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Association between methylenetetrahydrofolate reductase polymorphisms and non-syndromic cleft lip with or without palate susceptibility: an updated systematic review and meta-analysis

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Abstract

Methylenetetrahydrofolate reductase (MTHFR) polymorphisms are thought to be involved in the development of cleft lip with or without cleft palate (NSCL/P), but published results are contradictory. We therefore designed an updated meta-analysis to pool eligible studies and to evaluate further the possible relations between MTHFR polymorphisms (c.677C > T and c.1298A > C) and susceptibility to NSCL/P. A comprehensive search based on PubMed, Medline, Web of Science, and Embase databases was made up to February 2018. Twenty-three case-control and 10 case-parent trio studies (including 1149 cases and 1161 controls) were retrieved. Odds ratio (OR) with 95% CI were used to estimate the pooled strength of association under different genetic models. The Q test and I^2 test were used to estimate heterogeneity among studies, the quality of which was assessed using the Newcastle-Ottawa scale. In the MTHFR c.677C > T polymorphism group, there were significant overall results for the recessive (OR 1.231, 95%CI 1.092 to 1.387) and homozygote (OR 1.252, 95%CI 1.078 to 1.456) models. Subgroup analysis by subjects and ethnicity identified only associations in European mothers for the recessive model and the homozygote model. For the c.1298A > C group, there were no significant results for either European or Asian patients for all genetic models. The MTHFR c.677C > T polymorphism might increase susceptibility to NSCL/P in European mothers, but was negatively associated in Asian patients, and the MTHFR c.1298A > C polymorphism is not involved in the development of NSCL/P in either European or Asian patients.

Keywords: Meta-analysis; MTHFR; NSCL/P; polymorphism; susceptibility

Introduction

Non-syndromic cleft lip with or without cleft palate (NSCL/P) is one of the most common congenital birth defects worldwide,[1] and because of the differences in geographic regions, races, and ethnic origins, the distribution and incidence of NSCL/P is heterogeneous around the globe. About 1 in every 700 newborns is affected, with the prevalence varying across geographical regions and racial and ethnic origins. Generally, the live-birth prevalence of NSCL/P is highest among Asians and Native Americans, often as high as 1/500. In Europeans, the live-birth prevalence is about 1/1000, and the lowest rates are among people of African descent (about 1/2500).[1,2]

For the last few decades it has been widely accepted that both environmental and genetic factors are vital in the aetiology.[3] Numerous candidate genes have been identified as potential causative of NSCL/P, such as MTHFR, msh homeobox homolog 1(MSX1), interferon regulatory factor 6 (IRF6), transforming growth factor α (TGF-α),

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small ubiquitin-like modifier 1(SUMO1), forkhead box E1 (FOXE1), and B-cell CLL/lymphoma 3 (BCL3).[4–10]

Among the candidate genes mentioned above, MTHFR polymorphisms have recently attracted a great deal of attention and have been extensively studied. MTHFR is of great importance in the regulation of available folate for the remethylation of homocysteine, and the role of folate has been described for the prevention of neural tube defects.[11,12] Folate deficiency or sequence variation in folate pathway genes are associated with neural tube defects, including NSCL/P.[13–15] Even though plenty of case-control and case-parent trio studies have been done to evaluate the correlation between MTHFR c.677C > T and c.1298A > C polymorphisms and NSCL/P, the outcomes are controversial and inconsistent. Previous research has identified a total of 39 genome-wide loci for significant risks of NSCL/P across different populations. Of these, 37 loci were identified by genome-wide association studies, meta-analyses of these data, or follow-up studies,[16] but a genome-wide association signal has not so far been detected in MTHFR. Some have reported a positive association,[17–19] but others have not.[20–24]

To elucidate the relation between MTHFR c.677C > T and c.1298A > C polymorphisms and susceptibility to NSCL/P, several meta-analyses have been done,[25–29] but the relation, particularly among Europeans and Asians, remains unclear. Recently, new studies with larger sample sizes have been published.[30–35] This prompted us to reassess the contribution of the MTHFR c.677C > T and c.1298A > C polymorphisms to susceptibility to NSCL/P in a large new independent sample. We have therefore updated the meta-analysis based on 23 case-control and 10 case-parent trio studies.

Methods

Eligibility criteria

Eligible studies were independently identified by two investigators. Each study was required to meet the following criteria: it was a published paper that evaluated the association between MTHFR c.677C > T and c.1298A > C polymorphisms and NSCL/P. It should be a case-control or cohort study, with distributions of genotypes available for both cases and controls, and it should be in human subjects. Studies were excluded if they were: conference reports, animal studies or studies of fundamental mechanisms, reviews, comments, editorials, case reports, or papers that did not report original data. They were also excluded if they gave insufficient data, described non-relevant topics, and contained overlapping participants or republished data. Language was not a restriction.

Search strategy

The present meta-analysis was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklists and guidelines.[36] The PubMed, Web of Science, Medline, and Embase electronic databases were searched comprehensively up to February 2018 using restricted words such as Medical Subject Headings (MeSH) terms combined with free words. The main search terms were: "cleft lip," "cleft palate," "congenital," "methylenetetrahydrofolate reductase," and "MTHFR polymorphisms." All studies reporting the association between the MTHFR polymorphism and NSCL/P were identified. The potentially relevant studies were supplemented through manual searches of eligible papers.

Extraction of data

Two investigators independently examined the studies included, and extracted the data required. Any disagreement was submitted to the third senior author. Together they rechecked the original data and had a discussion until a consensus was reached. The following information was collected and summarised in a table of basic characteristics (Table 1, Supplemental data online only), including name of the first author, year of publication, country, ethnic group, type of study, number of cases and controls, distribution of genotypes for MTHFR polymorphisms, source of sample, method of genotyping, and Hardy–Weinberg equilibrium. If data of an included study were missing or insufficient, authors were also contacted and asked to provide data by e-mail.

Quality of the method of assessment

The quality of the method of assessment was assessed using the Newcastle-Ottawa scale (Table 2, Supplemental data, online only). The scale consisted of three categories containing eight items in total, including adequate definition of cases, representativeness of cases, selection and definition of controls, quality control of impact factor, assessment of exposure, and non-response rate. A score of 0–9 (expressed as stars) was allocated to each study. Studies achieving higher scores were considered to be of higher quality.

Statistical analysis

To assess the quality of the study, the Hardy–Weinberg equilibrium (HWE) for distribution of the frequencies of the allele was examined for each study, using the chi-squared test in control groups. Probabilities of less than 0.05 were accepted as significant. Odds ratios (OR) corresponding to the 95% CI were used to estimate the strength of association between MTHFR polymorphisms and the risk of NSCL/P. Pooled OR were used to examine the homozygote contrast, heterozygote, recessive, dominant, and over-dominant models.[37,38] The significances of pooled OR were detected using the Z test. Statistical heterogeneity across the eligible studies was tested using a Q-test based on the chi-squared statistic with the level of significance set at $p < 0.10$.[39] Heterogeneity was quanti-

fied using the I^2 metric. When $I^2 < 50\%$ the heterogeneity was considered not obvious, and a fixed-effects model was used. Otherwise, the random-effects model was used ($I^2 > 50\%$).[40] Sensitivity analyses were made based on the "leave-one-out" method to detect the influence of each single study.[41] Funnel plots and Egger's linear regression test were used to screen for potential publication bias.[42,43] All statistical analyses in this study were made using Stata/SE 12.0 software (Stata Corp LP).

Results

Study characteristics

Based on the inclusion and exclusion criteria, 328 studies were initially retrieved from four online databases. After we had removed duplicates and irrelevant studies, we made a primary screening of the abstracts. Fifty studies were then selected for a full-text review, resulting in a total of 33 studies being included in the final analysis. A PRISMA flow chart describing the search process is shown in Fig. 1. Examination of the references listed for these studies and for the reviews yielded no further studies for evaluation.

The basic characteristics of the selected studies are shown in Table 1, and the Newcastle Ottawa scores for quality assessment in Table 2. Among the 33 studies included, 32[17–24,30–34,44–62] investigated the MTHFR c.677C > T polymorphism, 20[18,19,24,30–32,34,44–47,51,54,55,57–62] investigated the MTHFR c.1298A > C polymorphism, and one reported the MTHFR c.677C > T polymorphism.[59] Fourteen studies had been done only on patients with NSCL/P, and two[46,57] only on the patients' mothers. The remaining 18 studies included a mixture of patients with NSCL/P and their mothers. Nearly all the studies included used a standard polymerase chain reaction (PCR) method for genotyping. The sources of DNA included blood samples (stored or peripheral) and buccal cells. Studies from 15 countries were included. Among them, 11 studies were on Asians and the rest were on Europeans. The major ethnicity of the country included was considered to integrate the OR of each study effectively. However, strictly speaking, most countries' ethnicities were actually mixed as a result of decades of assimilating immigrations.

Hardy–Weinberg equilibrium (HWE)

For all polymorphisms, frequencies of genotypes and HWE of control groups in each included study were re-examined based on the chi-squared test. Studies on the MTHFR c.677C > T group,[34,35] and the MTHFR and c.1298A > C group[30] were excluded because the p values of their control groups were out of HWE (less than 0.05).

Quantitative data synthesis

The pooled OR of the overall meta-analysis and results of heterogeneity testing for all genetic models are listed in Tables 3 and 4 (Supplemental data, online only). Significant results are shown in bold. No significant heterogeneity was detected in the c.1298A > C polymorphism group for any genetic model (Table 3). On the contrary, in the MTHFR c.677C > T group, significant heterogeneity was detected in both dominant and homozygous models (Table 4).

The Forest plot of the association under the dominant model is shown in Figs. 2 and 3. In the Forest plot, the diamonds indicate the study effect, and the middle line (RR = 1) stands for "no significance". The overall results and subgroup results showed no significant risk of NSCL/P and MTHFR polymorphisms (Figs. 2 and 3). For the other genetic models, the results are summarised in Tables 3 and 4, Supplemental data, online only. For studies that investigated the association between the MTHFR c.1298A > C polymorphism and risk of NSCL/P, all eligible ones were pooled in a random-effects model, but there were no significant results. For studies that investigated the relations between the MTHFR c.677C > T polymorphism and NSCL/P, there were significant overall results for the recessive model (OR 1.231, 95%CI 1.092 to1.387) and the homozygous model (OR 1.252, 95%CI 1.078 to 1.456), and a Z-test indicated that the pooled OR were significant.

Subgroup analyses

Subgroup analyses were based on ethnicity of the subjects in the study. Of the 33 studies included eight concerned Asians, and the rest were Europeans. For Asians there were no statistical associations between c.1298A > C polymorphism and the risk of NSCL/P in either patients or mothers. However, for all genetic models except the over-dominant model, the risks of NSCL/P in Asian patients or mothers were significant. As far as European patients were concerned, there was no significance for either the c.1298A > C or c.677C > T polymorphism. These two studies were not included in the pooled analysis as the corresponding OR did not provide much valuable information.

Publication bias

We did Begg's test and funnel plots to assess the publication bias of the studies. For the MTHFR c.677C > T group, funnel plots under the dominant model were not symmetrical (Fig. 4), and the funnel plot of the c.1298A > C group was probably asymmetrical as well (Fig. 5). The results of both Eggers and funnel plots indicate potential publication bias, which may result from an ethnicity bias, lack of phenotypic subdivision, or flawed study design. For the remaining genetic model, we detected no significant publication bias.

Table 1
Basic characteristics of the studies included.

Author name	Year	Country	Ethnicity[a]	Source of control	Source of DNA	Genotyping method	SNP type	Group	NOS Score	Environment assessment
Shaw	1998	USA	European	PB	Blood (stored)	PCR	c.677C > T	Case-control	7	Y
Tolarova	1998	Argentina	European	NA	NA	NA	c.677C > T, c.1298A > C	Case-control	4	NA
Martinelli	2001	Italy	European	PB	Blood	PCR	c.677C > T	Case-trio	7	N
Grunert	2002	Germany	European	NA	Blood	PCR	c.677C > T, c.1298A > C	Case-control	5	NA
van Rooij	2003	Netherlands	European	PB	Blood/Buccal cell	PCR	c.677C > T, c.1298A > C	Case-trio	7	Y
Shotelersuk	2003	Thailand	Asian	PB	Blood	PCR	c.677C > T, c.1298A > C	case-trios	6	N
Nurk	2004	Norway	European	HB	Blood	PCR-RFLP, MS-PCR	c.677C > T, c.1298A > C	Case-control	8	Y
Gasper	2004	USA	European	HB	Peripheral blood/Buccal cell	SNuPE test	c.677C > T	Case-trio	7	N
Pezzetti	2004	Italy	European	HB	Peripheral blood	PCR	c.677C > T, c.1298A > C	Case-trio	6	Y
Brandalize	2007	Brazil	European	HB	Peripheral blood	PCR	c.677C > T	Case-control	7	Y
Chevrie	2007	France	European	HB	Peripheral blood/Finger-prick blood	PCR	c.677C > T	Case-control	8	Y
Little	2008	UK	European	PB	Mouthwash sample/Buccal cell	MS-PCR	c.677C > T	Case-control	6	Y
Mills	2008	Ireland	European	HB	Blood/Buccal cell	PCR-RFLP	c.677C > T, c.1298A > C	Case-control	9	Y
Ali	2009	India	Asian	HB	Peripheral blood	PCR	c.677C > T, c.1298A > C	Case-trio	6	Y
Sożen	2009	Venezuela	European	PB	Blood	PCR	c.677C > T, c.1298A > C	Case-control	6	N
Guo	2009	China	Asian	HB	Peripheral blood	PCR	c.677C > T	Case-trio	5	N
Partica	2009	México	European	NA	Peripheral blood	PCR-RFLP	c.677C > T	Case-control	4	N
Mostowska	2010	Poland	European	HB	Peripheral blood	PCR-RFLP	c.677C > T	Case-control	6	N
Han	2011	China	Asian	HB	Peripheral blood	PCR-RFLP	c.677C > T	Case-control	5	N
Aida	2012	Turkey	European	NA	Peripheral blood	PCR	c.677C > T, c.1298A > C	Case-trio	7	Y
Lopez	2013	Mexico	European	HB	Peripheral blood	PCR-RFLP	c.677C > T, c.1298A > C	Case-control	9	Y
Alar	2013	Turkey	European	NA	Peripheral blood	PCR-RFLP	c.677C > T	Case-control	6	Y
Kumari	2013	India	Asian	PB/HB	Peripheral blood	PCR-RFLP	c.677C > T, c.1298A > C	Case-control	6	Y
Lu	2013	China	Asian	HB	Blood	PCR	c.1298A > C	Case-trio	7	Y
Bezerra	2014	Brazil	Asian	PB	Peripheral blood	PCR-RELP	c.677C > T, c.1298A > C	Case-control	7	Y
Jahanbin	2014	Iran	European	PB	Peripheral blood	PCR-RFLP	c.677C > T, c.1298A > C	Case-control	7	Y
Murthy	2014	India	Asian	HB	Peripheral blood	PCR-RFLP	c.677C > T, c.1298A > C	Case-control	7	N
de Aguiar	2015	Brazil	European	HB	Buccal cells	qPCR	c.677C > T, c.1298A > C	Case-control	6	N
Fakhim	2015	Iran	European	NA	Peripheral blood	PCR-RFLP, ARMS-PCR	c.677C > T, c.1298A > C	Cross-sectional	7	N
Ebadifar	2015	Iran	European	HB	Peripheral blood	PCR	c.677C > T, c.1298A > C	Case-control	5	Y
Chau	2016	Chile	European	HB	Peripheral blood	qPCR	c.677C > T	Case-control	6	N
Wang	2016	China	Asian	HB	Blood	PCR-RFLP	c.677C > T, c.1298A > C	Case-control	5	N

NA: not available; Y: yes; N: no; PB: public-based; HB: hospital-based; PCR: polymerase chain reaction; PCR-RFLP: PCR-restricted fragment length polymorphisms; qPCR: Real-time PCR; MS-PCR: Methylation-Specific PCR; SNuPE; Single Nucleotide Primer Extension; ARMS-PCR: amplification refractory mutation system-polymerase chain reaction; A: adenine; C: cytosine; T: thymine.

[a] Most countries' ethnicities (e.g. USA) are actually mixed in light of decades of assimilating immigrations.

Table 2
Scale for methodological quality assessment.

Study, Year	Selection				Comparability[a]	Exposure			Scores
	Adequate definition of cases	Representativeness of the cases	Selection of controls	Definition of controls	Control for important factors	Assessments of exposure	Same method of ascertainment for cases *vs*. controls	Non-response rate	
Shaw et al.,1998	☆	☆	☆	☆	☆	☆	☆	–	7
Tolarova et al.,1998	☆	☆	☆	–	–	–	☆	–	4
Martinelli et al.,2001	☆	☆	☆	☆	☆	☆	☆	☆	7
Grunert et al.,2002	☆	☆	–	☆	☆	–	☆	–	5
van Rooij et al., 2003	☆	☆	☆	☆	☆	☆	☆	–	7
Shotelersuket al.,2003	☆	☆	☆	☆	☆	–	☆	☆	6
Nurk et al.,2004	☆	☆	☆	☆	☆☆	☆	☆	–	8
Gasper et al.,2004	☆	☆	–	☆	☆☆	–	☆	☆	7
Pezzetti et al.,2004	☆	☆	–	–	☆	☆	☆	☆	6
Brandalize et al.,2007	☆	☆	–	☆	☆	☆	☆	☆	7
Chevrier et al.,2007	☆	☆	–	☆	☆☆	☆	☆	☆	8
Little et al.,2008	☆	–	–	☆	☆	☆	☆	☆	6
Mills et al.,2008	☆	☆	☆	☆	☆☆	☆	☆	☆	9
Ali et al.,2009	☆	☆	–	–	☆	☆	☆	☆	6
Sožen et al.,2009	☆	☆	–	☆	☆	–	☆	☆	6
Guo et al.,2009	☆	–	☆	–	☆	–	☆	☆	5
Partica et al.,2009	☆	☆	–	–	☆	–	☆	–	4
Mostowska et al.,2010	☆	☆	–	☆	☆	–	☆	☆	6
Han et al.,2011	☆	☆	–	–	☆	–	☆	☆	5
Aida et al.,2012	☆	☆	–	☆	☆	☆	☆	☆	7
Lopez et al.,2013	☆	☆	☆	☆	☆☆	☆	☆	☆	9
Alar et al.,2013	☆	☆	–	☆	☆	☆	☆	–	6
Kumari et al., 2013	☆	☆	☆	–	☆	☆	☆	–	6
Lu et al.,2013	☆	☆	–	☆	☆	☆	☆	☆	7
Bezerra et al., 2014	☆	☆	–	–	☆☆	☆	☆	☆	7
Jahanbin et al., 2014	☆	☆	☆	☆	☆	☆	☆	–	7
Murthy et al., 2014	☆	☆	☆	☆	☆	–	☆	☆	7
de Aguiar et al., 2015	☆	☆	–	☆	☆	–	☆	☆	6
Fakhim et al. 2015	☆	☆	–	☆	☆☆	–	☆	☆	7
Ebadifar et al., 2015	☆	☆	–	–	☆	☆	☆	–	5
Chau et al.,2016	☆	☆	–	☆	☆	–	☆	☆	6
Wang et al.,2016	☆	☆	–	–	☆	–	☆	☆	5

[a] A maximum of 2 stars can be allotted in each category.

Table 3
Pooled results of the MTHFR c.1298A > C polymorphism for NSCL/P studies.

Study (n)	Recessive			Homozygote			Heterozygote			Dominant			Over dominant		
	Test of association	Test of heterogeneity		Test of association	Test of heterogeneity		Test of association	Test of heterogeneity		Test of association	Test of heterogeneity		Test of association	Test of heterogeneity	
	OR (95%CI)	I^2%	*P*	OR (95%CI)	I^2%	*P*	OR (95%CI)	I^2%	*P*	OR (95%CI)	I^2%	*P*	OR (95%CI)	I^2%	*P*
Caucasian-patients	0.898(0.696–1.16)	10.8%	0.343	0.936(0.731–1.199)	3.00	0.412	1.08(0.943–1.236)	0.00	0.911	1.048(0.921–1.191)	0.00	0.949	0.918(0.805–1.046)	0.00	0.823
Caucasian-mothers	1.033(0.803–1.327)	0.00	0.455	0.98(0.756–1.272)	0.00	0.521	0.948(0.82–1.095)	1.80	0.419	0.953(0.832–1.091)	0.00	0.565	1.056(0.91–1.225)	8.20	0.367
Asian-mothers	0.626(0.292–1.34)	0.00	0.479	0.634(0.27–1.487)	15.70	0.276	1.034(0.529–2.017)	69.50	0.07	0.945(0.767–1.164)	73.10	0.065	0.927(0.521–1.648)	61.00	0.109
Asian-patients	1.059(0.824–1.359)	0.00	0.77	1.05(0.806–1.367)	0.00	0.66	0.935(0.748–1.17)	49.7	0.05	0.945(0.767–1.164)	47.4	0.06	1.074(0.873–1.321)	46.3	0.07
Overall	0.972(0.847–1.117)	0.00	0.634	0.969(0.839–1.119)	0.00	0.671	0.993(0.91–1.084)	11.30	0.293	0.986(0.912–1.064)	1.70	0.439	1.003(0.921–1.93)	13.2	0.264

MTHFR: Methylenetetrahydrofolate reductase; OR: Odds ratio; CI: confidence interval.

Table 4
Pooled results for the MTHFR c.677C > T polymorphism in different genetic models.

Study (n)	Recessive			Homozygote			Heterozygote			Dominant			Over dominant		
	Test of association	Test of heterogeneity		Test of association	Test of heterogeneity		Test of association	Test of heterogeneity		Test of association	Test of heterogeneity		Test of association	Test of heterogeneity	
	OR (95%CI)	I^2 %	*P*	OR (95%CI)	I^2 %	*P*	OR (95%CI)	I^2 %	*P*	OR (95%CI)	I^2 %	*P*	OR (95%CI)	I^2 %	*P*
Caucasian-patients	1.138 (0.967–1.340)	21.3	0.196	1.145 (0.939–1.396)	36.1	0.059	1.010 (0.912–1.118)	11.7	0.312	1.035 (0.925–1.158)	29.9	0.108	1.020 (0.936–1.110)	0.0	0.616
Caucasian-mothers	**1.336 (1.093–1.634)**	22.0	0.198	**1.336 (1.033–1.727)**	40.7	0.042	0.954 (0.796–1.144)	53.2	0.005	1.020 (0.847–1.227)	59.8	0.01	1.134 (0.977–1.316)	39.9	0.046
Asian-patients	1.497 (0.895–2.505)	30.5	0.206	1.755 (0.973–3.163)	33.5	0.185	1.256 (0.956–1.652)	47.8	0.088	1.262 (0.921–1.730)	62.6	0.020	0.840 (0.660–1.071)	41.3	0.13
Asian-mothers	1.013 (0.555–1.851)	0.00	0.513	0.924 (0.408–2.092)	8.00	0.337	1.322 (0.652–2.680)	74.7	0.019	1.332 (0.668–2.655)	75.1	0.018	0.733 (0.392–1.370)	72.4	0.027
Overall	**1.231 (1.092–1.387)**	19.1	0.121	**1.252 (1.078–1.456)**	37.7	0.007	1.038 (0.941–1.146)	48.8	0.00	1.076 (0.973–1.191)	56.0	0.00	1.012 (0.930–1.102)	39.6	0.004

MTHFR, Methylenetetrahydrofolate reductase; OR, Odds ratio; CI, confidence interval.
*Significant results are shown in bold.

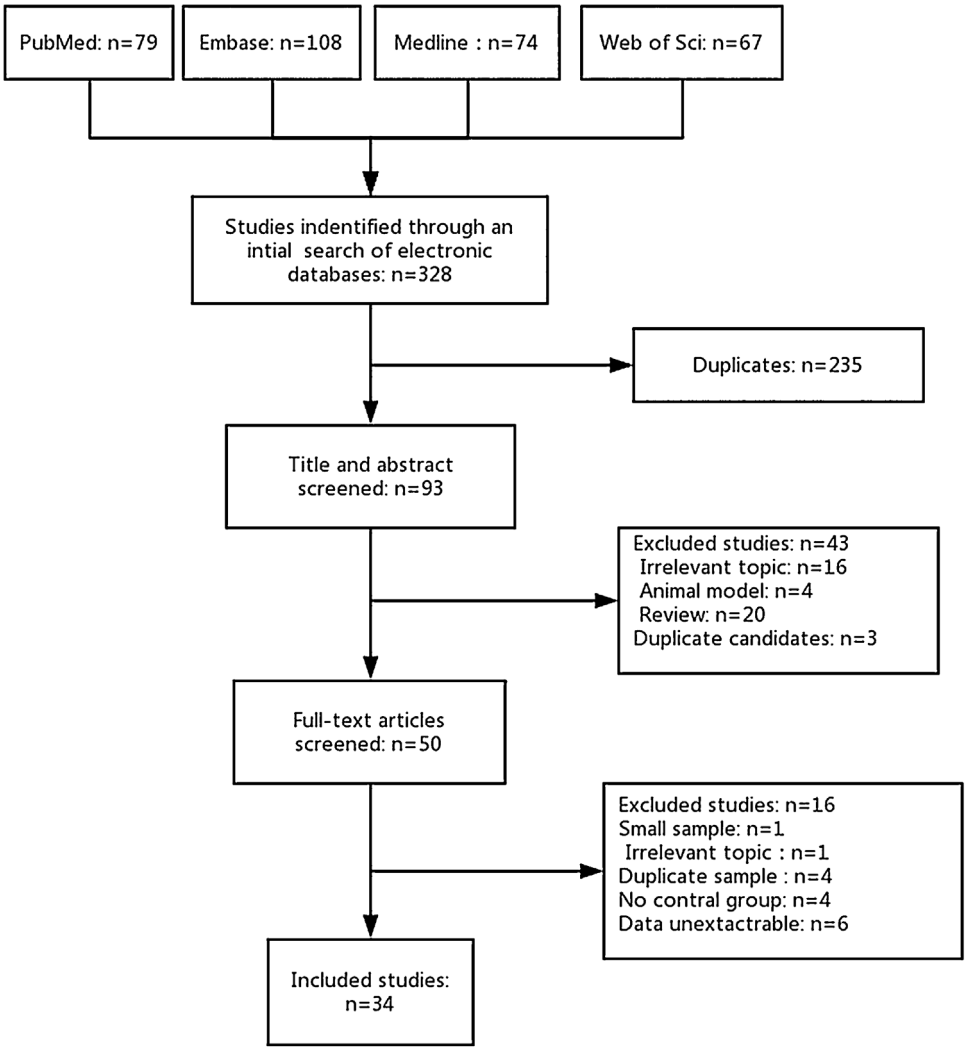

Fig. 1. Flow diagram of studies identified, included, and excluded.

Sensitivity analyses

Three studies, two on the MTHFR c.677C > T group and one on the MTHFR c.1298A > C group, were removed from this meta-analysis because the genotypic distribution in the control groups deviated slightly from the HWE. By alternating exclusion of the included studies one by one, the pooled results of each genetic subgroup were not substantially changed.

Discussion

We have investigated the association between MTHFR c.677C > T and c.1298A > C polymorphisms and NSCL/P based on 23 case-control and 10 case-parent trio studies that involved a total of 1149 cases and 1161 controls. The pooled results showed a positive significant association between Europeans and the MTHFR c.677C > T polymorphism. Subgroup analyses by race further identified a significant association in European mothers, which indicates that in the MTHFR c.677C > T polymorphism in European mothers, the T > C polymorphism-might increase the risk of giving birth to children with NSCL/P. However, it should be noted that significant results were obtained in only two certain genetic models - the recessive (TT compared with CT + CC) model and the homozygotic model.

As a gene-environment disease, NSCL/P had no definite genetic model to be found, so the choice of a different genetic model will to a certain extent influence the results. To ensure choice of genetic models, we used the method suggested by Thakkinstian et al, which defined effects of genes between each genotype as "odds ratio". [37] Based on the odds ratios, which are TT compared with CC ($OR_1 = 1.24$), CT compared with CC ($OR_2 = 0.98$) and TT compared with CT ($OR_3 = 1.25$), the recessive model is suggested. That is consistent with our results, and indicates that they are robust. As for the c.677C > T polymorphism in Asians, we found

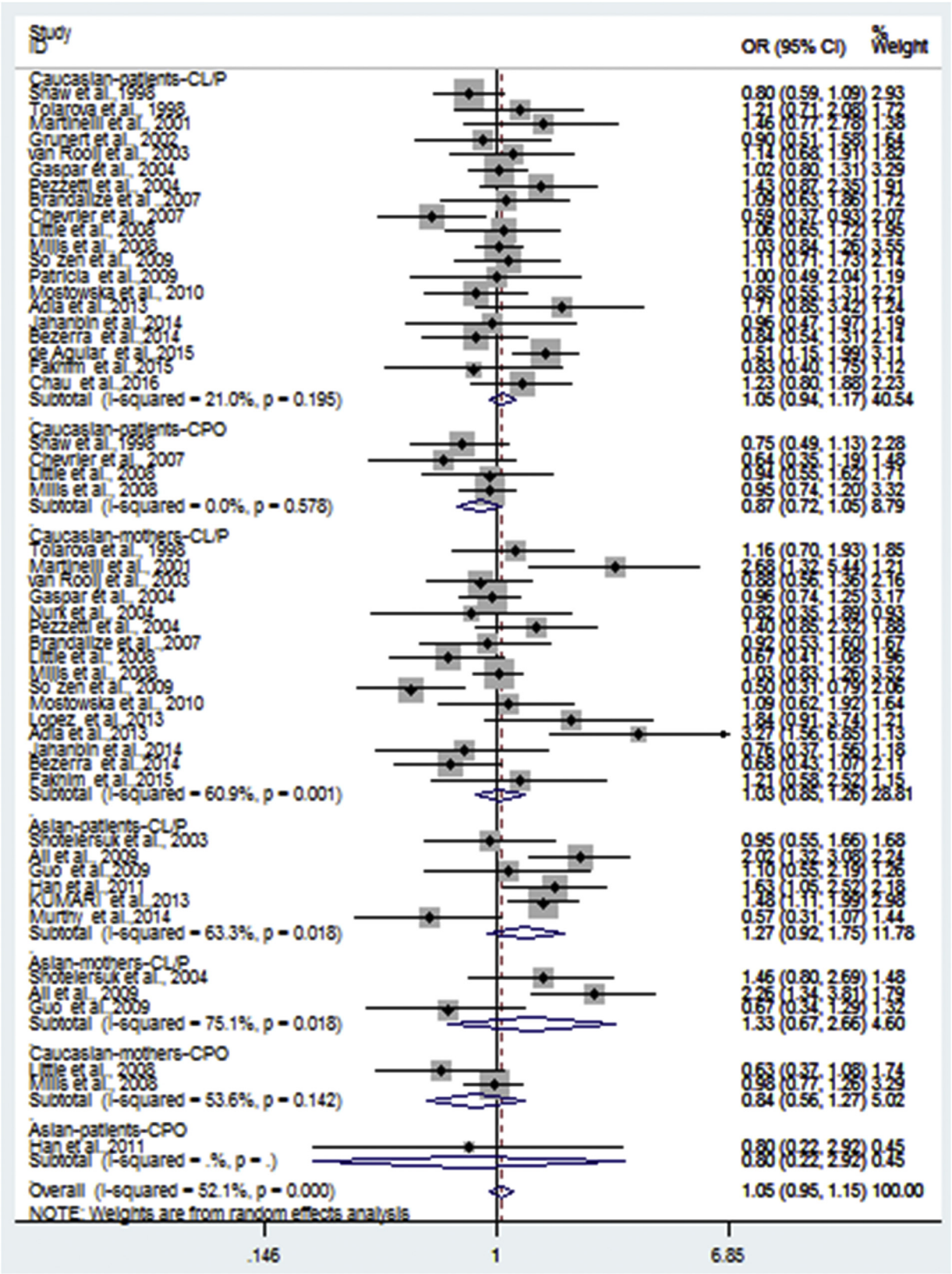

Fig. 2. Forest plot and meta-analysis of the association between the MTHFR c.677C > T polymorphism and risk of non-syndromic cleft lip, with or without cleft palate, in the dominant model (CT + TT/CC). MTHFR = methylenetetrahydrofolate reductase; C = cytosine; T = thymine.

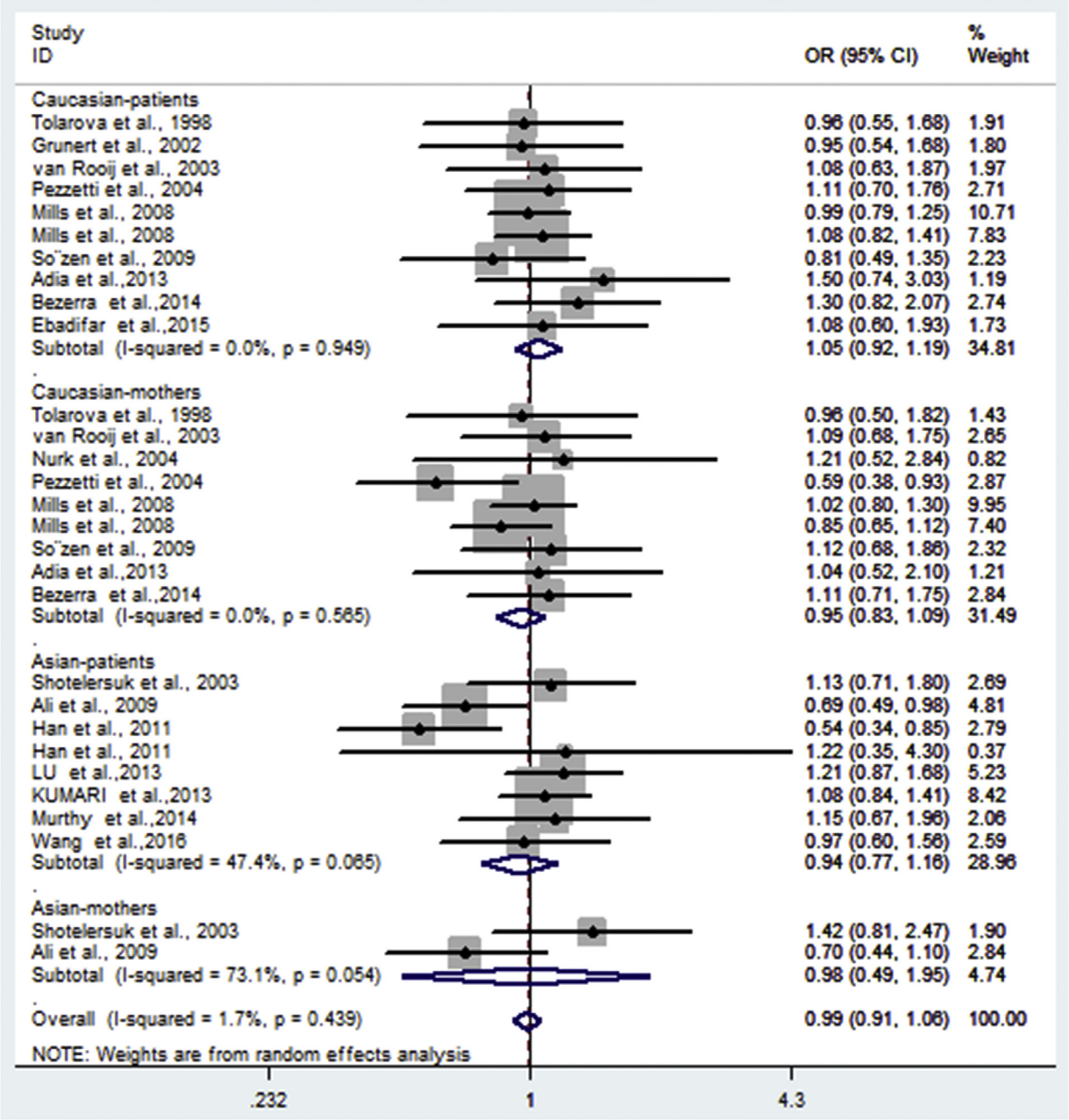

Fig. 3. Forest plot and meta-analysis of the association between the MTHFR c.1298A > C polymorphism and risk of non-syndromic cleft lip, with or without cleft palate, in the dominant model (CC + AC compared with AA). MTHFR = methylenetetrahydrofolate reductase; A = adenine; C = cytosine.

no significant results. In addition, no significant association between c.1298A > C polymorphism and NSCL/P was detected in either Europeans or Asians.

In the MTHFR c.677C > T polymorphism group, there was significant heterogeneity in both dominant and heterozygotic models. Subgroup analyses indicated that the heterogeneity comes mainly from European mothers and Asian mothers, which may be the result of the genetic differences between Europeans and Asians, or the genetic differences between mothers and patients with NSCL/P. A random effect model was therefore chosen to alleviate the heterogeneity, but could not eliminate it. In contrast, no significant heterogeneity was seen for the c.1298A > C group.

So far, five meta-analyses have been published about MTHFR polymorphisms as risk factors for NSCL/P. Johnson and Little published a review, the aim of which was to detect the association between folate intake and clefts in 2008, but they could not provide strong evidence for the association.[63] Similarly, Verkleij-Hagoort et al reported no independent relation between MTHFR c.677C > T and c.1298A > C polymorphisms and NSCL/P.[25] Two studies reported the risk of NSCL/P from the c.677C > T polymorphism in Asians.[26,27] Luo et al investigated both Asian and European ethnicities,

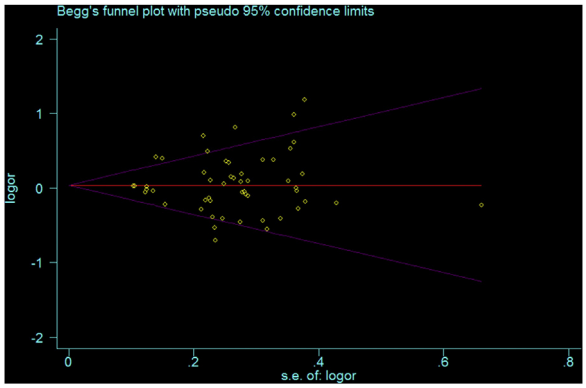

Fig. 4. Funnel plots showing the meta-analysis of the association between the MTHFR c.677C > T polymorphism and risk of cleft lip with or without cleft palate in the dominant model (CT + TT/CC). MTHFR: methylenetetrahydrofolate reductase; C = cytosine; T = thymine.

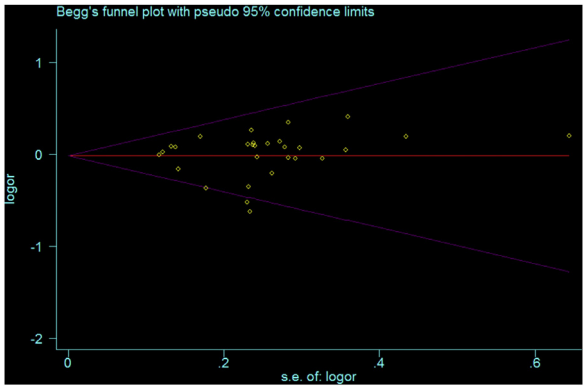

Fig. 5. Funnel plots showing the meta-analysis of the association between the MTHFR c.1298A > C polymorphism and risk of cleft lip with or without cleft palate in the dominant model (CC + AC compared with AA). MTHFR: methylenetetrahydrofolate reductase; A = adenine; C = cytosine.

but could show only that an MTHFR c.677C > T genotype might be related to the risk of having offspring with NSCL/P.[28] Our results are consistent with those of Luo et al. As an updated meta-analysis, newly published studies were added and groups were subdivided not only by ethnicity but also by subject and phenotype.[30–34,56–59,60,62,64] The aetiology of NSCL/P is not exactly the same. An early review showed that the risk of having NSCL/P is not equal even in the same group with the same folic acid supplement.[65] An analysis based on the phenotype of NSCL/P is therefore necessary.

The present meta-analysis pooled the latest and largest numbers of studies available, but some limitations should be taken into consideration. First, almost all of the studies have been conducted in Asians and Europeans. Secondly, the sample sizes are still relatively small. Thirdly, subgroups were not categorised by age because of lack of data. Fourthly, publication bias might exist for the published reports only in science citation index journals, which were included in this study. Finally, because MTHFR has not been reported with a significant signal in any genome-wide association studies so far, the lack of MTHFR on a genome-wide association signal of NSCL/P might emerge as bias. To assess the role of MTHFR variations associated with NSCL/P, future researches on genotyping of a large population with varied phenotypic measures, duration, and area, using genome-wide association studies are needed. The conclusions drawn from the present study should therefore be interpreted with caution.

Taken together, our meta-analysis suggests that the MTHFR c.677C > T polymorphism in European mothers might increase children's susceptibility to NSCL/P, but is negatively associated with NSCL/P in Asian patients. There was also no significant association between the c.1298A > C polymorphism and NSCL/P detected in European and Asian patients. In the future, larger-sample and multicentre collaborative studies, particularly genome-wide association studies and meta-analyses of these data, or follow-up studies between MTHFR polymorphisms and NSCL/P, are awaited to confirm and update the findings of this analysis.

Ethics statement/confirmation of patients' permission

Ethics approval not required. The patientsl permission was obtained.

Conflict of interest

We have no conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bjoms.2019.06.016.

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