval of the version to be published. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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Additional file

Additional file 1: Appendix S1. The Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement of our study.
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