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REVIEW ARTICLE

Strong Association of C677T Polymorphism of Methylenetetrahydrofolate Reductase Gene With Nosyndromic Cleft Lip/Palate (nsCL/P)

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Abstract Methylenetetrahydrofolate reductase (MTHFR) is essential for DNA biosynthesis and the epigentic process of DNA methylation. It has been reported that abnormal DNA methylation contributes to the pathogenesis of congenital anomalies. There were many published case control studies assessing the associations of MTHFR C677T polymorphism with risks of nosyndromic cleft lip with and without palate (nsCL/P), but with inconsistent results. To derive a more precise estimation of the relationship, a meta-analysis was performed. Eligible articles were identified by search of databases including PubMed, Science Direct, Google Scholar and Springer Link up to December, 2015. Finally, a total of 22 studies with 3724 nsCL/P cases and 5275 controls were included in the present meta-analysis. Odds ratios (ORs) with corresponding 95% confidence intervals (95% CIs) were pooled to assess the association. Subgroup analysis based on ethnicity was also performed. All statistical analyses were done by MIX program. Meta-analysis results suggested that MTHFR C677T polymorphism contributed to the increased nsCL/P risk in overall population using four genetic models except homozygote model (for T vs. C: OR = 1.24, 95% CI = 1.1–1.4; for TT + CT vs. CC: OR = 1.29, 95% CI = 1.04–1.59; for CT vs. CC: OR = 1.26, 95% CI = 0.98–1.63; for TT vs. CC: OR = 1.02, 95% CI = 0.74–1.4; for TT vs. CT + CC: OR = 1.36, 95% CI = 1.05–1.74). In conclusion, results of present meta-analysis suggested that MTHFR C677T polymorphism is significantly associated with nonsyndromic orofacial cleft.

Keywords nsCL/P · MTHFR · C677T · Folate · Meta-analysis · Polymorphism

Abbreviations

nsCL/P	Nonsyndromic cleft lip with or without cleft palate
MTHFR	Methylenetetrahydrofolate reductase

Introduction

Nonsyndromic cleft lip with or without cleft palate (nsCL/P) is a common congenital defect with the prevalence rate of 1 in 300–2000 birth depending upon ethnicity, and socioeconomic status [1–3]. Twin and family studies suggested that genetic factors play an important role in the etiology of nsCL/P [4]. The risk of recurrence in first-degree relatives of affected persons is about 40-folds greater than in the general population, which also suggests a strong genetic component [5–7]. Its frequency is highest in Asian and Native American populations of Asian genetic origin, intermediate in Caucasian population and the lowest in African and African-American populations [8].

Perinatal intake of folic acid and multivitamins is suggested to provide protection from neural tube defects (NTD) and nsCL/P birth defects [9–17]. Several studies reported that perinatal supplementation of folic acid reduces the risk of neural tube defects (NTD) [18, 19] led to speculation that folic acid supplementation might also

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reduces the risk of craniofacial closure defects such as nsCL/P [9–11, 20].

A number of observational studies [10, 11, 19, 21–23] although not all have reported that folate deficiency during conception period results in nsCL/P in offspring. Some studies have found a decline in cleft rates since food fortification with folic acid began [24], although others have not found a change [25]. These observations have led investigators to look for associations between facial clefts and folate enzyme genes, including methylenetetrahydrofolate reductase (MTHFR) [26–35].

MTHFR enzyme catalyzes the reduction of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-THF), which donates methyl group for the conversion of homocysteine to methionine. Several polymorphisms were reported in MTHFR gene, out of which C677T variant is most clinically important polymorphism. The C677T variant has been associated with decreased activity of MTHFR, and increased homcysteine plasma level [36]. A hypofunctional MTHFR leads to lower S-adenosyl-L-methionine levels and consequently to hypomethylation. MTHFR C677T polymorphism is reported as risk factor for several diseases and disorders like- neural tube defects [37], Down syndrome [36, 38], congenital heart defects [39], and cardiovascular diseases etc. Globally, the prevalence of 677T allele ranged from 24.1 to 64.3% among Europeans, 2–48% among North Americans, 7% among South Americans, 0–35.5% among Africans and 2–63.1% in Asians [40–44].

Since the first report in 1998 that the MTHFR C677T variant genotype was found more commonly among nsCL/P cases than controls [45], several case–control and epidemiological studies have been published to determine the exact role of MTHFR gene in the etiology of oral clefts. However, the results remain conflicting rather than conclusive. So, to shed some light on this association present meta-analysis was carried out of all available studies relating the C677T polymorphism of the MTHFR gene to the risk of nsCL/P.

Methods

Identification of Studies

Literature search for eligible studies was conducted on PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Google Scholar (http://scholar.google.com), Science Direct (http://www.sciencedirect.com), and Springer Link (http://link.springer.com) databases up to December 31, 2015, using keywords "methylenetetrahydrofolate reductase", "MTHFR", "C677T" and "cleft lip and palate".

Data Extraction

The following information were collected from studies: first author family name, year of publication, name of journal, country name, ethnicity, number of cases and controls, numbers of different MTHFR genotypes in nsCL/P cases and controls.

Inclusion Criteria

The study inclusion criteria were that (1) the study should be case–control study, (2) study should be published as full papers, and (3) complete information of different MTHFR genotype number should be reported in the study. The study exclusion criterion were (1) only cases were studied, (2) review papers, editorial, letter to editor and (3) containing overlapping data and (4) no enough information to estimate OR with 95% CI.

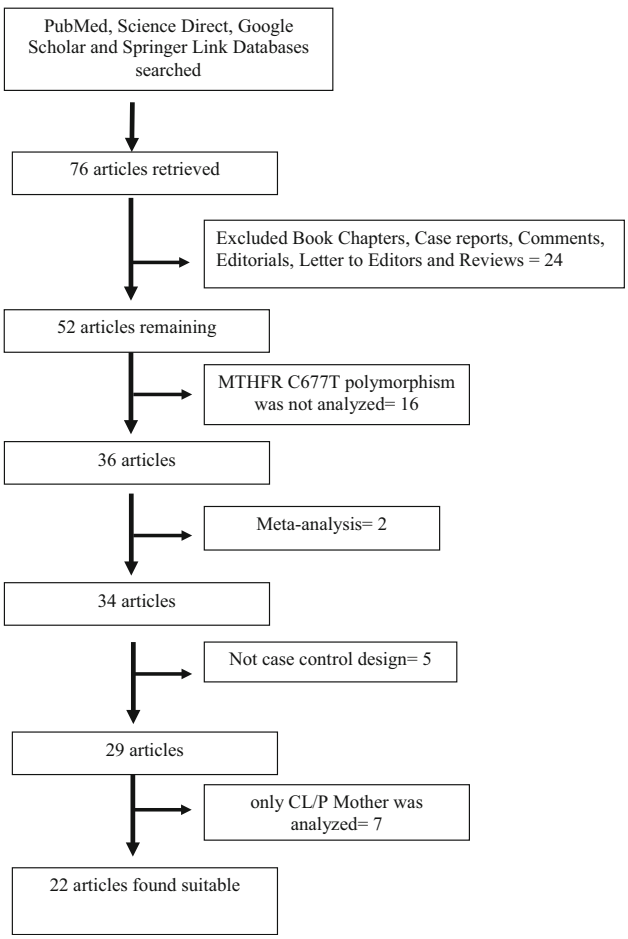

Fig. 1 Flow chart of the selection of studies

Statistical Analysis

Odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were used as the measure of effect to evaluate the strength of association between the MTHFR C677T polymorphism and nsCL/P risk. The meta-analysis examined the association between C677T polymorphism and nsCL/P using all five genetic models like- allele contrast/additive (T vs. C), homozygote (TT vs. CC), recessive (TT vs. CT + CC), dominant (TT + CT vs. CC) and co-dominant (CT vs. CC) models. The pooled OR was performed by using both fixed effects (FE) (Mantel–Haenszel) and random effects (DerSimonian and Laird) models [46, 47]. The heterogeneity between studies was tested by the Q-statistic [48, 49] and was quantified with the I^2 metric, which is independent of the number of studies in the meta-analysis [50, 51]. I^2 takes values between 0 and 100% with higher values denoting greater degree of heterogeneity [52]. When there is higher heterogeneity between studies, then the pooled OR is preferably estimated using the random effects model. Further subgroup analysis according to ethnicity and sensitivity analysis were performed to explore potential heterogeneity. To assess the study quality, control population of individual study was tested for Hardy–Weinberg equilibrium (HWE) using an online program available at https://ihg.gsf.de/cgi-bin/hw/hwa1.pl.

Publication Bias

An estimate of publication bias was carried out by the Begg's funnel plot, in which the standard error of log (OR) and precision of each study was plotted against its log (OR) [53]. The funnel plot asymmetry was further assessed by the method of Egger's linear regression test [54]. All analyses were performed by MIX version 1.7 [55]. A p value less than 0.05 was considered statistically significant.

Results

Present meta-analysis was carried out according to the Moose guidelines. The article search concerning the association of MTHFR C677T polymorphism with nsCL/P resulted in 76 studies in total. After examination of abstracts, 24 studies were excluded, which were review, editorials and comments etc. Full text of remaining 52 articles were examined and again 30 article were excluded. Out of 30 articles, sixteen articles were irrelevant for

Table 1 Characteristics of twenty-two studies included in the present meta-analysis

Study	Ethnicity	Country	Case/control	Case genotype			Control genotypes			*P* value of HWE
				CC	CT	TT	CC	CT	TT	
Shaw et al. (1998)	Caucasian	USA	310/383	143	127	40	156	178	49	0.87
Tolarova et al. (1998)	Caucasian	Argentina	111/106	43	49	19	46	52	8	0.19
Gasper et al. (1999)	Caucasian	Brazil	77/103	30	39	8	49	49	5	0.09
Wyszynski et al. (2000)	Caucasian	USA	259/327	114	109	36	129	154	44	0.85
Martinelli et al. (2001)	Caucasian	Italy	116/106	64	22	30	46	43	17	0.20
Blanton et al. (2002)	Caucasian	USA	75/50	–	–	–	–	–	–	–
Grunert et al. (2002)	Caucasian	Germany	66/184	34	26	6	90	69	25	0.05
Shoteresuk et al. (2003)	Asian	Thailand	109/202	84	25	0	154	46	2	0.47
van Roij et al. (2003)	Caucasian	Netherlands	105/128	54	45	6	70	54	4	0.09
Gasper et al. (2004)	Caucasian	Brazil	644/269	327	269	48	213	17	39	0.00
Pezzetti et al. (2004)	Caucasian	Italy	110/289	28	58	24	95	151	43	0.17
Brandalize et al. (2007)	Caucasian	Brazil	114/100	49	46	19	45	41	14	0.35
Chevrier et al. (2007)	Caucasian	France	168/148	54	81	33	66	60	22	0.17
Mills et al. (2008)	Caucasian	Ireland	492/1599	217	221	54	715	721	163	0.34
Ali et al. (2009)	Asian	India	323/214	–	–	–	–	–	–	–
Guo et al. (2009)	Asian	China	96/103	19	53	24	22	57	24	0.27
Sozen et al. (2009)	Caucasian	USA	179/138	81	80	18	66	65	7	0.07
Chorna et al. (2011)	Caucasian	Ukraine	33/50	12	17	4	22	26	2	0.09
Semic-Jusufagic et al. (2012)	Asian	Turkey	56/76	25	28	3	44	24	8	0.10
Kumari et al. (2013)	Asian	India	467/469	327	125	15	364	100	5	0.52
Aslar et al. (2013)	Asian	Turkey	80/125	13	57	10	59	62	4	0.01
Estandia-Ortega et al. (2014)	Caucasian	Mexico	132/370	39	55	38	143	172	55	0.77

present meta-analysis and two were published meta-analyses on the same topic, 2 articles were not case control study and in 7 studies maternal MTHFR C677T polymorphism was reported. Flow diagram of study selection was given in Fig. 1.

Selected Studies

Twenty-two case-control eligible studies on MTHFR C677T polymorphism and nsCL/P were identified through literature search and selection based on the inclusion and exclusion criteria and included in the present meta-analysis [17, 26, 27, 29, 30, 33, 34, 45, 56–69]. All these twenty-two studies were performed in different countries—Argentina [45], Brazil [27, 56, 60], China [64], France [17], Germany [58], India [62, 68], Ireland [61], Italy [29, 59], Mexico [69], Netherlands [34], Thailand [33], Turkey [66, 67], Ukraine [65], and USA [26, 30, 57, 63]. Details of included studies are given in Table 1.

Characteristics of Included Studies

The studies were published between 1998 and 2014. Two studies [56, 67] departed from HWE. Two studies did not reported MTHFR genotypes in cases and controls [30, 62], reported only allele numbers in cases and controls. The smallest case sample size was 3365 and highest sample size was 64,456.

Table 2 Summary estimates for the odds ratio (OR) of MTHFR C677T in various allele/genotype contrasts, the significance level (*p* value) of heterogeneity test (Q test), and the I^2 metric and publication bias *p* value (Egger and Beggs Tests)

Genetic models	Fixed effect OR (95% CI), p	Random effect OR (95% CI), p	Heterogeneity *p* value (Q test)	I^2 (%)	Publication bias (*p* of Egger's test)	Publication bias (*p* value of Beggs test)
All studies						
Allele contrast (T vs. C)	1.2 (1.12–1.28), <0.0001	1.24 (1.1–1.4), 0.0006	<0.0001	66.26	0.40	1
Co-dominant (Ct vs. CC)	1.24 (1.13–1.37), <0.0001	1.26(0.98–1.63), 0.007	<0.0001	83.34	0.32	0.16
Homozygote (TT vs. CC)	0.8 (0.69–0.91), 0.001	1.02 (0.74–1.4), 0.09	<0.0001	75.14	0.03	0.04
Dominant (TT + CT vs. CC)	1.26 (1.15–1.38), <0.0001	1.29 (1.04–1.59), 0.02	<0.0001	78.17	0.50	0.34
Recessive (TT vs. CT + CC)	1.24 (1.07–1.43), 0.002	1.36 (1.05–1.74), 0.02	0.0004	59.32	0.18	0.14
Asian studies						
Allele contrast (T vs. C)	1.5 (1.27–1.74), <0.0001	1.49 (1.1–1.9), 0.006	0.02	63.39	0.63	1
Co-dominant (Ct vs. CC)	1.5(1.21–1.88), 0.0002	1.6 (1.03–2.5), 0.03	0.02	66.85	0.6	0.8
Homozygote (TT vs. CC)	1.32 (0.81–2.1), 0.3	1.2 (0.52–2.7), 0.65	0.07	53.49	0.48	1
Dominant (TT + CT vs. CC)	1.55 (1.25–1.92), <0.0001	1.6 (1.01–2.5), 0.04	0.008	70.68	0.72	0.46
Recessive (TT vs. CT + CC)	1.5 (0.98–2.3), 0.06	1.5 (−0.67 to 3.38),0.31	0.05	56.81	0.89	1
Caucasian studies						
Allele contrast (T vs. C)	1.15 (1.06–1.23), 0.0002	1.17 (1.03–1.33), 0.01	0.0005	62.31	0.55	0.093
Co-dominant (Ct vs. CC)	1.19 (1.06–1.32), 0.002	1.17 (0.85–1.59), 0.32	<0.0001	85.5	0.50	0.28
Homozygote (TT vs. CC)	0.75 (0.65–0.87), 0.0002	0.98 (0.68–1.42), 0.92	<0.0001	78.19	0.02	0.09
Dominant (TT + CT vs. CC)	1.20 (1.1–1.33), 0.0003	1.2 (0.95–1.5), 0.1	<0.0001	79.74	0.74	0.55
Recessive (TT vs. CT + CC)	1.21 (1.04–1.4), 0.01	1.3 (1.0–1.72), 0.04	0.0008	61.94	0.13	0.13

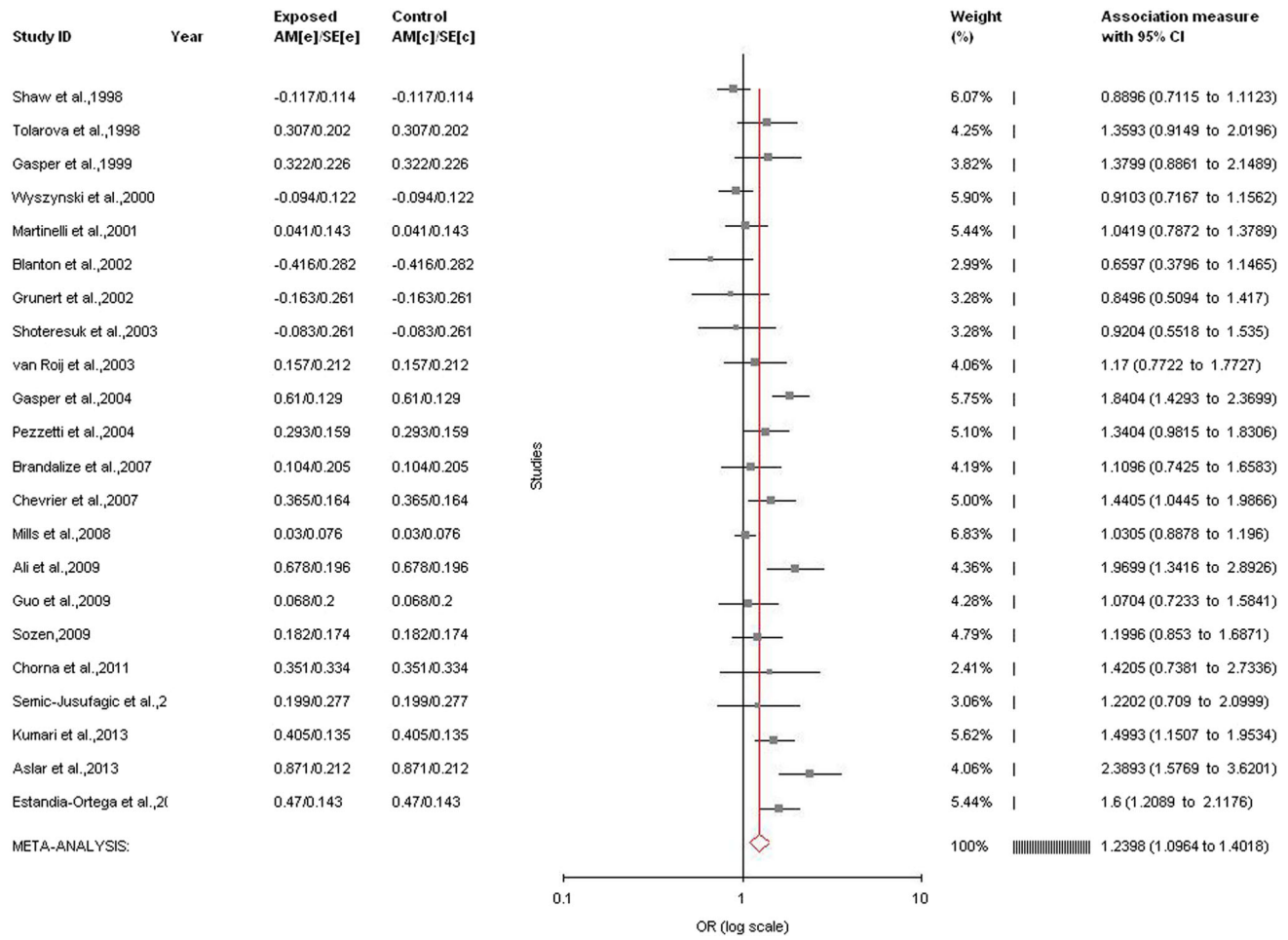

Fig. 2 Forest plot for the association between MTHFR C677T polymorphism and nsCL/P for allele contrast model (T vs. C) with random effect model in total studies

In included studies, total cases were 3274 with CC (1757), CT (1532) and TT (435), and controls were 5275 with CC (2594), CT (2141), and TT (540). In controls genotype percentage of CC, CT and TT were 49.2, 40.59 and 10.24% respectively. In cases genotype percentage of CC, CT and TT were 47.18, 41.14 and 11.68% respectively. Six studies did not show any association [26, 29, 30, 33, 57, 58] and odds ratio was above one in other sixteen studies.

Meta-Analysis

Allele contrast meta-analysis (T vs. C) of twenty-two studies indicated a strong significant association between MTHFR C677T polymorphism and nsCL/P susceptibility with both fixed (OR = 1.20, 95% CI = 1.12–1.28, $p < 0.0001$) and random (OR = 1.24, 95% CI = 1.1–1.4, $p = 0.0006$) effect models (Table 2, Fig. 2).

Significant association was detected between the MTHFR C677T polymorphism and the susceptibility to nsCL/P using other genetic models except homozygote model adopting random effect model (for CT vs. CC (co-dominant/heterozygote): OR = 1.26, 95%CI = 0.99–1.63, $p = 0.007$; for TT + CT vs. CC (dominant): OR = 1.29, 95% CI = 1.04–1.59, $p = 0.02$; for TT vs. CC (homozygote): OR = 1.02, 95% CI = 0.74–1.4, $p = 0.09$; for TT vs. CT + CC (recessive): OR = 1.36, 95% CI = 1.05–1.74, $p = 0.02$) (Table 2; Fig. 3). Significant association was also found in fixed effect models using all genetic models except homozygote (for CT vs. CC: OR = 1.24, 95%CI = 1.13–1.37, $p < 0.0001$; for TT + CT vs. CC: OR = 1.26, 95% CI = 1.15–1.38, $p < 0.0001$; for TT vs. CC: OR = 0.80, 95% CI = 0.69–0.91, $p = 0.001$; for TT vs. CT + CC: OR = 1.24, 95% CI = 1.07–1.43, $p = 0.002$; for) (Table 2).

A significant between studies heterogeneity was existed in allele contrast (Pheterogeneity < 0.0001, Q = 62.24, $I^2 = 66.26\%$, t2 = 0.05, z = 3.4), genotype homozygote (Pheterogeneity < 0.0001, Q = 76.44, $I^2 = 75.14\%$, t2 = 0.37, z = 0.13), dominant (Pheterogeneity < 0.0001,

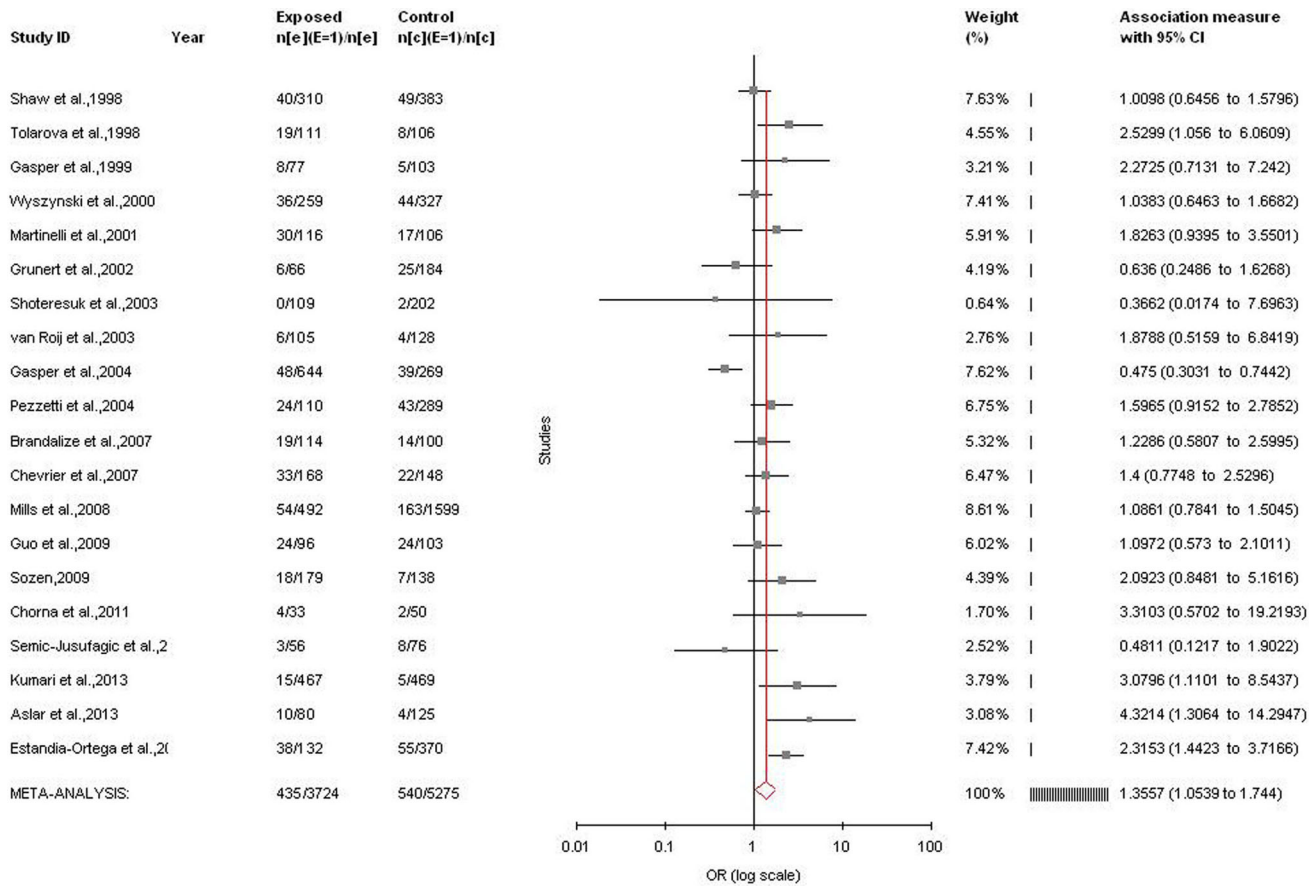

Fig. 3 Forest plot for the association between MTHFR C677T polymorphism and nsCL/P for recessive model (TT vs. CT + CC) with random effect model in total studies

Q = 87.02, I^2 = 78.17%, t2 = 0.17, z = 2.34) and recessive (Pheterogeneity = 0.002, Q = 46.71, I^2 = 59.32%, t2 = 0.16, z = 2.37) comparisons.

Sensitivity Analysis

Sensitivity analysis was performed by exclusion of the studies with small sample size and studies deviated from Hardy–Weinberg equilibrium. Genotypes distribution in control population of two studies 56,67 was not in HW equilibrium and sample size in one study 65 was less than 50. Exclusion of these three studies, heterogeneity between studies was decreased (Pheterogeneity = 0.003, Q = 38.35, I^2 = 53.07%, t2 = 0.03, z = 2.7) and OR was also decreased (T vs. C: OR = 1.17, 95% CI = 1.04–1.22, p = 0.0003).

Subgroup Analysis

Sub-group analysis based on ethnicity was also performed. In Asian population (number of studies = 6; 808/975 cases/controls), allele contrast meta-analysis showed significant association adopting both fixed (ORT vs. C = 1.5; 95% CI = 1.27–1.74; p = < 0.0001; I^2 = 63.39%; Pheterogeneity = 0.02; PPb = 0.63) and random (ORT vs. C = 1.49; 95% CI = 1.1–1.94; p = 0.006) effect models. Combined mutant genotypes also showed significant association with fixed (ORTT + CT vs. CC = 1.55; 95% CI = 1.25–1.9; p < 0.0001; I^2 = 70.68%; Pheterogeneity = 0.008; PPb = 0.72) and random (ORTT + CT vs. CC = 1.6; 95% CI = 1.01–2.5; p = 0.04) effect models (Table 2).

Results of Caucasian studies (number of studies = 16; 2916/4300 cases/controls) meta-analysis also indicated significant association with both fixed (ORT vs. C = 1.15; 95% CI = 1.06–1.23; p = 0.0002) and random (ORT vs. C = 1.17; 95% CI = 1.03–1.33; p = 0.01) effect models. However, higher between studies significant heterogeneity was also observed (I^2 = 62.31%; Pheterogeneity = 0.0005). The combined mutant genotype showed statistically significant association with fixed effect model (ORTT + CT vs. CC = 1.20; 95% CI = 1.1–1.33; p = 0.0003; I^2 = 79.74%; Pheterogeneity < 0.0001) and

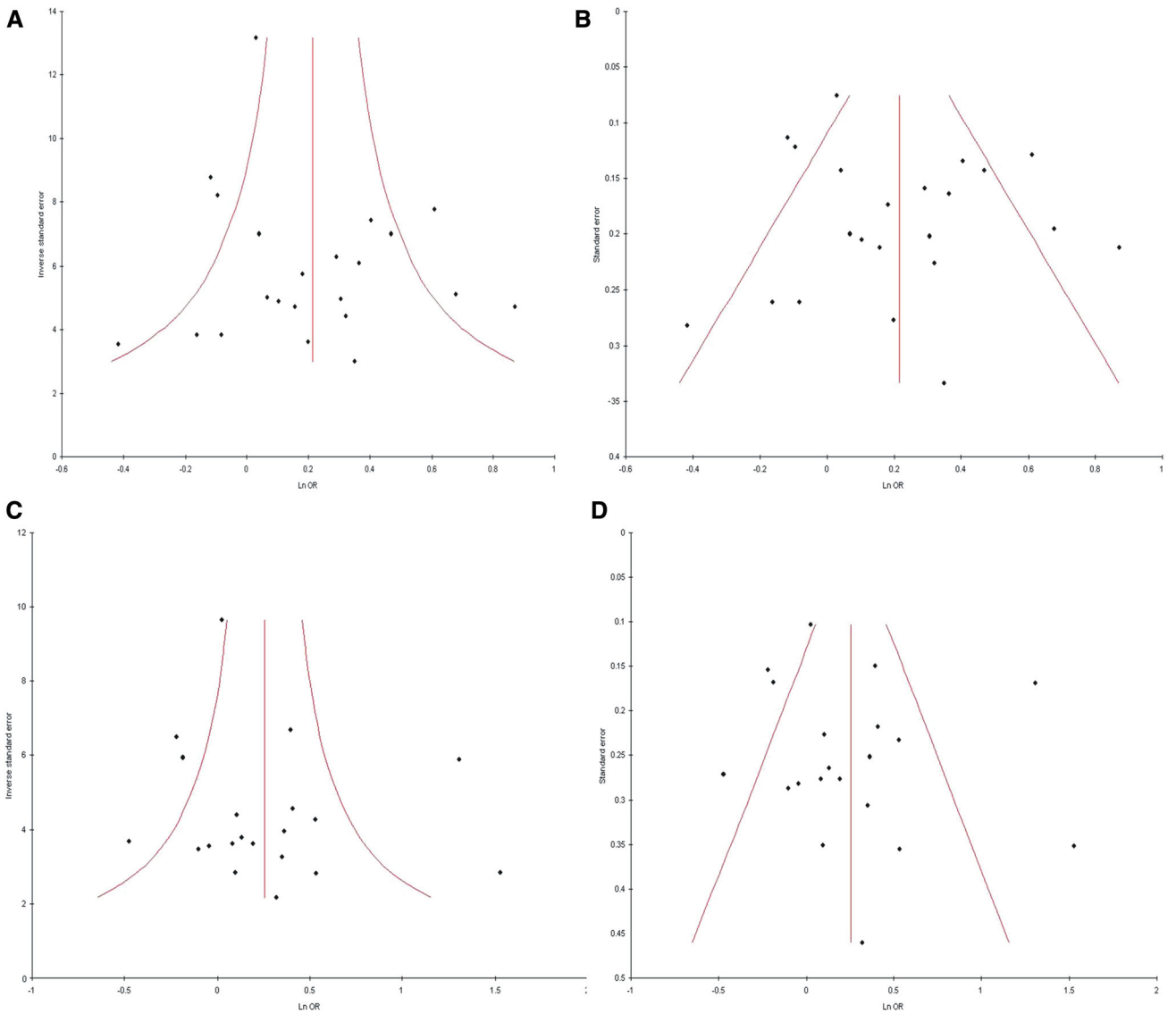

Fig. 4 Funnel plots A. precision versus OR (T vs. C), B. standard error versus OR (T vs. C), C. precision versus OR (TT + CT vs. CC), D. standard error versus OR (TT + CT vs. CC)

insignificant association with random effect (ORTT + CT vs. CC = 1.2; 95% CI = 0.95–1.5; $p = 0.1$) (Table 2).

Publication Bias

Except homozygote model publication bias could not be observed in all genetic models by Begg's and Egger's test. Funnel plots did not reveal any evidence of asymmetry, and except homozygote model p value were more than 0.05 (Begg's $p = 1.0$, Egger's $p = 0.40$ for T vs. C; Begg's $p = 0.04$, Egger's $p = 0.03$ for TT vs. CC; and Begg's $p = 0.16$, Egger's $p = 0.32$ for CT vs. CCA; Begg's $p = 0.34$, Egger's $p = 0.50$ for TT + AC vs. CC; Begg's $p = 0.14$, Egger's $p = 0.18$ for TT vs. CT + CC) (Table 2; Fig. 4).

Discussion

A number of population-based epidemiological studies have suggested that inadequate intake of folic acid [12, 14, 16, 57, 70, 71], or exposure to folic acid antagonists [72] during pregnancy might predispose to orofacial clefts. Conversely, multivitamin supplementation during pregnancy may reduce the risk of orofacial clefts. Folic acid and its derivatives are essential for DNA synthesis and methylation which are required for normal cell division and gene expression during fetal development. Genetic polymorphism in folate/homocysteine pathway genes like MTHFR and MTRR increases concentration of homocysteine (hyperhomocysteinemia), which has been identified as risk factors for certain congenital defects (e.g. NTD,

congenital heart defects) and late-age disorders (e.g. cardiovascular, cancers) [73].

There are strong experimental evidences showing that folate deficiency and/or dysfunctional MTHFR gene is risk factor for nsCL/P like- (1) in murine model, knockout of MTHFR gene induced apoptosis in embryonic palatal mesenchymal cells (MEPM) and prevent growth [74], (2) supplementation with folic acid was sufficient to reverse the teratogenic effect of the silenced MTHFR gene (3) exposure to folic acid antagonists [72] during pregnancy might predispose to orofacial clefts, (4) number of population-based epidemiological studies have suggested that inadequate intake of folic acid [12, 14, 16, 57, 70, 71], during pregnancy might predispose to orofacial clefts and (5) Munger et al. [75] showed that folic acid could reduce the risk of facial clefts in mouse models [75].

Meta-analysis is a useful strategy for elucidating genetic factors in different diseases/disorders. Several meta-analysis were published which evaluated risk of MTHFR polymorphism for different disease and disorders- like-congenital heart defects [39], Down syndrome [76]; recurrent pregnancy loss [77]; stroke [78]; psychiatric disorders [79–81]; Alzheimers disease [82]; and cancer [83–86].

Four meta-analyses were published so far investigated MTHFR polymorphisms as risk factor for cleft lip and palate [87–90]. Out of four meta-analyses, two were investigated MTHFR A1298C polymorphism as risk factor [88, 89] whereas other two meta-analyses ncluded C677T polymorphism [87, 90]. Verkleij-Hagoort et al. [87] published a meta-analysis of eight studies and reported no association (OR = 1.0; 95% CI = 0.9–1.2). In a recent meta-analysis, Zhao et al. [90] carried out meta-analysis of nine Asian studies and reported significant association between C677T polymorphism and CL/P risk (OR = 1.41; 95% CI = 1.23–1.61). Previous meta-analyses [87, 90] included only eight and nine case–control studies, which were too little to confirm the association between MTHFR C677T polymorphism and nsCL/P risk. Hence to provide the more comprehensive assessment, present meta-analysis was carried out to update the existing meta-analyses by including studies published in the interim not included in the previous meta-analyses. Present meta-analysis has included data from twenty-two case control studies with 3592 nsCL/P cases and 4905 controls.

The present meta-analysis showed a higher heterogeneity between studies, which may be due to differences in the ethnicity of studied populations and study design. There are few limitations in the present meta-analysis like crude ORs used in without adjustment; other factors such as folate intake etc. were not considered. nsCL/P may be due to several other gene polymorphisms involved in the homocysteine and folate metabolic pathway which were not considered in this meta-analysis. In addition, present meta-analysis has strength also like (1) absence of publication bias and (2) inclusion of larger number of studies.

In conclusion, present meta-analysis indicated that MTHFR gene C677T polymorphism is associated with the higher risk of nsCL/P. Simultaneously, subgroup analyses based on ethnicity further confirmed this association. In future, well designed studies with larger samples are necessary to validate association between this polymorphism and role of micronutrient factors like folate and B12 in the susceptibility of nsCL/P. However, results of the present meta-analysis should be interpreted cautiously, owing to the higher heterogeneity among studies.

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Compliance with Ethical Standards

Conflict of interest The author declares that she has no conflict of interest.

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