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Review Article

Methylenetetrahydrofolate reductase rs1801133 C>T polymorphism is association with nonsyndromic cleft lip with or without cleft palate susceptibility: a meta-analysis

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Abstract: Background: The potentially functional polymorphism rs1801133 C>T in *methylenetetrahydrofolate reductase* (*MTHFR*) gene was proposed to be associated with nonsyndromic cleft lip with or without cleft palate (nsCL/P) susceptibility. The purpose of this meta-analysis was to evaluate the association between this polymorphism and nsCL/P risk in three groups: Group A, nsCL/P patients vs. healthy controls; Group B, mothers with nsCL/P progeny vs. mother controls with healthy progeny; and Group C: father with nsCL/P progeny vs. father controls with healthy progeny. Methods: All case-control studies up to January 10, 2016 were identified by searching EMBASE and PubMed. The nsCL/P risk associated with the polymorphism was estimated by ORs together with their 95% CIs for each study, respectively. Results: A total of 37 case-control studies from 28 articles were included in our meta-analysis. In overall meta-analysis, we found no association between any genotypes and nsCL/P in Group A and Group C, whereas the *MTHFR* rs1801133 TT genotype significantly increased nsCL/P risk in Group B. In subgroup analysis stratified by ethnicity, we summarized the association between the genotypes, which indicate that the *MTHFR* rs1801133 TT genotype was a dangerous factor for nsCL/P in Asians for Group A. For Group B, we found that *MTHFR* rs1801133 TT genotype of mothers in Caucasians might raise progeny nsCL/P morbidity. Conclusion: *MTHFR* rs1801133 TT genotype is a dangerous factor for nsCL/P in Asians for Group A. And *MTHFR* rs1801133 TT genotype of mothers in Caucasians may raise progeny nsCL/P morbidity.

Keywords: Methylenetetrahydrofolate reductase, polymorphism, NsCL/P, risk, meta-analysis

Introduction

Cleft lip with or without cleft palate (CL/P) is one of the most common orofacial congenital defects worldwide [1]. The incidence of CL/P is about 1/500 to 1/2500, but this rate varies among different populations and socio-economic groups [2, 3]. CL/P includes nonsyndromic CL/P (nsCL/P), which is not associated with other defects, and syndromic CL/P, which is usually associated with other defects in different organs. Approximately 70 percent of CL/P is thought to be nsCL/P [4, 5]. NsCL/P is the research object of this meta-analysis. NsCL/P pathogenesis remains poorly understood, but it is acknowledged that both environmental and genetic factors may constitute its multifactorial aetiology. Environmental factors, such as folic acid and vitamin deficiency, maternal hormonal disorders, hypoxia, psychiatric medications use, overweight, smoking and maternal obesity, can affect the nsCL/P incidence [6].

Folic acid, the oxidized and more bioavailable form of folate, exists in multi-vitamins and food supplements and plays important roles in metabolism of amino acids as well as the synthesis and methylation of DNA [7]. Many studies have been performed to determine the role

of folic acid in the etiology of nsCL/P. But it is difficult to investigate the specific effects of folic acid separate from the effects of other kind of vitamins found in multivitamins or supplements. Attention has been distracted to the effects of polymorphisms of genes involved in folic acid metabolism. One of the first folic acid metabolism genes studied about association with nsCL/P was *methylenetetrahydrofolate reductase (MTHFR)*. MTHFR is a key enzyme in folic acid metabolism and regulates the intracellular folic acid pool for synthesis and methylation of nucleic acid. MTHFR catalyzes 5, 10-methylenetetrahydrofolate into 5-methyltetrahydrofolate [8]. The gene of *MTHFR* is located at chromosome 1p36.3 and includes 11 exons with a total length of 2.2 kb. Several *MTHFR* single nucleotide polymorphisms (SNPs) have been established. The polymorphism rs1801133 C>T (C677T) of *MTHFR* gene can cause reduced enzyme activity [9]. It transforms cytosine to thymine at nucleotide position 677 in exon 4 and then entails a substitution of alanine to valine in the catalytic domain [10], which results in the loss of the enzyme quaternary structure and an enhancive dissociation of FAD cofactor.

It has been hypothesized that nsCL/P may be associated with *MTHFR*. Thus, the relationship between *MTHFR* rs1801133 C>T polymorphism and the risk of nsCL/P in patients has been widely studied. Recently, some case-control studies explored this issue; however, the findings were conflicting. Maternal folic acid deficiency is a definite risk factor for nsCL/P and may increase its morbidity in progeny [11]. Thus, it was hypothesized that variants of *MTHFR*, involved in folic acid metabolism pathway, were associated with the risk of nsCL/P. Some studies have focused on the relationship between maternal *MTHFR* rs1801133 C>T polymorphism and morbidity of nsCL/P, but the conclusions are inconsistent [12-21].

Recently, two meta-analysis studies have been performed on the association of *MTHFR* rs1801133 C>T with susceptibility to nsCL/P [22, 23]. However, no meta-analysis summarized the association between paternal *MTHFR* rs1801133 C>T polymorphism and susceptibility to nsCL/P in offspring. To solve the problems of inadequate statistical power and disputable results, we conducted this meta-analysis using evidence from published data to explore the association between nsCL/P and *MTHFR* rs1801133 C>T polymorphism. As far as we know, this is the first meta-analysis to evaluate the association of *MTHFR* rs1801133 C>T polymorphism with nsCL/P risk in three groups: Group A: nsCL/P patients vs. healthy controls, Group B: mothers with nsCL/P progeny vs. mother controls with healthy progeny, and Group C: father with nsCL/P progeny vs. father controls with healthy progeny.

Materials and methods

Article search

A comprehensive publication search for studies on the association of the *MTHFR* rs1801133 C>T polymorphism with the susceptibility to nsCL/Ps was conducted in the EMBASE and Pubmed databases up to January 10, 2016. The search terms were: 'Methylenetetrahydrofolate reductase' or 'MTHFR' and 'polymorphism' or 'mutation' or 'variant' or 'SNP' and 'cleft'. All studies were only about human subjects and published in English or Chinese. Manual search of all references quoted in these published reviews or studies was supplemented to the results of electronic retrieval.

Inclusion and exclusion criteria

In this meta-analysis, the major inclusion criteria were: (a) all candidate studies focused on the relationship between *MTHFR* rs1801133 C>T polymorphism and the susceptibility of nsCL/P; (b) all candidate studies were designed as case-control studies; (c) all candidate studies provided sufficient available genotype data of *MTHFR* rs1801133 C>T polymorphism in cases and controls; (d) all candidate studies evaluate *MTHFR* rs1801133 C>T polymorphism and nsCL/P risk. The major exclusion criteria were: (a) not related to *MTHFR* rs1801133 C>T polymorphism and nsCL/P risk; (b) not case-control study; (c) repeated studies; (d) no available data; (e) reviews, comments, letters and conference papers; (f) inconsistent with Hardy-Weinberg equilibrium (HWE). The inclusion and exclusion procedures are shown in **Figure 1**.

Data extraction

Three investigators (Y. Wang, G. Zheng and M. Kang) independently extracted the data from

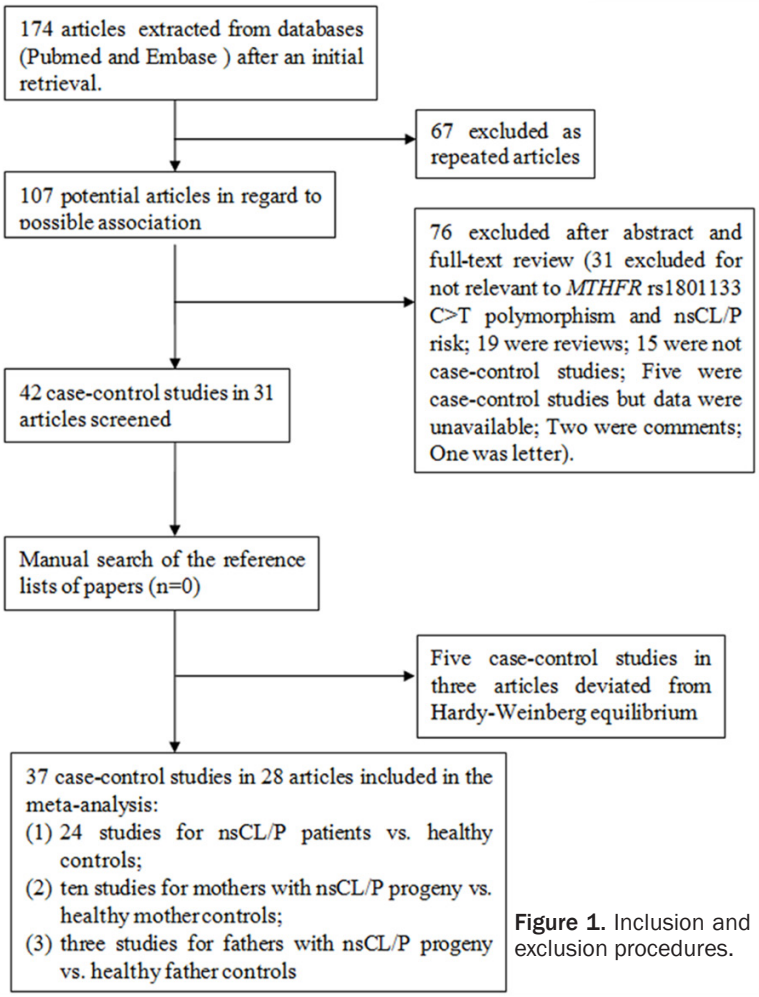

Figure 1. Inclusion and exclusion procedures.

all eligible studies according to the selection criterion mentioned above. Disagreements were solved through further discussion until agreements were reached among all reviewers. The following information was extracted: the first author's name, the sample size, year of publication, country of origin, the source of control group, HWE in controls, ethnicity, and number of controls and cases.

Statistical analysis

All statistical analyses were conducted by the STATA software (Version 12.0). A P value < 0.05 (Two-sided) was regarded statistically significant. HWE was evaluated using the χ^2 test for the observed genotype frequencies of the control groups in each study. The strength of association between *MTHFR* rs1801133 C>T polymorphism and nsCL/P risk was estimated by the crude odds ratios (ORs) with 95% (confidence intervals) CIs. Pooled ORs of three groups (nsCL/P patients vs. healthy controls, mothers with nsCL/P progeny vs. mother controls without nsCL/P progeny, and fathers with nsCL/P progeny vs. father controls without nsCL/P progeny were calculated in allele model (T vs. C), homozygote model (TT vs. CC), dominant model (TT + CT vs. CC), and recessive model (TT vs. CT + CC). The meta-analysis results also included an examination for publication bias, an analysis of the sensitivity, and a test for heterogeneity. In terms of possible heterogeneity between studies, the I^2 metric and Cochran's Q-statistic were conducted [24, 25]. P < 0.10 or I^2>50% were considered significant heterogeneity. If heterogeneity test results turned out P < 0.1 or I^2>50%, the pooled ORs were analyzed using the random effect model [26]; otherwise, the fixed effect model was used [27]. To determine the source of heterogeneity, subgroup analyses stratified by ethnicity, source of controls and sample sizes were performed. Sensitivity analyses were conducted using the step by step exclusion method to assess whether each individual study influenced the final results. The publication bias was examined using Begg's funnel plot and Egger's test [28]. For publication bias, P value < 0.10 (Two-sided) was regarded statistically significant.

Results

Study characteristics

In total, 174 potentially correlative publications yielded initially after retrieving according to the eligible criteria. **Figure 1** exhibited the detailed study extraction processing. Finally, a total of 37 case-control studies from 28 articles were

Table 1. Characteristics of Studies in the Meta-analysis

Study	Year	Ethnicity	No. of cases/ controls	Source of case	Genotype Method	Objective of study
Group A: 4452 nsCL/P patients vs. 5632 healthy controls in 24 studies						
Abdollahi-Fakhim S et al.	2015	Caucasians	65/50	Hospital-based	RFLP-PCR	Group A
Bezerra JF et al.	2015	Mixed	140/175	Population-based	RFLP-PCR	Group A
Jahanbin A et al.	2014	Caucasians	45/101	Population-based	PCR-RFLP	Group A
Estandia-Ortega B et al.	2014	Caucasians	132/370	Hospital-based	KASPar assay system	Group A
Murthy J et al.	2014	Asians	123/141	Hospital-based	RFLP-PCR	Group A
Mostowska A et al.	2014	Caucasians	174/176	Population-based	RFLP-PCR	Group A
Kumari P et al.	2013	Asians	318/281	Population-based	RFLP-PCR	Group A
Semic-Jusufagic A et al.	2012	Caucasians	56/169	Population-based	PCR	Group A
Chorna LB et al.	2011	Caucasians	33/50	Hospital-based	RFLP-PCR	Group A
Han Y et al.	2010	Asians	200/213	Hospital-based	RFLP-PCR	Group A
Guo JZ et al.	2009	Asians	97/104	Hospital-based	PCR	Group A
Sozen MA et al.	2009	Mixed	179/138	Population-based	PCR	Group A
Ali A et al.	2008	Asians	646/428	Population-based	RFLP-PCR	Group A
Mills JL et al.	2008	Caucasians	536/1000	Hospital-based	RFLP-PCR	Group A
Little J et al.	2008	Caucasians	190/248	Population-based	MS-PCR	Group A
Brandalize AP et al.	2007	Mixed	114/100	Hospital-based	RFLP-PCR	Group A
Chevrier C et al.	2007	Caucasians	168/148	Hospital-based	RFLP-PCR	Group A
Wan WD et al.	2006	Asians	76/60	Hospital-based	RFLP-PCR	Group A
Pezzetti F et al.	2004	Caucasians	110/289	Hospital-based	RFLP-PCR	Group A
Gaspar DA et al.	2004	Mixed	424/644	Hospital-based	SNuPE test	Group A
Shotelersuk V et al.	2003	Asians	109/202	Population-based	RFLP-PCR	Group A
van Rooij IA et al.	2003	Caucasians	130/150	Hospital-based	RFLP-PCR	Group A
Gaspar DA et al.	1999	Caucasians	77/113	Hospital-based	PCR	Group A
Shaw GM et al.	1998	Mixed	310/282	Population-based	RFLP-PCR	Group A
Group B: 1080 mothers with nsCL/P progeny versus 1263 mother controls with healthy progeny in 10 studies						
Bezerra JF et al.	2015	Mixed	140/175	Population-based	RFLP-PCR	Group B
Ibarra-Lopez JJ et al.	2013	Caucasians	88/116	Hospital-based	RFLP-PCR	Group B
Wang SM et al.	2012	Asians	89/64	Hospital-based	RFLP-PCR	Group B
Chorna LB et al.	2011	Caucasians	33/50	Hospital-based	RFLP-PCR	Group B
Bufalino A et al.	2010	Mixed	106/184	Hospital-based	RFLP-PCR	Group B
Vujkovic M et al.	2010	Caucasians	198/191	Hospital-based	RFLP-PCR	Group B
Guo JZ et al.	2009	Asians	97/104	Hospital-based	PCR	Group B
Brandalize AP et al.	2007	Mixed	100/100	Hospital-based	RFLP-PCR	Group B
van Rooij IA et al.	2003	Caucasians	170/189	Hospital-based	RFLP-PCR	Group B
Gaspar DA et al.	1999	Caucasians	59/90	Hospital-based	PCR	Group B
Group C: 334 fathers with nsCL/P progeny versus 345 father controls with healthy progeny in 3 studies						
Wang SM et al.	2012	Asians	89/64	Hospital-based	RFLP-PCR	Group C
Guo JZ et al.	2009	Asians	97/104	Hospital-based	PCR	Group C
van Rooij IA et al.	2003	Caucasians	158/177	Hospital-based	RFLP-PCR	Group C

RFLP-PCR: polymerase chain reaction-restriction fragment length polymorphism. MS-PCR : mutagenically seperated PCR. SNuPE test: single nucleotide primer extension test. PCR: polymerase chain reaction.

included in our meta-analysis. The following shows we have included: (a) 24 studies for Group A: 4452 nsCL/P patients vs. 5632 healthy controls; (b) 10 studies for Group B: 1080 mothers with nsCL/P progeny vs. 1263 mother controls with healthy progeny; (c) 3

Table 2. Distribution of *MTHFR* rs1801133 C>T polymorphisms genotype and allele among multiple cancer patients and controls

		Case			Control			Case		Control		
Study	Year	CC	CT	TT	CC	CT	TT	C	T	C	T	nsCL/P
Group A: 4452 nsCL/P patients vs. 5632 healthy controls in 24 studies												
Abdollahi-Fakhim S et al.	2015	38	25	2	27	22	1	101	29	76	24	Yes
Bezerra JF et al.	2015	74	54	12	85	70	20	202	78	240	110	Yes
Jahanbin A et al.	2014	20	16	7	46	41	14	56	30	133	69	Yes
Estandia-Ortega B et al.	2014	38	55	39	55	172	143	131	133	282	458	Yes
Murthy J et al.	2014	104	19	0	107	31	3	227	19	245	37	Yes
Mostowska A et al.	2014	81	65	17	78	77	16	227	99	233	109	Yes
Kumari P et al.	2013	327	126	15	364	100	5	780	156	828	110	Yes
Semic-Jusufagic A et al.	2012	25	28	3	44	24	8	78	34	112	40	Yes
Chorna LB et al.	2011	12	17	4	22	26	2	41	25	70	30	Yes
Han Y et al.	2010	46	106	35	74	110	29	198	176	258	168	Yes
Guo JZ et al.	2009	19	53	24	22	57	24	91	101	101	105	Yes
Sozen MA et al.	2009	81	80	18	66	65	7	242	116	197	79	Yes
Ali A et al.	2008	225	87	11	176	36	2	537	109	388	40	Yes
Mills JL et al.	2008	217	221	54	715	721	163	655	329	2151	1047	Yes
Little J et al.	2008	39	47	10	94	101	29	125	67	289	159	Yes
Brandalize AP et al.	2007	49	46	19	45	41	14	144	84	131	69	Yes
Chevrier C et al.	2007	66	60	22	54	81	33	192	104	189	147	Yes
Wan WD et al.	2006	13	49	14	31	20	9	75	77	82	38	Yes
Pezzetti F et al.	2004	28	58	24	95	151	43	114	106	341	237	Yes
Gaspar DA et al.	2004	213	172	39	327	269	48	720	296	1174	454	Yes
Shotelersuk V et al.	2003	84	25	0	154	46	2	193	25	354	50	Yes
van Rooij IA et al.	2003	54	45	6	70	54	4	153	57	194	62	Yes
Gaspar DA et al.	1999	33	39	8	49	49	15	105	55	147	79	Yes
Shaw GM et al.	1998	143	127	40	155	178	49	719	345	859	463	Yes
Group B: 1080 mothers with nsCL/P progeny versus 1263 mother controls with healthy progeny in 10 studies												
Bezerra JF et al.	2015	82	53	5	107	64	4	217	63	278	72	Yes
Ibarra-Lopez JJ et al.	2013	14	42	32	30	55	31	70	106	115	117	Yes
Wang SM et al.	2012	10	41	38	15	39	10	61	117	69	59	Yes
Chorna LB et al.	2011	16	9	2	22	26	2	41	13	70	30	Yes
Bufalino A et al.	2010	49	50	7	95	72	17	148	64	262	106	Yes
Vujkovic M et al.	2010	58	52	13	50	51	7	168	78	151	65	Yes
Guo JZ et al.	2009	26	49	22	20	57	25	101	93	97	107	Yes
Brandalize AP et al.	2007	44	45	21	38	52	10	133	87	128	72	Yes
van Rooij IA et al.	2003	78	55	15	84	74	12	211	85	242	98	Yes
Gaspar DA et al.	1999	30	19	10	37	40	13	79	39	114	66	Yes
Group C: 334 fathers with nsCL/P progeny versus 345 father controls with healthy progeny in 3 studies												
Wang SM et al.	2012	31	50	8	17	37	10	112	66	71	57	Yes
Guo JZ et al.	2009	26	44	26	21	57	22	96	96	99	101	Yes
van Rooij IA et al.	2003	64	58	12	73	72	9	186	82	218	90	Yes

HWE: Hardy-Weinberg equilibrium.

studies for Group C: 334 fathers with nsCL/P progeny vs. 345 father controls with healthy progeny. The characteristics of all studies on association between *MTHFR* rs1801133 C>T polymorphism and nsCL/Ps risk are shown in **Table 1**. **Table 2** showed the distribution of

Table 3. Summary results on the associations between *MTHFR* rs1801133 C>T polymorphisms and risk of nsCL/P in Group A

	No. of study	T vs. C			TT vs. CC			TT + CT vs. CC			TT vs. CT + CC		
		OR (95% CI)	*P*	*P* (Q-test)	OR (95% CI)	*P*	*P* (Q-test)	OR (95% CI)	*P*	*P* (Q-test)	OR (95% CI)	*P*	*P* (Q-test)
Total	24	1.06 (0.95-1.19)	0.280	< 0.001	1.15 (0.90-1.47)	0.252	0.002	1.07 (0.91-1.26)	0.395	< 0.001	0.94 (0.80-1.10)	0.234	0.229
Ethnicity													
Caucasians	12	0.97 (0.84-1.12)	0.666	0.034	0.93 (0.67-1.30)	0.683	0.028	0.95 (0.77-1.17)	0.606	0.020	0.96 (0.80-1.16)	0.704	0.319
Mixed	5	0.99 (0.89-1.10)	0.802	0.350	1.09 (0.83-1.42)	0.538	0.355	0.95 (0.81-1.11)	0.505	0.626	1.14 (0.88-1.48)	0.313	0.457
Asians	7	1.30 (0.99-1.71)	0.061	0.002	1.96 (1.35-2.85)	< 0.001	0.170	1.45 (0.99-2.12)	0.053	0.298	1.45 (1.04-2.01)	0.028	0.298
Number of cases													
< 300	10	1.11 (0.96-1.29)	0.168	0.108	1.27 (0.90-1.81)	0.179	0.395	1.20 (0.87-1.66)	0.266	0.011	1.08 (0.79-1.49)	0.625	0.711
≥ 300	14	1.04 (0.91-1.20)	0.542	< 0.001	1.10 (0.81-1.50)	0.528	< 0.001	1.02 (0.85-1.23)	0.830	< 0.001	1.11 (0.90-1.38)	0.333	0.066
Source of Control													
Hospital based	13	1.04 (0.89-1.21)	0.641	< 0.001	1.16 0.82-1.62)	0.400	0.001	1.04 (0.82-1.32)	0.720	< 0.001	1.07 (0.91-1.25)	0.438	0.291
Population based	9	1.10 (0.95-1.32)	0.278	0.004	1.12 (0.86-1.45)	0.404	0.154	1.12 (0.90-1.38)	0.323	0.012	1.14 (0.88-1.46)	0.323	0.193

Table 4. Summary results on the associations between *MTHFR* rs1801133 C>T polymorphisms and risk of nsCL/P in Group B

	No. of study	T vs. C			TT vs. CC			TT + CT vs. CC			TT vs. CT + CC		
		OR (95% CI)	*P*	*P* (Q-test)	OR (95% CI)	*P*	*P* (Q-test)	OR (95% CI)	*P*	*P* (Q-test)	OR (95% CI)	*P*	*P* (Q-test)
Total	10	1.12 (0.94-1.33)	0.205	0.076	1.44 (1.07-1.94)	0.016	0.144	1.01 (0.84-1.21)	0.906	0.179	1.52 (1.18-1.97)	0.001	0.211
Ethnicity													
Caucasians	5	1.07 (0.88-1.29)	0.505	0.334	1.50 (0.98-2.30)	0.064	0.759	0.93 (0.71-1.20)	0.569	0.210	1.51 (1.03-2.20)	0.033	0.985
Mixed	3	1.11 (0.89-1.39)	0.334	0.954	1.31 (0.74-2.29)	0.353	0.429	1.10 (0.83-1.46)	0.508	0.723	1.34 (0.79-2.27)	0.279	0.193
Asians	2	1.36 (0.52-3.58)	0.536	0.001	1.90 (0.24-15.40)	0.543	0.002	1.23 (0.35-4.34)	0.750	0.021	1.87 (0.43-8.11)	0.401	0.004
Number of cases													
< 300	7	1.15 (0.88-1.50)	0.315	0.020	1.46 (0.84-2.55)	0.179	0.038	1.03 (0.72-1.47)	0.874	0.062	1.50 (0.96-2.35)	0.076	0.063
≥ 300	3	1.06 (0.85-1.31)	0.607	0.896	1.48 (0.83-2.62)	0.044	0.953	0.98 (0.75-1.28)	0.880	0.759	1.57 (0.90-2.74)	0.109	0.976
Source of Control													
Hospital based	8	1.14 (0.91-1.43)	0.263	0.035	1.47 (0.91-2.38)	0.113	0.063	0.98 (0.72-1.35)	0.902	0.459	1.53 (1.15-2.02)	0.003	0.100
Population based	2	1.05 (0.81-1.36)	0.712	0.649	1.42 (0.70-2.85)	0.328	0.881	0.66 (0.33-1.31)	0.922	0.935	1.51 (0.76-2.99)	0.236	0.935

Table 5. Summary results on the associations between *MTHFR* rs1801133 C>T polymorphisms and risk of nsCL/P in Group C

Genetic model	OR (95% CI)	*P*	*P* (Q-test)
T vs. C	0.95 (0.75-1.19)	0.633	0.443
TT vs. CC	0.93 (0.55-1.57)	0.786	0.238
TT + CT vs. CC	0.83 (0.60-1.16)	0.285	0.594
TT vs. CT + CC	1.13 (0.71-1.80)	0.597	0.228

MTHFR rs1801133 C>T polymorphisms genotype and allele among multiple cancer patients and controls.

Results of the overall and subgroup analysis

The main results on the relationship between *MTHFR* rs1801133 C>T polymorphism and nsCL/P risk are listed in (**Tables 3**-**5**). **Table 3** is about Group A (nsCL/P patients vs. healthy controls), **Table 4** about Group B (mothers with nsCL/P progeny vs. mother controls with healthy progeny), and **Table 5** about Group C (fathers with nsCL/P progeny vs. father controls with healthy progeny). Overall, *MTHFR* rs1801133 TT genotype significantly increased nsCL/P risk in Group B [TT vs. CC (OR, 1.44; 95% CI, 1.07-1.94; *P* = 0.016), TT vs. CT + TT (OR, 1.52; 95% CI, 1.18-1.97; *P* = 0.001)] (**Table 4**; **Figure 2** for TT vs. CC, **Figure 3** for TT vs. CT + TT). No association was found between *MTHFR* rs1801133 C>T polymorphism and nsCL/P risk in Group A [T vs. C (OR, 1.06; 95% CI, 0.95-1.19; *P* = 0.280), TT vs. CC (OR, 1.15; 95% CI, 0.90-1.47; *P* = 0.252), TT + CT vs. CC (OR, 1.07; 95% CI, 0.91-1.26; *P* = 0.395), TT vs. CT + CC (OR, 0.94; 95% CI, 0.80-1.10; *P* = 0.234)] (**Table 3**; **Figure 4**) and Group C [T vs. C (OR, 0.95; 95% CI, 0.75-1.19; *P* = 0.633), TT vs. CC (OR, 0.93; 95% CI, 0.55-1.57; *P* = 0.786), TT + CT vs. CC (OR, 0.83; 95% CI, 0.60-1.16; *P* = 0.285), TT vs. CT + CC (OR, 1.13; 95% CI, 0.71-1.80; *P* = 0.597)] (**Table 5**; **Figure 5**).

As Asians, Caucasians and mixed populations were contained in our study, subgroup analyses were performed based on different ethnicities. The results showed that *MTHFR* rs1801133 TT genotype was a dangerous factor for nsCL/P in Asians in Group A [TT vs. CC (OR, 1.96; 95% CI, 1.35-2.85; *P* < 0.001) and TT vs. CT + TT (OR, 1.45; 95% CI, 1.04-2.01; *P* = 0.028)] (**Table 3**; **Figure 6** for TT vs. CC, **Figure 7** for TT vs. CT + TT). *MTHFR* rs1801133 TT genotype of mothers in Caucasians may raise progeny nsCL/P morbidity [TT vs. CT + CC (OR, 1.51; 95% CI, 1.03-2.20; *P* = 0.033)] (**Table 4**; **Figure 8**).

Publication bias

In this meta-analysis, Begg's Funnel plot and Egger's test were conducted to detect potential publication bias. In all comparison models of Group A and Group B, the funnel plot shape appeared to be symmetrical supported by Egger's test [Group A: T vs. C: Begg's test *P* = 0.747, Egger's test *P* = 0.535; TT vs. CC: Begg's test *P* = 0.568, Egger's test *P* = 0.392; TT + CT vs. CC: Begg's test *P* = 0.568, Egger's test *P* = 0.638; TT vs. CT + CC: Begg's test *P* = 0.503, Egger's test *P* = 0.513 (**Figure 9**); Group B: T vs. C: Begg's test *P* = 0.592, Egger's test *P* = 0.850; TT vs. CC: Begg's test *P* = 0.721, Egger's test *P* = 0.675; TT + CT vs. CC: Begg's test *P* = 0.721, Egger's test *P* = 0.925; TT vs. CT + CC: Begg's test *P* = 1.000, Egger's test *P* = 0.826 (**Figure 10**)].

Sensitivity analyses

One-way sensitivity analyses were performed through sequential omission of an individual study at a time. No individual study significantly influenced the pooled ORs in each group (Group A: **Figure 11**, Group B: **Figure 12**, data not shown).

Heterogeneity test

Large heterogeneities among the studies were identified in Group A and Group B. Meanwhile, we implemented subgroup analyses to evaluate the source of heterogeneity. For Group A, the results indicated that Asians, Caucusus, large-sample size of cases (≥ 300) and hospital-based subgroups may contribute to the major heterogeneity (**Table 3**). For Group B, heterogeneities were derived from Asian population, small-sample size of cases (< 300) and hospital-based subgroups (**Table 4**).

Discussion

It is well known that nsCL/P is one of the most prevalent congenital malformations and has a high mortality rate. The pathogenesis of nsCL/P is difficult to be attributed to any single factor, either environmental or genetic factors. The folic acid metabolism pathway plays an impor-

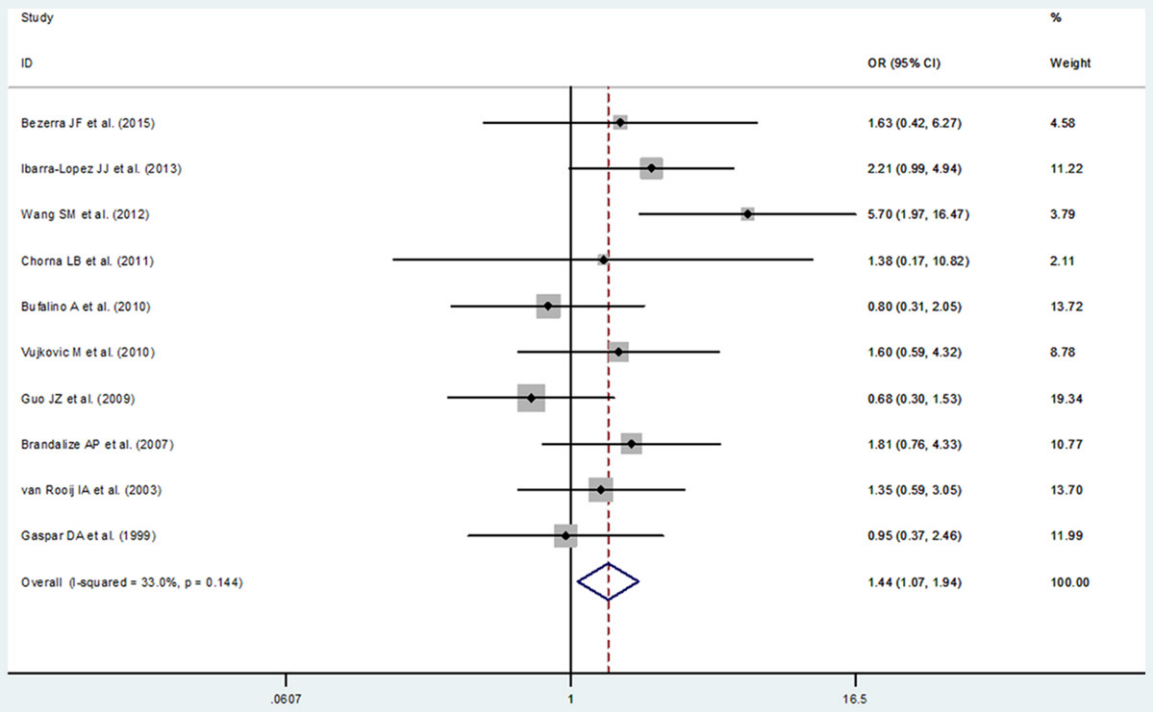

Figure 2. Group B (nsCL/P patients vs. healthy controls): fixed effect forest plot of homozygote model (TT vs. CC) for the association between the risk of nsCL/P and *MTHFR* rs1801133 C>T polymorphism.

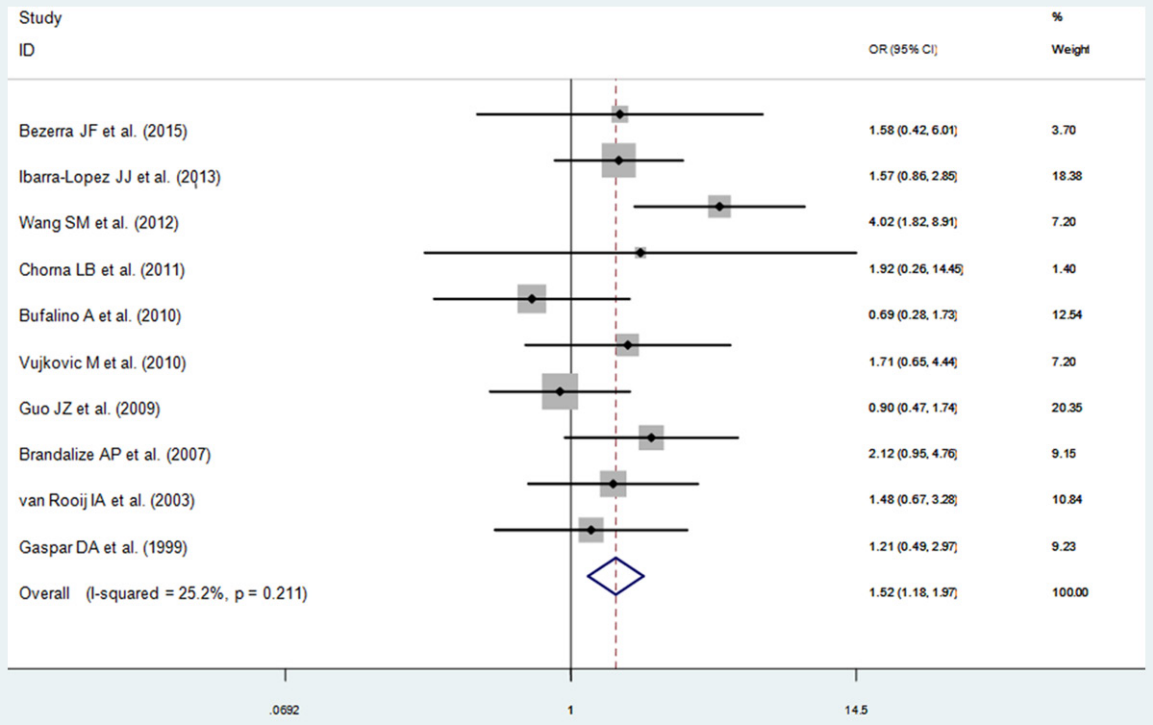

Figure 3. Group B (nsCL/P patients vs. healthy controls): fixed effect forest plot of recessive model (TT vs. CT+CC) for the association between the risk of nsCL/P and *MTHFR* rs1801133 C>T polymorphism.

tant role in DNA synthesis, DNA methylation, cell division, and tissue growth [29]. Therefore, a defective folic acid metabolism may result in impaired DNA methylation or DNA synthesis

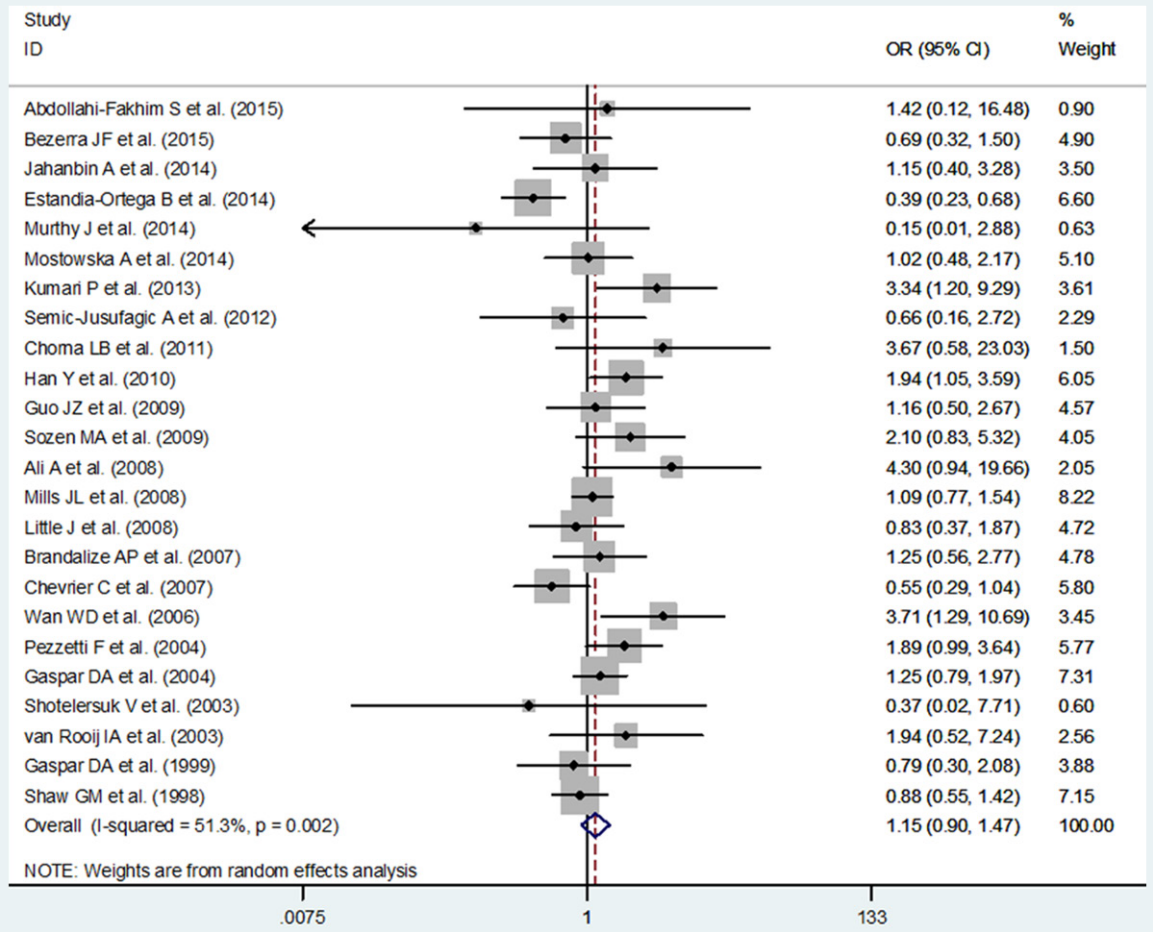

Figure 4. Group A (nsCL/P patients vs. healthy controls): random effect forest plot of homozygote model (TT vs. CC) for the association between the risk of nsCL/P and *MTHFR* rs1801133 C>T polymorphism.

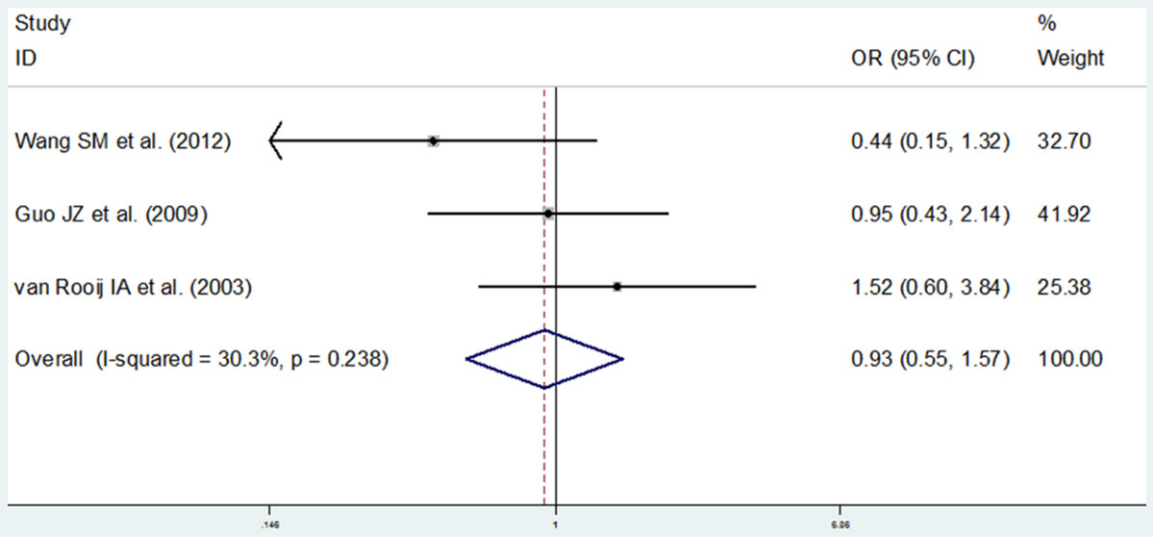

Figure 5. Group C (father with nsCL/P progeny vs. father controls with healthy progeny): fixed effect forest plot of homozygote model (TT vs. CC) for the association between the risk of nsCL/P and *MTHFR* rs1801133 C>T polymorphism.

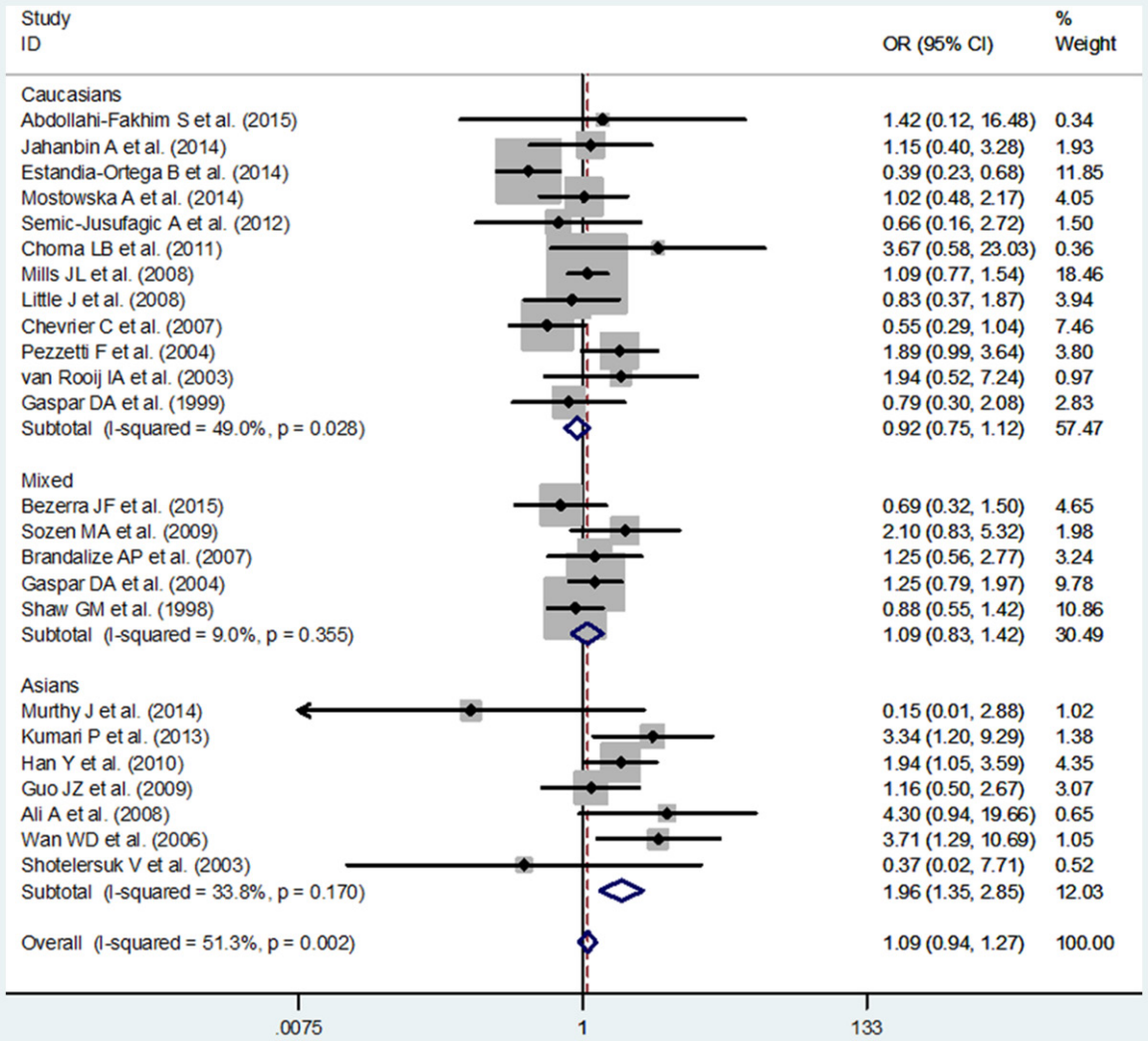

Figure 6. fixed effect forest plot of homozygote model (TT vs. CC) for the association between the risk of nsCL/P and *MTHFR* rs1801133 C>T polymorphism in Asian subgroup (Group A).

involved in the nsCL/P pathogenesis. MTHFR is a critical enzyme in folic acid metabolism. *MTHFR* rs1801133 C>T polymorphism is the most studied mutation among several single nucleotide polymorphisms of *MTHFR* gene. Previous studies reported that heterozygote CT of rs1801133 C>T polymorphism has 65% normal enzyme activity and lowers 10% red blood cell folic acid level; homozygous variant TT has only 30% normal enzyme activity and lower 18% red blood cell folic acid level [30, 31]. Additionally, individuals with TT mutation have decreased plasma folic acid and vitamin B12 levels and augmented homocysteine level [32, 33]. To date, many studies have focused on the correlation between *MTHFR* rs1801133 C>T polymorphism and nsCL/P risk. The potential implications of *MTHFR* rs1801133 C>T polymorphism in the pathogenesis of nsCL/P have been extensively studied; however, the results remain contradictory. In this meta-analysis, we collected a comprehensive data from previous studies to derive a more precise estimate of the relationship between *MTHFR* rs1801133 C>T polymorphism and susceptibility to nsCL/P. To the best of our knowledge, this is the first meta-analysis to comprehensively assess the association of *MTHFR* rs1801133 C>T polymorphism with susceptibility to nsCL/P in three groups.

In overall meta-analysis, we found no association between any genotypes and nsCL/P in Group A and Group C, whereas *MTHFR*

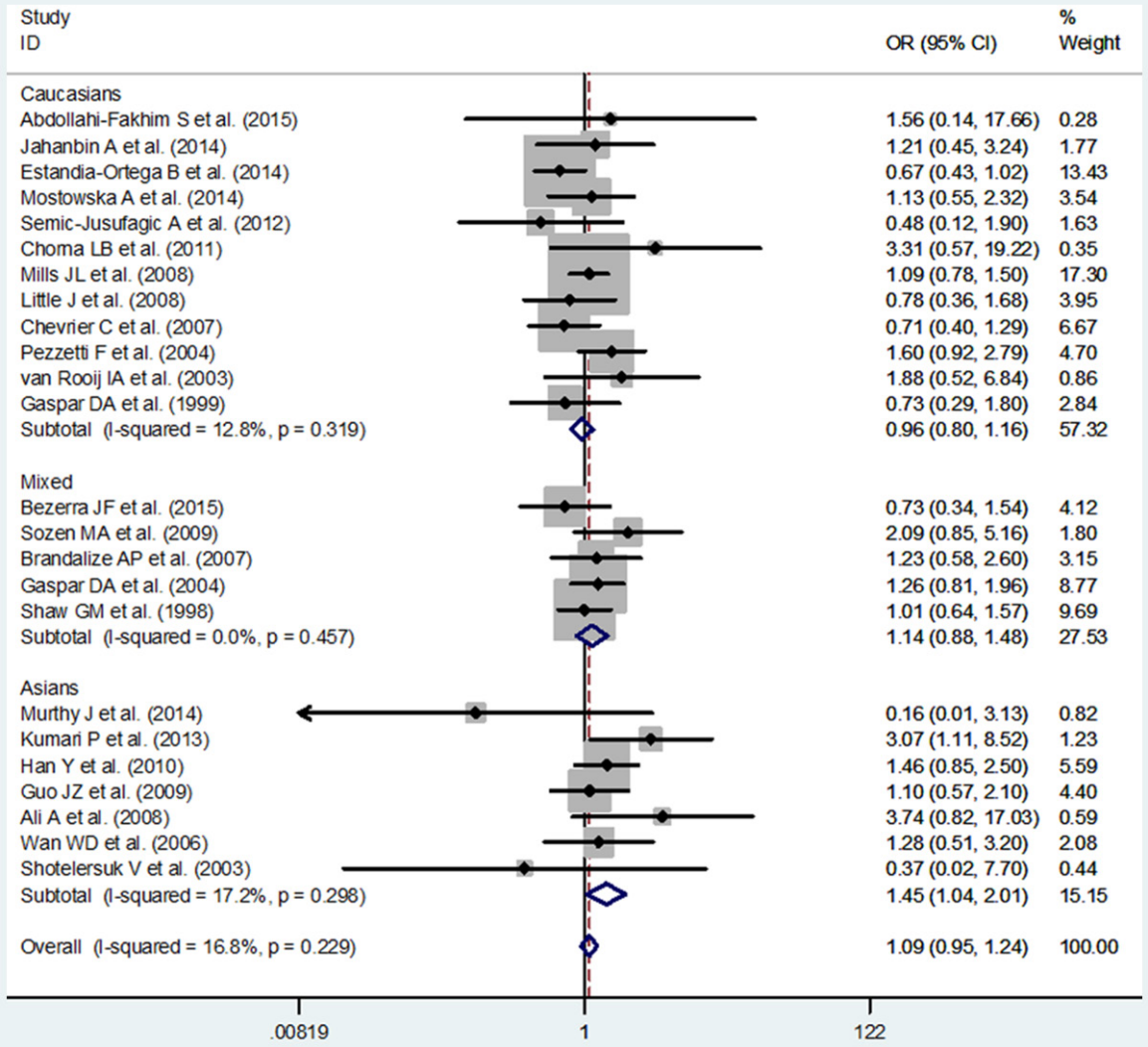

Figure 7. fixed effect forest plot of recessive model (TT vs. CT+CC) for the association between the risk of nsCL/P and *MTHFR* rs1801133 C>T polymorphism in Asian subgroup (Group A).

rs1801133 TT genotype significantly increased nsCL/P risk in Group B [TT vs. CC (OR, 1.44; 95% CI, 1.07-1.94; *P* = 0.016), TT vs. CT + TT (OR, 1.52; 95% CI, 1.18-1.97; *P* = 0.001)]. Our results in Group B were partially coincident with the results of meta-analysis conducted by Pan X *et al.* [22], which showed that the TT genotype in mothers raised the possibility of having nsCL/P progeny 1.25 times (OR, 1.25; 95% CI, 1.05-1.49) more than the CC genotype.

Several studies had showed that multivitamin supplementation during pregnancy is related to a reduction of oral clefts morbidity [11, 34, 35]. As a key enzyme in folic acid metabolism, *MTHFR* rs1801133 C>T polymorphism of mother may play a very important role and then affect oral clefts morbidity. It was reported that the mutation rate of *MTHFR* rs1801133 C>T polymorphism of mothers with nsCL/P offspring was higher than those of without nsCL/P offspring and the frequency of genotype CT and TT of mothers with nsCL/P offspring were 2.75 fold and 2.51 fold of those of without nsCL/P offspring respectively [36]. Pezzettil *et al*. found that the women with lower MTHFR activity had origins in mutation of allelic gene of *MTHFR* rs1801133 C>T polymorphism [37]. Folic acid supplementation of mothers may prevent nsCL/P of progeny partially [38]. Mutation of *MTHFR* rs1801133 C>T polymorphism of matrix could lead to decrease of MTHFR activity in placenta, which caused disorder of generation of 5-methyltetrahydrofolic acid, indirect methyl

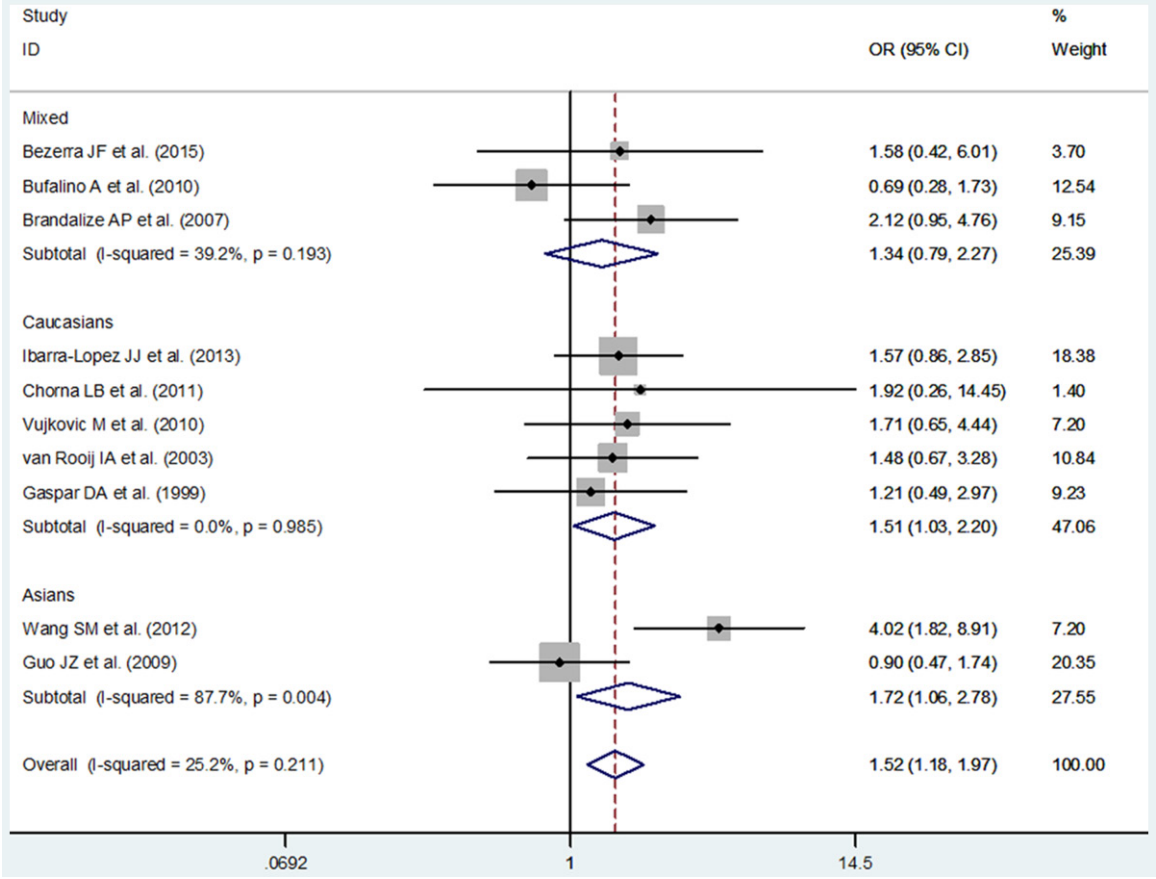

Figure 8. Fixed effect forest plot of recessive model (TT vs. CT+CC) for the association between the risk of nsCL/P and mother's *MTHFR* rs1801133 C>T polymorphism in Caucasians subgroup (Group B).

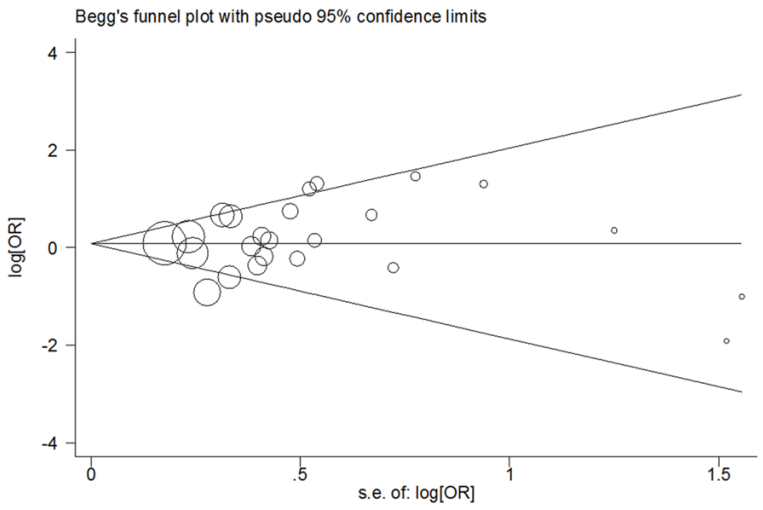

Figure 9. Group A (nsCL/P patients vs. healthy controls): Begg's funnel plot of homozygote model (TT vs. CC) for the association between the risk of nsCL/P and *MTHFR* rs1801133 C>T polymorphism (random-effects model).

donor, and methylation deficiency of DNA and proteins. These may be involved in the etiologies of nsCL/P and similar to our results.

In subgroup analysis stratified by ethnicity, we found *MTHFR* rs1801133 TT genotype was a dangerous factor for nsCL/P in Asians for Group A. For Group B, we found that the *MTHFR* rs1801133 TT genotype of mothers in Caucasians may raise progeny nsCL/P morbidity. Because the number of studies and sample size is relatively small, subgroup analysis stratified by ethnicity was not conducted for Group C. In general, native Americans and Asian populations possess the highest recorded prevalence rates (1 in 500); European-derived populations, both white and Hispanic, possess intermediate

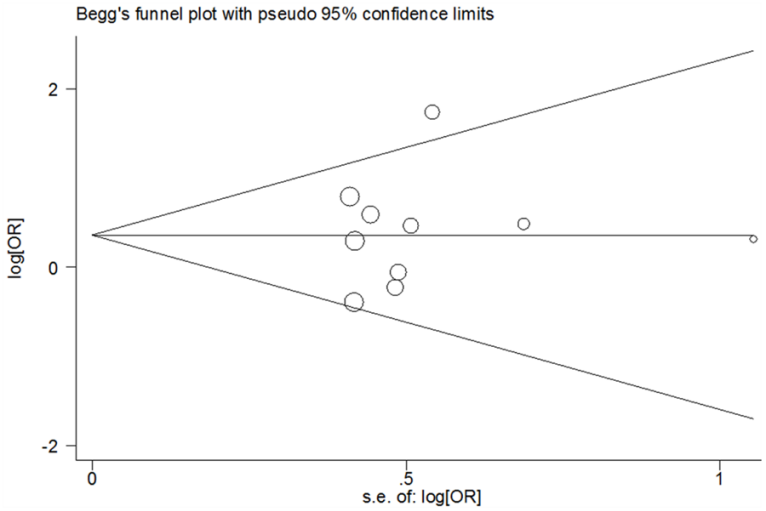

Figure 10. Group B (mothers with nsCL/P progeny vs. mother controls with healthy progeny): Begg's funnel plot of homozygote model (TT vs. CC) for the association between the risk of nsCL/P and *MTHFR* rs1801133 C>T polymorphism (fixed-effects model).

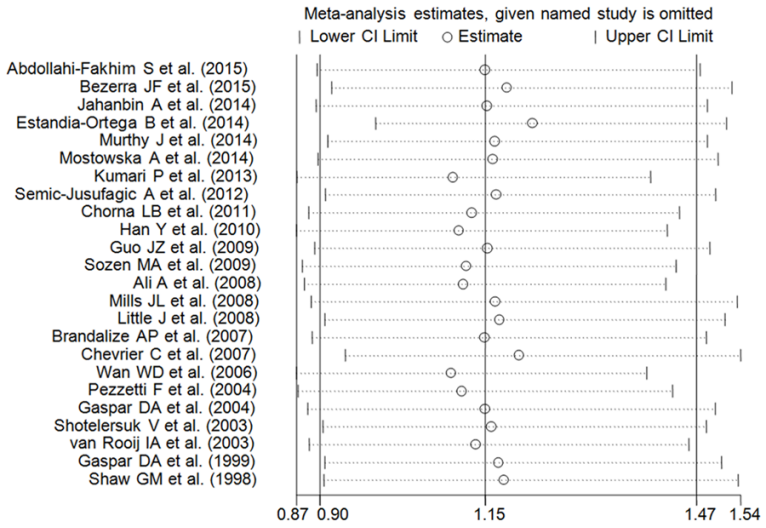

Figure 11. Group A (nsCL/P patients vs. healthy controls): Sensitivity analysis of homozygote model (TT vs. CC) for the association between the risk of nsCL/P and *MTHFR* rs1801133 C>T polymorphism (random-effects model).

recorded prevalence rates (1 in 1000), and African-derived populations possess the lowest recorded prevalence rates (1 in 2500) [13]. These observations indicated that the relative contribution of individual susceptible genes may vary across different ethnicities [39]. Tolarova *et al*. [40] first observed that homozygotes 677 T allele were three folds more frequent in nsCL/P Argentinean patients than in controls. Studies from China [41-43] and India [44, 45] supported the role of child's *MTHFR* rs1801133 C>T polymorphism with risk of nsCL/P in Asian populations. Its distinctive roles in nsCL/P pathogenesis may be due to the following factors: *MTHFR* rs1801133 C>T mutant is associated with decreased MTHFR activity, increases plasma homocysteine levels, and lowers plasma folic acid, which consequently contribute to nsCL/P [46]. By virtue of meta-analysis from the independent studies, we obtained a more reliable conclusion about the influence of *MTHFR* rs1801133 C>T polymorphism on nsCL/P risk. All studies we included from Mexico [13], Ukraine's western region [15], Dutch [17, 20], America [21] and England [38] supported that maternal *MTHFR* rs1801133 C>T polymorphism was a risk of nsCL/P in Caucasians.

Our meta-analysis has some clear merits. Firstly, this is the first meta-analysis that summarized the association between *MTHFR* rs1801133 C>T polymorphism and nsCL/P risk touched upon three groups, which may get a comprehensive conclusion. Secondly, sensitivity analysis showed no single study strongly affected the aggregated results, suggesting sufficient stability and reliability of the pooled results. Finally, no publication bias was reached, indicating the reliability of our results.

Nevertheless, several limitations should be mentioned. Firstly, the publications included in our meta-analysis were only derived from electronic databases, and unpublished articles failed to be retrieved. Secondly, we only focused on rs1801133 C>T polymorphism in *MTHFR* gene, but did not consider other polymorphisms or other genes. Thirdly, In Group C, only three studies were included in our meta-analysis, and this was less convincing. Finally, environmental factors, such as serum folic acid levels, play important roles in influence of *MTHFR*

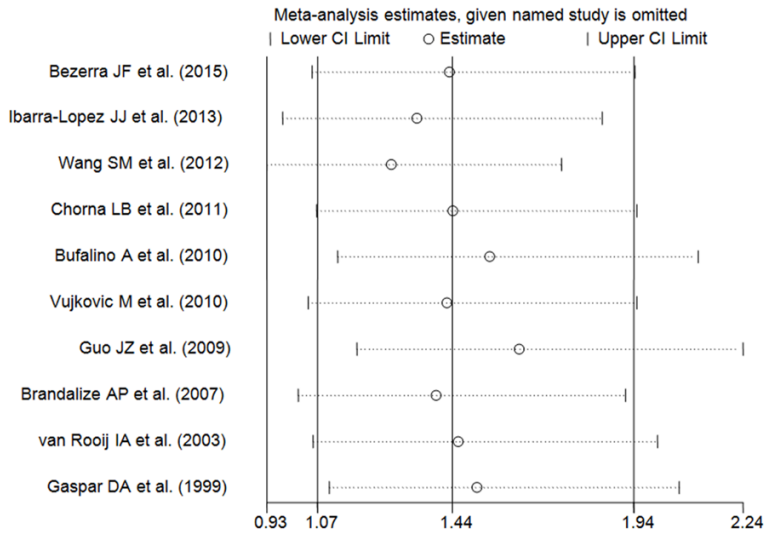

Figure 12. Group B (mothers with nsCL/P progeny vs. mother controls with healthy progeny): Sensitivity analysis of homozygote model (TT vs. CC) for the association between the risk of nsCL/P and *MTHFR* rs1801133 C>T polymorphism (fixed-effects model).

rs1801133 C>T polymorphism on nsCL/P risk; thus, these factors should be also considered.

In conclusion, this meta-analysis indicates the following inferences: (a) the *MTHFR* rs1801133 TT genotype is a risk factor for nsCL/P in Asians for Group A and (b) the *MTHFR* rs1801133 TT genotype of mothers in Caucasians may raise progeny nsCL/P morbidity. Therefore, for practical reasons, more large-scale and well-designed studies are needed to validate or refute the findings of this preliminary study.

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Disclosure of conflict of interest

None.

Abbreviations

nsCL/P, nonsyndromic cleft lip with or without cleft palate; CI, confidence interval; OR, odds ratio; MTHFR, methylenetetrahydrofolate reductase; HWE, Hardy-Weinberg equilibrium; SNP, single nucleotide polymorphism.

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