www.jpnim.com Open Access eISSN: 2281-0692
Journal of Pediatric and Neonatal Individualized Medicine 2025;14(2):e140201
doi: 10.7363/140201
Received: 2024 Dec 05; revised: 2025 Mar 12; accepted: 2025 Mar 31; published online: 2025 Aug 19

Review

Overview of neural tube defects: history, folate-homocysteine cycle, *MTHFR* C677T polymorphism and preventions

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Abstract

Neural tube defects (NTDs) are common significant congenital malformations caused by very early disruptions in the development of the brain and spinal cord. They are caused by a neurulation failure, which happens about the 28th day after conception. Folates and vitamin B12 (vitB12) deficiencies, as well as hyperhomocysteinemia and *MTHFR* C677T polymorphism, are the common risk factors studied for the occurrence of these birth defects. In this study, we reported the roles of folates, vitB12, homocysteine, *MTHFR* gene and their relation with the appearance of NTDs. We also discuss prevention strategies, such as food fortification with folic acid, to encourage developing countries to adopt these recommendations in order to prevent these birth defects in subsequent pregnancies.

Keywords

Neural tube defects, folates, vitamin B12, homocysteine, *MTHFR* C677T polymorphism, prevention, educational interventions, food fortification.

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How to cite

Nasri K. Overview of neural tube defects: history, folate-homocysteine cycle, *MTHFR* C677T polymorphism and preventions. J Pediatr Neonat Individual Med. 2025;14(2):e140201. doi: 10.7363/140201.

Introduction

Neural tube defects (NTDs) are congenital malformations of the central nervous system, which appear during the 4th week of embryonic development [1].

Sometimes the closure of the neural tube is not completed and the neuropores, cranial and caudal, remain open. These two embryopathological events result in NTDs, with anencephaly and spina bifida being the most well-known [2].

Several factors have been studied as potential causes of NTDs.

Overall, NTDs are currently considered multifactorial genetic anomalies, implying that both genetic and environmental factors may play a role in the appearance of these birth defects [3].

Numerous studies have highlighted a relationship between NTDs and a folate deficiency at the begining of pregnancy. Indeed, 50% to 70% of NTDs are prevented by the periconceptional intake of folic acid [4-6].

Folic acid may not reduce the risk of NTDs with the same degree in all ethnic groups [7, 8]. This suggests that genetic factors may be involved and raises the suspicion of the influence of folate metabolism in particular through gene polymorphism. As a result, a lot of studies have been done to determine the genetic and biochemical causes of NTDs.

In this review, we documented the functions of folates, vitamin B12 (vitB12), homocysteine (Hcy), 5,10-methylene-tetrahydrofolate reductase gene (*MTHFR*) and their relationship with the development of NTDs. We also provide ways to prevent these birth anomalies in future pregnancies, like fortifying foods with folic acid. This will help developing nations to follow these guidelines.

Folates

The origin of the name folates comes from the Latin *folium*, which means leaf, because these substances were isolated from spinach leaves in 1941 by Mitchell and his collaborators.

Folate is a water-soluble vitamin that belongs to group B. It is also called folic acid (the synthetic form of folate), pteroylmonoglutamate, folacin, vitamin BC, vitamin B9, and *Lactobacillus casei* factor [9].

Nutritional intake

Humans cannot synthesize folates, so intake is dependent on food sources. Yeast extracts, liver, kidney, green leaves, vegetables and citrus fruits are foods rich in folates. Bread, potatoes, and dairy products are medium-level sources, but because they are consumed in large quantities, they contribute significantly to folates intake [10].

The majority of these folates (80%) are reduced methylated (60%) and formylated (20%) derivatives of protein-bound folylpolyglutamates. Only 20% is provided in the form of unsubstituted derivatives. Furthermore, a small quantity can be directly provided by the bacterial flora inhabiting the digestive tract [11].

Normal plasma folate levels are 5 to 15 µg/L. In the United States, the recommended nutritional intake for adults is 400 µg/day of dietary folate equivalent, 600 µg/day during pregnancy and 500 µg/day during lactation. In Europe, the recommended intakes are: 100-300 µg/day from birth to puberty, 200-400 µg/day in adults and 400 µg/day in pregnant women [12].

The role of folates

During the transmethylation reaction, N_5-methyltetrahydrofolic acid provides a methyl group that will be transferred to Hcy with the aid of vitB12 (a cofactor for methionine synthase). Tetrahydrofolic acid is regenerated as a result of this reaction, and methionine is produced, which can subsequently be converted to S-adenosylmethionine.

The latter is the universal donor of methyl groups for methylation reactions in the body (DNA, proteins, lipids, etc.) leading to the formation of S-adenosylhomocysteine [13].

Folate deficiency

Folate deficiency is one of the most common vitamin deficiencies in the world and poses a public health problem in both developing and industrialized countries. Folate deficiency can occur at any age and is often the result of malnutrition, malabsorption, alcoholism or the use of certain medications.

Also, genetic alterations in folate absorption, transport or metabolism can lead to folate deficiency [14].

Prevalence of folate deficiency

Folate deficiency is defined for plasma folate concentrations below 3 ng/ml and erythrocyte folate concentrations below 150 ng/ml. According to data collected in the United States during the Second National Health and Nutrition Examination Survey (NHANES II), folate deficiency would reach approximately 10% of Americans aged between 20 and 44 years [15]. In agreement with these data, an epidemiological study, carried out on 423 healthy Americans, has shown that 23% of studied subjects had serum folate concentrations lower than 3 ng/ml despite a dietary folate intake greater than 200 µg/day corresponding to currently recommended intakes [16].

Vitamin B9 (folate) deficiency is also common among elderly people, with reported prevalences of up to 25% in nursing homes for dependent elderly people [17].

Vitamin B12

Nutritional data: food sources and needs

VitB12 or cobalamin (Cbl) was discovered in 1948. Foods containing Cbl are of animal origin such as kidneys, offal, liver, meat, milk, eggs, seafood and fish, in addition to some rhizome plants [18].

VitB12 is obtained exclusively from dietary sources, with daily requirements estimated between 2 and 5 µg. A balanced diet usually provides quantities significantly higher than physiological needs [19].

In the United States, the intake recommended by the Food and Drug Administration (FDA) is 2.4 µg per day for adults [20], and between 1 and 2 µg per day for children. Requirements increase significantly during periods of growth, hypermetabolic state, gestation and breastfeeding [21]. They are 2.6 µg during pregnancy and 2.8 µg during breastfeeding. The reserves, mainly hepatic, are significant (more than 1.5 mg), explaining the delay of 5 to 10 years between the onset of a B12 deficiency and the appearance of clinical manifestations [20].

Role of cobalamins

As mentioned previously, methionine synthase catalyzes the methyl-Cbl dependent (re)methylation of Hcy to methionine within the methionine cycle; a reaction required to produce this essential amino acid and generate S-adenosylmethionine, the most important cellular methyl-donor.

Blocking this reaction has two consequences:

1. blocking the regeneration of methionine, which disrupts the metabolism of sulfur amino acids and explains the increased urinary excretion of Hcy;
2. the absence of regeneration of tetrahydrofolate which prevents the reconstitution of N_5-methylene tetrahydrofolic acid 4, a thymidylate synthetase coenzyme. This results in a blockage of DNA synthesis since dUMP (deoxyuridylate) cannot be converted into dTMP (thymidylate) explaining partly the medullary megaloblastosis caused by Cbl deficiency [22].

Vitamin B12 deficiency

Tab. 1 shows the main definitions of vitB12 deficiency proposed in the literature, in the absence of a standardized and formally reproducible vitB12 biological assay and well-established standards [20, 22-25].

In developing countries, prevalences of vitB12 deficiency of more than 40% have been reported [22].

The etiology of this deficiency was mainly dominated by poor dietary intake in a population made up mainly of children.

On the other hand, in industrialized countries, maldigestion of dietary Cbls was constantly pre-

Table 1. Definitions of vitamin B12 (vitB12) deficiency.

Definitions	Reference
VitB12 < 200 pg/ml + clinical (neurological) signs and/or hematological abnormalities compatible with vitB12 deficiency	Andrès et al., 2004 [22]
VitB12 on two occasions < 200 pg/ml (or 150 pmol/L)	Andrès et al., 2005 [20]
VitB12 < 200 pg/ml + total Hcy > 13 µmol/L or methyl malonic acid > 0.4 µmol/L (in the absence of renal insufficiency, folate and vitamin B6 deficiencies and/or the presence of a heat-labile mutant of methyl tetrahydrofolate reductase)	Klee, 2000 [24]
VitB12 < 148 pmol/L	Gyawali et al., 2023 [25]

Hcy: homocysteine; vitB12: vitamin B12.

dominant compared to other etiologies of Cbl deficiency and mainly affects patients of advanced age [26].

Socio-economic conditions and their impact on the standard of living and diet of patients certainly explain this observation [27].

In addition, it has been reported that a female predominance has been objectified in certain studies but this remains to be verified [28].

VitB12 deficiency is common in the elderly, with a prevalence of 12% among non-institutionalized elderly subjects in the Framingham cohort [29].

Some studies observed higher prevalences among hospitalized or institutionalized elderly subjects [30], while other studies did not report differences between "community" or "institutionalized" elderly populations [31].

However, certain studies have shown the influence of other factors, particularly ethnic factors, on the etiological profile of vitB12 deficiency. In an American study of 202 subjects, Latin American, African American origins and advanced age were highlighted as independent risk factors. In an Indian series, a greater frequency of the deficit was noted in patients of Indian origin compared to patients of other ethnicities [32].

The etiology of vitB12 deficiency is dominated in adults and the elderly by two causes. The classic Biermer disease (which corresponds to an illness due to a deficiency in vitB12, itself resulting from a lack of secretion of intrinsic factor) represents 20% to 50% of cases. The syndrome of non-dissociation of vitB12 from its carrier proteins or maldigestion of dietary vitB12, described in the 1990s, represents more than 60% of cases [20].

Homocysteine

Hcy is an amino acid discovered in 1932 by Du Vigneaud during his study on the chemistry of sulfur amino acids [33].

It is a sulfur amino acid derived from the demethylation of methionine provided by a diet rich in animal proteins [34].

Plasma levels are considered normal between 5 and 15 µmol/L. It does not exceed 15 µmol/L in healthy subjects [34].

Biological functions of homocysteine

Hcy has major biological functions such as an intermediate in the methionine cycle and a substrate for folate recycling [35].

Hyperhomocysteinemia

Under physiological conditions, a balance between Hcy formation and degradation is present and approximately 50% of Hcy is remethylated to methionine. In the case of an excess of Hcy, the latter passes into the blood circulation, thus causing an increase in the plasma level of Hcy (this is hyperhomocysteinemia – HyperHcy), or an increase in the level of Hcy in the urine (in this case we speak about hyperhomocystinuria) [36].

The definition of HyperHcy is established according to an arbitrary threshold corresponding to the 95th percentile of the distribution of Hcy concentration in a population considered normal. Fasting Hcy level is normally between 5 and 15 µmol/L in adults. In people over 60 years old, it must be less than 20 µmol/L. In pregnant women and children, the limit is reduced to 10 µmol/L.

Fasting HyperHcy is classified into three categories: moderate (16-30 µmol/L), intermediate (31-100 µmol/L), and severe (> 100 µmol/L) [34].

Folate, cobalamin, homocysteine and neural tube defects

When a famine struck the Dutch in 1944, it was observed that the incidence of spina bifida doubled, raising the first suspicion that the etiology of NTDs could be linked to diet. This suggested that vitamin deficiencies, especially folate, are at least partially responsible [37].

The specific role of folates in the development of NTDs was identified in 1964 by Hibbard, who observed metabolic abnormalities in mothers of children with myelomeningoceles and found that children of folate-deficient mothers had a higher rate of these birth defects (3%) compared to controls (1.6%) [38].

Smithells et al., in 1976, demonstrated that these mothers had a decrease in erythrocyte folates during the first trimester of pregnancy. So, they speculated that periconceptional folic acid supplementation would reduce the risk of NTD recurrence [39].

Thus, in 1980, they carried out the first non-randomized intervention study, supplementing women who had already given birth to a child with NTDs with a multivitamin complex containing 360 µg of folic acid. Compared to women who did not utilize supplements, the risk of having another child with NTDs was 86% lower for these mothers [40].

Folate's ability to prevent NTDs was clearly established in 1990s. A prospective, randomized, double-blind trial conducted in 1991 examined the relative risk of recurrent NTDs in women who received 4 mg of folic acid daily or a combination of seven other vitamins. The results showed a relative risk of 0.28 (95% CI: 0.12-0.71). Of the 33 centers that took part in this study, 17 were situated in the high-incidence nation of the United Kingdom, while the remaining centers were spread across 6 other nations, including France.

In 1999, the Centers for Disease Control and Prevention (CDC) reported that supplementation with 0.4 mg led to a reduction in the incidences of NTDs of 79% in northern China compared to 41% in southern China [41].

Several studies have suggested the protective effect of folate consumption, including descriptive epidemiological studies, case-control studies, cohort studies, and randomized and nonrandomized intervention studies [4, 42, 43]. In a study carried out in Hungary, the prevalence of all malformations was reduced by half in the group supplemented with 0.8 mg of folic acid compared to the group receiving mineral supplementation, with no NTDs cases recorded for the folic acid supplemented group [6, 44].

In another randomized trial, folic acid supplementation reduced the risk by 75%, while other vitamins were associated with a 20% of reduction [4].

In a large retrospective study concerning women during the first half of pregnancy, Kirke et al. [45] found significantly lower levels of (plasma, erythrocyte) folate, and serum vitB12 in mothers of fetuses with NTDs. This suggests that an associated vitB12 deficiency may also be involved [46].

These studies strongly support widespread folic acid supplementation as a preventive measure against NTDs.

Then, these findings prompted us to deepen our understanding of folate metabolism, and to look for possible disturbances in subjects with NTDs or their parents.

Several hypotheses have attempted to provide an explanation [47, 48]. None is completely verified, but they all point to a folate deficiency at a critical moment in the embryonic development of the central nervous system which can act at the maternal level or directly on the embryo.

In embryonic brain cell cultures, studies have shown that decreasing folate has inhibited the proliferation of astrocytic and neuronal stem cells [49]. Similarly, the use of methotrexate, an inhibitor of folate metabolism, has decreased proliferation and increased apoptosis of stem cells by modifying the quantity and pairing of nucleotides to DNA during the S phase of the cell cycle [50].

The mechanism of action of folates appears to be twofold: on the one hand, as a cofactor in the biosynthesis of DNA and RNA, and on the other hand, as a donor of methyl radicals in the methylation cycle which converts Hcy to methionine [51].

The intervention of folates in the metabolism of Hcy may be an element which partly explains their preventive effect [52].

Indeed, low serum levels of folate and vitB12, but especially high levels of Hcy, have been observed in women carrying or having carried fetuses with NTDs. In particular, an Irish study observed that in pregnant women of NTDs fetuses, serum Hcy levels were significantly higher than those of healthy fetuses. This suggested a deficiency in the remethylation of Hcy, which requires a methyl group delivered by 5-methyltetrahydrofolate [53].

In 1991, Steegers-Theunissen et al. reported elevated Hcy levels in women who carried a fetus with NTDs and suggested that maternal HyperHcy was a risk factor for this birth defect [54].

The association of NTDs with HyperHcy has been the subject of various publications [55, 56].

In this sense, vitB12 deficiency also causes an elevation of Hcy in the fetus and increases developmental alterations in its nervous system during gestation [57]. In infants, vitB12 deficiency can cause various problems, such as severe hypotonia and apathy, which could reflect a deficiency in myelination [58]. Furthermore, hereditary transcobalamin II deficiency causes neurological abnormalities characterized by intellectual delay, ataxia, etc. [59].

HyperHcy is certainly a sensitive marker of folate decline, but genetic causes are also responsible [60].

The hypothesis of enzyme polymorphisms occurring at this level of the metabolic chain is raised, firstly the 677 C>T (C677T) mutation of *MTHFR* [61].

Studies have shown that the non-closure of the neural tube is not due to a direct action of Hcy, but to an abnormal functioning of methionine synthase.

The alteration of the enzyme would certainly cause an accumulation of Hcy but also a deficit of

methionine necessary for the synthesis of myelin. Therefore, there is an association between neural tube closure anomalies and HyperHcy, but these pathologies most often result from a deficiency in methyl groups [56].

Another *MTHFR* polymorphism (1298 A>C [A1298C]) has been shown to be associated with the occurrence of NTDs.

The association of these 2 polymorphisms, C677T and A1298C, could increase the risk of NTDs [62], and would also be responsible for spontaneous abortions [63].

More recently, the possible responsibility of autoimmune mechanism was raised in 9 mothers (out of 12 studied) who had a child affected by NTDs. These mothers carried anti-folic acid receptor antibodies whose pathogenicity has been demonstrated in animals [64].

Although the majority of studies have shown a protective effect of folic acid supplementation, some analyzes have failed to demonstrate this role.

These studies highlighted that, in these women, the problem is more complex than a simple folate deficiency. The protective effect of folic acid could be the correction of increased needs by genetic predisposition [65].

MTHFR C677T genetic polymorphism and neural tube defects

The *MTHFR* C677T polymorphism consists of a substitution of a cytidine by a thymidine at position 677 on exon 4, in the catalytic domain of the enzyme. At the protein level, this autosomal mutation results in a modification of a valine to alanine, leading to a drop in enzymatic activity, which varies depending on the allelic status, of the order of 30-40% in heterozygotes and 60-70% in homozygotes.

When this polymorphism is present in the homozygous form, the enzyme becomes less active and more thermolabile, causing a rise in Hcy levels and a decrease in folate levels [66].

The frequency of C677T homozygosities varies between geographic regions. A high frequency is observed particularly in Europe (between 10% and 20%) unlike other continents (Africa: < 1%) [67].

In the Maghreb countries, the results showed an allelic frequency of 17.8% in Tunisia [68], and 8.5% in the Algerian population of the Batna region [69].

The C677T variant is the first genetic risk factor proposed to cause this anomaly. In the Netherlands (1995), Van der Put et al. were the first to demonstrate that C677T mutation in the *MTHFR* gene is more prevalent in NTDs patients and their mothers compared to controls. The C677T mutation was associated with a 2.9- to 3.7-fold increased risk in infants with spina bifida and their mothers [70]. This observation was further supported by two studies from the United States and Ireland, in which NTDs were 2 to 7 times more common in infants with homozygous mutation [71].

Many studies have reported a high prevalence of homozygosity (TT) in mothers of an infant with NTDs compared to controls [72-74]. This confirmed that homozygosity for the *MTHFR* C677T gene polymorphism could be a risk factor for NTDs.

Prevention

Educational interventions

The educational programs were first launched in the United States and Canada. This intervention is in use in most other countries. In some studies, very high folic acid utilization has been demonstrated among high socioeconomic and cultural status women (Prevention program for reducing risk for neural tube defects – South Carolina, 1992-1994, 1995). A Canadian study revealed that the number of NTD cases did not decrease while educational initiatives were in place [75].

Folic acid fortification

According to current recommendations from Health Canada, women should ingest at least 400 µg of folic acid per day to reduce the risk of NTDs in the fetus.

Since the majority of people cannot obtain this amount of folate from unfortified foods, many nations, notably the United States, United Kingdom, and Canada, have implemented programs to boost folic acid intake. These programs involve promoting the use of oral folic acid supplements and fortifying grain products with folic acid. Foods fortified with folic acid have been required in an effort to enhance the daily consumption of folic acid for all women of reproductive age. Supplementing fortified cereals with acid folic has been mandatory since 1998 [76].

Foods enriched with folic acid

To correct dietary folic acid insufficiency in North America, the enrichment of flour and cereal products (including ready-to-eat cereals and pasta),

at a rate of 150 μg of folic acid / 100 g, became mandatory in 1998.

Foods were fortified to increase daily folic acid intake by an average of approximately 100 μg. An American study demonstrated an increase in erythrocyte folate concentrations from 527 nmol/L to 741 nmol/L in 38,000 women of childbearing age after the introduction of folic acid fortification [77].

Furthermore, a large pharmacokinetic modeling study carried out in China demonstrated the beneficial effect of even higher levels of erythrocyte folate (up to 1,500 nmol/L) [78].

Fortification of foods with folic acid has been shown to be very effective in reducing the prevalence of NTDs at birth. Indeed, after folic acid enrichment, the prevalence of spina bifida at birth decreased more than 50% in Canada and that of other NTDs subtypes approximately a third [79].

Furthermore, the East-West gradient in NTDs levels was reduced considerably after the adoption of folic acid fortification [79]. Similar reductions of 50% to 70% in NTDs at birth have been reported in other countries where mothers increased their folic acid intake [80].

Use of supplements was the main predictor of optimal rates and was correlated with favorable socioeconomic status [81].

Oral folic acid supplements

Despite the abundance of data demonstrating the effectiveness of folic acid enrichment in reducing NTDs, a high proportion of women still have a folic acid deficiency at the begining of pregnancy. Health Canada and the Public Health Agency of Canada recommend that women of childbearing age take a daily supplement of 0.4 mg of folic acid to reduce the risk of NTDs [82].

This recommendation is supported by detailed guidelines from the Society of Obstetricians and Gynecologists of Canada (SOGC). In accordance with these recommendations, healthy women should consume a diet high in folic acid, which includes taking 0.4-1.0 mg of folic acid-containing multivitamin supplements daily, for at least 2 to 3 months prior to conception, during pregnancy, and during the postpartum phase (for at least 4 to 6 weeks and as long as breastfeeding is continued) [82].

According to the SOGC, women with a family history of NTDs or other health issues should increase their intake of foods high in folate and take a daily multivitamin supplement that contains 5 mg of folic acid from at least 3 months prior to conception until 10 to 12 weeks after conception [82].

In comparison to the prevalence observed from January 1995 to December 1996 in the United States, data noted between January 1998 and December 1999 showed a 31% decrease in the prevalence of spina bifida and a 16% decrease in the prevalence of anencephaly [83].

The overall increase in folate levels among women of childbearing age was notable, but it was less pronounced among high-risk groups, such as those of Mexican-American origin and low-socioeconomic status. Oral intake of folic acid in the fully absorbable medicinal form must be prescribed by a doctor. In addition, free distribution of folate supplements to women of childbearing age, particularly those who are disadvantaged and at high risk, is strongly recommended [84].

Conclusion

Numerous studies have shown that folic acid deficiency, particularly in the early stages of pregnancy, is linked to the development of NTDs. However, even if this prevention is not complete, it has been demonstrated that women taking this vitamin supplement have a protective effect during the periconceptional phase. This is why developing countries must adopt initiatives to increase folic acid intake. Efforts must be made by specialists to educate women of childbearing age about the importance of taking folic acid supplements, especially in the periconception period.

Abbreviations

Cbl: cobalamin

CDC: Centers for Disease Control and Prevention

FDA: Food and Drug Administration

Hcy: homocysteine

HyperHcy: hyperhomocysteinemia

MTHFR: 5,10-methylene-tetrahydrofolate reductase gene

NHANES II: Second National Health and Nutrition Examination Survey

NTDs: neural tube defects

SOGC: Society of Obstetricians and Gynecologists of Canada

VitB12: vitamin B12

Declaration of interest

The Author has no competing interests to declare.

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