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Meta Analysis
Cleft Lip and Palate

# Maternal biomarkers of methylation status and non-syndromic orofacial cleft risk: a meta-analysis

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*Abstract.* Animal models have shown evidence of the role of maternal methyl donor status and its metabolism (one-carbon metabolism) in normal embryonic maxillofacial development. Nevertheless, studies in humans have shown conflicting results for the association of maternal methylation status biomarkers in the aetiology of the main craniofacial birth defects: non-syndromic orofacial clefts (NSOFCs). The aim of this study was to perform a meta-analysis assessing the relationship between maternal levels of methylation status biomarkers (plasma and erythrocyte folates and plasma vitamin B12 and homocysteine) and the risk of NSOFCs. A literature search of the conventional and grey medical–scientific databases identified 12 studies considering these variables. Based on standardized differences between means among cases and controls (Cohen's *d* test), evidence was found of an association only with high plasma homocysteine ($d = 0.37$; $P = 0.026$) when single effects were pooled. In addition to its usefulness as a marker of poor methyl-donor intake and/or metabolism, homocysteine appears to have a teratogenic effect. Although the results are based on a relatively small number of reports and/or studies of small sample sizes showing between-study heterogeneity, these problems were resolved by including an additional analysis. Therefore these findings constitute a real contribution towards explaining the complex aetiology of orofacial clefts.

Key words: orofacial clefts; folate; vitamin B12; homocysteine; meta-analysis.

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Orofacial clefts (OFCs) are the most common birth defects affecting the craniofacial structures. The average prevalence of OFCs varies according to ethnic origin, geographic location, and socioeconomic status, among other factors.[1] OFCs are generally classified as cleft palate only (CPO), cleft lip only (CL), or cleft lip with cleft palate (CLP). The latter two categories are historically referred to as cleft lip with or without cleft palate (CL/P).[2] Approximately 70% of OFCs are non-syndromic (NSOFCs), occurring as isolated conditions without any other apparently structural or cognitive abnormality. The remaining 30% are defects found as part of more than 300 recognizable genetic syndromes.[1,2] The

 

prevalence rates, complexity of their rehabilitation plus medical costs, and the emotional burden on patients and their families make OFCs a worldwide public health problem. In this context, OFC patients present a wide variety of medical complications in early processes such as feeding, speaking, and hearing and in their social integration. These problems can be corrected totally or in part by maxillofacial and plastic surgery and by dental, audiologist, and psychological therapies beginning in the first months of life and extending beyond 18 years of age, according to the severity of the malformation.[3]

The aetiology of NSOFCs can be explained by the interaction between functionally altered genes and environmental factors, with the relationship with folate/one-carbon metabolism being probably the best example of this genetic–environmental interaction.[3,4] It has been reported that maternal periconceptional use of folic acid supplementation has beneficial effects in preventing the occurrence of NSOFCs.[5,6] Additionally, the interaction between foetal and maternal genotypes of functional variants within the folate metabolism genes and maternal intake of folic acid has also been demonstrated in the risk of NSOFCs.[7,8]

Folates are involved in the transfer of one-carbon units (methyl groups) to molecules involved in several biological processes, such as DNA synthesis and methylation, an epigenetic mechanism of genetic expression control.[9] Through evidence found in animal models, it has been postulated that a maternal methyl group deficiency can alter the DNA methylation status of the offspring, thereby playing a role in the aetiology of birth defects.[10] A deficit of methyl groups affects embryo and foetal cells with high proliferation rates, including those from the neural tube and neural crests.[4] The latter cell population, after neural tube closure, migrates to the ventral area and differentiates through processes regulated by epigenetic mechanisms. Notably, they contribute to bone and cartilage development of the craniomaxillofacial structures.[11] In addition, murine models have shown the importance of epigenetic mechanisms in secondary palate development.[9]

Consequently, some authors have evaluated the association of maternal methylation status and the risk of NSOFCs in the offspring, based on plasma and erythrocyte folate levels as direct biomarkers. Some studies have reported that mothers of cases have significantly lower levels of plasma folate in comparison to mothers of controls.[12–14] The same relationship has been described for erythrocyte folate levels.[12,13] However, associations between both of these biomarkers and cleft risk are not found in other reports.[7,15,16] The plasma level of vitamin B12 is another biomarker of maternal methylation status. This vitamin is closely related to one-carbon metabolism and is considered as another dietary methyl donor; it acts as an enzymatic co-factor for enzymatic reactions in methyl group transfer.[17] Positive and negative results for the association between maternal plasma levels of vitamin B12 and NSOFCs are described in the literature.[7,15,16,18]

S-adenosylmethionine is the principal methyl group donor in humans, which, after demethylation, is converted to homocysteine in a two-step enzymatic reaction.[17] Elevated plasma levels of this latter molecule are considered a good and sensitive marker of an impaired folate/one-carbon metabolism status, being associated with low levels of methyl donors such as folates and vitamin B12.[17,19] Consequently, some reports have shown significantly higher plasma levels of homocysteine in mothers of NSOFC cases in comparisons to control mothers, while other studies have not found significant differences between the two groups of mothers.[13–15,19,20]

Thus, the results of studies on the relationship between maternal levels of folate, vitamin B12, and homocysteine in the plasma and folate within erythrocytes and the risk of NSOFC in the offspring are controversial. In order to contribute towards resolving this controversy, it was decided to perform meta-analyses for these variables based on a literature search of several databases, with a comparison of the mean values among case mothers and control mothers.

## Materials and methods

### Literature search and study quality assessment

A search of the following scientific literature databases was conducted: PubMed, EMBASE, Cochrane Library, Web of Science, SpringerLink, and Scielo. In addition, grey literature databases were also searched (GreyNet, GreyLit, OpenGrey, LILACS, and POPLINE) (Fig. 1). The literature search was conducted through 1 October 2015 with no date restrictions for early studies and including the ''Related articles'' option, based on the terms ''cleft lip palate'' OR ''cleft palate'' OR ''orofacial clefts'' AND ''maternal homocysteine'' OR ''maternal folate'' OR ''maternal vitamin B12'' and restricted to English and Spanish languages. This search was performed independently by two authors (RB and JS), who identified the authors, year of publication, sample sizes, and mean values and standard deviations (SD) or median values and interquartile range (IQR) for the four selected biomarkers in NSOFC mothers and control mothers (offspring without any structural birth defect). After discarding reports according to the criteria described in the flowchart (Fig. 1), a quality assessment was performed on the selected studies using the Newcastle–Ottawa Scale, which considers three categories: selection of cases and controls, comparability of these groups, and the ascertainment of either the exposure or outcome. The maximum score that can be awarded is 9 points. A study with a poor quality score (<5 points) will have limitations for inclusion in a meta-analysis, with a high risk of bias.[21]

### Statistical analyses

The meta-analyses were performed comparing the mean values of four folate/one-carbon metabolism biomarkers in mothers of NSOFC cases and control mothers. When data were expressed as the median and IQR, the mean value and SD were estimated using the formulae described by Hozo et al.[22] In order to pool the results of the individual studies, Cohen's *d* test (with 95% confidence interval, CI) was used to standardize differences between means for each determination among cases and controls, especially when the groups presented different variances for a measurement.[23] This statistic represents the effect size of a variable on a trait even when different scales have been used in the two groups. Its calculation and interpretation are relatively simple and it can be considered as the standard for effect size estimation.[23,24]

The presence of heterogeneity among the selected studies was assessed based on the Cochran $Q$ statistic, which is calculated by summing the squared deviations for the effect of each study related to the pooled effect.[25] In addition, heterogeneity was quantified by means of the $I^2$ statistic, which represents the percentage of between-study variability explained by heterogeneity.[25] Thus, the combined effect was estimated using the fixed-effects or random-effects method according to the absence ($I^2 < 50$) or presence of heterogeneity ($I^2 > 50$), respectively.[26] In the presence of between-study heterogeneity, it was attempted to detect the source by