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# *BMP4* rs17563 polymorphism and nonsyndromic cleft lip with or without cleft palate

## A meta-analysis

Yue-Hua Li, MD[a], Jiaomei Yang, PhD[b], Ju-Lei Zhang, PhD[a], Jia-Qi Liu, MD, PhD[a], Zhao Zheng, MD, PhD[a,*], Da-Hai Hu, MD, PhD[a,*]

**Abstract**

**Background:** Previous studies have investigated the relationship between human bone morphogenetic protein 4 gene (*BMP4*) rs17563 polymorphism and nonsyndromic cleft lip with or without cleft palate (NSCL/P). However, the results remained inconsistent. Therefore, we conducted a meta-analysis to assess the effect of *BMP4* rs17563 polymorphism on NSCL/P.

**Methods:** Electronic searches in 5 databases were conducted to select all eligible studies up to March 2017. Odds ratios (ORs) with the corresponding 95% confidence intervals (CIs) were calculated to estimate the association. Sensitivity analysis was performed to evaluate the results stability by excluding each study in turn. Publication bias was assessed by Begg funnel plots and Egger test.

**Results:** A total of 11 case–control studies were included in the meta-analysis. The pooled frequency of the minor allele C for *BMP4* rs17563 was lower in Asians (pooled frequency = 0.33, 95% CI: 0.29–0.37) than in Brazilian population (pooled frequency = 0.47, 95% CI: 0.40–0.54). The overall results showed no significant association of *BMP4* rs17563 polymorphism with NSCL/P risk. However, the results turned out to be different when stratified by ethnicity. *BMP4* rs17563 polymorphism was associated with a higher risk of NSCL/P among Asian ethnicity (C vs T: OR = 1.33, 95% CI: 1.02–1.73; CC vs TT: OR = 2.10, 95% CI: 1.28–3.43; CC vs TT + TC: OR = 2.16, 95% CI: 1.34–3.47) and among Caucasian population (TC vs TT: OR = 3.36, 95% CI: 2.03–5.54; TC + CC vs TT: OR = 3.71, 95% CI: 2.43–5.69). Among Brazilian population, *BMP4* rs17563 polymorphism exerted a significantly protective effect on NSCL/P (C vs T: OR = 0.70, 95% CI: 0.58–0.84; CC vs TT: OR = 0.54, 95% CI: 0.33–0.88; TC vs TT: OR = 0.55, 95% CI: 0.44–0.69; TC + CC vs TT: OR = 0.56, 95% CI: 0.45–0.69).

**Conclusion:** The results suggest that the C allele of *BMP4* rs17563 may be a risk factor for NSCL/P among Asians and Caucasians, and may be a protective factor for NSCL/P in Brazilian population. Future large-sample studies with appropriate designs among specific populations are warranted to evaluate the association.

**Abbreviations:** *BMP4* = bone morphogenetic protein 4 gene, CIs = confidence intervals, HWE = Hardy–Weinberg equilibrium, MAF = minor allele frequency, NSCL/P = nonsyndromic cleft lip with or without cleft palate, ORs = odds ratios, SNPs = single nucleotide polymorphisms.

**Keywords:** *BMP4*, meta-analysis, nonsyndromic cleft lip with or without cleft palate, polymorphism, rs17563

## 1. Introduction

Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is among the most common human congenital abnormalities worldwide. The live-birth prevalence of NSCL/P varied by geographic origin and ethnic groups, with 1/500 in Asians and American Indians, 1/1000 in European populations, and 1/2500 in African descent.[1] NSCL/P can cause poor health outcomes because of the effects on speaking, feeding, hearing, appearance, and social integration. Although rehabilitation can be achieved to various degrees through surgery, dental treatment, and psychosocial intervention, NSCL/P inevitably poses a great burden to the families and society.[2]

It has been suggested that both genetic predisposition and environmental exposures may contribute to the occurrence of NSCL/P. However, the molecular mechanisms remain poorly understood. Several potentially causal genes for NSCL/P have been identified over the past years, including *TGF-α*, *IRF6*, *MTHFR*, and *MSX1*.[3–6]

The bone morphogenetic protein 4 gene (*BMP4*) is among the strong candidate genes for NSCL/P risk.[7] In a *BMP4* knockout

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*Y-HL and JY contributed equally to this work.*

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*[a] Department of Burns and Cutaneous Surgery, Xijing Hospital, The Fourth Military Medical University, [b] Department of Epidemiology and Health Statistics, School of Public Health, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi, China.*

*[*] Correspondence: Zhao Zheng, Department of Burns and Cutaneous Surgery, Xijing Hospital, The Fourth Military Medical University, Xi'an, Shaanxi, China (e-mail: zhengzhao123@msn.com); Da-Hai Hu, Department of Burns and Cutaneous Surgery, Xijing Hospital, The Fourth Military Medical University, Xi'an, Shaanxi, China (e-mail: xjburnlab@126.com).*



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mouse model, a CL/P phenotype was observed in embryos, indicating a role for the *BMP* signal pathway in lip and palate fusion.[8] Animal studies also showed that chick embryos deficient in *BMP4* exhibited craniofacial malformations, indicating the importance of *BMP4* for craniofacial development.[9] Moreover, *BMP4* overexpression was observed in maxillary prominence of mouse embryon, which suggested an important function of *BMP4* in mediating lip fusion.[10]

The *BMP4* T538C (rs17563) polymorphism is among the most functional single nucleotide polymorphisms (SNPs) in *BMP4*. The T→C sequence variation at 538 nucleotide position results in an amino acid change of Val/Ala (V152A) in the polypeptide. Several studies have focus on the effect of *BMP4* rs17563 polymorphism on NSCL/P risk among humans, and their results were later summarized by a meta-analysis in 2014.[11] However, in this previous meta-analysis, the number of included publications is limited (only 6) and the association among ethnic groups other than Chinese and Brazilian populations remains unknown. Moreover, more relevant human genetic association studies have been published among different populations since then, and the association results are not consistent.[12–15] We, therefore, conducted an updated meta-analysis to assess the association between *BMP4* rs17563 polymorphism and NSCL/P risk. The allele frequencies of *BMP4* rs17563 among groups were also pooled in the current meta-analysis, which, to our knowledge, have not been investigated before.

## 2. Methods

### 2.1. Literature search strategy

Electronic searches in the PubMed, Embase, China National Knowledge Infrastructure, Wanfang, and China Biology Medicine databases were conducted up to March 2017. The following search terms were used: ("cleft lip" or "cleft palate" or "orofacial cleft" or "oral cleft") and ("*BMP4*" or "bone morphogenetic protein 4"). The reference lists from reviews and retrieved studies were also scanned to find potential publications. Only articles in English or Chinese were included.

### 2.2. Eligibility criteria

Two reviewers (Y-HL and JY) independently excluded irrelevant studies by scanning all titles and abstracts of the retrieved articles, and they further independently read the full texts to select articles that met the eligibility criteria. Discrepancies were resolved by discussion with the third reviewer (ZZ). A study was included if it met the following inclusion criteria: case–control or cohort design; the outcome of interest was NSCL/P; the studied polymorphisms included *BMP4* rs17563; sufficient data of genotype distributions to calculate the odds ratios (ORs) and 95% confidence intervals (CIs). Articles without sufficient data of genotype distributions were excluded if authors did not provide information after 3 contacts. Animal studies and reviews were not included. Case-only and family-based studies were also removed. If multiple articles with similar or overlapped data were present, only the study with the most comprehensive information, such as the largest population or the longest study period, was included.

### 2.3. Data extraction

Data extraction was conducted independently by 2 reviewers (Y-HL and JY) through a standardized form. The following information was extracted from the included studies: first author's surname, publication year, study type, study country, ethnicity, source of controls, sample size, genotyping method, and the genotype distributions in cases and controls. Disagreements between the 2 reviewers were settled by discussion with the third reviewer (DH).

### 2.4. Statistical analysis

The Hardy–Weinberg equilibrium (HWE) in the control group was calculated by Chi-square test, and $P < .05$ was considered as a significant deviation from HWE. The allele frequencies were assessed in each study, and pooled separately by ethnicity and disease groups. The strength of the association between *BMP4* rs17563 polymorphism and NSCL/P was evaluated by crude ORs with 95% CIs under the following 5 genetic models: the allele model (C vs T), the homozygote model (CC vs TT), the heterozygote model (TC vs TT), the dominant model (TC + CC vs TT), and the recessive model (CC vs TT + TC). The significance of the pooled OR was determined by the $Z$ test, with $P < .05$ regarded statistically significant. Heterogeneity across studies was assessed using both the Cochrane $Q$ test and $I^2$ statistic.[16] $P < .1$ or $I^2 > 50\%$ was suggestive of statistically significant heterogeneity. A random-effects model (DerSimonian and Laird method) was used when significant heterogeneity existed; otherwise, a fixed-effects model (Mantel–Haenszel method) was applied.

Subgroup analysis was conducted according to ethnicity, source of controls, and genotyping method. Sensitivity analysis was performed to evaluate the results stability by excluding each study in turn. In addition, publication bias was assessed by Begg funnel plots[17] and Egger test[18] at the $P < .05$ level of significance. All statistical analyses were conducted using STATA version 12.0 (Stata Corp, College Station, TX).

## 3. Results

### 3.1. Study characteristics

The flow chart of study selection is displayed in Fig. 1. Among the 68 citations retrieved from the electronic databases, 14 had the potential to be included after screening the titles or abstracts. Three articles of these were further excluded after reviewing the full texts because 2 articles did not investigate the *BMP4* rs17563 mutation and one study did not provide sufficient data even after our 3 contacts. One study by de Araujo et al[14] had insufficient data but the author provided additional data after our request. Thus, a total of 11 eligible publications were finally included in the present meta-analysis.[13–15,19–26] The characteristics of the included studies are presented in Table 1. Of these studies, seven were hospital-based case–control designs, and 4 were population-based case–control designs. There were 6 studies conducted in Asians, 2 in Caucasians, and 3 in mixed population. The mixed population in the present study refers to the Brazilian population, which include European, African, Asian, American Indian, and their mixed descendants. Two genotyping methods including polymerase chain reaction–restriction fragment length polymorphism and TaqMan were utilized in the studies. The included studies had a total of 1633 cases and 1992 controls. The genotype distribution in the control group was consistent with HWE in all studies except for one.[13]

### 3.2. Meta-analysis results

According to the allele number in groups, the pooled minor allele frequency (MAF) of the C allele in control group were

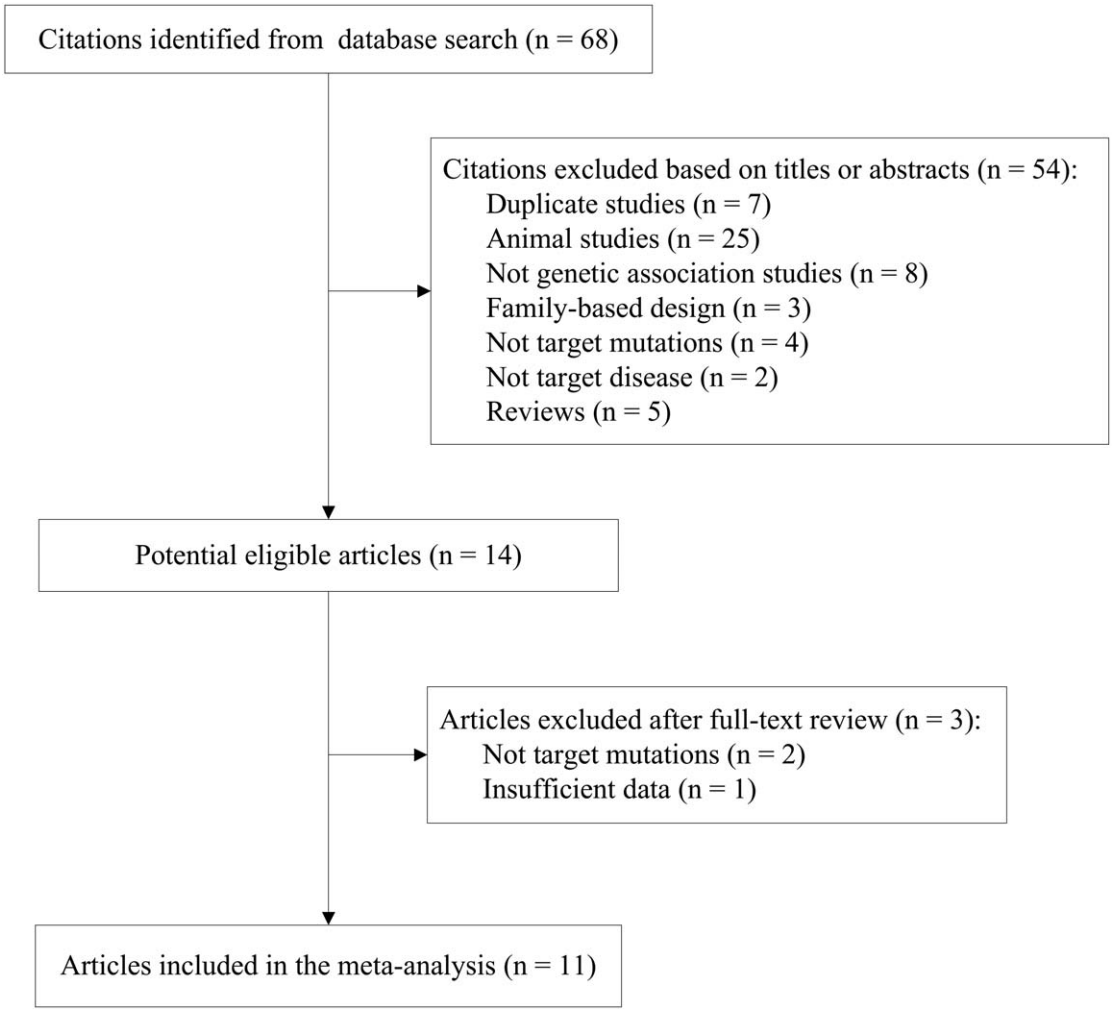


**Figure 1.** Flow diagram of study selection.

0.33 (95% CI: 0.29–0.37) in Asians, 0.34 (95% CI: 0.17–0.51) in Caucasians, and 0.47 (95% CI: 0.40–0.54) in mixed population (Table 2), indicating a lower MAF C in Asians than in mixed population among normal people. The pooled MAF of the C allele in NSCL/P group were 0.40 (95% CI: 0.33–0.47) in Asians, 0.55 (95% CI: 0.46–0.63) in Caucasians, and 0.37 (95% CI: 0.34–0.40) in mixed population (Table 2), which suggested a higher MAF C in Caucasians than in mixed population among NSCL/P patients.

**Table 1**

**Characteristics of the studies included in the meta-analysis.**

| First author, year | Country | Ethnicity | Source of controls | Genotyping method | Study size | | Genotype distribution (TT/TC/CC) | | *P* for HWE in controls |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | In cases | In controls | In cases | In controls | |
| Lin et al | China | Asian | Hospital-based | PCR-RFLP | 200 | 200 | 74/90/36 | 89/94/17 | .26 |
| Lu[20] | China | Asian | Hospital-based | PCR-RFLP | 40 | 40 | 12/17/11 | 10/25/5 | .09 |
| Araujo et al[21] | Brazil | Mixed | Population-based | PCR-RFLP | 123 | 246 | 49/53/21 | 52/130/46 | .35 |
| Wang et al[22] | China | Asian | Hospital-based | PCR-RFLP | 65 | 65 | 21/32/12 | 32/28/5 | .74 |
| Antunes et al[23] | Brazil | Mixed | Hospital-based | TaqMan | 382 | 436 | 176/147/59 | 150/224/62 | .14 |
| Hao[24] | China | Asian | Hospital-based | PCR-RFLP | 165 | 52 | 91/61/13 | 20/27/5 | .34 |
| You et al[25] | China | Asian | Population-based | PCR-RFLP | 116 | 123 | 40/40/36 | 46/66/11 | .06 |
| Jin et al[26] | China | Asian | Hospital-based | PCR-RFLP | 154 | 190 | 66/70/18 | 104/69/17 | .26 |
| Savitha et al[13] | India | Caucasian | Hospital-based | PCR-RFLP | 100 | 100 | 32/18/50 | 68/13/19 | <.001 |
| de Araujo et al[14] | Brazil | Mixed | Population-based | TaqMan | 182 | 354 | 70/81/31 | 95/173/86 | .68 |
| Saket et al[15] | Iran | Caucasian | Population-based | PCR-RFLP | 106 | 186 | 16/74/16 | 65/83/38 | .23 |

HWE = Hardy–Weinberg equilibrium, PCR-RFLP = polymerase chain reaction–restriction fragment length polymorphism.

**Table 2**

**Estimated pooled frequencies of minor and major alleles for *BMP4* rs17563 by ethnicity and disease groups in the meta-analysis.**

| | In control group | | | | | In NSCL/P group | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. of allele | C allele | | T allele | | No. of allele | C allele | | T allele | |
| First author, Year | | Number | Frequency | Number | Frequency | | Number | Frequency | Number | Frequency |
| Asian | | | | | | | | | | |
| Lin et al | 400 | 128 | 0.32 | 272 | 0.68 | 400 | 162 | 0.41 | 238 | 0.60 |
| Lu[20] | 80 | 35 | 0.44 | 45 | 0.56 | 80 | 39 | 0.49 | 41 | 0.51 |
| Wang et al[22] | 130 | 38 | 0.29 | 92 | 0.71 | 130 | 56 | 0.43 | 74 | 0.57 |
| Hao[24]' | 104 | 37 | 0.36 | 67 | 0.64 | 330 | 87 | 0.26 | 243 | 0.74 |
| You et al[25] | 246 | 88 | 0.36 | 158 | 0.64 | 232 | 112 | 0.48 | 120 | 0.52 |
| Jin et al[26] | 400 | 128 | 0.32 | 272 | 0.68 | 400 | 162 | 0.41 | 238 | 0.60 |
| Pooled frequency (95% CI) | | 0.33 (0.29–0.37) | | 0.67 (0.63–0.71) | | | 0.40 (0.33–0.47) | | | |
| Caucasian | | | | | | | | | | |
| Savitha et al[13] | 200 | 51 | 0.26 | 149 | 0.75 | 200 | 118 | 0.59 | 82 | 0.41 |
| Saket et al[15] | 372 | 159 | 0.43 | 213 | 0.57 | 212 | 106 | 0.50 | 106 | 0.50 |
| Pooled frequency (95% CI) | | 0.34 (0.17–0.51) | | 0.66 (0.49–0.83) | | | 0.55 (0.46–0.63) | | | |
| Mixed ethnicity | | | | | | | | | | |
| Araujo et al[21] | 492 | 258 | 0.52 | 234 | 0.48 | 246 | 95 | 0.39 | 151 | 0.61 |
| Antunes et al[23] | 872 | 348 | 0.40 | 524 | 0.60 | 764 | 265 | 0.35 | 499 | 0.65 |
| Araujo et al[14] | 708 | 345 | 0.49 | 363 | 0.51 | 364 | 143 | 0.39 | 221 | 0.61 |
| Pooled frequency (95% CI) | | 0.47 (0.40–0.54) | | 0.53 (0.46–0.61) | | | 0.37 (0.34–0.40) | | 0.63 (0.60–0.66) | |

CI = confidence interval, NSCL/P = nonsyndromic cleft lip with or without cleft palate.

The overall results suggested no association of *BMP4* rs17563 polymorphism with NSCL/P risk, and significant heterogeneity existed in all genetic models (Table 3). However, the results turned out to be different when stratified by ethnicity. Among Asian ethnicity, *BMP4* rs17563 polymorphism was associated with a higher risk of NSCL/P (C vs T: OR = 1.33, 95% CI: 1.02–1.73; CC vs TT: OR = 2.10, 95% CI: 1.28–3.43; CC vs TT + TC: OR = 2.16, 95% CI: 1.34–3.47), and no significant heterogeneity was observed under the homozygote model ($I^2$ = 42.2%, $P$ = .12). Among Caucasian population, *BMP4* rs17563 polymorphism may contribute to an increased risk of NSCL/P (TC vs TT: OR = 3.36, 95% CI: 2.03–5.54, Fig. 2; TC + CC vs TT: OR = 3.71, 95% CI: 2.43–5.69), and heterogeneity was absent under the heterozygote model ($I^2$ = 0%, $P$ = .70) and dominant model ($I^2$ = 0%, $P$ = .36). In contrast, among Brazilian population, *BMP4* rs17563 polymorphism exerted a significantly protective effect on NSCL/P (C vs T: OR = 0.70, 95% CI: 0.58–0.84; CC vs TT: OR = 0.54, 95% CI: 0.33–0.88; TC vs TT: OR = 0.55, 95% CI: 0.44–0.69, Fig. 2; TC + CC vs TT: OR = 0.56, 95% CI: 0.45–0.69), and heterogeneity was not statistically significant under the allele model ($I^2$ = 39.9%, $P$ = .19), heterozygote model ($I^2$ = 0%, $P$ = .51), or dominant model ($I^2$ = 12.0%, $P$ = .32). Subgroup analysis by source of controls yielded a significant association in hospital-based studies under the recessive model (CC vs TT + TC: OR = 1.87, 95% CI: 1.17–3.00), while no associations were observed in population-based studies under any genetic models. When stratified by genotyping type, significant protective effect of *BMP4* rs17563 polymorphism on NSCL/P was found in TaqMan subgroup (C vs T: OR = 0.75, 95% CI: 0.64–0.88; TC vs TT: OR = 0.59, 95% CI: 0.46–0.75; TC + CC vs TT: OR = 0.60, 95% CI: 0.48–0.76), and heterogeneity was absent under the 3 genetic models (all $I^2$ = 0%). In fact, these 2 studies using TaqMan genotyping method were both conducted in Brazilian population.

### 3.3. Sensitivity analysis

The association between *BMP4* rs17563 polymorphism and NSCL/P risk did not materially alter after excluding each study in turn under any genetic models. Although the genotype distribution in one included study was not in HWE, the pooled ORs did not qualitatively change when it was removed. The sensitivity analysis revealed the statistical robustness of our results.

**Table 3**

**Summary results of the association between *BMP4* rs17563 polymorphism and NSCL/P risk in the meta-analysis.**

| | | C vs T | | | CC vs TT | | | TC vs TT | | | TC + CC vs TT | | | CC vs TT + TC | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | OR (95% CI) | $I^2$ (%) | $P_h$ | OR (95% CI) | $I^2$ (%) | $P_h$ | OR (95% CI) | $I^2$ (%) | $P_h$ | OR (95% CI) | $I^2$ (%) | $P_h$ | OR (95% CI) | $I^2$ (%) | $P_h$ |
| Overall | 11 | 1.20 (0.87–1.65) | 89.8 | <.001 | 1.45 (0.82–2.57) | 85.7 | <.001 | 0.98 (0.66–1.46) | 83.8 | <.001 | 1.12 (0.73–1.73) | 88.3 | <.001 | 1.47 (0.93–2.32) | 81.5 | <.001 |
| Ethnicity | | | | | | | | | | | | | | | | |
| Asian | 6 | **1.33 (1.02–1.73)** | 59.7 | .030 | **2.10 (1.28–3.43)** | 42.2 | .124 | 0.98 (0.65–1.46) | 62.7 | .020 | 1.18 (0.82–1.69) | 58.4 | .035 | **2.16 (1.34–3.47)** | 46.6 | .095 |
| Caucasian | 2 | 2.36 (0.77–7.22) | 94.1 | <.001 | 3.16 (0.99–10.07) | 79.7 | .027 | **3.36 (2.03–5.54)** | 0 | .695 | **3.71 (2.43–5.69)** | 0 | .356 | 1.72 (0.29–10.21) | 93.6 | <.001 |
| Mixed | 3 | **0.70 (0.58–0.84)** | 39.9 | .189 | **0.54 (0.33–0.88)** | 63.2 | .066 | **0.55 (0.44–0.69)** | 0 | .505 | **0.56 (0.45–0.69)** | 12.0 | .321 | 0.77 (0.51–1.16) | 58.4 | .091 |
| Source of controls | | | | | | | | | | | | | | | | |
| Hospital-based | 7 | 1.38 (0.89–2.13) | 90.1 | <.001 | 1.85 (0.96–3.58) | 80.3 | <.001 | 1.05 (0.65–1.69) | 81.1 | <.001 | 1.26 (0.72–2.19) | 88.3 | <.001 | **1.87 (1.17–3.00)** | 65.8 | .008 |
| Population-based | 4 | 0.96 (0.59–1.55) | 89.7 | <.001 | 0.99 (0.36–2.77) | 894 | <.001 | 0.90 (0.40–2.02) | 89.8 | <.001 | 0.93 (0.43–2.02) | 90.2 | <.001 | 1.01 (0.44–2.31) | 87.5 | <.001 |
| Genotyping method | | | | | | | | | | | | | | | | |
| PCR-RFLP | 9 | 1.36 (0.93–1.97) | 88.4 | <.001 | 1.81 (0.93–3.51) | 82.9 | <.001 | 1.12 (0.69–1.82) | 82.6 | <.001 | 1.31 (0.79–2.18) | 86.6 | <.001 | 1.71 (0.97–3.02) | 80.7 | <.001 |
| TaqMan | 2 | **0.75 (0.64–0.88)** | 0 | .334 | 0.64 (0.39–1.06) | 55.4 | .134 | **0.59 (0.46–0.75)** | 0 | .621 | **0.60 (0.48–0.76)** | 0 | .852 | 0.85 (0.50–1.45) | 68.5 | .075 |

*BMP4* = bone morphogenetic protein 4 gene, CI = confidence interval, N = number of studies involved, NSCL/P = nonsyndromic cleft lip with or without cleft palate, OR = odds ratio, PCR-RFLP = polymerase chain reaction–restriction fragment length polymorphism, $P_h$ = *P*-value for heterogeneity.

Bold values indicated statistically significant.

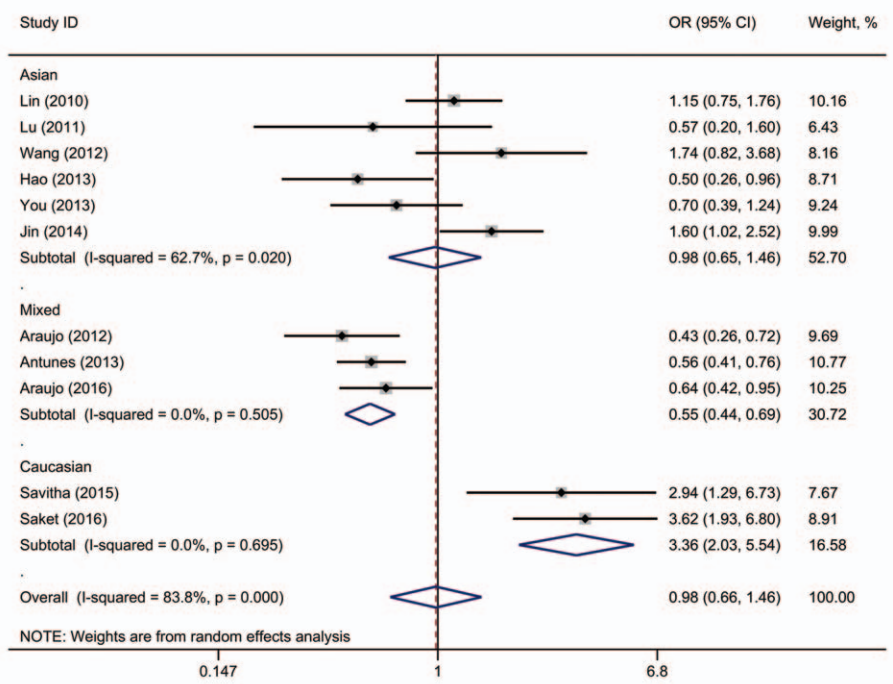


**Figure 2.** Forest plot of the association between *BMP4* rs17563 polymorphism and NSCL/P risk under the heterozygote model (TC vs TT).

### 3.4. Publication bias

No publication bias was detected for the association of *BMP4* rs17563 polymorphisms with NSCL/P risk according to the Egger test ($P = .14$ for C vs T, $P = .21$ for CC vs TT, $P = .22$ for TC vs TT, $P = .21$ for TC + CC vs TT, and $P = .14$ for CC vs TT + TC), and the funnel plots seemed no evidence of obviously asymmetrical (Fig. 3 for the heterozygote model).

## 4. Discussion

In the present study, we conducted an updated meta-analysis to assess the effect of *BMP4* rs17563 polymorphism on NSCL/P risk. The MAF of the C allele for *BMP4* rs17563 was lower in Asians than in Brazilian population. The pooled results showed no association of *BMP4* rs17563 polymorphism with NSCL/P risk. However, the subgroup analysis by ethnicity suggested that the C allele of *BMP4* rs17563 may be a risk factor for NSCL/P in Asians and Caucasians, while a protective effect of the *BMP4* rs17563 C allele on NSCL/P was found in Brazilian population.

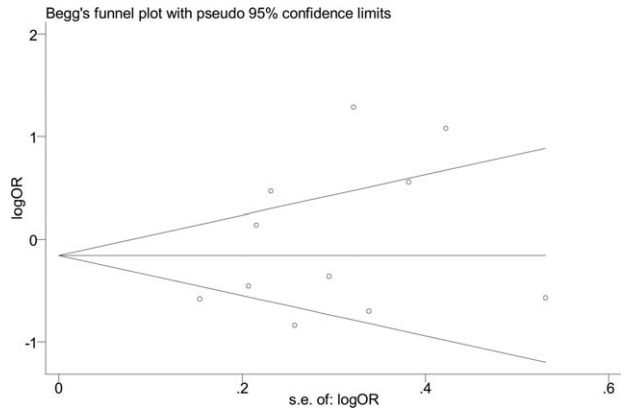


**Figure 3.** Funnel plot of the association between *BMP4* rs17563 polymorphism and NSCL/P risk under the heterozygote model (TC vs TT).

Our overall results and the results of subgroup analysis on Asians and Brazilian population are consistent with the previous meta-analysis, which included a total of 6 studies conducted in Chinese and Brazilian populations.[11] We confirmed the previous findings by expanding the number of included studies to 11 with a larger sample size. Not only was the effect of *BMP4* rs17563 polymorphism on NSCL/P among Caucasians evaluated, but also the pooled allele frequencies among groups were additionally provided in the current meta-analysis.

Development of the lip and palate occurs in early pregnancy and entails a complex process of cell growth, differentiation, migration, and apoptosis.[1] Evidence suggests that both genetic and nongenetic factors are involved in the etiology of NSCL/P. Environmental risk factors, maternal smoking, alcohol drinking, and poor nutrition during the periconceptional period may increase the risk of NSCL/P.[1] Genetic studies of NSCL/P have identified several causative genes including *TGF-α*, *IRF6*, *MTHFR*, and *MSX1*, although the results differ among populations. Moreover, gene–environment interactions have been investigated as important factors for NSCL/P etiology. For example, the study by Lin et al reported a synergistic effect between *BMP4* rs17563 variation and maternal passive smoking.

*BMP4*, a member of the transforming growth factor-beta superfamily, has distinct functions in embryonic development, including craniofacial development.[9] The presence of a CL/P phenotype in *BMP4* knockout mice revealed the role of *BMP4* in lip and palate fusion.[8] Chick embryos deficient in *BMP4* also exhibited craniofacial malformations.[9] Moreover, experimental research showed that *BMP4* could be overexpressed in the maxillary prominence,[10] which formed the lateral parts of the upper lip and the secondary palate.[27] These studies suggest that *BMP4* is a strong candidate gene for NSCL/P.

*BMP4* rs17563 polymorphism, resulting in an amino acid change of Val/Ala (V152A) in the polypeptide, is one of the most functional SNP in *BMP4*. Since it was first reported as a promising candidate SNP of human *BMP4*,[28] more and more researches have focused on it. The candidate gene association study on *BMP4* rs17563 mutation and NSCL/P risk was first reported in 2008,[29] in which a higher risk of NSCL/P was observed in 538C carriers compared with the noncarriers. More studies conducted in different populations have reported the effect of *BMP4* rs17563 polymorphism on NSCL/P since then; however, the results were inconsistent. In the current meta-analysis, we found that the C allele at *BMP4* rs17563 may increase the risk of NSCL/P in Asians and Caucasians, while a protective effect of the *BMP4* rs17563 C allele on NSCL/P was observed in Brazilian population. The different results suggested that *BMP4* rs17563 polymorphism contributed differently to NSCL/P risk among ethnicities, which may be partly due to the ethnic variations in genetic backgrounds and environmental factors.

Some limitations of our study should be acknowledged. First, publication bias may exist because only published articles in English and Chinese were included, although the Egger tests and funnel plots did not show it. Second, the sources of controls among studies were not consistent. Some studies were population-based designs, while others were hospital-based designs. The hospital-based controls may not be representative of the general population because of the potential selection bias. Third, the sample size was still relatively small to reveal the exact effect of *BMP4* rs17563 polymorphism on NSCL/P risk. Fourth, our results were based on single-factor estimates. Maternal risk factors for NSCL/P such as maternal smoking, alcohol drinking, and environmental exposure could not be adjusted because most included articles did not consider them and no sufficient data were present. Gene–-environment and gene–gene interactions were also not taken into account in the present study for the same reason.

## 5. Conclusion

In conclusion, our meta-analysis suggests that the C allele of *BMP4* rs17563 may be associated with an increased risk of NSCL/P among Asians and Caucasians. However, the *BMP4* rs17563 C allele may be a protective factor for NSCL/P in Brazilian population. In the future, large-sample studies with appropriate designs are warranted to evaluate the association among specific populations, and assess gene–gene and gene–-environment interactions. Such studies with these efforts may contribute to our comprehensive understanding of the relationship between *BMP4* rs17563 polymorphism and NSCL/P risk.

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