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Maternal methylenetetrahydrofolate reductase (MTHFR) gene A1298C polymorphism and risk of nonsyndromic Cleft lip and/or Palate (NSCL/P) in offspring: A meta-analysis

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ABSTRACT

Objective: Methyleneterahydrofolate reductase (MTHFR) A1298C polymorphism has been reported a risk factor for nonsyndromic cleft/palate (NSCL/P) in several published articles but results were inconclusive. To confirm the association between maternal MTHFR A1298C polymorphism and NSCL/P risk, a meta-analysis was conducted. **Method:** Case control articles for maternal MTHFR A1298C polymorphism and NSCL/P risk were identified by search of databases including PubMed, Google Scholar, Elsevier and Springer Link for the period up to December, 2013. Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated to assess the association. **Results:** Meta-analysis of ten included studies showed that there was no significant association between maternal MTHFR A1298C polymorphism and risk of NSCL/P under five genetic models (for C versus A, OR= 1.007, 95 % CI= 0.89-1.13, P=0.90; for CC versus AA, OR=0.851, 95 % CI = 0.63-1.15, P=0.30.; for AC versus AA, OR= 1.033, 95 % CI= 0.88-1.21, P= 0.69; for CC+AC versus AA, OR= 1.005, 95 % CI= 0.86-1.17, P=0.94; for CC versus AC+AA, OR= 0.86, 95 % CI= 0.64-1.15, P= 0.32). **Conclusion:** In conclusion, results of present meta-analysis demonstrate that maternal MTHFR A1298C polymorphism may not be a risk factor for developing NSCL/P in offspring. Further studies with large sample sizes are needed to evaluate the association of maternal MTHFR A1298C polymorphism with NSCL/P in more detail.

Key words: Homocysteine, Orofacial cleft, Cleft lip/palate, Methylenetetrahydrofolate reductase, MTHFR, A1298C

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INTRODUCTION

Nonsyndromic cleft lip with or without cleft palate (NSCL/P)(OMIM 119530) is one of the most common congenital malformation with the global prevalence ranging between 1 in 300 and 1 in 2000 depending upon geographical origin, ethnicity, and socioeconomic status.[1] The frequency of NSCL/P has been found to decrease in the offspring of mothers who have received prenatal dietary supplementation with multivitamins,[2-6] suggesting that one or more vitamins may be of importance in the pathogenesis or prevention of NSCL/P. The effectiveness of maternal folic acid supplementation during pregnancy in reducing the frequency of neural tube defects (NTD)[7,8] led to speculation that folic acid might play a similar role in craniofacial closure defects such as NSCL/P.[2,3] Twin and family studies suggested that genetic factors play an important role in the etiology of NSCL/P. Furthermore, it has become increasingly clear that NSCL/P is a genetically complex trait, which is likely to be determined by several susceptibility loci acting in a multiplicative fashion, including genes such as: methylenetetrahydrofolate reductase (MTHFR).

MTHFR is key enzyme of folate and methionine metabolic cycles. It catalyzes the conversion of 5,

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10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate reductase, which donates methyl group for the conversion of homocysteine to methionine. The methyl cycle supplies 1-carbon units critical for a variety of methylation reactions essential for proper gene expression and maternal and paternal imprinting by methylated DNA. The human MTHFR gene is 20 kb long (20,336 bp) and mapped at 1p36.3 (OMIM 607093), having 11 exons. Several single nucleotide polymorphisms (SNPs) in the MTHFR gene have been identified. Among which the most commonly studied polymorphisms are C677T in exon 4 and A1298C in exon 7.[9,10] These two polymorphisms were shown to be associated with reduced enzyme activity.

A1298C allele frequency differs greatly in various ethnic groups of the world. The prevalence of the A1298C homozygote variant genotype ranges from 7 to 12% in White populations from North America and Europe. Lower frequencies have been reported in Hispanics (4 to 5 %), Chinese (1 to 4 %) and Asian populations (1 to 4%).[11,12] Several studies have explored the impact of maternal MTHFR A1298C polymorphism NSCL/P, but the association is conflicting.[13-17] To better understand these issues, a comprehensive meta-analysis was conducted to clarify the quantitative association between the maternal MTHFR A1298C polymorphism and risk of NSCL/P.

METHODS

Article search

Comprehensive search was conducted in the Pubmed, Google Scholar, Elsevier and Springer Link databases from their inception through December, 2013. Following search terms were used: 'MTHFR', 'A1298C', 'methylenetetrahydrofolate reductase', 'polymorphism', 'Cleft lip', and Cleft palate' and 'NSCL/P'. There was no language limitation. All references cited in those included studies were also reviewed to identify additional published articles not indexed in common databases.

Inclusion criteria were following: (1) study should be published in a peer-reviewed journal; (2) studies had sufficient data to calculate the odds ratio (OR) with a confidence interval (CI) and a P-value, (3) study should be case control, (4) should used the relevant genotyping protocols or provided reference to them, (5) used healthy individuals as controls. The major reasons for exclusion of studies were (1) only case studies, (2) review, conference abstract, letter to editor and editorials, and (3) containing overlapping data.

Data extraction

Information was carefully extracted from all eligible studies according to the inclusion criteria listed above. The following data were collected from each study: first author's family name, year of publication, country, ethnicity, genotyping method, sample size, and numbers of genotype A1298C for both cases and controls, respectively.

Statistical analysis

In the meta-analysis of MTHFR A1298C polymorphism, the overall association of the C allele with risk of NSCL/P was evaluated in comparison with the A allele (Allele contrast model). Also, the contrasts of homozygote CC versus AA (homozygote model), AC versus AA (co-dominant model), CC versus AC+AA (recessive model) and CC+AC versus AA (dominant model) were examined.

The associations were indicated as a pooled odds ratio (OR) with the corresponding 95% confidence interval (CI). The heterogeneity between studies was tested using the Q-statistic, which is a weighted sum of the squares of the deviations of individual study OR estimates from the overall estimate.[18,19] When the ORs are homogeneous, Q follows a chi-squared distribution with r – 1 (r is the number of studies), degrees of freedom (df). When $P<0.50$ then the heterogeneity was considered to be statistically significant. Heterogeneity was quantified with the I^2 metric ($I^2 = (Q – df)/Q$), which is independent of the number of studies in the meta-analysis. I^2 takes values of between 0 and 100%, with higher values denoting a greater degree of heterogeneity.[19,20] The pooled OR was estimated using fixed effects (FE)[21] and random effects (RE)[22] models. Random effects modeling assume a genuine diversity in the results of various studies, and it incorporates a between-study variance into the calculations. Hence, when there is heterogeneity between studies then the pooled OR is preferably estimated using the RE model.[19,23]

Publication bias

Funnel plots were drawn to estimate the publication bias, in which the standard error (SE) of log (OR) of each study and precision of log (OR) of each study were plotted against its log (OR). The funnel plot asymmetry was assessed with Egger's test.[24] Publication bias $P<0.05$ was considered statistically significant. All analyses were performed using the computer program MIX version 1.7.[25] A p value less than 0.05 was considered statistically significant, and all the p values were two sided.

RESULTS

Study characteristics

Details of the included studies were given in table 1. Total ten studies were found to be suitable for the inclusion in the present meta-analysis.[13-17,26-30] All these ten studies were performed in different countries- Argentina,[26] India,[16] Norway,[13] Ireland,[15] Italy,[14] Netherlands,[28] Thailand,[27]

Ukraine,[29] Turkey, [30] and Venezuela.[17] In one study[16] reported only allele numbers (Table 1). In only seven studies[13,14,17, 27-30] odds ratio was above one.

Summary statistics

The frequencies of the genotypes MTHFR 1298AA and 1298AC were the highest in both cases and controls, and allele A was the most common (Table 2). The prevalence of allele C was 26.48% and 31.99% for the CL/P case mothers and control groups, respectively. The percentage frequency of CC genotype among case mothers and controls was 6.77% and 10.29%, respectively whereas prevalence of AC heterozygote among case mothers was 39.84% and 43.43% in controls. The prevalence of AA homozygote among CL/P case mothers and controls was 53.39% and 46.28%, respectively. In all the studies, distribution of genotypes in the control group was in Hardy Weinberg Equilibrium.

Meta-analysis

Mutant allele (C) did not show any significant association with NSCL/P in both fixed effect (p = 0.90, OR=1.007, 95% CI = 0.89-1.13) and random effect (p =0.90, OR= 1.007, 95% CI =0.89-1.13) models (Figure 1). In cumulative analysis using fixed and random effect models, the association of mutant 'C' allele with NSCL/P remained insignificant statistically with the addition of each study. Odds ratio for mutant genotypes (CC+AC) showed no association with NSCL/P adopting both fixed (p =0.94, OR= 1.005, 95% CI =0.86-1.117) and random (p = 0.94, OR= 1.005, 95% CI =0.86-1.17) effect models. Similarly no significant association was found with other three genetic models (for CC vs AA: OR= 0.851, 95% CI= 0.63-1.15, p= 0.30; for AC vs AA: OR= 1.03, 95% CI= 0.88-1.21, p= 0.69; for CC vs AC+AA: OR= 0.86, 95% CI= 0.64-1.15, p= 0.32)(Table 3).

Publication bias

Publication bias could not be observed in all five genetic models by using of Begg's and Egger's test. P values of Egger's test were insignificant in all five models (for C vs A (allele contrast model): p= 0.34; for CC vs AA (homozygote model): p= 0.34; AC vs AA(co-dominant model): p value= 0.99;for CC+AC vs AA(dominant model): p= 0.69; For CC vs AC+AA(recessive model): p= 0.44). Funnel plots were showed in Figure 2 and all funnel plots were symmetrical.

DISCUSSION

There is considerable evidence suggesting that folate related genes play a role in the etiology of nonsyndromic facial clefts. Nonsyndromic clefts are complex traits and it is likely that genetic factors interact with environmental

Table 1: Characteristics of eleven studies included in the present meta-analysis

Study	Country	Year	Case	Control	References
Tolarova et al.	Argentina	1998	108	103	Am J Hum Genet, 63:A27.
Shoteresuk et al.	Thailand	2003	109	202	J Med Genet, 40:e64.
Van Roij et al.	The Netherlands	2003	94	115	Am J Epidemiol, 157:583-591.
Nurk et al.	Norway	2004	22	14452	Am J Med 117: 26-31.
Pezzetti et al.	Italy	2004	110	289	Hum Mutat, 24:104-105.
Mills et al.	Ireland	2008	407	1050	Birth defects research, 82:636-643.
Ali et al.	India	2009	323	214	Genetic Testing and Molecular Biomarkers, 13 (3).
Sozen et al.	Venezuela	2009	179	138	J. Genet. Genomics, 36: 283-288.
Chorna et al.	Ukraine	2011	33	50	Cytology and Genetics, 45: 177-181.
Semic-Jusufagic et al.	Turkey	2012	56	76	The Turkish Journal of Pediatrics, 54: 617-625

Table 2: The distributions of MTHFR A1298C genotypes and allele frequencies for CLP case mothers and control mothers

Study ID	Genotype						Alleles			
	AA		AC		CC		A		C	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Tolarova et al., 1998	56	50	27	25	3	3	139	125	33	31
Shoteresuk et al., 2003	30	108	33	80	4	14	93	296	41	108
van Roij et al., 2003	57	76	52	67	16	16	166	219	84	99
Nurk et al., 2004	9	6598	10	6332	3	1522	28	19528	16	9376
Pezzetti et al., 2004	57	121	36	130	11	38	150	372	58	206
Mills et al., 2008	179	519	164	439	23	92	522	1477	210	623
Ali et al., 2009							175	295	57	133
Sozen et al, 2009	119	101	47	33	2	4	285	235	51	41
Chorna et al., 2011	12	24	13	22	2	4	37	70	17	30
Semic-Jusufagic et al., 2012	25	36	24	36	5	4	74	108	34	44

Table 3: Summary estimates for the odds ratio (OR) of MTHFR A1298C in various allele/genotype contrasts, the significance level (p value) of heterogeneity test (Q test), and the I^2 metric: overall analysis, and publication bias p-value (Egger test)

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)
Allele Contrast (C vs A)	1.007 (0.89-1.13), 0.90	1.0074 (0.89-1.13),0.90	0.73	0	0.34
Co-dominant (AC vs AA)	1.0333 (0.88-1.21), 0.69	1.0349 (0.88-1.21),0.68	0.49	0	0.99
Homozygote (CC vs AA)	0.8515 (0.63-1.15), 0.30	0.862 (0.63-1.17),0.34	0.76	0	0.336
Dominant (CC+AC vs AA)	1.005 (0.86-1.17), 0.94	1.005 (0.86-1.17),0.94	0.50	0	0.69
Recessive (AA+AC vs CC)	0.8605 (0.64-1.1546), 0.32	0.8709 (0.65-1.17),0.36	0.80	0	0.44

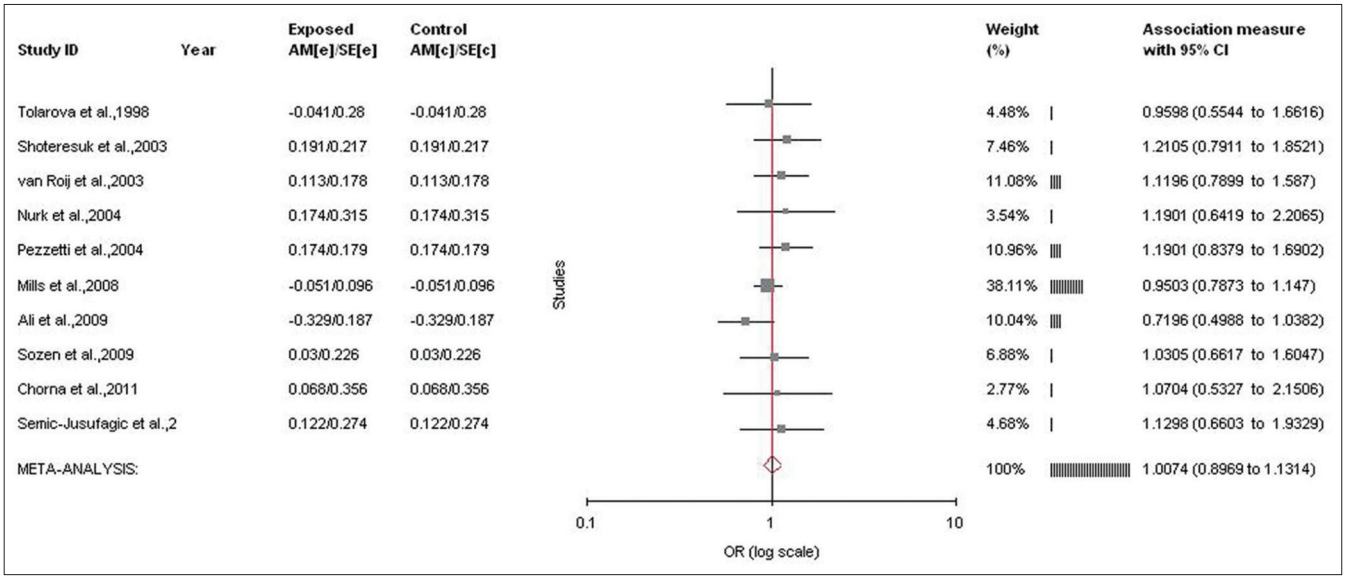

Figure 1: Forest plots for the association between MTHFR A1298C polymorphism and cleft lip for additive model (C vs A) with Random effect model

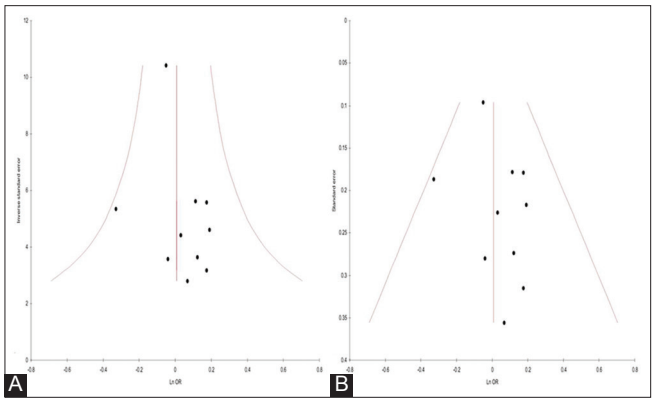

Figure 2: Funnel plots (A) precision versus OR (C vs. A), (B) standard error versus OR (C vs. A)

factors. Folate has long been considered one such factor in number of observational studies.[2-5,7,8,15,31] Maternal folic acid supplementation in early pregnancy has been suggested to play a role in the prevention of nonsyndromic orofacial cleft, i.e. cleft lip with or without cleft palate (CL/P).

Martinelli et al[32] suggested that the variants of the MTHFR gene, considered to be a cause of reduced efficiency of folate utilization, could be an important risk factor for CL/P. Wong et al.[33] observed that maternal hyperhomocysteinemia may be a risk factor for having CL/P offspring. Considering that one of the effects of the reduced MTHFR activity is the hyperhomocysteinemia. Insufficient DNA synthesis and methylation by the mother could damage the developing embryo.

Meta-analysis is a powerful tool for analyzing cumulative data of studies where the individual sample sizes are small and the statistical power low.[34] Several meta-analysis studies illustrate the utility of the technique in identifying genes of small effects like MTHFR with phenotypes like –NTD,[35] Down syndrome,[36] Cardiovascular disease,[37] Migraine,[38] Schizophrenia,[39] bipolar disorder,[40] and depression.[41]

The present meta-analysis (including 1019 case mothers and 16494 controls) was performed to assess the relationship between MTHFR A1298C polymorphism and NSCL/P with ten published case control studies, but no significant association was found in the total population. The main strength of the present study was that heterogeneity and publication bias were not observed. As with all meta-analyses, present analysis had also several limitations that must be acknowledged.

Firstly, sample sizes of some included studies are rather small and they do not have adequate power to detect the possible risk for MTHFR A1298C polymorphism.[13,29,30] Secondly, main analysis was based on unadjusted estimates owing to the lack of adjusted estimates. However, a more precise analysis could be performed if adjusted estimates were available in all studies. Further owing to the limited evidence available on other folate gene polymorphisms; this review was restricted to the only one MTHFR polymorphism. Finally, data were not stratified by folate intake, ethinicity, and other suspected factors. Therefore, a more precise analysis should be conducted if enough data were available.

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