

NONSYNDROMIC CLEFT LIP AND/OR PALATE. THE ROLE OF FOLIC ACID

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Abstract

The nonsyndromic cleft lip and/or palate (NSCL/P) is a common congenital orofacial defect of multifactorial origin with involvement of genetic and nutritional factors. The presence of genetic polymorphisms in enzymes that metabolize folic acid and vitamin supplements used to prevent these anomalies have not yet been well established in the literature. The aim of this study was to conduct a review of the literature about the role of folate and gene polymorphisms in the metabolic pathway of folic acid as regards the occurrence of NSCL/P. We reviewed theoretical material that addressed data on these topics. According to the studies analyzed in the literature, there was no consensus with regard to the protective effect of supplemental folic acid taken in the periconceptual period in the prevention of NSCL/P. The studies that evaluated defects in genes involved in folate metabolism, found an association of some polymorphisms with NSCL/P in different populations.

Keywords: Folic acid. Polymorphism. Cleft palate. Cleft lip.

INTRODUCTION

Nonsyndromic cleft lip and palate NSCL/P are frequent orofacial malformations, of complex etiology, which involve diverse genetic and environmental factors, among which nutritional deficiency is outstanding.

The teratogenic effect of vitamin deficiencies has been known since 1914. In 1961, the influence of folate and the vitamin B complex on the facial development of mice was investigated.⁽¹⁾ Folate or vitamin B₉ is a hydrosoluble vitamin of the B

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Complex, considered an essential nutrient, acquired by means of diet and one that plays an important role in the synthesis of DNA, tDNA and amino acids.⁽²⁾ This premise becomes particularly important during gestation, because alterations that involve folate metabolism in pregnant women, for example the low ingestion of folic acid and the presence of genetic polymorphisms, may have an influence on the development of various congenital defects, particularly those of the neural tube (NTD).⁽³⁾ However, with regard to NSCL/P the results are still controversial.⁽⁴⁾

Population-based studies have shown a reduction in cases of cleft lip and palate since fortification of food products with folic acid was introduced.^(5,6) Other researches have been unable to confirm this association.^(7,8) Multicentric studies conducted in Latin America and in the Irish population have demonstrated the involvement of genes *MTHFR* and *MTHFD1*, both associated with the metabolic routes of folic acid, in the occurrence of NSCL/P.^(9,10)

Bearing in mind the importance of vitamin supplements during pregnancy and their possible role in the etiology of NSCL/P, the aim of this study was to evaluate the results of the literature on the action of folic acid in the prevention of orofacial clefts. In addition, the probable participation of polymorphisms in genes related to folic acid metabolism as a cause of this anomaly was also reviewed.

FOLIC ACID IN THE PREVENTION OF OROFACIAL CLEFTS

Folate is a vitamin of the B group, soluble in water, present in legumes, green leafed vegetables such as spinach and turnip leaves, and some citric fruits. Folic acid is a more stable synthetic form of folate, and is frequently used in supplements and fortified foods. The bioavailability of folic acid is approximately 70% higher than the folate naturally contained in foods, although there are variations depending on the methodology used for measurement.⁽¹¹⁾

Folic acid and its metabolites are essential for the normal development of the fetus during embryogenesis.⁽¹²⁾ The quantity of folic acid required during pregnancy increases around 5 to 10 times the

amount needed outside the gestational period.⁽¹³⁾ Folic acid plays an important role in the metabolism and conversion of proteins into energy. This vitamin is also essential for the biosynthesis of nitrogen bases and amino acids during cell division and tissue growth.⁽¹⁴⁾

Maternal nutrition, especially pre-conceptional vitamin supplementation with folic acid, has been recognized as an important component for the beginning of fetal development. Studies of the case-control type have demonstrated that the use of folic acid reduces the occurrence of neural tube diseases (NTD) by at least 60%.⁽¹⁴⁾ This vitamin supplementation also plays an important role in the prevention of orofacial/oral clefts. Talarova & Harris, in 1995,⁽³⁾ found a reduction of 65% in the risk of occurrence of CL/P in children who used vitamin supplements during pregnancy. For Canfield and collaborators, in 2005,⁽¹⁵⁾ after cereals were supplemented with folic acid, a reduction in the occurrence of DTN and cleft palates was verified.

It is believed that the effect of folic acid on the prevention of NSCL/P is dose dependent. Various studies in different populations have observed that women who ingested high doses of folic acid had a reduced risk of having children with NSCL/P.⁽¹⁶⁾

Another factor that appears to have an influence on the prevention of NSCL/P is the period of folic acid ingestion. When this supplementation was taken before conception and maintained after fertilization, a reduction in the birth of children with NSCL/P was observed in various populations.⁽¹⁷⁾

A Research of meta-analysis that evaluated five prospective studies of the case control type in the period from 1964 to 2004 about folic acid consumption during pregnancy and the reduction in risk of oral clefts, found that in the case control studies, there was a reduction of 20 to 29% in the risk of having a child with any type of oral fissure. While in prospective studies the reduction was from 45 to 49%.⁽⁸⁾

In another meta-analysis study searching articles in all languages between January and July 2005, the protective effect of folic acid and supplements enriched with multivitamins on congenital anomalies was evaluated. The protective effect of folic acid and supplements on neural tube defects and other anomalies was verified. The results of this review

showed that maternal prenatal consumption of folic acid containing multivitamins was associated with diminished risk of various congenital anomalies, and not only neural tube defects. These data had important implications for public health, because up to 2005, only the use of folic acid without supplemental vitamins had been encouraged.⁽¹⁶⁾

In a more recent meta-analysis published by Johnson & Little⁽¹⁷⁾ a reduction of around 18% in the risk of the occurrence of CL/P was estimated, with the use of supplements containing folic acid, but without any significant reduction in CP. A reduction of around 23% in the risk of CL/P with the use of multivitamins was also found. Thus a world-wide consensus was established that women of a reproductive age must receive supplemental vitamins containing folic acid.

Although the above-mentioned researches have shown evidence of the protective effect of folic acid on the prevention of DTN and NSCL/P, other studies have presented contradictory results. IN a case-controlled study in Scotland and England, no impact was observed with the use of vitamin supplements and folic acid on the reduction of CL/P and CP.⁽⁷⁾ This association may not have been verified due to the small sample of the study.

The most significant study with regard to the lack of association of the use of folic acid and the occurrence of cleft lip and palate was published in 2010 by De Regil et al.⁽¹⁸⁾ In a systematic review, all the

randomized clinical trials were included, on the effect of pre-conceptional supplementation of folate alone or in combination with other vitamins and minerals. The review showed that folic acid of itself alone or in combination with vitamins and minerals prevented the formation of NTD, but had no impact on the prevention of other congenital defects, especially on NSCL/P.

THE METABOLIC PATHWAY OF FOLIC ACID

Folate acts on various reactions in the transfer of a carbon, including the biosynthesis of purine and Thymidilate, metabolism of amino acids and process of oxidation. The biosynthesis of purine and thymidilate are requisites for DNA and RNA synthesis, with the dependent folate reactions being essential for fetal growth and development. These reactions are also involved in the metabolism of homocysteine, since the plasma level of homocysteine is regulated by the quantity of folate obtained in the diet.⁽¹⁹⁾

The metabolic pathways of homocysteine and folic acid are directly related (Figures 1-3). Folic acid acts in two cycles: On that involves DNA biosynthesis, essential for cell division, and the other, methylation, essential for the supply of methyl groups for cell methyltransferases.⁽²⁰⁾

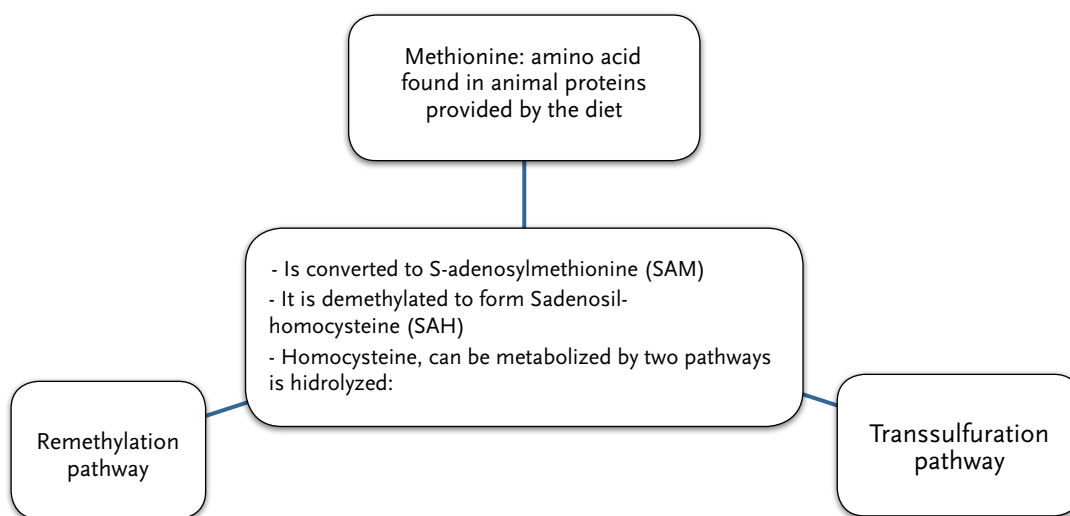


Figure 1. Metabolic pathway of folic acid

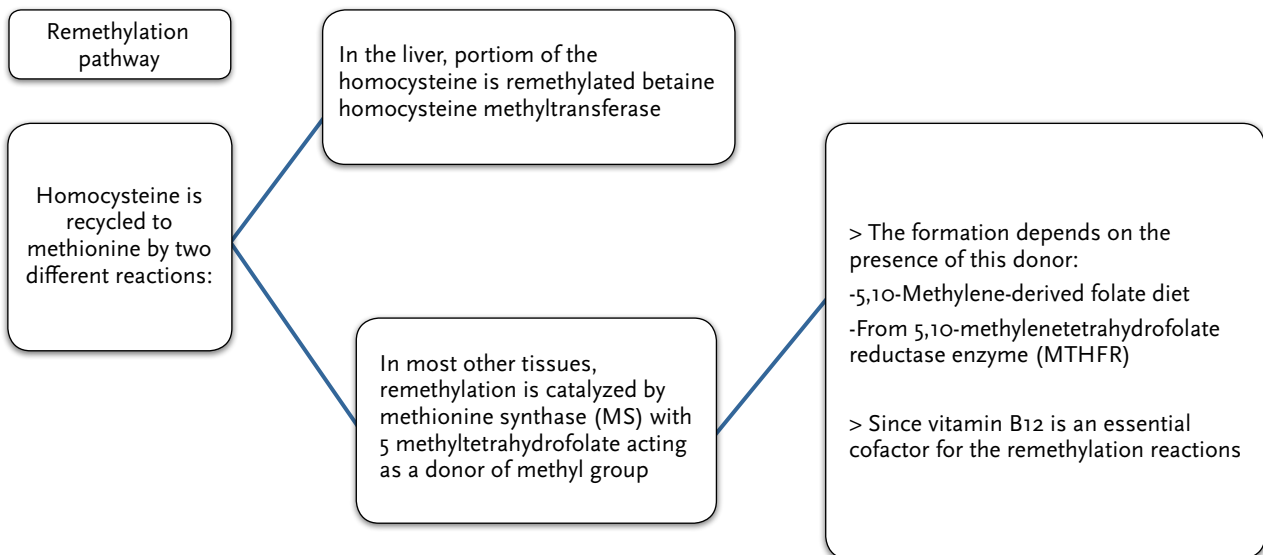
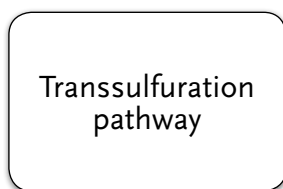
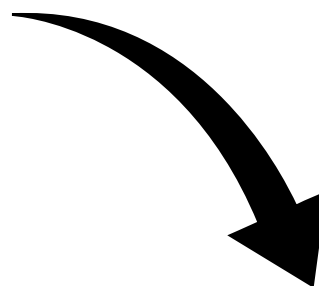


Figure 2. Remethylation Pathway



- The homocysteine-cystathionine is irreversibly catabolized by cystathionine-beta-synthase enzyme (CBS)
- It's a reaction dependent on vitamin B6
- Resulting in the formation of cystathionine, which is then metabolized to cysteine



The metabolism of homocysteine depends on the influence of concentrations of folate (folic acid), cobalamin (vitamin B12) and pyridoxine (vitamin B6).

Figure 3. Transsulfuration Pathway

Folic acid is reduced from the active form called tetrahydrofolate (THF) to 5,10-methylenetetrahydrofolate. The enzyme methylenetetrahydrofolate reductase (MTHFR) converts the 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, which is the circulating form of folate. The product of this reaction are the methyl groups used in the synthesis of methionine, necessary for the methylation of DNA.(21)

In the next stage of this cycle the enzyme methionine synthase, coded by gene MTR, catalyzes the remethylation of homocysteine into methionine. This reaction is necessary for the production of S-adenosylmethionine (SAM), the universal donor of the methyl groups for the methylation of DNA. Vitamin B12 acts as co-factor for the reaction. Cobalamine oxidizes over the course of time and

the enzyme methionine synthase becomes inactive. The functional regeneration of methionine synthase requires the action of another enzyme, methionine synthase reductase, codified by gene *MTRR*. Another enzyme, cystathionine β -synthase, which is dependent on vitamin B6, catalyzes the first transsulfuration reaction in the metabolism of homocysteine, and is regulated by SAM.⁽²²⁾

The methyl groups formed from this metabolism are essential to many biochemical processes. They are necessary for DNA replication, and the hypomethylation of DNA is associated with chromosomal instability and errors during segregation.⁽²³⁾

POLYMORPHISM IN GENES OF THE FOLIC ACID PATHWAY

At present genetic studies seek to associate genetic polymorphisms with causal factors in diverse types of congenital defects. This genetic alteration is characterized by the occurrence of at least two alleles in one locus, since the rare variant must be found at a frequency higher than 1% in the population.⁽²⁴⁾ This genetic mutation may be found in the child that has the disease, in the parents that may transmit this defective gene or it may happen due to the influence of the environment.

In molecular investigation into cleft lip and palate, polymorphisms in genes related to craniofacial development, DNA repair and folic acid metabolism have been studied, especially in the last few years.⁽²⁴⁾

Polymorphisms of genes *MTHFR*, *MTHFD*, *MTR*, *MTRR*, *RFC1*, *FOLR*, *BHMT* and *CBS* have been associated with the development of NSCL/P. These polymorphisms may alter the regulating mechanism of serum folate resulting in congenital anomalies, especially NSCL/P.^(25,26,27) It has been found that polymorphisms in the genes that participate in the regulation of plasma levels of an amino acid denominated homocysteine (*MTHFR*, *MTR* and *MTRR*) of mothers is related to the occurrence of NSCL/P in their children.^(26,28)

Polymorphism C677T of gene *MTHFR* has been found in different frequencies, according to ethnic

and regional variations in individuals with NTD. In the Brazilian population the prevalence of this polymorphism was higher than the result presented in the European population.⁽²⁹⁾

Han et al. 2011⁽³⁰⁾ investigated associations with the risk of occurrence of NSCL/P in a population in the South of China, and showed that the genotypes *MTHFR* 677 were associated with increased risk of cleft lip with or without cleft palate. Pan et al. 2012,⁽³¹⁾ in a meta-analysis study with 17 case control studies conducted in an Asiatic population also verified that genotypes of *MTHFR* C677T and A1298C were related to the development of NSCL/P. Zhu et al 2006,⁽³²⁾ in 170 trios, verified a moderate association of polymorphisms of gene *MTHFR* and NSCL/P in a population in the North of China, but not in populations in the South of the same country.

However, other investigators have observed different results with regard to some polymorphisms of genes that regulate folic acid metabolism. In a study with trios, it was found that polymorphisms C677T and A1298C of gene *MTHFR* were not risk factors for some types of orofacial malformations. However, should the mother have the genotype *MTHFR* 677TT or *MTHFR* 1298CC and low periconceptional ingestion of folic acid occurs, the risk would be increased.⁽³³⁾ Boyles et al. 2008⁽²³⁾ also found no evidence of the risk of variant C677T of gene *MTHFR* (rs1801133). In the population of Central Europe, similar results were demonstrated with the same polymorphism. It was suggested that the allele T does not make an important contribution to the occurrence of NSCL/P.⁽³³⁾ Sozen et al. 2009⁽³⁴⁾ found no association of risk for NSCL/P in the genotype variants of gene *MTHFR* C677T and A1298C in the North of Venezuela.

Bhaskar et al. 2011,⁽⁴⁾ in a study reviewing articles published up to October 2010, about polymorphisms of genes related to the metabolism of folate and homocysteine and their associations with NSCL/P, found no strong association between the risk of NSCL/P and any known gene related to folate metabolism.

In addition to the genes *MTHFR* e *MTHFD1*, Mostowska et al. 2006⁽¹⁾ in Poland, investigated genotype variants of genes *MTR* and *RFC1*. They observed that mothers with genotype *MTR* A2756G or genotype GG had 2.195 times the risk of generating

a child with NSCL/P (IC 95% 1.189-4.050, $p= 0.011$). When this same gene was evaluated in the Brazilian population, no significant association was found.⁽³⁵⁾

Another polymorphism of gene *MTHFD1*, namely G1958A, was related as a maternal risk factor for NSCL/P in 2008. In a Latin American group, Blanton et al. 2011⁽³⁶⁾ verified the association of risk of genes *MTR*, *BHMT2*, *MTHFS* and *SLC19A1* with the occurrence of NSCL/P.

FINAL CONSIDERATIONS

The review of the literature showed a possible role of folic acid in the prevention of defects related to the neural tube. However, in more recent researches, its protective effect on the prevention of NSCL/P was not observed. Genetic studies have demonstrated that polymorphisms related to the metabolic pathway of folic acid in certain genes are associated with NSCL/P in different populations.

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