Two SNPs rs1801133 and rs1801394 in folate pathway are associated with the risk of nonsyndromic cleft lip with or without cleft palate

Qiuyan Li(Former Corresponding Author)

Harbin Medical University <https://orcid.org/0000-0002-6707-0172>

Lidan Xu

Harbin Medical University

Xueyuan Jia

Harbin Medical University

Komal Saleem

Harbin Medical University

Tahir Zaib

Harbin Medical University

Wenjing Sun

Harbin Medical University

Songbin Fu(New Corresponding Author) (✉ fusb@ems.hrbmu.edu.cn)

<https://orcid.org/0000-0003-0382-9888>

Research article

Keywords: NSCL/P; SNP; MTHFR; MTRR; TCN2; BHMT; Meta-analysis

Posted Date: October 29th, 2019

DOI: <https://doi.org/10.21203/rs.2.13322/v2>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background Prenatal intake of folic acid is important for prevention of nonsyndromic cleft lip with or without cleft palate (NSCL/P). Associated genes in folate pathway are major enzymes of folic acid metabolism that is crucial for preventing birth defects. The present study aims to investigate the association between four single nucleotide polymorphisms (SNPs) in folate pathway genes and the risk of NSCL/P. **Methods** Comprehensive bioinformatics analysis was used to predict the functional pathogenicity of genetic variation. The PubMed, Embase database and Google Scholar were intensively searched by two researchers to ascertain all relevant studies. Stata 11.0 software was used to analyze the results. Subgroup analysis was carried out to assess the influence of genetic background. Sensitivity analysis, regression analysis and publication analysis were also conducted to enhance the strength of our results. **Results** It is estimated that the probability of two missense mutation rs1801133 in *MTHFR* and rs1801394 in *MTRR* are more likely to be damaging by bioinformatics analysis. A total of 34 publications were included in the present study analysis. Our results showed a significant association between rs1801133 and risk of NSCL/P in two genetic models: TT allele vs CC allele (OR=1.333 95%CI=1.062-1.674, P =0.013), and recessive model (OR=1.325 95%CI=1.075-1.634, P =0.008). A significant association between rs1801394 and the risk of NSCL/P in Asian (GG allele vs AA allele, OR=0.520 95%CI=0.321-0.841, P =0.008) was also observed. Meta-regression, sensitivity analysis, and publication bias analysis confirmed that the results of the present study were statistically significant. **Conclusions** The present study highlights two SNPs rs1801133 in *MTHFR* and rs1801394 in *MTRR*, associated with the increasing risk of NSCL/P. Further, larger studies should be performed to confirm these findings.

Background

Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is one of the most common birth defects, characterized by craniofacial abnormality due to incomplete separation between the nasal and oral cavities [1]. NSCL/P can influence the quality of life by affecting communication problems and contributing to dysphagia. Cleft lip and palate occur in approximately one in 500-700 live births worldwide. This rate varies substantially across different ethnic groups and geographical areas (http://www.who.int/oral_health/publications/factsheet/en/). Cleft lip is a hereditary disease with polygenic inheritance, however, the underlying genetic cause and fundamental molecular mechanism of the disease remains still elusive.

Although folic acid and multivitamin supplementation in prescribed period of pregnancy has been indicated as an effective method to prevent the risk of oral facial cleft. The significance of genetic variants in folate pathway and folate metabolism involved in disease pathogenesis is not clear [2, 3]. Recently, many efforts have been made to find the genetic polymorphisms in folate pathway genes such as *MTHFR*, *MTRR*, *TCN2*, and *BHMT* and their susceptibility to cleft lip [4-10]. *MTHFR* plays an important role in primary circulation of folate and catalyzing the reaction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The substrate and metabolites are important for DNA biosynthesis, cell division and process during development. Currently, there is no targeted therapy for NSCL/P patients carried with *MTHFR* mutations, while there are some reports on other genetic diseases. In 2017, Martinez Sagner *et al.* reported successful management of hereditary angioedema during pregnancy in a patient carried with heterozygous *MTHFR* mutation [11]. Lahiri *et al.* reported successful conservative treatment of myocardial infarction in a teenager carried with *MTHFR* mutation [12]. Recently, Al-Eitan *et al.* also showed that *MTHFR* polymorphism was associated with treatment response in Jordanian population with epilepsy [13]. *MTRR* and *TCN2* are essential in maintaining the levels of activated vitamin B12, and *BHMT* is vital for catalyzing betaine to dimethyl glycine (DMG), which are involved in remethylating homocysteine (Hcy) to methionine (Met) (Figure 1). The four genetic missense variations C677T in *MTHFR* (rs1801133), A66G in *MTRR* (rs1801394), C776G in *TCN2* (rs1801198), and G716A in *BHMT* (rs3733890) have influence on protein function (Table 1), and have been reported to be associated with cleft lip. However, there are different conclusions regarding the influence of these SNPs in different populations [4-10, 14-36].

Here, therefore, we conduct a systematic review and performed an updated meta-analysis of all related studies published before April 2019, to provide more precise statistical results. Our study could provide basic data for exploring effective therapeutic strategies for NSCL/P.

Methods

2.1 Literature search

The present meta-analysis was conducted according to the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis 2009 (PRISMA2009). All published studies before April 2019 were searched using the PubMed database, Embase database, and Google Scholar with the following terms: "NSCL/P", "cleft lip", "SNP", "polymorphism", "genetic", "variant", "MTHFR", "MTHFR C677T", "rs1801133", "MTRR", "MTRR A66G", "rs1801394", "TCN2", "TCN2 C776G", "rs1801198", "BHMT", and "rs3733890". Relevant references of related articles were also included.

2.2 Inclusion criteria

All studies were intensively reviewed by two researchers. Studies were included in the meta-analysis if they met the following criteria: (1) original observational study of human participants; (2) an association study between rs1801133 and/or rs1801194 and/or rs1801398 and/or rs3733890 and NSCL/P; (3) case-control study or cohort study; (4) allele data were available; (5) largest sample size or sufficient useful data were included in duplicate publications from the same population; and (6) studies were published in English.

2.3 Quality score assessment

Study quality was assessed to guarantee the strength of results and conclusions. The Newcastle-Ottawa scale (NOS) score was calculated to assess the quality of studies. A maximum of nine scores, including selection, comparability and exposure items, could be awarded [37]. Any variances in comparison were decided by a third researcher.

2.4 Data extraction

The data were extracted independently by two researchers from all included studies using an integrated and standardized form. The following information was extracted: (1) first author name; (2) publication year; (3) population ethnicity; and (4) genotype distribution.

2.5 Computational and Statistical analysis

Polyphen2, SIFT, CADD, phyloP, and LRT were used for bioinformatics prediction. The Hardy-Weinberg equilibrium (HWE) test was calculated by the chi-square test. The distribution of allelic frequencies in controls were considered to deviate from HWE when $P < 0.05$. STATA (11.0; Stata Corporation, College Station, TX, USA) software was used to calculate the results of meta-analysis. Heterogeneity across individual studies was assessed by Cochran's Q test and I^2 statistic ($P < 0.10$ and $I^2 > 50\%$ indicated evidence of heterogeneity). The fixed-effects model (Mantel-Haenszel method) was used to estimate the pooled OR when there was no evidence of the heterogeneity; otherwise, the random-effects model (DerSimonian and Laird method) was used. The odd ratios (ORs), with corresponding 95% confidence intervals (95% CIs), were calculated to assess the association between four genetic SNPs of folate pathway and NSCL/P risk. Four genetic models were performed in the present meta-analysis. Using rs1801133 C>T as an example: (1) allele model, T allele vs. C allele; (2) dominant model, (CT+TT vs. CC); (3) recessive model, (TT vs. CT+CC); and (4) genotype model, (CT vs. CC; CT vs. TT). The same genetic models were performed for "rs1801394", "rs1801198", and "rs3733890". A P value of $P < 0.05$ was established as the significant threshold for each genetic model. Two subgroups, including Caucasian and Asian, based on ethnicity were analyzed to reduce the heterogeneity and influences from the genetic background. Meta-regression and one-way sensitivity analysis were also included in the meta-analysis. Egger's regression test was used to evaluate the publication bias with quantitative analysis [38]. The trim and fill method was also used when publication bias exists.

Results

3.1 Study characteristics

Based on the search strategy, 926 publications were identified in the initial search. After evaluating the titles and abstracts, 801 publications were excluded, and 125 full-text publications were further reviewed. By applying the inclusion criteria, 34 publications were used for the final meta-analysis (Figure. 2). A total of 30 publications with 5517 cases and 7770 controls were included in the rs1801133 group; ten publications with 1767 cases and 2029 controls were included in the rs1801394 group; six publications with 1815 cases and 898 controls were included in the rs1801198 and five studies with 1253 cases and 1562 controls were included in the rs3733890 group. A total of seven studies in the control group (not excluded) were found to deviate from HWE. A total NOS assessment score of three or lower, four to six, and seven or greater were defined as low, medium and high quality, respectively. The main characteristics of the included publications are shown in Table 2.

3.2 Associations between the four SNPs of folate pathway gene and NSCL/P in the overall population

The meta-analysis results showed that there was a significant association between rs1801133 and NSCL/P risk in two genetic models: TT allele vs CC allele (OR=1.333 95%CI=1.062-1.674, $P=0.013$) and recessive model (OR=1.325 95%CI=1.075-1.634, $P=0.008$) (Table 3, Figures 3-4). There was no statistically significant association between rs1801394 of the *MTRR*, rs1801198 of the *TCN2*, rs3733890 of the *BHMT* and NSCL/P risk in the overall population (Tables 4-6).

3.3 Subgroup analysis by ethnicity stratification

To assess the role of genetic background in the meta-analysis, we carried out a subgroup analysis, where the overall population was divided into two subgroups, (i) Asian and (ii) Caucasian. The subgroup analysis showed that there was significant association between rs1801394 and NSCL/P risk in Asian (GG allele vs AA allele, OR=0.520 95%CI=0.321-0.841, $P=0.008$), but no associations in Caucasian (Table 4).

3.4 Meta-regression and influence analysis

The following covariates were considered for meta-regression: publication year, sample size and HWE in controls. The results showed that there was no influence for the four SNPs ($P > 0.05$). To identify the degree to which a single study affected the overall OR estimates, one-way sensitivity analysis was performed by repeating the meta-analysis, sequentially excluding one study at a time. The results showed that no study was found to exert an excessive influence on the pooled effect.

3.5 Publication bias

There was publication bias for rs1801133 in the Asian population in genotype model CT vs CC (Table 3). Trim and fill results showed that the adjusted risk estimate unchanged, which confirmed that the results of present study are statistically robust.

Discussion

NSCL/P is a multifactorial disease caused by genetic and environmental factors. In previous years, various genomic susceptibility regions have been identified in association studies, linkage studies, family sequencing studies, and animal experiments suggesting that gene mutations influence the development of maxillofacial area. However, the underlying biological mechanisms remain unclear.

Folic acid is an important factor that influences the metabolism and the synthesis of nucleotides and amino acids. Previous studies have suggested that folic acid plays an important role in decreasing the risk of NSCL/P [2, 3]. Folic acid metabolism is a complex process and many genes are involved in the pathway, such as *MTHFR*, *MTRR*, *TCN2*, and *BHMT*. However, there are no consistent results regarding the association between the genetic variations of these genes and NSCL/P in different populations. To clarify these inconsistent results, we carried out the meta-analysis in this study.

The present meta-analysis results demonstrated a significant association between rs1801133 and NSCL/P risk in two genetic models: TT allele vs CC allele (OR=1.333 95%CI=1.062-1.674, $P=0.013$) and recessive model (OR=1.325 95%CI=1.075-1.634, $P=0.008$). There were a significant association found between rs1801394 and NSCL/P risk in Asian (GG allele vs AA allele, OR=0.520 95%CI=0.321-0.841, $P=0.008$).

TCN2, encode transcobalamin2, transports vitamin B12 to cells, have been reported to be associated with multiple diseases, such as cancer, Alzheimer and other congenital abnormalities [39-41]. In 2006, Martinelli *et al.* found that the C776G in *TCN2* was associated with risk of cleft lip, but subsequent studies didn't get the significant results [10]. Similarly, the present study didn't find the significant association between the C776G and NSCL/P.

BHMT, a zinc dependent cytosolic enzyme, is important for homocysteine metabolism and methionine synthesis. In 2010, Mostowska *et al.* first found that rs3733890 of the *BHMT* was associated with NSCL/P, and other studies also indicated its association with coronary artery disease and neural tube defects [23]. In the present study, we found no evidence showing rs3733890 playing any significant role [23]. We inferred several factors may contribute to the result. First, we found a relative high value of heterogeneity among studies, which cause a different distribution of genotype. Second, the number of included studies and sample size are relatively small. So the subgroup analysis was not conducted based on ethnicity.

MTRR plays a vital role in functional regeneration of methionine synthase, and it may be associated with increasing the congenital heart disease risk [18]. But the meta-analysis conducted by Zhang *et al.* in 2013 and Lei *et al.* in 2018 showed no association between rs1801394 and the risk of NSCL/P [42, 43]. In the present study, we found a significant association between rs1801394 and the NSCL/P risk in Asian, but no association in Caucasian. The data from 1000 genomes and ExAC database was shown in Supplementary table 1.

MTHFR is an important enzyme in homocysteine metabolism and C677T rs1801133 is one of the most important functional polymorphisms. Prediction by bioinformatics tools showed that the change of genetic variant will influence the protein function and predispose to cause the disease (Table 1). However, the association between rs1801133 and the risk of NSCL/P is inconsistent. The allelic frequencies vary in different ethnic groups and the minor allele frequency (MAF) of *MTHFR* rs1801133 in Asian are lower than that in European and American, so it is very valuable to summarize and analyze by systematic statistical methods. In the present study, we included 30 studies including 5517 cases and 7770 controls and found T allele can increase the risk of NSCL/P.

The strength of this meta-analysis is that it expands to a large number of related studies, and the most updated publications were included. To guarantee the quality of the included studies, a strict procedure for search strategy, literature inclusion, data extraction, and quality assessment was performed, which could avoid potential influences and increase the strength of the results. Meta-regression and sensitivity analysis were also performed to strengthen the conclusions. We confirmed the previous investigation by summarizing a larger number of closely related studies.

There are some limitations in the present meta-analysis. Firstly, studies published only in English were included in the meta-analysis, and studies published in other languages were excluded. Secondly, environmental factors also contribute to NSCL/P, and in the present study, non-genetic factors and other potential interactions, including environment-gene interactions, were limited. Additionally, some potential covariates (e.g., age, sex, folate etc) were not included in the analysis due to insufficient information.

Conclusion

In this study, we successfully identified two SNPs rs1801133 in *MTHFR* and rs1801394 in *MTRR*, associated with the increasing risk of NSCL/P. Further well-designed studies are required to established these findings.

Abbreviations

HWE: Hardy-Weinberg equilibrium

NOS: Newcastle-Ottawa scale

NSCL/P: Nonsyndromic cleft lip with or without cleft palate

OR: Odd ratio

SNP: Single nucleotide polymorphism

Declarations

Availability of data and materials

All the data in the present research is contained in this manuscript

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author information

Qiuyan Li and Lidan Xu contributed equally to this work.

Affiliations

Laboratory of Medical Genetics, Harbin Medical University, Harbin, China. Key Laboratory of Preservation of Human Genetic Resources and Disease Control in China (Harbin Medical University), Ministry of Education, China.

Qiuyan Li, Lidan Xu, Xueyuan Jia, Komal Saleem, Tahir Zaib, Wenjing Sun, Songbin Fu

Editorial Department of International Journal of Genetics, Harbin Medical University, Harbin, China

Qiuyan Li

Corresponding authors

Correspondence to Songbin Fu or Wenjing Sun

Funding

This work was supported by the National Key Research and Development Program (#2016YFC1000504, to SF).

Authors' Contributions

All authors have contributed to the paper. Q.L, L.X, W.S, and S.F conceived and designed the study; Q.L and L.X, X.J, K.S, T.Z extracted and analyzed the data. Q.L, L.X, X.J, K.S, T.Z, W.S, and S.F drafted the manuscript. All authors revised and approved the final draft.

Acknowledgements

Thank you for the invaluable devotion of all participants in this study.

References

1. Zuccherro TM, Cooper ME, Maher BS, Daack-Hirsch S, Nepomuceno B, Ribeiro L, Caprau D, Christensen K, Suzuki Y, Machida J et al: Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. *N Engl J Med* 2004, 351(8):769-780.
2. Tolarova M: Periconceptional supplementation with vitamins and folic acid to prevent recurrence of cleft lip. *Lancet* 1982, 2(8291):217.
3. Wehby GL, Murray JC: Folic acid and orofacial clefts: a review of the evidence. *Oral Dis* 2010, 16(1):11-19.
4. Wang W, Jiao XH, Wang XP, Sun XY, Dong C: MTR, MTRR, and MTHFR Gene Polymorphisms and Susceptibility to Nonsyndromic Cleft Lip With or Without Cleft Palate. *Genet Test Mol Biomarkers* 2016, 20(6):297-303.
5. Aslar D, Ozdiler E, Altug AT, Tastan H: Determination of Methylenetetrahydrofolate Reductase (MTHFR) gene polymorphism in Turkish patients with nonsyndromic cleft lip and palate. *Int J Pediatr Otorhinolaryngol* 2013, 77(7):1143-1146.
6. Bezerra JF, Oliveira GH, Soares CD, Cardoso ML, Ururahy MA, Neto FP, Lima-Neto LG, Luchessi AD, Silbiger VN, Fajardo CM et al: Genetic and non-genetic factors that increase the risk of non-syndromic cleft lip and/or palate development. *Oral Dis* 2015, 21(3):393-399.
7. Waltrick-Zambuzzi M, Tannure PN, Vieira TC, Antunes LS, Romano FL, Zambuzzi WF, Granjeiro JM, Kuchler EC: Genetic Variants in Folate and Cobalamin Metabolism-Related Genes in Nonsyndromic Cleft Lip and/or Palate. *Braz Dent J* 2015, 26(6):561-565.
8. Karas Kuzelicki N, Smid A, Kek T, Eberlinc A, Gersak K, Mlinaric-Rascan I: Common polymorphism in the glycine N-methyltransferase gene as a novel risk factor for cleft lip with or without cleft palate. *Int J Oral Maxillofac Surg* 2018, 47(11):1381-1388.
9. Hu Y, Chen E, Mu Y, Li J, Chen R: BHMT gene polymorphisms as risk factors for cleft lip and cleft palate in a Chinese population. *Biomed Environ Sci* 2011, 24(2):89-93.
10. Martinelli M, Scapoli L, Palmieri A, Pezzetti F, Baciliero U, Padula E, Carinci P, Morselli PG, Carinci F: Study of four genes belonging to the folate pathway: transcobalamin 2 is involved in the onset of non-syndromic cleft lip with or without cleft palate. *Hum Mutat* 2006, 27(3):294.
11. Martinez Saguer I, Escuriola Ettingshausen C: Successful management of hereditary angioedema during pregnancy in a patient with heterozygous MTHFR mutation. *Ann Allergy Asthma Immunol* 2017, 118(6):734-735.
12. Lahiri S, Cuglievan B, Gutierrez JL, Pefkarou A: Successful conservative treatment of myocardial infarction in a teenager with MTHFR mutation. *Int J Cardiol Heart Vasc* 2017, 15:24-25.
13. Al-Eitan LN, Al-Dalalah IM, Mustafa MM, Alghamdi MA, Elshammari AK, Khreisat WH, Aljamal HA. Effects of MTHFR and ABCC2 gene polymorphisms on antiepileptic drug responsiveness in Jordanian epileptic patients. *Pharmgenomics Pers Med* 2019, 12: 87-95.

14. Brandalize AP, Bandinelli E, Borba JB, Felix TM, Roisenberg I, Schuler-Faccini L: Polymorphisms in genes MTHFR, MTR and MTRR are not risk factors for cleft lip/palate in South Brazil. *Braz J Med Biol Res* 2007, 40(6):787-791.
15. Jin LL, Chen EJ, Hou W, Liu XH, Hu Y: The Association between Folate Pathway Genes and Cleft Lip With or Without Cleft Palate in a Chinese Population. *Biomed Environ Sci* 2015, 28(2):136-139.
16. Aşlar D, Taştan H: Prevalence of MTHFR, MTR and MTRR gene polymorphisms in Turkish patients with nonsyndromic cleft lip and palate, vol. 16; 2014.
17. Wyszynski DF, Diehl SR: Infant C677T mutation in MTHFR, maternal periconceptional vitamin use, and risk of nonsyndromic cleft lip. *Am J Med Genet* 2000, 92(1):79-80.
18. Verkleij-Hagoort A, Blik J, Sayed-Tabatabaei F, Ursem N, Steegers E, Steegers-Theunissen R: Hyperhomocysteinemia and MTHFR polymorphisms in association with orofacial clefts and congenital heart defects: a meta-analysis. *Am J Med Genet A* 2007, 143A(9):952-960.
19. Sozen MA, Tolarova MM, Spritz RA: The common MTHFR C677T and A1298C variants are not associated with the risk of non-syndromic cleft lip/palate in northern Venezuela. *J Genet Genomics* 2009, 36(5):283-288.
20. Shotelersuk V, Ittiwut C, Siriwan P, Angspatt A: Maternal 677CT/1298AC genotype of the MTHFR gene as a risk factor for cleft lip. *J Med Genet* 2003, 40(5):e64.
21. Rafik A, Rachad L, Kone AS, Nadifi S: MTHFR C677T polymorphism and risk of nonsyndromic cleft lip with or without cleft palate in the Moroccan population. *Appl Clin Genet* 2019, 12:51-54.
22. Murthy J, Gurramkonda VB, Karthik N, Lakkakula BV: MTHFR C677T and A1298C polymorphisms and risk of nonsyndromic orofacial clefts in a south Indian population. *Int J Pediatr Otorhinolaryngol* 2014, 78(2):339-342.
23. Mostowska A, Hozyasz KK, Wojcicki P, Dziegielewska M, Jagodzinski PP: Associations of folate and choline metabolism gene polymorphisms with orofacial clefts. *J Med Genet* 2010, 47(12):809-815.
24. Mills JL, Molloy AM, Parle-McDermott A, Troendle JF, Brody LC, Conley MR, Cox C, Pangilinan F, Orr DJ, Earley M et al: Folate-related gene polymorphisms as risk factors for cleft lip and cleft palate. *Birth Defects Res A Clin Mol Teratol* 2008, 82(9):636-643.
25. Marini NJ, Yang W, Asrani K, Witte JS, Rine J, Lammer EJ, Shaw GM: Sequence variation in folate pathway genes and risks of human cleft lip with or without cleft palate. *Am J Med Genet A* 2016, 170(11):2777-2787.
26. Little J, Gilmour M, Mossey PA, Fitzpatrick D, Cardy A, Clayton-Smith J, Hill A, Duthie SJ, Fryer AE, Molloy AM et al: Folate and clefts of the lip and palate—a U.K.-based case-control study: Part II: Biochemical and genetic analysis. *Cleft Palate Craniofac J* 2008, 45(4):428-438.
27. Kumari P, Ali A, Sukla KK, Singh SK, Raman R: Lower incidence of nonsyndromic cleft lip with or without cleft palate in females: is homocysteine a factor? *J Biosci* 2013, 38(1):21-26.
28. Jiang C, Yin N, Zhao Z, Wu D, Wang Y, Li H, Song T: Lack of Association Between MTHFR, MTR, MTRR, and TCN2 Genes and Nonsyndromic CL+/-P in a Chinese Population: Case-Control Study and Meta-Analysis. *Cleft Palate Craniofac J* 2015, 52(5):579-587.
29. Han Y, Pan Y, Du Y, Tong N, Wang M, Zhang Z, Wan L, Wang L: Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and nonsyndromic orofacial clefts susceptibility in a southern Chinese population. *DNA Cell Biol* 2011, 30(12):1063-1068.
30. Gaspar DA, Pavanello RC, Zatz M, Passos-Bueno MR, Andre M, Steman S, Wyszynski DF, Mاتيoli SR: Role of the C677T polymorphism at the MTHFR gene on risk to nonsyndromic cleft lip with/without cleft palate: results from a case-control study in Brazil. *Am J Med Genet* 1999, 87(2):197-199.
31. Estandia-Ortega B, Velazquez-Aragon JA, Alcantara-Ortigoza MA, Reyna-Fabian ME, Villagomez-Martinez S, Gonzalez-Del Angel A: 5,10-Methylenetetrahydrofolate reductase single nucleotide polymorphisms and gene-environment interaction analysis in non-syndromic cleft lip/palate. *Eur J Oral Sci* 2014, 122(2):109-113.
32. Ebadifar A, KhorramKhorshid HR, Kamali K, Salehi Zeinabadi M, Khoshbakht T, Ameli N: Maternal Supplementary Folate Intake, Methylenetetrahydrofolate Reductase (MTHFR) C677T and A1298C Polymorphisms and the Risk of Orofacial Cleft in Iranian Children. *Avicenna J Med Biotechnol* 2015, 7(2):80-84.
33. Chevrier C, Perret C, Bahuau M, Zhu H, Nelva A, Herman C, Francannet C, Robert-Gnansia E, Finnell RH, Cordier S: Fetal and maternal MTHFR C677T genotype, maternal folate intake and the risk of nonsyndromic oral clefts. *Am J Med Genet A* 2007, 143A(3):248-257.
34. Ali A, Singh SK, Raman R: MTHFR 677TT alone and IRF6 820GG together with MTHFR 677CT, but not MTHFR A1298C, are risks for nonsyndromic cleft lip with or without cleft palate in an Indian population. *Genet Test Mol Biomarkers* 2009, 13(3):355-360.
35. Abdollahi-Fakhim S, Asghari Estiar M, Varghaei P, Alizadeh Sharafi M, Sakhinia M, Sakhinia E: Common Mutations of the Methylenetetrahydrofolate Reductase (MTHFR) Gene in Non-Syndromic Cleft Lips and Palates Children in North-West of Iran. *Iran J Otorhinolaryngol* 2015, 27(78):7-14.
36. Murthy J: Genetic variant in MTRR A66G, but not MTR A2756G, is associated with risk of non-syndromic cleft lip and palate in Indian population. *J Oral Maxillofac Surg Med Pathol* 2015.
37. Stang A: Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* 2010, 25(9):603-605.
38. Egger M, Davey Smith G, Schneider M, Minder C: Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997, 315(7109):629-634.
39. Martinelli M, Scapoli L, Mattei G, Ugolini G, Montroni I, Zattoni D, Rosati G, Solmi R: A candidate gene study of one-carbon metabolism pathway genes and colorectal cancer risk. *Br J Nutr* 2013, 109(6):984-989.
40. Oussalah A, Levy J, Filhine-Tresarrieu P, Namour F, Gueant JL: Association of TCN2 rs1801198 c.776G>C polymorphism with markers of one-carbon metabolism and related diseases: a systematic review and meta-analysis of genetic association studies. *Am J Clin Nutr* 2017, 106(4):1142-1156.
41. Pawlik P, Mostowska A, Lianeri M, Sajdak S, Kedzia H, Jagodzinski PP: Folate and choline metabolism gene variants in relation to ovarian cancer risk in the Polish population. *Mol Biol Rep* 2012, 39(5):5553-5560.

42. Zhang T, Lou J, Zhong R, Wu J, Zou L, Sun Y, Lu X, Liu L, Miao X, Xiong G: Genetic variants in the folate pathway and the risk of neural tube defects: a meta-analysis of the published literature. PLoS One 2013, 8(4):e59570.

43. Lei W, Xia Y, Wu Y, Fu G, Ren A: Associations Between MTR A2756G, MTRR A66G, and TCN2 C776G Polymorphisms and Risk of Nonsyndromic Cleft Lip With or Without Cleft Palate: A Meta-Analysis. Genet Test Mol Biomarkers 2018, 22(8):465-473.

Tables

Table 1 Information of four SNPs in the present study

SNP	Gene	Codon	Polyphen2		SIFT		CADD		PhyloP		LRT	
			score	prediction	score	prediction	score	prediction	score	prediction	score	prediction
rs1801133	<i>MTHFR</i>	C677T	0.998	probability damaging	0.027	damage	25.0	damaging	9.137	conserved	0	delete
rs1801394	<i>MTRR</i>	A66G	1	probability damaging	0.064	tolerable	23.3	damaging	0.098	nonconserved	0	delete
rs1801198	<i>TCN2</i>	C776G	0.315	benign	0.09	tolerable	18.9	tolerable	0.081	nonconserved	0.027	neutr
rs3733890	<i>BHMT</i>	G716A	0.064	benign	0.218	tolerable	21.8	damaging	2.864	conserved	0.070	neutr

Table 2 Characteristics of included studies about associations between four SNPs of folate pathway gene and NSCL/P

Study	Year	Ethnicity	Genotype in case					Genotype in control					P value of HWE test	NOS score
<i>MTHFR</i> rs1801133			Total	CC	CT	TT		Total	CC	CT	TT			
Law et al.	1998	Caucasian	310	143	127	40	0.334	383	156	178	49	0.360	0.873	6
Polarova et al.	1998	Caucasian	111	43	49	19	0.392	106	46	52	8	0.321	0.195	6
Aspar et al.	1999	Caucasian	77	30	39	8	0.357	103	49	49	5	0.286	0.096	6
Wyszyński et al.	2000	Caucasian	259	114	109	36	0.349	327	129	154	44	0.370	0.854	6
Martinelli et al.	2001	Caucasian	64	22	30	12	0.422	106	46	43	17	0.363	0.205	6
Runert et al.	2002	Caucasian	66	34	26	6	0.288	184	90	69	25	0.323	0.052	6
Hotelersuk et al.	2003	Asian	109	84	25	0	0.115	202	154	46	2	0.124	0.478	6
van Rooij et al.	2003	Caucasian	105	54	45	6	0.271	128	70	54	4	0.242	0.091	6
Aspar et al.	2004	Caucasian	644	327	269	48	0.283	424	213	172	39	0.295	0.616	6
Dezzetti et al.	2004	Caucasian	110	28	58	24	0.482	289	95	151	43	0.410	0.174	6
Randalize et al.	2007	Caucasian	114	49	46	19	0.368	100	45	41	14	0.345	0.353	6
Hevrić et al.	2007	Caucasian	148	66	60	22	0.351	165	51	81	33	0.445	0.935	6
Little et al.	2008	Caucasian	96	39	47	10	0.349	224	94	101	29	0.355	0.819	6
Hills et al.	2008	Caucasian	492	217	221	54	0.334	1599	715	721	163	0.327	0.341	7
Li et al.	2009	Asian	323	225	87	11	0.169	214	176	36	2	0.093	0.916	6
Ozen et al.	2009	Caucasian	179	81	80	18	0.324	138	66	65	7	0.286	0.073	6
Łostowska et al.	2010	Caucasian	163	81	65	17	0.304	171	78	77	16	0.319	0.629	6
Badifar et al.	2010	Asian	61	21	18	22	0.508	215	114	72	29	0.302	0.003	6
Chan et al.	2011	Asian	187	46	106	35	0.471	213	74	110	29	0.394	0.236	6
Slar et al.	2013	Caucasian	80	13	57	10	0.481	125	59	62	4	0.280	0.010	6
Umari et al.	2013	Asian	467	327	125	15	0.166	469	364	100	5	0.117	0.518	6
Lurthy et al.	2014	Asian	123	104	19	0	0.077	141	107	31	3	0.131	0.672	6
Standia-Ortega et al.	2014	Caucasian	132	39	55	38	0.496	370	143	172	55	0.381	0.780	7
Yang et al.	2015	Asian	204	59	107	38	0.449	226	62	108	56	0.487	0.512	6
Rezerra et al.	2015	Caucasian	140	74	54	12	0.279	175	85	70	20	0.314	0.341	6
Abdollahi-Fakhim et al.	2015	Asian	121	38	58	25	0.446	103	27	54	22	0.476	0.605	6
Yang et al.	2016	Asian	147	28	66	53	0.585	129	19	97	13	0.477	<0.001	5
Marini et al.	2016	Caucasian	330	119	159	52	0.398	360	148	154	58	0.375	0.097	7
Maras Kuzelicki et al.	2018	Caucasian	103	45	45	13	0.345	199	85	96	18	0.332	0.214	7
Afik et al.	2019	Africa	52	44	8	0	0.077	182	97	74	11	0.264	0.526	6
<i>TRR</i> rs1801394			Total	AA	AG	GG		Total	AA	AG	GG			
Randalize et al.	2007	Caucasian	114	36	69	9	0.382	100	33	61	6	0.365	0.002	6
Łostowska et al.	2010	Caucasian	164	31	81	52	0.564	166	34	70	62	0.584	0.089	6
Slar et al.	2014	Caucasian	100	14	72	14	0.500	125	13	107	5	0.468	<0.001	6
Altrick-Zambuzzi et al.	2015	Caucasian	342	95	194	53	0.439	401	136	193	72	0.420	0.806	7
Yang et al.	2015	Asian	204	123	71	10	0.223	226	124	84	18	0.265	0.480	6
Rezerra et al.	2015	Caucasian	140	98	37	5	0.168	175	112	60	3	0.189	0.111	6
Lurthy et al.	2015	Asian	123	42	81	0	0.329	141	65	76	0	0.270	<0.001	5
Yang et al.	2016	Asian	147	71	26	50	0.429	129	29	59	41	0.547	0.380	5
Marini et al.	2016	Caucasian	330	160	134	36	0.312	367	175	161	31	0.304	0.478	7
Maras Kuzelicki et al.	2018	Caucasian	103	14	56	33	0.592	199	30	111	58	0.570	0.051	7
<i>FCN 2</i> rs1801198			Total	CC	CG	GG	AF	Total	CC	CG	GG	AF		
Martinelli et al.	2006	Caucasian	218	85	110	23	0.358	289	89	150	50	0.433	0.330	5
Hills et al.	2008	Caucasian	316	99	153	64	0.445	1097	347	532	218	0.441	0.243	7
Łostowska et al.	2010	Caucasian	163	46	88	29	0.448	181	48	103	30	0.450	0.044	6
Chan et al.	2015	Asian	429	76	215	138	0.572	461	75	231	155	0.587	0.475	5
Altrick-Zambuzzi et al.	2015	Caucasian	359	139	160	60	0.390	440	179	199	62	0.367	0.576	7
Marini et al.	2016	Caucasian	330	135	140	55	0.379	366	160	155	51	0.351	0.177	7
<i>HMT</i> rs3733890			Total	GG	GA	AA		Total	GG	GA	AA			
Łostowska et al.	2010	Caucasian	174	95	76	3	0.236	176	82	75	19	0.321	0.766	6
Gu et al.	2011	Asian	166	90	56	20	0.289	268	130	118	20	0.295	0.334	5
Chan et al.	2015	Asian	481	219	202	60	0.335	554	265	245	44	0.301	0.222	5
Marini et al.	2016	Caucasian	330	140	150	40	0.348	366	156	163	47	0.351	0.665	7
Maras Kuzelicki et al.	2018	Caucasian	102	42	51	9	0.338	198	98	84	16	0.293	0.734	7

Note: AF: allele frequency of minor allele; HWE: Hardy-Weinberg equilibrium.

Table 3 Associations between rs1801133 of *MTHFR* and NSCL/P

Genetic model	I^2 (%)	P for heterogeneity	OR (95% CI)	P value	P for publication bias	Effects model
T allele vs C allele						
Overall	73.1	0	1.111 (0.992-1.244)	0.069	0.820	random
Caucasian	58.5	0.001	1.085 (0.976-1.206)	0.131	0.361	random
Asian	19.1	0	1.244 (0.961-1.611)	0.098	0.438	random
TT vs CC						
Overall	62.6	0	1.333 (1.062-1.674)	<i>0.013</i>	0.102	random
Caucasian	56.2	0.001	1.230 (0.976-1.551)	0.080	0.239	random
Asian	70.5	0.001	1.701 (0.949-3.049)	0.075	0.365	random
CT vs CC						
Overall	57.6	0	1.026 (0.901-1.169)	0.696	0.587	random
Caucasian	40.3	0.033	1.027 (0.904-1.166)	0.686	0.287	random
Asian	62.4	0.006	1.081 (0.821-1.422)	0.580	0.041	random
Dominant model						
Overall	66.2	0	1.075 (0.936-1.234)	0.305	0.804	random
Caucasian	52.8	0.003	1.067 (0.932-1.223)	0.347	0.208	random
Asian	67.9	0.002	1.172 (0.883-1.557)	0.272	0.089	random
Recessive model						
Overall	62.5	0	1.325 (1.075-1.634)	<i>0.008</i>	0.220	random
Caucasian	40.9	0.030	1.190 (0.989-1.433)	0.066	0.434	random
Asian	79.1	0	1.737 (0.940-3.210)	0.078	0.404	random

Note: Italic indicates statistically significance

Table 4 Associations between rs1801394 of *MTRR* and NSCL/P

Genetic model	I^2 (%)	P for heterogeneity	OR (95% CI)	P value	P for publication bias	Effects model
G allele vs A allele						
Overall	36.0	0.120	0.986 (0.895-1.085)	0.766	0.713	fixed
Caucasian	0	0.938	1.037 (0.928-1.158)	0.520	0.401	fixed
Asian	77.7	0.011	0.843 (0.694-1.023)	0.084	0.480	fixed
GG vs AA						
Overall	30.7	0.172	0.977 (0.781-1.223)	0.841	0.405	fixed
Caucasian	0	0.816	1.176 (0.909-1.520)	0.217	0.218	fixed
Asian	0	0.820	0.520 (0.321-0.841)	0.008	---	fixed
AG vs AA						
Overall	78.3	0	0.879 (0.630-1.227)	0.449	0.374	random
Caucasian	31.9	0.184	1.018 (0.813-1.275)	0.877	0.443	random
Asian	93.2	0	0.644 (0.211-1.968)	0.441	0.599	random
Dominant model						
Overall	69.4	0.001	0.921 (0.704-1.205)	0.550	0.586	random
Caucasian	0	0.514	1.046 (0.886-1.236)	0.595	0.353	random
Asian	90.3	0	0.746 (0.351-1.768)	0.506	0.908	random
Recessive model						
Overall	37.8	0.117	1.032 (0.853-1.248)	0.748	0.164	fixed
Caucasian	45.1	0.090	1.062 (0.858-1.315)	0.581	0.073	fixed
Asian	39.6	0.198	0.922 (0.605-1.406)	0.707	---	fixed

Note: Italic indicates statistically significance

Table 5 Associations between rs1801198 of *TCN2* and NSCL/P

Genetic model	I^2 (%)	P for heterogeneity	OR (95% CI)	P value	P for publication bias	Effects model
C allele vs G allele	38.8	0.147	0.990 (0.907-1.080)	0.821	0.541	fixed
CC vs GG	43.4	0.116	0.987 (0.824-1.181)	0.883	0.438	fixed
GC vs GG	0	0.820	0.966 (0.840-1.112)	0.631	0.196	fixed
Dominant model	0	0.428	0.976 (0.855-1.114)	0.717	0.248	fixed
Recessive model	27.4	0.229	1.002 (0.860-1.167)	0.982	0.747	fixed

Table 6 Associations between rs3733890 of *BHMT* and NSCL/P

Genetic model	I^2 (%)	P for heterogeneity	OR (95% CI)	P value	P for publication bias	Effects model
A allele vs G allele	61.0	0.036	0.994 (0.820-1.204)	0.948	0.409	random
AA vs GG	73.6	0.004	0.993 (0.558-1.764)	0.980	0.182	random
GA vs GG	23.7	0.264	0.966 (0.801-1.164)	0.715	0.961	random
Dominant model	33.2	0.200	0.983 (0.812-1.190)	0.861	0.709	random
Recessive model	75.1	0.003	1.002 (0.569-1.767)	0.994	0.192	random

Figures

Figure 1

Folate pathway.

Figure 2

Study flow diagram.

Figure 3

Forest plot for pooled ORs for the associations between TT vs CC model of rs1801133 and NSCL/P risk. Each square is proportional to the study-specific weight.

Figure 4

Forest plot for pooled ORs for the associations between recessive model of rs1801133 and NSCL/P risk. Each square is proportional to the study-specific weight.