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

Qiuyan Li (✉ liqiuyan@hrbmu.edu.cn)

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

Lidan Xu

Harbin Medical University

Xueyuan Jia

Harbin Medical University

Tahir Zaib

Harbin Medical University

Wenjing Sun

Harbin Medical University

Songbin Fu

Harbin Medical University

Research article

Keywords: TCN2; BHMT; MTRR; MTHFR; NSCL/P; SNP

Posted Date: August 21st, 2019

DOI: <https://doi.org/10.21203/rs.2.13322/v1>

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), genes participant in folate pathway are crucial for preventing birth defects. The present study aims to investigate the associations between four single nucleotide polymorphisms (SNPs) in folate pathway genes and the risk of NSCL/P. **Methods** Prediction by bioinformatics was conducted to assess the function of genetic variation. The PubMed, Embase database and Google Scholar were searched by two researchers to identify all relevant studies. Stata 11.0 software was used to calculate the results. Subgroup analysis was conducted to assess the influence of the genetic background. Sensitivity analysis, regression analysis and publication analysis were conducted to improve the strength of the results. **Results** Two genetic variations rs1801394 in *MTRR* gene and rs1801133 in *MTHFR* were predicted damaging. A total of 34 publications were included in the present analysis. The results showed that there were 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) and there were a 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). Meta-regression, sensitivity analysis, and publication bias analysis confirmed that the results of the present study were statistically robust. **Conclusions** This present study suggests that rs1801133 of the *MTHFR* gene and rs1801394 of the *MTRR* gene are risk factors for NSCL/P. Additional, 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, such as contributing to dysphagia and communication obstacles. Cleft lip and palate occur in approximately one per 500–700 of all births. 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 fundamental molecular mechanism causing the disease remains far from clear.

Folic acid supplementation in preconceptional period has been indicated as an effective method to prevent the risk of oral facial cleft. However, the precise mechanisms remain unknown. Within the past few years, great efforts have been done to find the genetic polymorphisms in folate pathway genes and susceptibility to cleft lip, such as the *MTHFR*, *TCN2*, *BHMT*, and *MTRR* genes (2-8). *MTHFR* plays an important role in primary circulation form of folate, catalyzing the reaction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The substrate and metabolites are vital for DNA biosynthesis, cell division and process during development. *MTRR* and *TCN2* are essential in maintaining the levels of activated vitamin B12, *BHMT* is vital for betaine to dimethyl glycine (DMG), they are involved in homocysteine (Hcy) to methionine (Met) (Figure 1). The C776G in *TCN2* gene (rs1801198), the G716A in *BHMT* gene (rs3733890), the A66G in *MTRR* gene (rs1801394), and the C677T in *MTHFR* gene (rs1801133) are four missense mutations and the change of them have influence for protein function (Table 1), and they have been reported to be associated with cleft lip. However, there are different conclusions regarding the influence of these gene SNPs in different populations (2-31).

Therefore, we undertook a systematic review and performed an updated meta-analysis of all studies published before 2019, aiming to provide more precise statistical results. This could help us to understand the mechanisms of disease pathogenesis and to explore effective prevention methods and therapeutic targets in the future.

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 up until April 2019 were searched using the PubMed database, the Embase database, and Google Scholar with the following terms: “cleft lip”, “NSCL/P”, “*TCN2*”, “*BHMT*”, “*MTRR*”, “*MTHFR*”, “*TCN2* C776G”, “*MTRR* A66G”, “*MTHFR* C677T”, “SNP”, “polymorphism”, “genetic”, “variant”, “rs1801198”, “rs3733890”, “rs1801394”, and “rs1801133”. Relevant references in associated articles were also included.

2.2 Inclusion criteria

All studies were independently 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 rs1801198 and/or rs3733890 and/or rs1801394 and/or rs1801133 and cleft lip; (3) case-control study or cohort study; (4) allele data were available; (5) the 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 the studies. A maximum of nine scores, including selection, comparability and exposure items, could be awarded (32). Any disagreements were decided by a third researcher.

2.4 Data extraction

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

2.5 Statistical analysis

Polyphen2, SIFT, CADD, phyloP, and LRT were used to bioinformatics prediction. The Hardy-Weinberg equilibrium (HWE) test was calculated by the chi-squared test. The distribution of allele frequencies in controls was 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 the 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 SNPs of the folate pathway gene 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 "rs1801198", "rs3733890", and "rs1801394". A P value of $P < 0.05$ was accepted 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 publication bias with quantitative analysis (33). 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 reading 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 six publications with 1815 cases and 898 controls were included in the rs1801198; five studies with 1253 cases and 1562 controls were included in the rs3733890 analysis; ten publications with 1767 cases and 2029 controls were included in the rs1801394 analysis; and 30 publications with 5517 cases and 7770 controls were included in the rs1801133 analysis. 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 the folate pathway gene and NSCL/P in the overall population

The meta-analysis results showed that there was no statistically significant association between rs1801198 of the *TCN2* gene, rs3733890 of the *BHMT* gene, rs1801394 of the *MTRR* gene and NSCL/P risk in the overall population (Table 3, Table 4, and Table 5). 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 6, Figure 3, and Figure 4).

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, which were Asian and Caucasian. The subgroup analysis showed that there were significant association between rs1801394 and NSCL/P risk in Asian (GG allele vs AA allele, OR=0.520 95%CI=0.321-0.841, $P=0.008$), and there was no associations in Caucasian (Table 5).

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 6). Trim and fill results showed that the adjusted risk estimate unchanged, which confirmed that the results of the present study are statistically robust.

Discussion

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

Folic acid is an important factor that influences the one carbon metabolism and the synthesis of nucleotides and amino acids. Previous studies have suggested that plays an important role in decreasing the risk of NSCL/P (34,35). Folic acid metabolism is a complex process and many enzymes are involved in the process, such as *TCN2*, *BHMT*, *MTRR*, and *MTHFR*. However, there are no consistent results regarding the association between the genetic variations of these genes and cleft lip in different populations. To clarify these inconsistent results, we carried out the meta-analysis in the present 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, $P=0.008$) and there were a significant association between

rs1801394 and NSCL/P risk in Asian (GG allele vs AA allele, OR=0.520 95%CI=0.321-0.841, $P=0.008$).

TCN2 gene, encode transcobalamin2, transports vitamin B12 to cells, have been reported to be associated with multiple diseases, such as cancer, Alzheimer, congenital abnormalities. In 2006, Martinelli *et al.* found that the C776G in *TCN2* was associated with risk of cleft lip, but subsequently studies didn't get the same conclusion (8). Similarly, the present study did not found the association between it and cleft lip. 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* gene associate with NSCL/P, and other studies also indicated that rs3733890 was associated in coronary artery disease and neural tube defects, but in the present study, we found no evidence showed rs3733890 plays an important role in the present study (18). 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 the included studies and the sample size are relatively small, so the subgroup analysis didn't be conducted based on ethnicity. MTRR plays a role in functional regeneration of methionine synthase, and it may be associated with increasing the congenital heart disease risk, but the meta-analysis conducted by Zhang in 2013 and Lei in 2018 showed it has no association between rs1801394 and the risk of defects and NSCL/P (36,37). In the present study, we found a significant association between rs1801394 and the NSCL/P risk in Asian, but no association in Caucasian. Combining ExAC and gnomAD database, it founds that minor allele frequency has differences in Asian and Caucasian (about 20% vs 40%). Considering only three studies in Asian, it should be further confirmed. MTHFR is an important enzyme in homocysteine metabolism and C677T rs1801133 is one of the most important functional polymorphism. Prediction by bioinformatics 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 cleft lip is inconsistent. 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 were 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. First, only studies published in English were included in the meta-analysis, and studies published in other languages were excluded. Second, environmental factors also contribute to cleft lip, 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) were not included in the analysis due to insufficient information.

Conclusion

Our study identified that the SNPs of rs1801133 of the *MTHFR* gene and rs1801394 of the *MTRR* gene are risk factors for NSCL/P. Combining genetic loci with environmental factors may be an important prevention and therapy method for NSCL/P, and further well-designed studies are warranted to confirm these results.

Declarations

Contributors

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 extracted and analyzed the data; and Q.L, L.X, X.J, T.Z, W.S, and S.F prepared the manuscript. All authors revised and approved the final draft.

Conflict of interest

The authors declare that there is no conflict of interest associated with this manuscript.

Funding

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

References

- [1] Zuccherro TM, Cooper ME, Maher BS, et al. Interferon regulatory factor 6 (irf6) gene variants and the risk of isolated cleft lip or palate [J]. *N Engl J Med*, 2004,351(8):769-780.
- [2] Wang W, Jiao XH, Wang XP, et al. Mtr, mtrr, and mthfr gene polymorphisms and susceptibility to nonsyndromic cleft lip with or without cleft palate [J]. *Genet Test Mol Biomarkers*, 2016,20(6):297-303.
- [3] Aslar D, Ozdiler E, Altug AT, et al. Determination of methylenetetrahydrofolate reductase (mthfr) gene polymorphism in turkish patients with nonsyndromic cleft lip and palate [J]. *Int J Pediatr Otorhinolaryngol*, 2013,77(7):1143-1146.
- [4] Bezerra JF, Oliveira GH, Soares CD, et al. Genetic and non-genetic factors that increase the risk of non-syndromic cleft lip and/or palate development [J]. *Oral Dis*, 2015,21(3):393-399.

- [5] Waltrick-Zambuzzi M, Tannure PN, Vieira TC, et al. Genetic variants in folate and cobalamin metabolism-related genes in nonsyndromic cleft lip and/or palate [J]. *Braz Dent J*, 2015,26(6):561-565.
- [6] Karas Kuzelicki N, Smid A, Kek T, et al. Common polymorphism in the glycine n-methyltransferase gene as a novel risk factor for cleft lip with or without cleft palate [J]. *Int J Oral Maxillofac Surg*, 2018,47(11):1381-1388.
- [7] Hu Y, Chen E, Mu Y, et al. Bhmt gene polymorphisms as risk factors for cleft lip and cleft palate in a chinese population [J]. *Biomed Environ Sci*, 2011,24(2):89-93.
- [8] Martinelli M, Scapoli L, Palmieri A, et al. 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 [J]. *Hum Mutat*, 2006,27(3):294.
- [9] Brandalize AP, Bandinelli E, Borba JB, et al. Polymorphisms in genes mthfr, mtr and mtrr are not risk factors for cleft lip/palate in south brazil [J]. *Braz J Med Biol Res*, 2007,40(6):787-791.
- [10] Jin LL, Chen EJ, Hou W, et al. The association between folate pathway genes and cleft lip with or without cleft palate in a chinese population [J]. *Biomed Environ Sci*, 2015,28(2):136-139.
- [11] Aşlar D, Taştan H. Prevalence of mthfr, mtr and mtrr gene polymorphisms in turkish patients with nonsyndromic cleft lip and palate [M]. 2014.
- [12] Wyszynski DF, Diehl SR. Infant c677t mutation in mthfr, maternal periconceptional vitamin use, and risk of nonsyndromic cleft lip [J]. *Am J Med Genet*, 2000,92(1):79-80.
- [13] Verkleij-Hagoort A, Blik J, Sayed-Tabatabaei F, et al. Hyperhomocysteinemia and mthfr polymorphisms in association with orofacial clefts and congenital heart defects: A meta-analysis [J]. *Am J Med Genet A*, 2007,143A(9):952-960.
- [14] 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]. *J Genet Genomics*, 2009,36(5):283-288.
- [15] Shotelersuk V, Ittiwut C, Siriwan P, et al. Maternal 677ct/1298ac genotype of the mthfr gene as a risk factor for cleft lip [J]. *J Med Genet*, 2003,40(5):e64.
- [16] Rafik A, Rachad L, Kone AS, et al. Mthfr c677t polymorphism and risk of nonsyndromic cleft lip with or without cleft palate in the moroccan population [J]. *Appl Clin Genet*, 2019,12(51-54).
- [17] Murthy J, Gurramkonda VB, Karthik N, et al. Mthfr c677t and a1298c polymorphisms and risk of nonsyndromic orofacial clefts in a south indian population [J]. *Int J Pediatr Otorhinolaryngol*, 2014,78(2):339-342.
- [18] Mostowska A, Hozyasz KK, Wojcicki P, et al. Associations of folate and choline metabolism gene polymorphisms with orofacial clefts [J]. *J Med Genet*, 2010,47(12):809-815.
- [19] Mills JL, Molloy AM, Parle-McDermott A, et al. Folate-related gene polymorphisms as risk factors for cleft lip and cleft palate [J]. *Birth Defects Res A Clin Mol Teratol*, 2008,82(9):636-643.
- [20] Marini NJ, Yang W, Asrani K, et al. Sequence variation in folate pathway genes and risks of human cleft lip with or without cleft palate [J]. *Am J Med Genet A*, 2016,170(11):2777-2787.
- [21] Little J, Gilmour M, Mossey PA, et al. Folate and clefts of the lip and palate—a u.K.-based case-control study: Part ii: Biochemical and genetic analysis [J]. *Cleft Palate Craniofac J*, 2008,45(4):428-438.
- [22] Kumari P, Ali A, Sukla KK, et al. Lower incidence of nonsyndromic cleft lip with or without cleft palate in females: Is homocysteine a factor? [J]. *J Biosci*, 2013,38(1):21-26.
- [23] Jiang C, Yin N, Zhao Z, et al. Lack of association between mthfr, mtr, mtrr, and tcn2 genes and nonsyndromic cl+/p in a chinese population: Case-control study and meta-analysis [J]. *Cleft Palate Craniofac J*, 2015,52(5):579-587.
- [24] Han Y, Pan Y, Du Y, et al. Methylenetetrahydrofolate reductase c677t and a1298c polymorphisms and nonsyndromic orofacial clefts susceptibility in a southern chinese population [J]. *DNA Cell Biol*, 2011,30(12):1063-1068.
- [25] Gaspar DA, Pavanello RC, Zatz M, et al. 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 [J]. *Am J Med Genet*, 1999,87(2):197-199.
- [26] Estandia-Ortega B, Velazquez-Aragon JA, Alcantara-Ortigoza MA, et al. 5,10-methylenetetrahydrofolate reductase single nucleotide polymorphisms and gene-environment interaction analysis in non-syndromic cleft lip/palate [J]. *Eur J Oral Sci*, 2014,122(2):109-113.
- [27] Ebadifar A, KhorramKhorshid HR, Kamali K, et al. Maternal supplementary folate intake, methylenetetrahydrofolate reductase (mthfr) c677t and a1298c polymorphisms and the risk of orofacial cleft in iranian children [J]. *Avicenna J Med Biotechnol*, 2015,7(2):80-84.

[28] Chevrier C, Perret C, Bahuaui M, et al. Fetal and maternal mthfr c677t genotype, maternal folate intake and the risk of nonsyndromic oral clefts [J]. *Am J Med Genet A*, 2007,143A(3):248-257.

[29] 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 [J]. *Genet Test Mol Biomarkers*, 2009,13(3):355-360.

[30] Abdollahi-Fakhim S, Asghari Estiar M, Varghaei P, et al. Common mutations of the methylenetetrahydrofolate reductase (mthfr) gene in non-syndromic cleft lips and palates children in north-west of iran [J]. *Iran J Otorhinolaryngol*, 2015,27(78):7-14.

[31] ; MJ, Gurramkonda; VB, Lakkakula BVKS. Genetic variant in mtrr a66g, but not mtr a2756g, is associated with risk of non-syndromic cleft lip and palate in indian population [J]. 2015.

[32] Stang A. Critical evaluation of the newcastle-ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses [J]. *Eur J Epidemiol*, 2010,25(9):603-605.

[33] Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test [J]. *BMJ*, 1997,315(7109):629-634.

[34] Tolarova M. Periconceptional supplementation with vitamins and folic acid to prevent recurrence of cleft lip [J]. *Lancet*, 1982,2(8291):217.

[35] Wehby GL, Murray JC. Folic acid and orofacial clefts: A review of the evidence [J]. *Oral Dis*, 2010,16(1):11-19.

[36] Zhang T, Lou J, Zhong R, et al. Genetic variants in the folate pathway and the risk of neural tube defects: A meta-analysis of the published literature [J]. *PLoS One*, 2013,8(4):e59570.

[37] Lei W, Xia Y, Wu Y, et al. Associations between mtr a2756g, mtrr a66g, and tcn2 c776g polymorphisms and risk of nonsyndromic cleft lip with or without cleft palate: A meta-analysis [J]. *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
rs1801198	<i>TCN2</i>	C776G	0.315	benign	0.09	tolerable	18.9	tolerable	0.081	nonconserved	0.027	neutral
rs3733890	<i>BHMT</i>	G716A	0.064	benign	0.218	tolerable	21.8	damaging	2.864	conserved	0.070	neutral
rs1801394	<i>MTRR</i>	A66G	1	probability damaging	0.064	tolerable	23.3	damaging	0.098	nonconserved	0	deleterious
rs1801133	<i>MTHFR</i>	C677T	0.998	probability damaging	0.027	damage	25.0	damaging	9.137	conserved	0	deleterious

Table 1 Information of four SNPs in the present study

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
TCN2 rs1801198			Total	CC	CG	GG	Total	CC	CG	GG		
Cartinelli et al.	2006	Caucasian	218	85	110	23	289	89	150	50	0.330	5
Hills et al.	2008	Caucasian	316	99	153	64	1097	347	532	218	0.243	7
Postowska et al.	2010	Caucasian	163	46	88	29	181	48	103	30	0.044	6
N et al.	2015	Asian	429	76	215	138	461	75	231	155	0.475	5
Altrick-Zambuzzi et al.	2015	Caucasian	359	139	160	60	440	179	199	62	0.576	7
Arini et al.	2016	Caucasian	330	135	140	55	366	160	155	51	0.177	7
HMT rs3733890			Total	GG	GA	AA	Total	GG	GA	AA		
Postowska et al.	2010	Caucasian	174	95	76	3	176	82	75	19	0.766	6
U et al.	2011	Asian	166	90	56	20	268	130	118	20	0.334	5
N et al.	2015	Asian	481	219	202	60	554	265	245	44	0.222	5
Arini et al.	2016	Caucasian	330	140	150	40	366	156	163	47	0.665	7
Aras Kuzelicki et al.	2018	Caucasian	102	42	51	9	198	98	84	16	0.734	7
TRR rs1801394			Total	AA	AG	GG	Total	AA	AG	GG		
Randalize et al.	2007	Caucasian	114	36	69	9	100	33	61	6	0.002	6
Postowska et al.	2010	Caucasian	164	31	81	52	166	34	70	62	0.089	6
Slar et al.	2014	Caucasian	100	14	72	14	125	13	107	5	<0.001	6
Altrick-Zambuzzi et al.	2015	Caucasian	342	95	194	53	401	136	193	72	0.806	7
Ang et al.	2015	Asian	204	123	71	10	226	124	84	18	0.480	6
Ezerra et al.	2015	Caucasian	140	98	37	5	175	112	60	3	0.111	6
Lurthy et al.	2015	Asian	123	42	81	0	141	65	76	0	<0.001	5
Ang et al.	2016	Asian	147	71	26	50	129	29	59	41	0.380	5
Arini et al.	2016	Caucasian	330	160	134	36	367	175	161	31	0.478	7
Aras Kuzelicki et al.	2018	Caucasian	103	14	56	33	199	30	111	58	0.051	7
THFR rs1801133			Total	CC	CT	TT	Total	CC	CT	TT		
Haw et al.	1998	Caucasian	310	143	127	40	383	156	178	49	0.873	6
Blarova et al.	1998	Caucasian	111	43	49	19	106	46	52	8	0.195	6
Aspar et al.	1999	Caucasian	77	30	39	8	103	49	49	5	0.096	6
Yszynski et al.	2000	Caucasian	259	114	109	36	327	129	154	44	0.854	6
Cartinelli et al.	2001	Caucasian	64	22	30	12	106	46	43	17	0.205	6
Runert et al.	2002	Caucasian	66	34	26	6	184	90	69	25	0.052	6
Hotellersuk et al.	2003	Asian	109	84	25	0	202	154	46	2	0.478	6
Van Rooij et al.	2003	Caucasian	105	54	45	6	128	70	54	4	0.091	6
Aspar et al.	2004	Caucasian	644	327	269	48	424	213	172	39	0.616	6
Ezzetti et al.	2004	Caucasian	110	28	58	24	289	95	151	43	0.174	6
Randalize et al.	2007	Caucasian	114	49	46	19	100	45	41	14	0.353	6
Hevriar et al.	2007	Caucasian	148	66	60	22	165	51	81	33	0.935	6
Little et al.	2008	Caucasian	96	39	47	10	224	94	101	29	0.819	6
Hills et al.	2008	Caucasian	492	217	221	54	1599	715	721	163	0.341	7
Li et al.	2009	Asian	323	225	87	11	214	176	36	2	0.916	6
Ozen et al.	2009	Caucasian	179	81	80	18	138	66	65	7	0.073	6
Postowska et al.	2010	Caucasian	163	81	65	17	171	78	77	16	0.629	6
Badifar et al.	2010	Asian	61	21	18	22	215	114	72	29	0.003	6
An et al.	2011	Asian	187	46	106	35	213	74	110	29	0.236	6
Slar et al.	2013	Caucasian	80	13	57	10	125	59	62	4	0.010	6
Umari et al.	2013	Asian	467	327	125	15	469	364	100	5	0.518	6
Lurthy et al.	2014	Asian	123	104	19	0	141	107	31	3	0.672	6
Standia-Ortega et al.	2014	Caucasian	132	39	55	38	370	143	172	55	0.780	7
Ang et al.	2015	Asian	204	59	107	38	226	62	108	56	0.512	6
Ezerra et al.	2015	Caucasian	140	74	54	12	175	85	70	20	0.341	6
Bdollahi-Fakhim et al.	2015	Asian	121	38	58	25	103	27	54	22	0.605	6
Ang et al.	2016	Asian	147	28	66	53	129	19	97	13	<0.001	5
Arini et al.	2016	Caucasian	330	119	159	52	360	148	154	58	0.097	7
Aras Kuzelicki et al.	2018	Caucasian	103	45	45	13	199	85	96	18	0.214	7
Afik et al.	2019	Africa	52	44	8	0	182	97	74	11	0.526	6

Table 3 Associations between rs1801198 of *TCN2* gene 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 4 Associations between rs3733890 of *BHMT* gene and NSCL/P

Genetic model	<i>I</i> ² (%)	<i>P</i> for heterogeneity	<i>OR</i> (95% <i>CI</i>)	<i>P</i> value	<i>P</i> 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

Table 5 Associations between rs1801394 of *MTRR* gene and NSCL/P

Genetic model	<i>I</i> ² (%)	<i>P</i> for heterogeneity	<i>OR</i> (95% <i>CI</i>)	<i>P</i> value	<i>P</i> 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 6 Associations between rs1801133 of *MTHFR* gene 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

Figures

Figure 2

final meta-analysis

Figure 3

Figure 4