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REVIEW ARTICLE

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Folate intake, markers of folate status and oral clefts: An updated set of systematic reviews and meta-analyses

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Abstract

Background: There has been a longstanding debate about the role of folate in the etiology of orofacial clefts (OFCs). Studies of different measures of nutritional intake or folate status have been done to investigate the possible role of folate in the prevention of OFC. Only one knowledge synthesis has attempted to bring together different types of evidence. The aim of the present work was to update it.

Methods: Evidence for associations between OFC and dietary folate, supplement use, folic acid fortification, biomarkers of folate status, and variants of *MTHFR* (C677T and A1298C) were included. Potentially eligible articles were systematically identified from PubMed, Medline, Embase, and Web of Science (2007–2020) and combined using random-effects meta-analysis when appropriate. Quality assessments were conducted using the Newcastle-Ottawa scale and Cochrane's risk of bias tool.

Results: Sixty-four studies published since the previous knowledge synthesis were identified, with eight of these identified through a supplementary search from October, 2018 to August, 2020. There was an inverse association between folic acid-containing supplement use before or during pregnancy and cleft lip with or without cleft palate (CL/P) (OR 0.60, 95% CI 0.51–0.69), with considerable between-study heterogeneity. The prevalence of CL/P showed a small decline post-folic acid fortification in seven studies (OR 0.94, 95% CI 0.86–1.02). No association was found between OFC and genetic markers of folate status. The coronavirus-19 pandemic has threatened food availability globally and therefore there is a need to maintain and even enhance surveillance concerning maternal intake of folate and related vitamins.

Conclusions: The risk of non-syndromic OFC was reduced among pregnant women with folic acid-containing supplements during the etiologically relevant period. However, high heterogeneity between included studies, incomplete reporting of population characteristics and variation in timing of exposure and supplement types mean that conclusions should be drawn with caution.

KEYWORDS

folate intake, multivitamin supplements, non-syndromic clefts, folic acid fortification, *MTHFR*

1 | INTRODUCTION

1.1 | Rationale

Both orofacial clefts (OFCs) and neural tube defects are caused by developmental failures of midline body structures (Khoury, Cordero, Mulinare, & Opitz, 1989; Oyen, Boyd, Poulsen, Wohlfahrt, & Melbye, 2009) and both involve neural crest cell migrations (Copp, 2005; Ji et al., 2020; Kousa, Mansour, Seada, Matoo, & Schutte, 2017). These similarities have led to interest in the possible role of folate in the prevention of OFCs, high levels of which are known to prevent neural tube defects. Because of the difficulties of nutritional assessment, it may be relevant to consider associations between OFCs and multiple indicators of folate exposure including dietary intake, supplement use, folic acid fortification, blood folate concentrations, and genetic variants known to affect blood folate concentrations. Only one knowledge synthesis has attempted to bring together these different types of evidence (Johnson & Little, 2008), but this was published over a decade ago. Since then, systematic reviews have synthesized evidence on associations with single types of nutritional exposure-maternal folic acid supplementation (Jahanbin, Shadkam, Miri, Shirazi, & Abtahi, 2018); mandatory folic acid fortification (Millacura, Pardo, Cifuentes, & Suazo, 2017); and maternal levels of methylation status biomarkers (Blanco, Colombo, Pardo, & Suazo, 2016). Each of these studies has one or more limitations such as lack of a comprehensive search strategy, incomplete reporting of decisions about the review process and analyses, incomplete data extraction, and overlap between primary studies. The rationale for considering different aspects of nutritional exposure in a single review is that these aspects are interdependent. For example, folic acid fortification, as a population-level intervention, may affect the interpretation of analysis of dietary folate intake and folic acid supplements. In addition, the effect of folic acid supplements sometimes cannot be distinguished from that of multivitamin supplements or dietary folate intake. Blood levels of folate may reflect not only intake, but also genetic variation and blood levels of related nutrients.

1.2 | Research objectives

The objective of this study was to synthesize the evidence for associations between folate and non-syndromic OFCs using systematic reviews and meta-analyses. Measures of folate considered were supplement use, dietary folate intake, folic acid fortification, biomarkers of folate status, and *MTHFR* genetic variants. The protocol for this review was submitted for registration in the International Prospective Register of Systematic Reviews (PROSPERO) on February 8, 2019 (CRD42019123126).

2 | METHODS AND ANALYSIS

2.1 | Outcomes

The primary outcome was non-syndromic OFCs. This included cleft lip with or without cleft palate (CL/P) and cleft palate only (CPO), both separately and combined.

Studies in which either or both parents had themselves been born with a non-syndromic cleft, or in which the mother had previously given birth to an affected child, were included and analyzed separately because of the possibility that these cases had an unrecognized genetic syndrome.

2.2 | Exposures, genotypes, and comparators

2.2.1 | Folate acid/multivitamin supplement

Women who reported taking folic acid supplements at any time during the 3 months before pregnancy to the end of pregnancy were compared with women who did not. Folic acid is often taken as a part of a multivitamin supplement, therefore women who took multivitamins were also included in the analysis. Analyses were stratified by type of supplement (multivitamin vs. folic acid supplement) when possible.

2.2.2 | Dietary folate intake

Studies of estimated dietary folate intake were included. Separate analyses were conducted for studies that included or excluded folic acid supplements from the dietary folate assessment. The highest quantile of maternal folate intake was compared with the lowest quantile, using quantiles defined by the authors of each study. *p*-values for tests of trend and for differences in mean dietary folate intake between mothers of cases and mothers of unaffected infants were also extracted. If *p*-values were not presented, they were calculated when possible.

2.2.3 | Folic acid fortification

Mandatory folic acid fortification is a population-level intervention. Potentially eligible study designs included

before-after comparisons, interrupted time series (Ramsay, Matowe, Grilli, Grimshaw, & Thomas, 2003), and systematic reviews of such studies.

2.2.4 | Biochemical markers of folate status

Observational studies and systematic reviews in which investigators compared the plasma (serum) or erythrocyte (red cell) folate concentrations of mothers of children with oral clefts with those of mothers of unaffected children were eligible for inclusion. Comparisons were made between the highest and lowest quantiles of folate, as defined by the authors of each study. *p*-values from dose–response relationships and for differences between mean levels of folate between case and control mothers were extracted; if they were not presented, they were calculated when possible.

2.2.5 | Variants of genes involved in folate metabolism or transport

Studies were included if a MTHFR genotype or haplotype (i.e., the combination of haplotypes inherited from the mother and father) frequencies in cases, case mothers, or case fathers were compared with frequencies in controls or their parents.

The two most common variants of the MTHFR gene are located at nucleotides 677 (C677T) and 1,298 (A1298C). These variant genotypes are associated with increased thermolability and substantial diminution of activity of the enzyme in vitro (Narayanan et al., 2004). The C677T polymorphism leads to major conformational changes within the tertiary structure of MTHFR, resulting in a significant reduction in its FAD-binding affinity while the A1298C mutation alters the MTHFR activity to a lesser extent (Lakkakula et al., 2020).

Homozygosity for the C677T variant (TT genotype) is consistently associated with lower blood levels of folate and higher levels of homocysteine than those observed for CT heterozygotes and CC homozygotes (Jin et al., 2018; Tsang et al., 2015). Aggregate evidence is suggestive that homozygosity of heterozygosity for the A1298C variant (CC and AC genotypes) is associated with increased blood folate levels (Xin et al., 2018). Therefore, for studies of *MTHFR* polymorphisms, individuals homozygous for either of the common variants (677CC or 1298AA) were chosen as the reference group. In the consideration of haplotypes, only studies of the *MTHFR* C677T-*MTHFR* A1298C haplotype were included. Articles reporting results from transmission disequilibrium tests (TDT) of case-parent trio data for *MTHFR* C677T, A1298C, or haplotypes were also included. *p*-values for differences in transmission were extracted. Studies reporting parent-of-origin effects were considered separately.

Studies examining interactions between *MTHFR* and folate intake were also included. Studies were grouped by polymorphism (C677T, A1298C), individuals genotyped (infant, mother, father) and exposure (dietary folate intake, folic acid-containing supplements).

2.3 | Information sources

Four databases were searched including Medline (via Ovid), PubMed, Embase (via Ovid) and Web of Science Citation Index Expanded (SCI-EXPANDED), from January 1, 2007 to October 20, 2018. The Canadian Agency for Drugs and Technologies in Health (CADTH) gray matters resource guide was also searched to identify evidence in the gray literature (CADTH., 2010). Reference lists of included studies and relevant review articles identified through the search were checked for additional references. We carried out a supplementary search from October 2018 to August 2020.

2.4 | Search strategy

Both medical subject headings (MeSH) and text keywords for non-syndromic clefts and each folate category were used to develop the search strategies based on the original review (Johnson & Little, 2008). There was no restriction by language. The search strategy was developed and finalized in Medline (Appendix S1), and then adapted to the syntax of the other databases. The search strategy was peer reviewed by the Health Science Librarian (LS).

2.5 | Study selection

Included studies had information on non-syndromic CL/P, CPO, or both types of clefts combined. When two or more studies recruited participants or extracted data from the same population during the same time period, the study with the most relevant primary outcome was included; if there was more than one, the study with the largest sample size or the most recent was included.

Articles could be selected for more than one review topic, and inclusion and exclusion of the article were assessed independently for each topic.

The processes of screening were conducted in duplicate by Y. Z. and V. S. Any disagreement arising was

resolved by discussion between the two reviewers and a third reviewer (J. L.). Y. Z. and V.S. independently screened the titles and abstracts of all the materials obtained from the searching strategy to exclude articles that did not meet the eligibility criteria. YZ and VS also independently evaluated the potentially eligible studies with the full text and further excluded studies with reasons documented.

2.6 | Data collection process

Data extraction and quality assessment were conducted by Y. Z., Y. Y., and V.S., Y. Z., Y.Y., and V.S. independently extracted data from the included studies using a predefined extraction sheet. Three studies were piloted to finalize the data extraction sheet. Y. Z. reviewed all the data extraction and quality assessments sheets under the supervision of J. L. Any disagreements were resolved by discussion within two reviewers (Y. Z. and Y. Y. or Y. Z. and V. S.) and a third reviewer (Y. Z. or J. L.). The data extracted from each study are summarized in Appendix S2.

2.7 | Quality and risk of bias in individual studies

Y. Z. and Y. Y. independently assessed the quality and risk of bias of the included studies. Discrepancies between the two reviewers were resolved by discussion. If necessary, a third member of the team was consulted. The quality assessment for observational studies was carried out using the Newcastle-Ottawa Scale (NOS) (Wells et al., 2019). The included papers were categorized by NOS score, which awards "stars" for characteristics associated with high quality: selection criteria (six possible stars for cohort studies and four possible stars for case–control), comparability (three possible stars), and either outcome assessment for cohort studies (five possible stars) or exposure assessment for case–control studies (four possible stars). "High quality" studies were defined as those with ≥8 stars, "medium quality" with 6–7 stars, and "low quality" with <6 stars (Driscoll et al., 2016). The Cochrane Risk of Bias Tool was used to assess the quality of Randomized Control Trials (RCTs) following its recommended definitions for categorizing studies as having a "high," "medium," and "low" risk of bias. The National Heart, Lung, and Blood Institute (NHLBI) Quality Assessment Tool for Before-After Studies was used to assess quality of folic acid fortification before-and-after studies (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools).

2.8 | Data synthesis

Except when there were major differences in exposure assessment and categorizations of the variables, meta-analyses were done and incorporated both the newly identified studies and the studies identified by Johnson and Little (Johnson & Little, 2008). Meta-analysis was performed using Comprehensive Meta-Analysis (Version 2). When adjusted odds ratios (ORs) and 95% confidence intervals (CI) associated with exposure were available in the articles, they were extracted for each study and included in meta-analysis. Otherwise, crude ORs provided in the included studies, or calculated from the data presented in the primary study were extracted and included in meta-analysis. Pooled ORs were calculated using a random-effects model. Risk ratios (RRs) were extracted for analyses concerning folic acid fortification and were combined using a random-effects model.

The homogeneity of the estimates regarding magnitude and direction of effects and strength of evidence for heterogeneity was assessed by I^2 statistic (Higgins & Thompson, 2002). If there was substantial heterogeneity (≥ 75%), potential sources of heterogeneity were examined through subgroup analyses. For example, for studies of supplements, this included examining whether the study population was from a country with folic acid fortification, type of folic acid supplements (single supplements or multivitamins), the timing of folic acid supplements (e.g., pre-conceptional, during pregnancy) and type of cleft (CL/P or CPO). If appropriate, we undertook sensitivity analysis excluding studies with high risk of bias to determine if the bias could explain heterogeneity.

2.9 | Meta-Bias

For investigation of possible small study effects and publication bias, data from included studies were entered into a funnel plot asymmetry test when there were at least 10 studies in the meta-analysis to obtain sufficient power to distinguish chance from real asymmetry (Debray, Moons, & Riley, 2018). However, there were too few studies relating to dietary folate and folate biomarkers for this to be possible (Sterne, Egger, & Smith, 2001).

2.10 | Patient and public involvement

No patients were involved in this study.

3 | RESULTS

3.1 | Results of literature research

After the first round of screening of title and abstracts of the articles identified from the four databases, 150 potentially eligible papers were assessed and retrieved. We excluded 98 papers based on pre-defined inclusion and exclusion criteria (details of articles excluded are provided in Appendix S2), leaving 52 relating to maternal use of folic acid/ multi-vitamin supplements; five to fortification, four (Bezerra et al., 2015; Munger et al., 2011; Shaw et al., 2009; Vujkovic et al., 2010) to biomarkers of folate status and 19 to *MTHFR* (details of articles included are provided in Appendix S5, Table S1). Four additional articles (Godwin et al., 2008; Nazer H & Cifuentes O, 2014; Sayed, Bourne, Pattinson, Nixon, & Henderson, 2008; Souza & Raskin, 2013) on folic acid fortification were identified through searching reference lists bringing the total number of articles identified to 56 (Figure 1**).**

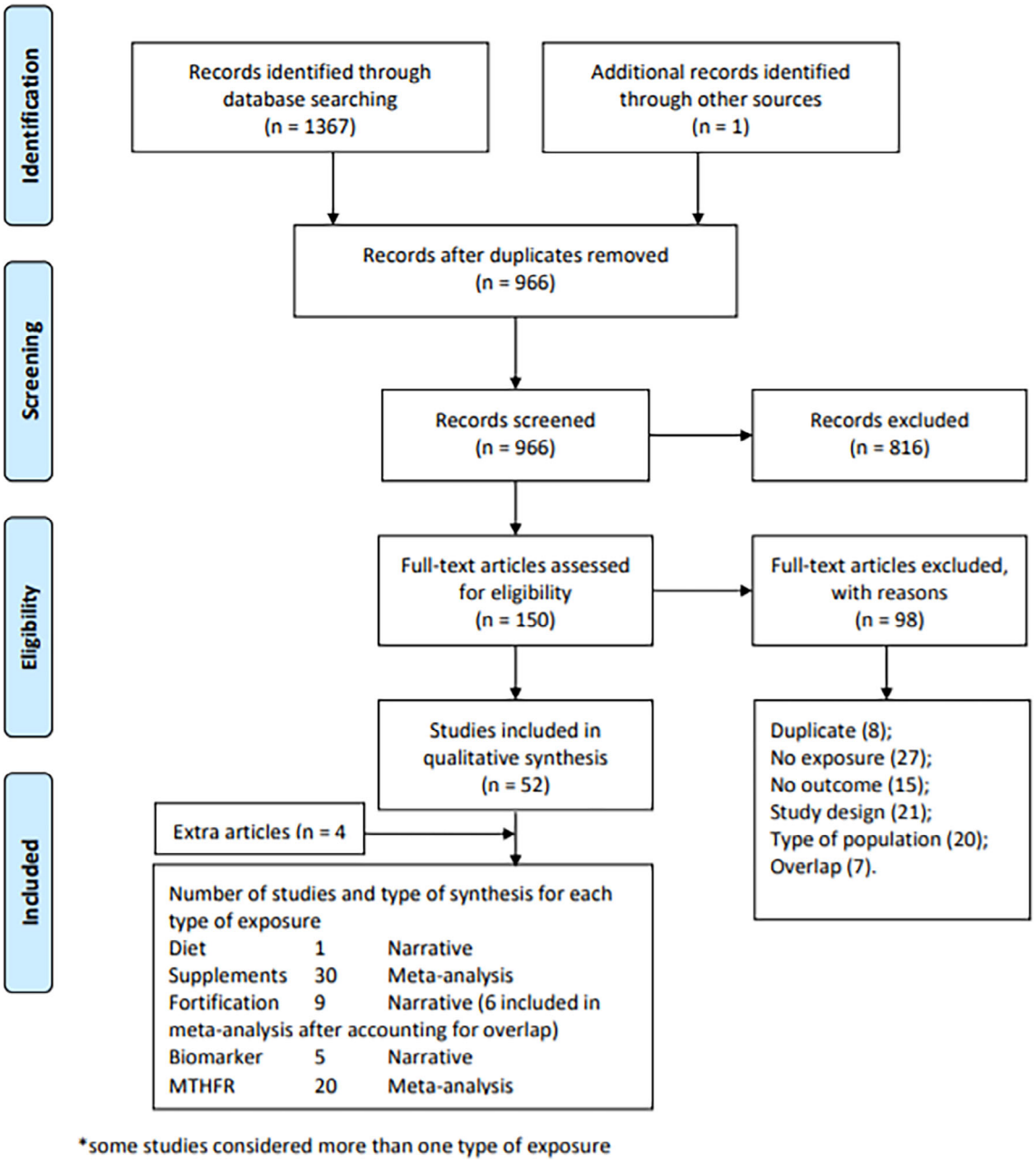


FIGURE 1 PRISMA flow diagram

3.2 | Characteristics of included studies

The characteristics of the studies identified since the original review (Johnson & Little, 2008) are presented in Appendix S5, Table S3, which is organized by components or indicators of exposure. Two (Fu, Chen, Huang, & Wu, 2007; Yuan, Lai, Zhou, & Qin, 2007) were published in Chinese, three (Davalos-Rodriguez et al., 2009; Nazer H & Cifuentes O, 2014; Paulos et al., 2016) in Spanish and 51 in English.

3.3 | Quality assessments

Forty case–control studies, two cohort studies and one cross-sectional study were assessed using the NOS (Appendix S4). Seventeen of the observational studies were rated "low quality" (score less than 6), 22 were rated "medium quality" (score between 6 and 7) and four were rated as "high quality" (score 8 or more). Using a hospital-based control group rather than a community-based control group and no report of participation/response rate were two major problems of the case–control studies. All cohort studies (Gildestad et al., 2015; Li et al., 2012) reported more than 25% loss to follow-up rate. One RCT (Wehby et al., 2013) was identified and rated as "Low" risk of bias according to the Cochrane risk of bias tool although the enrollment of all non-pregnant participants was terminated at all sites due to the lower than anticipated enrollment and pregnancy rates. In the absence of clear recommendation about how to assess the quality of before-after studies, these papers were assessed by the NHLBI Quality Assessment Tool for Before-After studies (Appendix S4). Two studies (López-Camelo, Castilla, & Orioli, 2010; Nazer H & Cifuentes O, 2014) were rated as "good" quality because all criteria of quality assessment tools were met. Other studies failed to provide clear justification of sample size, validity of the measurement of the effect of fortification and follow-up rate.

3.4 | Dietary folate intake

One population-based case–control study reported risk of non-syndromic clefts associated with periconceptional dietary nutrient intake stratified by vitamin supplement use in California during 1999 to 2003 (Wallenstein, Shaw, Yang, & Carmichael, 2013). Among mothers not using supplements, the odds of having offspring with CL/P was 60% lower for women with the highest quartile of dietary folate intake compared with those with the 25th to 74th percentile of dietary folate intake (Adjusted OR 0.4, 95% CI 0.2–0.9). For the mothers who used supplements, the odds of having offspring with CL/P was 20% higher and the difference was insignificant (Adjusted OR 1.2, 95% CI 0.7–2.0).

3.5 | Supplement use: Randomized controlled trials

One randomized controlled trial (Wehby et al., 2013) was conducted in Brazil during 2004 ~ 2011 where women who were born with oral clefts or who had a prior child with oral clefts and were trying to conceive, were randomized to receive 4 or 0.4 mg folic acid on a daily basis periconceptionally. The authors reported a significant decrease in the oral cleft recurrence rates in the 0.4 and 4 mg groups individually and combined, compared with the historic recurrence rate (6.3%) since the folic acid fortification program was implemented in this population ($p = .0009$ when combining the two folic acid groups). The cleft recurrence rate was 2.9% in the 0.4 mg folic acid group and 2.5% in the 4 mg group ($p = .59$ based on a one-sided Fisher's exact test).

3.6 | Observational studies of folic acid supplements or multivitamin containing folic acid

Twenty-two studies from the original review (Johnson & Little, 2008) were integrated with the 30 identified in the present study in the meta-analyses relating to maternal supplement use (Appendix S5, Table S1 and Table S3).

12076 CL/P cases, 4593 CPO cases and 2,452 OFC cases (from studies in which type of cleft was not distinguished) were included in the meta-analysis from studies identified for investigations concerning associations with maternal supplement use. Most of the studies were case–control studies.

For multivitamin supplements, 17 studies were conducted in Europe (no mandatory folic acid fortification), 15 in the Americas (all countries have mandatory fortification but three studies were done before implementation of this policy), 15 in Asia (no fortification policy, except for Vietnam), two in Australia and three studies in the Middle East (in Iran, which has mandatory fortification).

3.7 | Supplement use: Observational studies

Thirty-nine and twenty-one studies were included in the meta-analyses for CL/P and CPO separately while eight

did not provide information by type of cleft separately (Appendix S5).

Included studies reported on the effect of multivitamin or folic acid supplements during the periconceptional period (reported as pre-conceptional until 12 weeks after conception, that is, the end of the first trimester), after the first trimester, or during the whole pregnancy (Table 1). A separate analysis was conducted restricting to studies of supplement use during the etiologically-relevant time window (for CL/P, use beginning at any time between 1 month before conception and the first trimester, and for CPO, any use beginning at 1 month before and the fourth month after conception) (Werler, Hayes, Louik, Shapiro, & Mitchell, 1999). In a negative-control analysis to detect potential unmeasured confounding, data from women who took supplements after the first trimester were analyzed separately (Lipsitch, Tchetgen Tchetgen, & Cohen, 2010). Because it was difficult in some studies to determine whether multivitamin supplements contained folic acid, we conducted separate analyses for studies reporting on any vitamin supplements and multivitamin supplements containing folic acid.

Use of any supplements before conception or during pregnancy was associated with a significantly decreased risk of CL/P (OR 0.60, 95% CI 0.51–0.69; Figure 2 and Table 2) and a small decrease in risk of CPO (OR 0.88, 95% CI 0.79–0.99; Table 2). For the analysis restricted to use of folic acid only or multivitamins specified as containing folic acid, the effect estimates for CL/P and CPO were no different from those in the unrestricted analysis. High between-study heterogeneity was detected for the analysis of CL/P using the Cochran Q and I^2 statistics. Heterogeneity remained high after excluding multivitamin use for which folic acid content was not reported or when including only folic acid only supplements. The funnel plot for the association of any supplements and occurrence of CL/P was noticeably asymmetric, with a majority of the small studies clustering to the left of the mean, suggesting publication bias. The impression was confirmed by Egger's test ($p < .004$, two-tailed). The funnel plot remained asymmetric after restricting to studies of folic acid supplements only (Egger's test, $p = .038$, two-tailed).

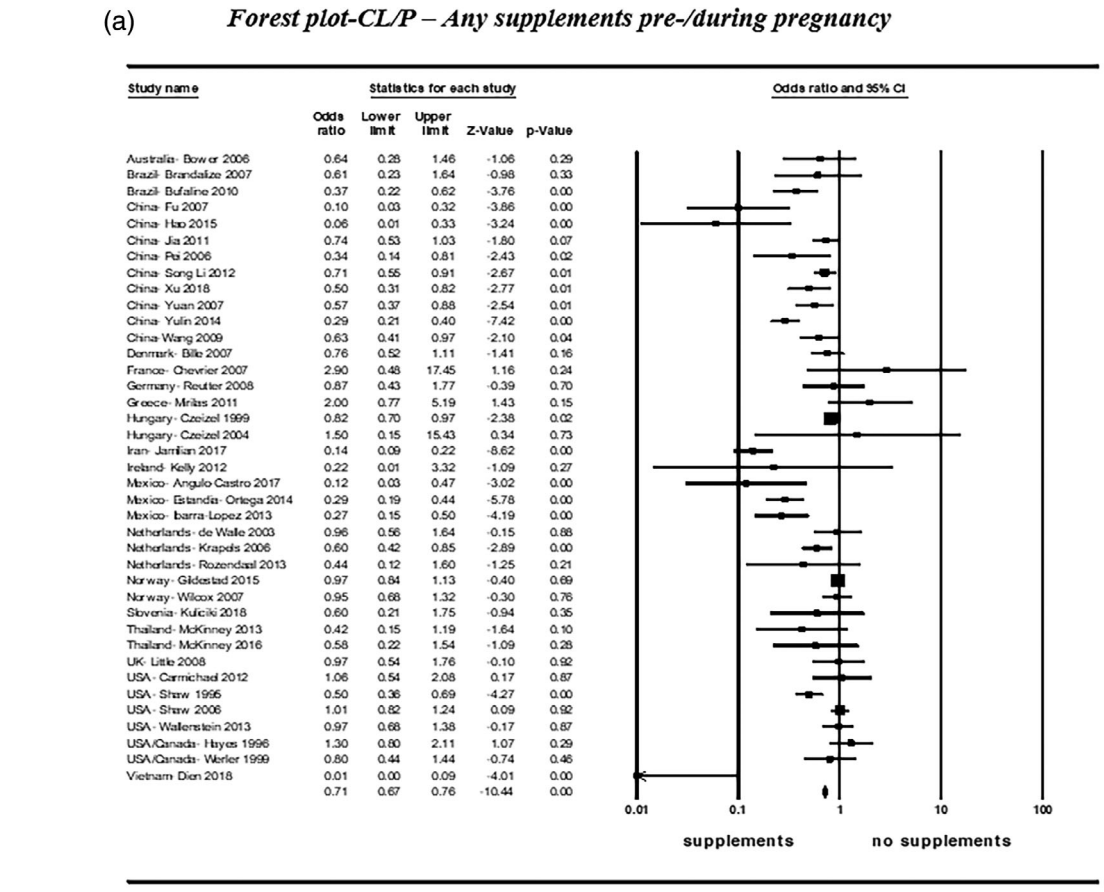
Use of supplements starting after the first trimester (after the ecologically relevant time window of folic acid supplements for CL/P, which is preconception until the first 3 months after pregnancy) showed no association with non-syndromic clefts (for CL/P OR 0.92, 95%CI 0.74–1.15; for CPO OR 0.92, 95% CI 0.66–1.26).

To help assess how the new evidence published after the previous knowledge synthesis may change conclusions, a meta-analysis was conducted for the studies included only through this updated search. Most of the studies identified through the updated search were conducted in low- and middle-income countries (e.g., Brazil,

TABLE 1 Summary of results from meta-analyses of all included studies

Number of studies	OR (95% CI)	I^2	Cochrane Q	Cochrane Q *p*-value	
Any supplement use	**49**	**0.60 (0.51–0.69)**	**80.7**	**248.087**	**<.01**
Folic acid or folic acid-containing multivitamins	39	0.58 (0.49–0.70)	82.8	220.662	<.01
Folic acid only	30	0.56 (0.45–0.70)	84.5	187.027	<.01
Use of supplements starting in the third month or later	4	0.92 (0.74–1.15)	30.0	4.283	.23
Any supplement use	**28**	**0.88 (0.79–0.99)**	**19.9**	**33.720**	**.17**
Folic acid or folic acid-containing multivitamins	21	0.89 (0.78–1.03)	32.8	29.758	.07
Folic acid only	18	0.89 (0.81–0.99)	6.1	18.102	.38
Use of supplements starting in the third month or later	2	0.92 (0.66–1.26)	5.0	1.052	.59
Any supplement use	**11**	**0.65 (0.47–0.90)**	**78.7**	**46.952**	**<.01**
Folic acid or folic acid-containing multivitamins	9	0.81 (0.61–1.08)	75.4	32.582	<.01
Folic acid only	9	0.68 (0.48–0.98)	79.7	39.500	<.01
Use of supplements starting in the third month or later	1	9.79 (3.47–27.5)	–	–	–

Note: The rows in bold represented the primary analysis of any supplement use, followed by subgroup analyses (i.e., folic acid or folic acid-containing multivitamins, folic acid only and use of supplements starting after the first trimester). Forest plots of all included studies concerning any supplement use before and during pregnancy were presented separately for CL/P (Appendix 5, Figure S2) and CPO (Appendix 5, Figure S3). Forest plots of all included studies concerning folic acid supplements only before and during pregnancy were presented separately for CL/P (Appendix 5, Figure S4) and CPO (Appendix 5, Figure S5).



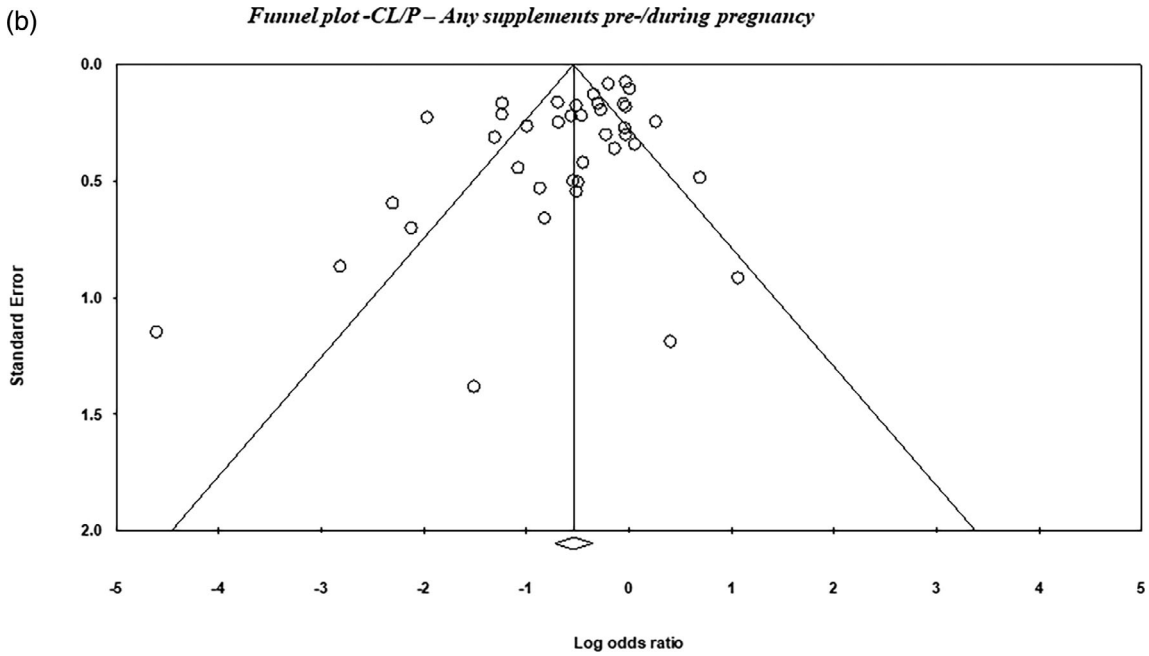


FIGURE 2 (a): Forest plot-CL/P-Any supplements pre-/during pregnancy, (b): Forest plot-CL/P-Any supplements pre-/during pregnancy

China, Iran, Mexico, Thailand, and Vietnam) while the original review (Johnson & Little, 2008) mainly included studies from high-income countries. The updated meta-analyses showed similar results to the original knowledge synthesis, again with high heterogeneity between studies.

TABLE 2 Results from meta-analyses of all included studies reporting adjusted ORs

Meta-analysis	Number of studies	OR (95% CI)	I^2	Cochrane Q	Cochrane Q *p*-value
CL/P					
Any supplement use	25	0.60 (0.49–0.73)	84.0	150.277	<.01
Folic acid or folic acid-containing multivitamins	17	0.62 (0.49–0.77)	81.8	87.690	<.01
Folic acid only	15	0.59 (0.45–0.78)	81.8	77.107	<.01
CPO					
Any supplement use	16	0.87 (0.74–1.03)	36.5	23.632	.07
Folic acid or folic acid-containing multivitamins	11	0.88 (0.72–1.09)	60.2	25.141	.01
Folic acid only	8	0.80 (0.67–0.97)	12.6	8.006	.33
OFC					
Any supplement use	7	0.86 (0.69–1.08)	56.6	13.820	.03
Folic acid or folic acid-containing multivitamins	7	0.90 (0.71–1.14)	52.3	12.582	.05
Folic acid only	5	0.87 (0.63–1.20)	65.2	11.495	.02

TABLE 3 Results from the random-effects meta-analysis of folic acid fortification in all included studies

Meta-analysis	Subgroup	Number of studies	OR (95% CI)	I^2	Cochrane Q	Cochrane Q *p*-value
CL/P	All	7	0.94 (0.86–1.02)	44.426	10.796	.10
CL/P	Optional	2	1.02 (0.93–1.12)	0.000	0.070	.79
CL/P	Compulsory	5	0.87 (0.76–1.00)	51.281	8.210	.08
CPO	All	7	1.01 (0.83–1.23)	79.759	29.643	<.01
CPO	Optional	2	1. 19 (1.03–1.38)	43	1.751	.19
CPO	Compulsory	5	0.90 (0.71–1.15)	54.524	8.796	.07
OFC	Compulsory	6	0.95 (0.85–1.06)	90.234	51.198	<.01

Note: Forest plots of all included studies concerning countries with folic acid fortification were presented separately for CL/P (Appendix 5, Figure S6), CPO (Appendix 5, Figure S7) and OFC (Appendix 5, Figure S8). Forest plots of all included studies concerning countries with mandatory folic acid fortification policy were presented separately for CL/P (Appendix 5, Figure S6a) and CPO (Appendix 5, Figure S7a).

3.8 | Folic acid fortification

The meta-analysis included data from United States, Australia, Canada (Alberta and Ontario), South America (Chile, Brazil, Argentina), Iran, and South Africa (Table 3). Except for Australia, which has a voluntary program, all the other countries had implemented compulsory fortification. The prevalence of CL/P showed a slight reduction associated with fortification in seven included countries (Botto et al., 2006; Canfield et al., 2005; López-Camelo et al., 2010; Souza & Raskin, 2013) (RR 0.94, 95% CI 0.86–1.02). In addition, there was no significant decline in the prevalence of CPO based on the meta-analyses of these countries (RR 1.01, 95% CI 0.83–1.23).

3.9 | Biomarkers—Concentrations of maternal red cell (erythrocyte) folate and serum folate

As shown in Table 4, three studies (Little et al., 2008b; Munger et al., 2004; Munger et al., 2011) were identified that assessed the association between erythrocyte folate status and CL/P, two (Little et al., 2008b; Munger et al., 2011) with CPO and two (van Rooij et al., 2003; Wong et al., 1999) with OFC. Results were varied, with both significant increased and decreased risks found for individuals with lower folate status. In a study in the U.S., (Munger et al., 2011) reported a significantly decreased risk of CPO in the offspring of mothers with

TABLE 4 Risk of oral clefts by quantiles of maternal red cell (erythrocyte) folate: studies reporting odds ratios and *p* value

Study	Location	Definition of lowest, highest quantile	OR (95% CI) highest vs. lowest quantile	*p*-value for trend if OR is presented	Adjusted
CL/P					
Munger et al., 2004	Philippines (NO)	Median, nmol/L tertiles: 1,189 vs. 596	0.46 (0.20–1.09)	.33	Maternal age, education, smoking, alcohol, time between birth and sample
		Mean, Cases vs. controls		<.05	
Munger et al., 2004	Philippines (D)	Median, nmol/L tertiles: 1,189 vs. 596	4.85 (2.24–10.50)	<.01	Maternal age, education, smoking, alcohol, time between birth and sample
		Mean, Controls vs. cases		<.01	
Little et al., 2008a	UK	μg/L quartiles: (584 to 2,228) vs. (103.5 to 323.5)	0.5 (0.18–1.18)	–	Sex, season of birth, ethnicity, maternal education
Munger et al., 2011	USA	nmol/L quartiles: (<36.7) vs. (≥74.1)	0.34 (0.21–0.56)	<.01	Maternal age, education, alcohol use, smoking, multivitamin use, and interval between delivery of the index child and maternal blood collection
CPO					
Little et al., 2008b	UK	μg/L quartiles: (607.5 to 2,228) vs. (107–355)	3.22 (1.14–9.10)	–	Sex, season of birth, ethnicity, maternal education
Munger et al., 2011	USA	nmol/L quartiles: (<36.7) vs. (≥74.1)	0.35 (0.18–0.68)	0.02	Maternal age, education, alcohol use, smoking, multivitamin use, and interval between delivery of the index child and maternal blood collection
All clefts					
Wong et al., 1999	Netherlands	Median, cases vs. controls		<.05	
van Rooij et al., 2003	Netherlands	nmol/L: above/below 394	0.9 (0.3–2.3)	–	Maternal age, education
		Median, cases vs. controls		.06	Maternal age
van Rooij et al., 2003	Netherlands	nmol/L: above/below 394	2.0 (0.3–11.5)	–	Maternal age, education; restricted to un-supplemented women

Abbreviations: D, davao; NO, negros occidental; .

highest compared with the lowest quartiles of erythrocyte folate (OR 0.49, 95%CI 0.24–0.97).

With regard to plasma folate, there were four studies of CL/P (Bezerra et al., 2015; Munger et al., 2004; Gary M. Shaw et al., 2009; Stoll, Dott, Alembik, & Koehl, 1999), one study (Munger et al., 2011) of CPO (Munger et al., 2011, and two of OFC (van Rooij et al., 2003; Wong et al., 1999). There was no consistency in the direction, magnitude, or statistical significance of association (Table 5), even when analysis was restricted to women not taking folic acid supplements (Stoll et al., 1999; van Rooij et al., 2003; Wong et al., 1999). The small number of studies and marked differences in exposure categorization between studies made it inappropriate to carry out meta-analysis.

3.10 | MTHFR (variants: C677T and A1298C)

Folates are essential vitamins needed for amino acid production and many other cellular development processes and insufficient intake might cause OFCs. One of the most studied genes involved in nsCLP, *MTHFR*, encodes the folate metabolism enzyme methylenetetrahydrofolate reductase (Reynolds et al., 2020). The enzyme converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary form of folate found in blood, and involved in the DNA and methylation cycles (World Health Organization, 2002). The two most common variants of the *MTHFR* gene are located at nucleotides 677 (C677T) and 1,298 (A1298C). These variant genotypes are associated with increased thermolability and substantial diminution of activity of the enzyme in vitro (Narayanan et al., 2004). Homozygosity for the C677T variant (TT genotype) is consistently associated with lower blood levels of folate and higher levels of homocysteine than those observed for CT heterozygotes and CC homozygotes (Jin et al., 2018; Tsang et al., 2015). Aggregate evidence is suggestive that homozygosity and heterozygosity for the A1298C variant (CC and AC genotypes) is associated with increased blood folate levels (Xin et al., 2018).

Thirteen studies from the review of Johnson and Little (Johnson & Little, 2008) were included together with the 20 identified in the present study in the meta-analyses relating to *MTHFR* C677T. There was no association between infant *MTHFR* C677T and CL/P or CPO (Table 6). Twenty-seven of the included studies examined the association of infant *MTHFR* C677T and CL/P and covered 20 different countries(Appendix S5, Table S1). For genetic markers (C677T) of folate status of infants, 13 studies were conducted in Europe, 8 (Bezerra et al., 2015; Brandalize et al., 2007; Davalos-Rodriguez et al., 2009; Estandia-Ortega et al., 2014; Gaspar et al., 2004; Ramírez-Chau, Blanco et al., 2016; Semic-Jusufagic et al., 2012; Shaw, Rozen, Finnell, Todoroff, & Lammer, 1998) in the Americas, five (Han et al., 2011; Murthy, Gurramkonda, Karthik, & Lakkakula, 2014; Shotelersuk, Ittiwut, Siriwan, & Angspatt, 2003; Wan et al., 2006; Zhu et al., 2006) in Asia and one (Ebadifar et al., 2015) in the Middle East.

With regard to the *MTHFR* C677T variant, the TT as compared with CC genotype of the index individual and their parents was positively but not significantly associated with CL/P, with substantial heterogeneity. The heterogeneity might be partially explained by differences in the folic acid fortification policy. Nine (Bezerra et al., 2015; Brandalize et al., 2007; Davalos-Rodriguez et al., 2009; Ebadifar et al., 2015; Estandia-Ortega et al., 2014; Gaspar et al., 2004; Ramírez-Chau, Blanco, Colombo, Pardo, & Suazo, 2016; Semic-Jusufagic et al., 2012; Shaw et al., 1998) studies conducted in countries with mandatory folic acid fortification (eight in the Americas and one in the Middle East) showed no association (OR 1.07, 95% CI 0.58–1.97), whereas there was an increase in 18 studies conducted in countries with no such implementation (5 studies conducted in Asia, and 13 in Europe) (OR 1.21, 95%CI 0.94–1.57).

Moreover, there was an increased risk of CL/P among children of parents with the *MTHFR* C677T TT genotype compared with those parents with the CC genotype (for mothers OR 1.18, 95% CI 0.91–1.53; for fathers OR 1.22, 95% CI 0.94–1.57) with medium and low heterogeneity between included studies.

Based on 15 studies of the association between *MTHFR* A1298C and CL/P or CPO (nine identified in the updated search and six in the original search) (Table 7), there was no increased risk of non-syndromic CL/P for infants with the *MTHFR* A1298C CC compared with AA genotype (OR 1.04, 95% CI 0.82–1.32), with low heterogeneity between included studies. Similarly, based on four studies(Han et al., 2011; Jugessur et al., 2003; Karas Kuželički et al., 2018; Mills et al., 2008), there was no association between this infant genotype and CPO (OR 0.85, 95% CI 0.46–1.56).

3.11 | MTHFR C677T/A1298C haplotypes

One study showed no association in the haplotype analysis of *MTHFR* C677T/A1298C haplotypes (Murthy et al., 2014). There was a decreased risk of CL/P for infants with the *MTHFR* C677T/A1298C haplotypes TA

TABLE 5 Risk of oral clefts by quantiles of maternal plasma folate: studies reporting odds ratios

Study	Location	Definition of highest, lowest quantile	OR (95% CI) highest vs. lowest	*p*-value for trend if OR is reported	Adjusted
CL/P					
Stoll et al., 1999	France	Mean, cases vs. controls		NS	Restricted to un-supplemented women
Munger et al., 2004	Philippines (NO)	Median, nmol/L tertiles: 20.6 vs. 8.3 Mean, cases vs. controls	0.89 (0.40–2.01)	.99 NS	Maternal age, education, smoking, alcohol, time between birth and sample
Munger et al., 2004	Philippines (D)	Median, nmol/L tertiles: 20.6 vs. 8.3 mean, cases vs. controls	2.70 (1.18–6.17)	.02 .05	Maternal age, education, smoking, alcohol, time between birth and sample
Shaw et al., 2009	USA	Median, nmol/L quartiles: 43.67 vs. 23.32	1.30 (0.64, 2.67)	.47	Maternal race/ethnicity and age
Munger et al., 2011	USA	Mean, cases vs. controls nmol/L quartiles: 74.1 vs. 36.7	0.78 (0.48–1.25)	.49 .2	Maternal race/ethnicity and age maternal age, education, alcohol use, smoking, multi-vitamin use, and interval between delivery of the index child and maternal blood collection
Bezerra et al., 2015	Brazil	Median, ng/L reference:7–24 median, controls vs. cases	2.18 (1.12, 5.67)	.003 .003	
CPO					
Munger et al., 2011	USA	nmol/L quartiles: 74.1 vs. 36.7	0.49 (0.24, 0.97)	.01	Maternal age, education, alcohol use, smoking, multi-vitamin use, and interval between delivery of the index child and maternal blood collection
All clefts					
Wong et al., 1999	Netherlands	Median, cases vs. controls		<.01	Restricted to un-supplemented women
van Rooij et al., 2003	Netherlands	nmol/L: above/below 7.5	1.2 (0.4–3.2)	–	Maternal age, education
van Rooij et al., 2003	Netherlands	nmol/L: above/below 7.5	0.7 (0.1–3.8)	–	Maternal age, education; restricted to un-supplemented women
van Rooij et al., 2003	Netherlands	Median: controls vs. cases		0.06	Restricted to un-supplemented women

TABLE 6 Results from the random-effects meta-analysis of MTHFR C677T (TT vs. CC) in all included studies

Meta-analysis subgroup	Number of studies	OR (95% CI)	I^2	Cochrane Q	Cochrane Q *p*-value
CL/P					
Infants	27	1.18 (0.96–1.51)	65.851	76.137	<.01
Mothers	18	1.18 (0.91–1.53)	55.837	38.494	<.01
Fathers	7	1.22 (0.94–1.57)	0.000	5.586	.47
CPO					
Infants	9	0.91 (0.60–1.38)	55.508	17.981	.02
Mothers	6	1.18 (0.91–1.53)	0.000	2.913	.71

TABLE 7 Results from the random-effects meta-analysis of MTHFR A1298C (CC vs. AA) in all included studies

Meta-analysis subgroup	Number of studies	OR (95% CI)	I^2	Cochrane Q	Cochrane Q *p*-value
CL/P					
Infants	15	1.04 (0.82–1.32)	15.285	16.526	0.28
Mothers	10	0.86 (0.65–1.13)	0.000	5.640	0.78
Fathers	5	0.92 (0.51–1.65)	53.579	8.617	0.07
CPO					
Infants	4	0.85 (0.46–1.56)	33.891	4.538	0.21
Mothers	3	1.09 (0.75–1.58)	0.000	0.053	0.97
Fathers	2	1.09 (0.71–1.66)	0.000	0.053	0.82

compared with CA (OR 0.58, 95% CI 0.25–1.36) and a slight increased risk of CL/P for infants with CC compared with CA (OR 1.07, 95% CI 0.64–1.78).

3.12 | Gene–environment interactions between MTHFR C677T and maternal folic acid supplements

Two articles reporting gene–environment interactions were found in the updated search (Ibarra-Lopez et al., 2013; Mossey et al., 2017). A synergistic effect of maternal homozygous and heterozygous T allele (CT + TT) and the lack of folic acid supplementation during the first trimester of pregnancy were reported on the increased risk of having a child with CL/P (OR 11.2, 95% CI 3.3–37.5) (Ibarra-Lopez et al., 2013). However, Mossey et al., found a suggestive increase in the risk of CL/P or CPO when comparing mothers with homozygous of T allele (TT) and no folic acid supplements to those who with common genotype (CC) taking folic acid supplements (for CL/P: OR 1.31, 95% CI 0.67–2.55; for CPO: OR 1.18, 95% CI 0.49–2.87) and no synergistic effect between these two exposures (Mossey et al., 2017).

3.13 | Results of supplementary search October 2018-August 2020

Eight articles were identified. Further detailes are provided in Appendix 6. These findings do not change our overall conclusions.

4 | DISCUSSION

The original review (Johnson & Little, 2008) indicated a protective role of multivitamin use in early pregnancy in prevention of child OFCs and our update further suggests that multivitamin supplements taken during the periconceptional period through the end of the first trimester is associated with reduced risk of non-syndromic oral clefts in the offspring. Mandatory folic acid fortification is associated with a decline in the prevalence at birth of CL/P and of CPO, but neither decline is statistically significant. Since CL/P and CPO are rare diseases with low prevalence and there was a few included studies, there may have been insufficient statistical power to detect a significant reduction. Only one study of the association between OFC and maternal dietary intake of folate was

identified. In the few studies of OFC and maternal levels of erythrocyte or plasma folate, there was no clear pattern of association. In aggregate, there was no association between CL/P or CPO and homozygosity for the *MTHFR* C677T or A1298C variants in the mother or index child, although there was substantial heterogeneity for the more widely studied C677T variant.

4.1 | Dietary folate

The one study of dietary folate identified in our search (Wallenstein et al., 2013) was conducted in California in a period soon after the implementation of fortification, so maternal diet could have been impacted by fortification. Un-supplemented women with the highest dietary intake had a reduced risk of having an affected offspring. In the original review (Johnson & Little, 2008), six studies were identified that measured dietary folate intake during pregnancy among CL/P case and control mothers and four of these also measured dietary folate in relation to CPO. Other than one study (van Rooij et al., 2003), which showed an apparent protective effect of high dietary folate intake after including women taking folic acid supplements, all studies included in the original review showed no significant effect of dietary folate intake in prevention of CL/P. We considered the possibility that these results might be affected by fortification policies. However, only one study was conducted in a setting in which mandatory folic acid fortification had been implemented (Shaw, Carmichael, Laurent, & Rasmussen, 2006), whereas the other studies were conducted in European countries where no such mandatory fortification has been adopted.

4.2 | Multivitamin and folic acid supplements

With regard to multivitamin supplements, the potentially protective effect was mainly apparent for CL/P, with weak inverse associations between supplementation and CPO and OFC. No difference in magnitude of association was apparent when restriction was made to multivitamin supplements specified as containing folic acid, or when the supplements contained folic acid only. However, most of the included studies were case–control studies. Because few studies reported on participation rates and most cases were identified in a single hospital, the potential risk of selection bias could not be well assessed. Misclassification of exposure was possible because of retrospective recall of exposure and between-study differences in timing of exposure and how exposure was defined. With regard to potential confounding, less than half of the studies adjusted for potential confounders, there was some attenuation in the magnitude of association when meta-analysis was restricted to studies in which such adjustment had been made.

Significant between-study heterogeneity was detected in several meta-analyses concerning the association between folic acid supplements and risk of CL/P or OFC. Heterogeneity remained high when we conducted subgroup analysis by the content of the supplements. However, in the sensitivity analysis restricting to studies in which adjusted ORs were available, there was only moderate heterogeneity between studies. As would be expected if potential confounding is an issue, there was attenuation of the apparent protective effect of multivitamin supplements when excluding unadjusted estimates.

Another potential source of heterogeneity is the study setting. Earlier studies were mostly done in higher-income countries (almost half of the included studies in the original review were conducted in the U.S.), whereas more recent studies were carried out in lower- and middle-income countries. We found that the magnitude of association was stronger in more recent studies, many of which were conducted in Asia and Europe, where there was no mandatory fortification.

Our update substantially increased the volume of evidence concerning supplements since the orginal systematic review published in 2008 (Johnson & Little, 2008). We included 30 extra studies, with 6,359 CL/P cases, 2007 CPO cases and 1,603 OFC cases in aggregate identified in the updated search. A total of 14,678 controls were identified from 26 case–control studies, while 1,120,371 participants were included from two cohort studies (Gildestad et al., 2015; Li et al., 2012) and 11,134 participants were included in the cross-sectional analysis (Kelly, O'Dowd, & Reulbach, 2012).

Our results were similar to the review of Jahanbin et al., (Jahanbin et al., 2018), despite searching an additional database (i.e., Embase), searching keywords in addition to MeSH terms, and not imposing a language restriction, and hence including 22 more studies. One study (Xu et al., 2018) was included in the meta-analysis of Jahanbin et al. (2018) but excluded in our analysis as it was a "Letter to editors" and so ineligible for inclusion.

4.3 | Folic acid fortification

Our analysis of the effects of folic acid fortification showed a non-statistically significant decrease in the prevalence of CL/P and CPO after implementation of a mandatory fortification policy (OR 0.87, 95%CI 0.76–1.00 and OR 0.90, 95% CI 0.71–1.15, respectively), whereas no

reduction was observed in Australia, which has a voluntary folic acid fortification program (for CL/P OR 1.02, 95%CI 0.93–1.12; for CPO OR 1.19, 95% CI 1.03–1.38). These studies were based on before-after comparisons, and it is difficult to exclude the possibility that changes in the distribution in the prevalence of other risk factors might account for changes in the prevalence at birth of either CLP or CPO.

Heterogeneity was substantial even though the number of included studies was small, especially with respect to the prevalence of CPO and OFC. This might be partially explained by differences in folic acid fortification policies. No preventive effect of optional fortification on CL/P and CPO was detected in Australia. In the United States, mandatory folic acid fortification of enriched grain products was implemented in 1998 and significant protective effects were reported in some studies (Canfield et al., 2005; Yazdy, Honein, & Xing, 2007). However, in some Latin America countries, Iran, and South Africa, mandatory folic acid fortification was only implemented in wheat, while maize remain an important food resource in Latin America (Ranum, Peña-Rosas, & Garcia-Casal,- 2014) and south Africa (Esterhuizen, 2020). This means that populations within those countries whose diets consist mostly of other grains, such as corn, maize, or rice, may not receive the full benefit of folic acid fortification. A recent systematic review also found that fortification of wheat or maize flour with folic acid (i.e., alone or in combination with other micronutrients) was associated with increased erythrocyte and serum/plasma folate concentrations (Centeno Tablante, Pachón, Guetterman, & Finkelstein, 2019). In 2016, the U.S. Food and Drug Administration approved folic acid fortification of corn masa flour; modeling studies suggested that such an intervention could reduce the disparity in prevalaence at birth of neural tube defects between Hispanic Americans and other ethnic groups (Tinker et al., 2013). Other explanations for high heterogeneity include variation in study quality, case ascertainment methods, and case definitions.

Our systematic review differs from that of another review (Millacura et al., 2017) in that searching was done for each specific type of cleft rather than OFC overall, and overlap between studies was taken into account.

4.4 | Erythrocyte and serum folate

We observed no clear pattern of association between biochemical markers of folate status (assessed after pregnancy and lactation) and risk of oral clefts. These studies assumed that blood levels after pregnancy and lactation reflect those in the preconceptional period. There is some empirical evidence to support this assumption (Little, Gilmour, Mossey, FitzPatrick, Cardy, Clayton-Smith, Hill, et al., 2008; Munger et al., 2004; Munger et al., 2011). The study conducted in the Philippines (Munger et al., 2004) showed a significant difference between the association between CL/P and erythrocyte folate level between the two study sites, which had marked differences in the prevalence of vitamin B_6 deficiency. Although the study was fairly small, it suggested that dysregulation of one-carbon metabolism might contribute to the risk for CL/P. The United States study (Munger et al., 2011) was carried out in Utah and during a time period when there was fortification, as was the Brazilian study (Bezerra et al., 2015). The other studies were conducted in Europe, where no fortification was implemented (Little et al., 2008a; Stoll et al., 1999; van Rooij et al., 2003; Wong et al., 1999). Erythrocyte folate is a better marker of folate status over a longer period than plasma folate, so might be less subject to the effects of day to day fluctuation, which would tend to attenuate association (Clifford, Noceti, Block-Joy, Block, & Block, 2005). Some studies (Little et al., 2008b; Munger et al., 2004; Munger et al., 2011) used a microbiological (*L. casei*) assay of folate status, which is considered to be the gold standard, whereas others used a radioimmunoassay which is known to overestimate folate level in individuals with the TT genotype compared with microbiological assay (Molloy et al., 1998; Sharp & Little, 2004).

4.5 | MTHFR genotype

Although substantial heterogeneity was identified between studies included for meta-analysis for the *MTHFR* C677T variant, based on substantially fewer studies, there was a marginal and insignificant inverse association between homozygosity for this variant in the index individual and CPO (OR 0.91, 95% CI 0.60–1.38); the pattern of association with maternal genotype was similar to that for CL/P. With regard to the *MTHFR* A1298C variant, no difference in risk of non-syndromic clefts were found between the CC and AA genotypes, with low heterogeneity between studies.

5 | APPLICABILITY OF RESULTS

5.1 | Sources of variation

There were large variations in the time (period, frequency and duration) and type of folic acid supplements (folic acid only supplements or multivitamin supplements that contains folic acid) as reported in Table 1.

Some studies only reported if supplements were used during the pre-conceptional period or during pregnancy irrespective of time period within it, which made it impossible to determine whether supplements had been used during preconception and the first trimester. If the studies included women who take supplements after this time window, our results might be biased toward null.

For most of the included studies, the components of supplements and extent of use were often incompletely reported, making it difficult to judge the effect of the exposure. Future researchers should be encouraged to use reporting tools such as the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement (von Elm et al., 2008) to provide more information concerning the interventions or exposures of interest in their papers. Another important factor to consider is the variability of covariates included in adjustment, making it difficult to compare results between studies. In addition, the variability of definition of reference and comparison groups between included studies that investigated biochemical markers of folate status precluded the conduct of meta-analyses for this question. Using standard cut-off points could help between-study comparisons, although variations in biomarker levels between populations could make this challenging.

5.2 | Other considerations

The biochemical markers and genetic factors reflecting folate status included in this paper had a narrow focus. In future work, it may be of value to consider a broader range of biomarkers and gene variants that would enable a more comprehensive assessment of one-carbon metabolism, of which folate is one element. Indeed, Blanco et al., reported a systematic review of the associations between OFC and biomarkers of maternal methyl donor status related to one-carbon metabolism and reported that a higher concentration of maternal plasma homocysteine with lower folate levels, was associated with an increased risk of non-syndromic OFC (Blanco et al., 2016).

5.3 | Limitations

While the present update and the original review (Johnson & Little, 2008) were systematic and comprehensive, there were some limitations. Firstly, in view of the small number of articles included regarding dietary intake and the different definition of quantiles in each study, meta-analysis was inappropriate, results were variable, and it was difficult to summarize the evidence for an association. Secondly, most of the included studies were case–control studies with either lack of transparent report on the participation rate or conducted within single hospitals, which makes the effect of potential selection bias difficult to appraise. Thirdly, less than half of the included studies reported adjusted results and in those that did, different potential confounders were considered. As a result, residual confounding is a concern, for example because women who take multivitamin supplements in early pregnancy likely have different characteristics, such as socioeconomic status (Camier et al., 2019), age, ethnicity and tobacco exposure (Wu, Buck, & Mendola, 1998) than women who do not take supplements or who start them later in pregnancy. Fourthly, unexplained high heterogeneity for some meta-analyses suggests the existence of other important variables we have not been able to capture in our study.

6 | FUTURE RESEARCH

Clearly randomized controlled trials would help address the problems posed by potential confounding (e.g., maternal age, BMI, education, smoking, alcohol intake, anti-epileptics drugs, etc.). Feasibility issues would include the size of the trials, which could be addressed by focusing on recurrence risk of clefts. It would be unethical to randomize pregnant women not to take any folic acid supplements because of its substantial protective role against neural tube defects, but we still could offer control group participants a low dose of multivitamin supplements, as (Wehby et al., 2013) suggested.

More rigorous studies with larger sample sizes, better reported description of both exposure and outcome are needed to further investigate the association between maternal intake of folate and folic acid, folic acid fortification, markers of folate status and occurrence of non-syndromic clefts. Cranial neural crest cells and orofacial epithelial cells are two major cell types that interact with various cell lineages and play key roles in orofacial development (Ji et al., 2020). The etiologies of Orofacial clefts (OFCs) are likely attribute to an interplay between genetic and environmental factors (Garland et al., 2020). Future research should also consider a broader range of epigenetic, microRNAs, environmental, and cellular mechanisms associated with one-carbon metabolism, dietary folate intake, and biochemical markers of one-carbon metabolism using biobanks.

Global changes in dietary patterns (e.g., as a result of global warming, or intended to reduce greenhouse gas emissions by changes in agriculture) and large-scale intervention program (e.g., intended to promote healthy

diets, prevent under-nutrition, prevent NTDs [Martinez et al., 2018]) may change population micronutrient status. Over 820 million people lack sufficient food resources and an even larger number suffer from micronutrient deficiency and diet-related obesity due to low quality diets (Willett et al., 2019). Furthermore, the 2020 coronavirus pandemic may have a profound global impact on the dietary pattern (Naja & Hamadeh, 2020). For example, the restriction on social and international interactions might affect the food diversity and even threaten the food resources and food security. These changes have potential implications for OFC, and therefore it will be important to maintain up to date surveillance on vitamin use and OFC.

7 | CONCLUSION

This systematic review of an updated set of manuscripts found evidence of 40% reduction in risk of CL/P (OR 0.60, 95% CI 0.51–0.69) and a 12% decrease in risk of CPO (OR 0.88, 95% CI 0.79–0.99) among women with multivitamin supplement use before and during pregnancy. However, these results should be interpreted with caution because of the high heterogeneity between the included studies, incomplete description of population characteristics, sources of bias unaccounted for in analyses, and variability of the covariates included in consideration of potential confounding.

CONFLICT OF INTEREST

All authors declare that they had no conflict of interest in conducting this review.

AUTHOR CONTRIBUTIONS

Yulai Zhou is the guarantor of the review. All authors contributed to the development of search strategies, eligibility criteria, and drafting of the protocol. Yulai Zhou and Vigigah Sinnathamby worked in duplicate to screen the titles and abstracts of all the materials obtained using the search strategy to exclude the articles that did not meet the eligibility criteria. Yulai Zhou and Vigigah Sinnathamby evaluated the potentially eligible studies at full text level and excluded further studies with documentation of the reason for exclusion. All authors contributed to the bias assessment strategy and data extraction criteria. Yulai Zhou and Yamei Yu independently extracted data from the included studies using a pre-defined extraction sheet. Yulai Zhou analyzed the data and drafted the results. All authors read, provided feedback, and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The study data is available from the corresponding author on the application.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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