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Folate Metabolism and Its Implications in Pregnancy

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Abstract

The impact of folate nutritional status on a variety of pregnancy outcomes has been acknowledged for a considerable period of time. Folate is increasingly recognized not just as a nutrient required to prevent megaloblastic anemia during pregnancy, but also as a vitamin necessary for reproductive health. Folate plays an important role in several metabolic processes including DNA synthesis and methylation. Changes in folate status can affect the stability and integrity of DNA or affect the methylation patterns of some tissues. Folate is required for cell division and cell maintenance, as it acts as a co-enzyme in the transfer and processing of carbon units and plays an important role in the synthesis of nucleotides (thymidine) that are essential for de novo construction or DNA repair. The purpose of this article is to examine the function of folic acid in human health especially in pregnancy and to evaluate the benefits, concerns, and epigenetic effects of maternal FA in light of recent discoveries that are crucial for the development of future research.

Introduction

Folate, also referred to as folic acid or vitamin B9, is an essential type of B vitamin. The demand for folate increases during periods of rapid cell division, such as pregnancy, as it plays a vital role in cell replication. On a population level, it is believed that the nutritional requirement for folate, similar to vitamin D, cannot be fully met through a varied diet alone, as advised by national health authorities. In many Western societies, folate intake through food sources like legumes, citrus fruits, and leafy green vegetables remains below recommended levels (Liew, 2016; Ohrvik & Witthoft, 2011; Subandrate et al., 2022; Talaulikar & Arulkumaran, 2011). An estimated additional intake of 50-180 µg of folate could help most people reach the recommended levels. Communities with lower socioeconomic status often face restricted access to foods high in folate. Additionally, natural dietary folate tends to be unstable, with approximately 30% of its vitamin activity typically lost during food processing. However, inadequate dietary folate intake remains the only directly modifiable risk factor for neural tube defects (NTDs) (Ohrvik & Witthoft, 2011; Cao et al., 2022; Isaković et al., 2022; Bhasker & Veleri, 2025).

Like other B vitamins, the primary role of folic acid in the body is as a coenzyme. Folic acid is converted into its active form, tetrahydrofolate (THF), within the body (Paduch-Jakubczyk & Dubińska, 2024; Carboni, 2022; Goossens et al., 2021). THF acts as a carrier of single-carbon units in various metabolic processes. In nucleotide metabolism, THF donates methenyl or

formyl groups, while in amino acid metabolism, it donates methyl groups. Folic acid is known to help prevent neural tube defects. Additionally, it is used for the prevention and treatment of megaloblastic anemia and certain malignancies. Folic acid deficiency has been associated with various health issues, including neural tube defects, megaloblastic anemia, some congenital disorders, and cardiovascular diseases (Shulpekova et al., 2021; Wald, 2022; Ekundayo et al., 2025). Consequently, many countries have implemented folic acid supplementation or fortification programs in food. These initiatives have successfully reduced the incidence of neural tube defects and megaloblastic anemia in several nations (Christensen et al., 2015; SACN, 2017; World Health Organization, 2015). The potential negative effects of folic acid supplementation or fortification have not been thoroughly assessed. According to data from the Scientific Advisory Committee on Nutrition (SACN), folate fortification may lead to harmful outcomes. Elevated serum folate levels have been linked to a higher risk of prostate cancer. High-dose folic acid treatment is believed to contribute to cognitive decline associated with vitamin B12 deficiency. Animal studies have shown that a folate-rich diet can lower the levels of the enzyme methylenetetrahydrofolate reductase and cause liver damage (De-Regil et al., 2015; SACN, 2017; Fardous & Heydari, 2023).

Folate concentrations in the blood typically fall between 5-15 ng/ml. According to the World Health Organization (WHO), normal serum folate levels are between 6-20 ng/ml. To maintain proper folate levels, an adequate intake through diet or supplementation is essential. If serum folate drops below 6 ng/ml, there is a potential risk for deficiency, and levels below 3 ng/ml confirm a deficiency. Folate levels are considered elevated if they exceed 20 ng/ml. The body needs folate in optimal amounts, as both deficiency and excess folate have been associated with various health conditions (De-Regil et al., 2015; World Health Organization, 2015). This paper will explore folate metabolism and related issues linked to folate deficiency during pregnancy.

Folic Acid

Folic acid is the synthetic version of vitamin B9 (folate), while tetrahydrofolate is another form of folate. Folic acid comprises three main components: pteridine derivatives, p-aminobenzoate (PABA), and glutamate. The glutamate in folate can range from one to eight residues. Folic acid, or pteroylmonoglutamate, which is folic acid with one glutamate molecule attached, has a core structure with a single glutamate (McNulty, 2022; Puspitasari et al., 2025). In food, folate exists as pteroylpolyglutamate, a form of folate with multiple glutamate molecules attached. This form is broken down into pteroylmonoglutamate, the single-glutamate form, in the intestines by an enzyme called folate conjugase. Absorption mostly happens through passive diffusion, a natural movement of nutrients, when folate intake is high, or with the help of special transport proteins like the proton-coupled folate transporter, folate receptors, and the reduced folate carrier. Once absorbed, pteroylmonoglutamate is changed back into pteroylpolyglutamate, the multiple-glutamate form, in body tissues so it can be stored and use (Liew, 2016; Shane, 2008; Subandrate et al., 2022). Folic acid is the inactive form of the vitamin and cannot function as a coenzyme in its original state. For it to be active in metabolism, folic acid is converted into dihydrofolate (DHF) and tetrahydrofolate (THF) through the action of the enzyme dihydrofolate reductase. THF is the active form of folate. It plays a key role in metabolism by carrying one-carbon units such as methyl, formyl, formimino, methylene, and methenyl groups. These one-carbon units attach to specific spots on the folic acid molecule, called the N5 or N10 positions (Subandrate et al., 2022).

Absorption of Folic Acid

The main sources of folate in mammals are foods such as leafy vegetables, legumes, citrus fruits, and liver. Folate from food is mostly found as polyglutamates, a form of folate where multiple glutamate molecules are attached. These polyglutamates cannot pass through cell membranes if the glutamate chain has more than three glutamate molecules. In the human small

intestine, where folate is absorbed, an enzyme called glutamate carboxypeptidase II, or GCPII, breaks down polyglutamates into monoglutamates, which are folate molecules with only one glutamate attached. This enzyme is located on the surface of intestinal cells. Once broken down, monoglutamyl folate is transported into the cells through three main pathways. The first pathway involves facilitated anion exchange, where a protein called the reduced folate carrier, or RFC, helps move folate into cells. This carrier prefers reduced folate, the active form of folate, over folic acid, the synthetic form. The second pathway uses folate receptors, which are proteins on the cell membrane that grab folate and bring it into the cell through a process called endocytosis. These receptors have a strong preference for folic acid. The third pathway is passive diffusion, where folate naturally moves into cells without needing energy or proteins. This process is more common when folate levels are very high, such as with supplements or medications (Nazki et al., 2014).

Folate Forms

Monoglutamate is the only form of folate present in the bloodstream and the only one able to cross cell membranes. However, once inside the cell, folate predominantly exists as polyglutamate. This conversion to polyglutamate is facilitated by the enzyme folypolyglutamate synthetase (FPGS). Polyglutamylation, the process of adding multiple glutamate molecules to folate inside cells, acts as a metabolic trap. This helps keep folate from being lost through efflux, or leakage out of the cell. Additionally, polyglutamylated folate, which has multiple glutamate molecules attached, works better as a substrate for intracellular folate-dependent enzymes compared to monoglutamate folate, which has only one glutamate. This means polyglutamylated folate is more efficient for the enzymes that rely on folate to carry out important cellular processes (Nazki et al., 2014; Pertiwi et al., 2022).

Enzymes Involved in Folate Metabolism

Folate metabolism consists of reducing carbon atoms at the formyl, methylene, or methyl oxidation stages, which are covalently linked to nitrogen at either the 5 or 10 positions. This complex metabolic pathway is regulated through a series of processes involving at least 30 different enzymes. Among them, several key enzymes play essential roles in ensuring the proper functioning of folate, facilitating processes like one-carbon unit transfer, nucleotide production, and supporting cellular activities such as DNA synthesis and repair (Nazki et al., 2014; Zheng & Cantley, 2019).

Dihydrofolate Reductase (DHFR)

The enzyme DHFR facilitates the reduction of dietary folate or dihydrofolate to THF, the main form of folate found in plasma. This process reduces folate compounds to create coenzymes that are essential in multiple metabolic processes (Nazki et al., 2014; Pertiwi et al., 2022; Zheng & Cantley, 2019).

C1-tetrahydrofolate (THF) Synthase

This enzyme is a complex made up of multiple enzymes, each with distinct functions: 10-formyl-THF synthetase, 5,10-methylenetetrahydrofolate cyclohydrolase, and 5,20-methylenetetrahydrofolate dehydrogenase. It facilitates the conversion between 10-formyl-THF and THF along with formate, between 10-formyl-THF and 5,10-methylenetetrahydrofolate, and between 5,10-methylenetetrahydrofolate and itself (Nazki et al., 2014; Pertiwi et al., 2022; Zheng & Cantley, 2019).

5,10-Methylenetetrahydrofolate Reductase (MTHFR)

5,10-MTHFR is a key enzyme in folate metabolism, responsible for catalyzing the irreversible transformation of 5,10-methyltetrahydrofolate into 5-methyltetrahydrofolate, the primary form of folate found in circulation. The 5,10-MTHF substrate is essential for DNA synthesis and

also acts as a source of one-carbon units derived from serine. This conversion is facilitated by the enzyme serine hydroxymethyltransferase, which mediates the interconversion of glycine and serine. The product, 5-methyltetrahydrofolate, then provides the methyl group required for methionine synthesis, which can impact DNA methylation when reduced (Nazki et al., 2014; Pertiwi et al., 2022; Zheng & Cantley, 2019).

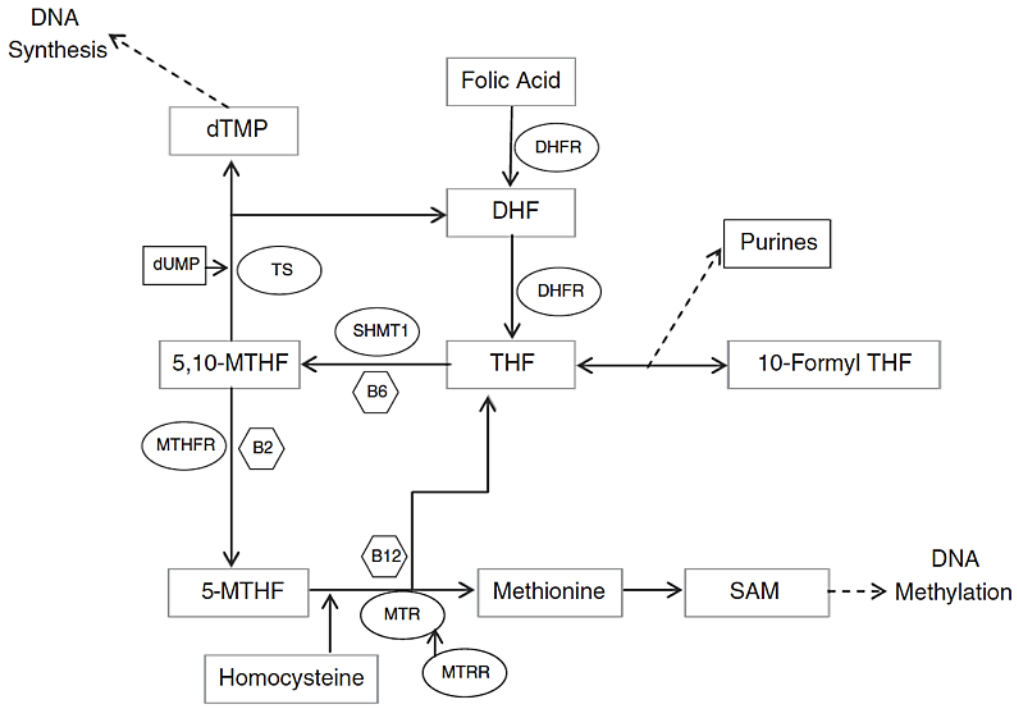

Figure 1. Folate metabolism (Nazki et al., 2014).

Thymidylate Synthase (TS)

Thymidylate synthase (TS) facilitates the transformation of Deoxyuridine monophosphate (dUMP) into deoxythymidine monophosphate (dTMP), with 5,10-MTHF serving as the methyl group donor. This reaction also produces dihydrofolate (DHF), which is then converted back to THF through the action of DHFR (Nazki et al., 2014).

Methionine Synthase Reductase (MTR)

MTR facilitates the remethylation of homocysteine into methionine by transferring a methyl group from 5-MTHF to homocysteine. This process is vital as it ensures the availability of methionine for producing S-adenosylmethionine, a key methyl group donor. Cobalamin serves as a cofactor in this reaction, forming a complex with Cbl(I) MTR that binds the methyl group from 5-MTHF, resulting in metCbl(III) MTR. This complex then transfers the methyl group to homocysteine, releasing Cbl(I) MTR. Additionally, Cbl(I) can be oxidized to the inactive Cbl(II) form. Methionine synthase reductase (MTRR) restores the Cbl(II) MTR complex by reductively adding a methyl group, using AdoMet as the donor (Nazki et al., 2014)

Cystathionine β-synthase (CBS) and Cystathionine γ-lyase (CTH)

Both are enzymes that catalyze the breakdown of homocysteine that cannot be converted into cysteine, with vitamin B6 being required for these reactions. CBS promotes the combination of homocysteine and serine to form cysteine, while CTH aids in the hydrolysis of cysteine into cysteine and α-ketobutyrate. Cysteine is crucial not only for protein synthesis but also as a precursor to glutathione, a strong antioxidant, and as an essential compound in the detoxification of xenobiotics (Nazki et al., 2014).

The Biochemical Role of Folic Acid

The purpose of this intricate biochemical process involving a single carbon transfer is to move carbon from amino acids like serine, glycine, and methionine as methyl groups for the synthesis of nucleotides and methylation reactions (Nazki et al., 2014; Zheng & Cantley, 2019).

Nucleotide Biosynthesis

Folate derivatives like 5,10-methylene-THF, 5,10-methylenetetrahydrofolate, and 10-formyl-THF are vital for DNA formation. During purine synthesis, 10-formyl-THF contributes a one-carbon unit to the carbon atoms at positions 2 and 8 of the purine ring. Additionally, 5,10-methylene-THF plays an important role in converting deoxyuridine monophosphate (dUMP) into deoxythymidine monophosphate (dTMP). This step is the only source of thymidine produced de novo and is a key rate-limiting process in DNA replication. As a result, a lack of folate in rapidly dividing cells hampers effective DNA synthesis. For example, disturbances in thymidylate production can lead to errors in inserting dUTP into DNA, causing strand breaks. If two breaks occur within 12 base pairs of each other, double-strand DNA damage may occur, leading to genomic instability and higher mutation rates. This misincorporation of uracil and the resulting double-strand breaks have been seen in cultured tumor cells and in the blood and tissues of both rodents and humans with low folate levels. Similarly, impaired purine synthesis reduces the cell's ability to produce and repair DNA. In cells grown in folate-deficient media with added hypoxanthine (a purine precursor that bypasses folate-dependent purine synthesis), chromosomal damage was notably lower compared to those grown in folate-deficient media alone (Nazki et al., 2014).

Methylation Pathway

5-Methyl-THF donates a methyl group to convert homocysteine into methionine, which is then used to produce S-adenosylmethionine (SAM), a methyl donor for processes like DNA, RNA, and protein methylation. SAM is transformed into S-adenosylhomocysteine (SAH), which inhibits methyltransferases. High SAM levels inhibit MTHFR, reducing 5-methyl-THF production and homocysteine remethylation. Low SAM levels, however, support remethylation. MTHFR activity can also be influenced by genetic variations (Nazki et al., 2014; Zheng & Cantley, 2019). A lack of folate can reduce the availability of carbon units essential for methylation reactions, leading to a buildup of homocysteine. High homocysteine levels are linked to cardiovascular diseases, neural tube defects, and Alzheimer's, although the exact mechanisms and causal connections remain unclear. The rise in homocysteine also leads to the accumulation of SAH, which inhibits SAM-dependent methyltransferases like DNMT. This reduction in SAM and increase in SAH can result in DNA hypomethylation and influence cancer development (Nazki et al., 2014).

Unsubstituted Folate

The DHF and THF forms of folate do not directly engage in metabolic processes but instead take up one-carbon units and transfer them into methylation pathways or DNA synthesis. Methotrexate acts as a competitive and irreversible inhibitor of DHF reductase, an enzyme responsible for converting folic acid and DHF into THF, thus blocking nucleotide biosynthesis and remethylation. The folate cycle is tightly controlled through feedback regulation and inhibition by its end products to optimize the use of dietary folate and maintain a balanced pool of intracellular folate (Nazki et al., 2014)

Folate Bioavailability

Several studies have aimed to estimate the bioavailability of food folate compared to folic acid, with results ranging from 10% to 98%, depending on the evaluation methods applied. To assess folate bioavailability, both long-term and short-term trials have been carried out. Long-term studies typically focus on measuring folate status indicators such as plasma folate levels, folate

concentrations in red blood cells (RBCs), and total plasma homocysteine. In contrast, short-term trials have assessed the bioavailability of folate and its active metabolites using the Area Under the Curve (AUC) method (Scaglione & Panzavolta, 2014). Various studies have shown that folic acid supplements have a higher relative bioavailability than folate from food, indicating that using folate supplements instead of natural food folate is less efficient in improving folate status. When considering supplementation, folate can be given either as folic acid or the natural form, 5-MTHF. Research has compared the effectiveness of these two forms in influencing folate-related parameters. One study revealed that supplementation with 6S-5-MTHF resulted in a notable increase in RBC and plasma folate levels, outperforming folic acid. This supports the use of 6S-5-MTHF as a safe and effective substitute for synthetic folic acid (Scaglione & Panzavolta, 2014).

Functional Polymorphism of Folate Metabolism Genes

Genomic polymorphisms refer to the presence of two or more variations of a particular DNA sequence, which can occur among individuals or different populations. The metabolism of folate can be influenced by these polymorphisms in the relevant genes (Nazki et al., 2014).

Down Syndrome

Down syndrome (DS) results from trisomy of chromosome 21, often due to errors in meiosis. The MTR A2756G allele, combined with high homocysteine levels, has been linked to DS risk, though another study found no such association. A Brazilian study found higher homocysteine levels in mothers of children with DS, with the MTHFR C677T allele linked to elevated homocysteine. No other polymorphisms showed an association with DS risk, but mothers of children with DS had more variant alleles overall. Additionally, higher homocysteine levels were found in DS mothers with the MTHFR 1298CC genotype compared to controls (Nazki et al., 2014)

Neural Tube Defects (NTDs)

Neural tube defects are common birth defects affecting the central nervous system, caused by incomplete neural tube closure within the first 28 days of pregnancy. NTDs include anencephaly, spina bifida, and encephalocele. While the exact cause is unclear, both genetic and environmental factors contribute. Most NTDs are isolated and occur sporadically, but about 10% are part of syndromes linked to chromosomal abnormalities. The recurrence risk in subsequent pregnancies is around 1% with 4 mg/day folic acid supplementation, and 3.5% with other vitamins, according to a UK study. Homozygous mothers have double the risk, while the risk increases 6-7 times if both mother and fetus are homozygous. Heterozygotes show a slight risk increase. Reduced MTHFR activity also limits S-adenosylmethionine for critical methylation, affecting cell proliferation during neural tube closure (Kancherla, 2023).

Research has demonstrated the role of folic acid in the early prevention of NTDs, indicating that disruptions in folate metabolism contribute to their development. Various studies across different populations have found that higher maternal homocysteine levels are associated with an increased risk of NTDs in offspring. However, a case-control study conducted in Brazil observed no notable differences in MTHFR polymorphisms between mothers, children with NTDs, and control groups (Félix et al., 2004; Kancherla, 2023; Nazki et al., 2014; Perez et al., 2003). The study suggests that factors other than the MTHFR mutations may be more influential in the development of NTDs. Notably, the study observed that participants in the case group had higher average blood folate levels compared to those in the control group, raising questions about the role of folate intake in NTD risk. Additionally, mothers in the NTD group had lower levels of vitamin B12, which was found to be associated with elevated homocysteine (t-Hcy) levels. Despite no significant differences in the distribution of MTHFR 677C>T and MTHFR 1298A>C genotypes between the case and control groups, the

homozygous TT genotype of MTHFR 677C>T was linked to higher t-Hcy levels (Félix et al., 2004).

These findings suggest that while *MTHFR* mutations might not be the main risk factor in this study, factors such as low vitamin B12 and elevated homocysteine may be more important contributors to NTD risk in this population. It is also possible that a combination of genetic factors, like the A1298C mutation, and environmental influences, such as inadequate folic acid intake, could have a more significant impact on disease development. Considering the small sample size, these results should be interpreted with caution. However, the study emphasizes the complexity of the relationship between genetic variations, nutrient levels, and NTDs, underscoring the need for further investigation into these contributing factors (Perez et al., 2003).

The main cause of high homocysteine levels and methionine deficiency is the MTHFR 677CT gene mutation, which reduces enzyme activity. This leads to decreased 5-MTHF production, raising homocysteine levels and delaying neural tube closure, contributing to NTDs. In addition to MTHFR 677CT, the A1298C variant of the MTHFR gene also contributes to reduced enzyme activity. This decline in enzyme function leads to increased intracellular homocysteine levels, which have been associated with a greater risk of central nervous system disorders, congenital abnormalities, and pregnancy complications (Shen, Gu, Tang, Shen, & Liu, 2024). Epidemiological research has indicated that the prevalence of the MTHFR C677T polymorphism differs across various geographical regions. The 677T allele of the MTHFR gene is more commonly found in Europe than in Africa, with its frequency showing a rising trend from northern to southern regions among European and North American populations (Binia et al., 2014; Yafei et al., 2012; Yang et al., 2013). Using neural tube defects in China as an example, their prevalence is higher in the northern region compared to the southern region, at 4.5‰ and 1.1‰, respectively. Furthermore, women in the North had lower plasma folate levels and daily dietary folate intake than those in the South. These findings suggest an intrinsic relationship between MTHFR C677T gene distribution, folic acid consumption, and certain health conditions. These connections are intricate and result from prolonged interactions between genetic factors and environmental influences (Ma, Wang, Jin, Li, & Ren, 2017; Meng et al., 2015; Shen et al., 2024).

Apart from the *MTHFR* C677T polymorphism, several other genetic variations contribute to different *MTHFR* alleles. Single nucleotide polymorphisms (SNPs) refer to genetic variations resulting from a single nucleotide change in the DNA sequence. A previous study identified 14 nonsynonymous SNPs, including 11 rare variants with a frequency of less than 1 percent and three common variants, namely C677T, A1298C, and G1793A, with C677T being the most extensively studied. The *MTHFR* A1298C variant occurs due to an A-to-C substitution at position 1298, leading to reduced enzyme activity, though its effects are milder compared to the C677T polymorphism. The frequency of the *MTHFR* A1298C genotype varies significantly across different populations (Lajin et al., 2012; Shen et al., 2024; Yang et al., 2013). Regarding the *MTHFR* A1298C polymorphism, its weaker impact on enzyme activity and lack of association with increased homocysteine levels have led most studies to find no direct link between this polymorphism alone and the occurrence of disorders. However, individuals with compound heterozygosity for both C677T and A1298C polymorphisms have shown elevated homocysteine concentrations. This interaction may partially explain previous findings suggesting a role of the A1298C mutation in the development of certain disorders (Chen et al., 2012; Wang et al., 2012; Yang et al., 2013).

In addition to genetic mutations, other recognized risk factors for neural tube defects include a lack of folate, maternal pregestational diabetes, obesity during pregnancy, the use of medications like valproic acid, and inadequate folate consumption (Endalifer & Diress, 2020; Evans et al., 2023). Anti-epileptic drugs (AEDs) disrupt folate metabolism in the embryo,

lowering plasma folate levels, and are among the most potent medications associated with the development of NTDs. These harmful effects are believed to stem from reduced folate absorption, competition between folate coenzymes and the drugs, and a higher requirement for folate as a coenzyme in the metabolism of AEDs. Vitamin A has been found to cause teratogenic effects in experimental animals. Research has shown that administering retinoic acid (RA) to pregnant rats results in reduced protein synthesis and the development of various neural tube defects (NTDs). Maternal obesity is also considered a risk factor for NTDs. Although the mechanisms linking obesity to NTDs remain unclear, factors such as elevated insulin levels, increased endogenous estrogen, and insulin resistance are believed to play a role in raising the risk. Smoking during pregnancy is a known risk factor for conditions like cleft lip and palate, congenital heart defects, low birth weight, placental abruption, sudden infant death syndrome, and Down syndrome. In a study examining serum folate levels among women with different MTHFR 677 genotypes (677TT, 677CC, and 677CT), those with the 677TT genotype who smoked had significantly lower folate levels, indicating that smoking harms both the mother's and the fetus's folate status (Kondo et al., 2017).

The Importance of Folate Diet and Synthetic Folic Acid

The polyglutamate form of folate, discovered by Lucy Wills in 1931, has been known for 82 years. She initially suggested calling it vitamin 11, likening it to vitamin B12. The monoglutamate form of this vitamin, known as FA, was later developed. However, "folate" is a broad term that includes all variations of pteroyl-L-glutamate, whether they are substituted or not, oxidized or reduced, and whether they are mono- or polyglutamate, including the synthetic FA. The distinction between dietary folate and synthetic FA is important in medical contexts to accurately estimate vitamin intake (Kancherla, 2023). Since humans cannot synthesize folate, the primary dietary sources include fresh and frozen leafy greens, citrus fruits and juices, liver, whole wheat bread, and beans. The body obtains this water-soluble vitamin from both dietary folate and synthetic FA. Red blood cell folate levels of 906 nmol/L or more are linked to a lower risk of NTDs. Achieving this concentration takes 8-12 weeks with the previous recommendation of 0.4 mg of FA, while 0.8 mg of FA can reach the desired levels in 4.2 weeks. Therefore, the recommended daily intake of folate/FA for sexually active women of reproductive age is 0.7 mg or 0.8 mg (De-Regil et al., 2015; Kancherla, 2023). Folate is essential for cell division, DNA synthesis, and repair, as well as gene regulation through DNA methylation. It also remethylates homocysteine into methionine. After ingestion, polyglutamate folate and synthetic monoglutamate FA are absorbed in the small intestine. Folate is converted to its active form, tetrahydrofolate (THF), which is then transported to the liver and body tissues. Homocysteine, produced from protein digestion, is toxic and is either metabolized into cystathionine through the trans-sulfuration pathway or remethylated back into methionine with the help of 5-MTHF and vitamin B12. A deficiency in folate-FA can contribute to NTDs (Kancherla, 2023).

Clinical Pharmacokinetic and Metabolic Considerations

Polyglutamate folate from food must be converted into its monoglutamate form by the conjugase enzyme for absorption, which functions best at a pH of 6-7. Changes in intestinal pH, due to conditions like atrophic gastritis or the use of medications like PPIs and H2 blockers, can reduce folate absorption. In these cases, folic acid supplementation is effective. However, drugs like methotrexate and trimethoprim inhibit DHFR, disrupting folate metabolism. In such situations, folic acid supplementation is ineffective, and alternatives like folinate or 5-MTHF are recommended (Czeizel et al., 2013). 5-MTHF offers significant advantages over folic acid, particularly in plasma folate levels. The DHFR-catalyzed reduction of folic acid to THF is slow and easily saturated, with human liver DHFR activity being only 52% of that in rats. This limits the benefits of high-dose folic acid, especially in individuals with lower DHFR activity. With increased folic acid intake through food fortification and supplements, unmetabolized folic acid

can accumulate in plasma. A key risk of folic acid supplementation is masking vitamin B12 deficiency, a risk that 5-MTHF reduces, as it does not cause hematological changes in B12-deficient individuals (Czeizel et al., 2013).

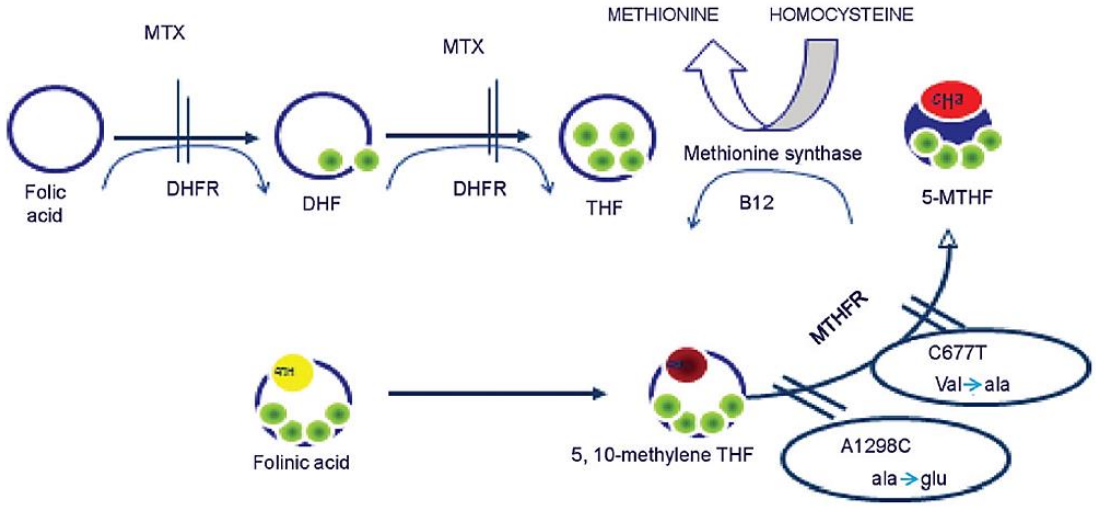

Figure 2. Genetic polymorphism of L-5-MTHFR and its implications for systemic folate depletion due to methotrexate (Czeizel et al., 2013).

The biological processes of folate during pregnancy form a vital point through which biochemistry, genetics, nutrition and public health systems create complex yet essential relationships with multiple contradictory elements. Every healthcare provider recognizes that folate supplementation prevents neural tube defects (NTDs) (De-Regil et al., 2015; Czeizel et al., 2013) yet the full spectrum of health effects from altered folate levels demands more complex insights.

Folate performs an indispensable role in embryogenesis because it enables nucleotide biosynthesis and DNA repair and supports methylation processes (Nazki et al., 2014; Zheng & Cantley, 2019). The study findings demonstrate why folate is essential for preserving cellular integrity while cells divide intensely such as in the first trimester of maternal pregnancy. The straightforward assumption that increased folic acid intake provides better results faces escalating scientific proof which shows that synthetic supplementation of folic acid and supraphysiological doses present unforeseen possibilities of harm (Christensen et al., 2015; SACN, 2017). Scientific evidence reveals that supplemental folic acid exposure causes four significant effects: it conceals vitamin B12 deficiency symptoms while generating pseudo-MTHFR deficiency conditions and potentially harmful liver damage and could increase the likelihood of prostate cancer development.

The metabolic system of folate stands out because it contains two opposite sides. The bodily compound functions as a necessary substance which stops fatal birth abnormalities from happening. Folate's metabolic mechanism has two aspects: a lifesaving function yet it shows sensitivity to various genetic factors which cancel possible benefits and potentially create health issues. The MTHFR C677T and A1298C polymorphisms reduce enzyme activity and impair homocysteine remethylation which results in hyperhomocysteinemia (a risk factor for NTDs, cardiovascular disease and neurodevelopmental disorders) according to Shen et al. (2024) and Felix et al. (2004) along with Kancherla (2023). Scientific research reveals the diverse population patterns of these gene variations because evolutionary forces shape their geographical distribution (Binia et al., 2014; Yang et al., 2013).

Additional analysis from Brazilian cohort data creates substantial confusion about MTHFR's contribution to medical conditions. The study of MTHFR genotypes demonstrated no differences between pregnancies with or without neural tube defects but the affected

pregnancies showed higher homocysteine levels and lower vitamin B12 concentrations (Perez et al., 2003). The data indicates a combined multiple cause explanation in which genetic makeup interacts with food macronutrient levels to produce variable outcomes. The evidence indicates that broad folic acid supplement advice does not consider how genetics and nutrient relationships affect individual groups.

This research study confirms that folate functions beyond being a dietary compound since it regulates epigenetic methylation processes during early development. Gene expression together with chromatin structure depends on S-adenosylmethionine (SAM)-dependent methylation pathways that function based on folate status (Nazki et al., 2014; Zheng & Cantley, 2019). The improper levels of folate create conditions for DNA hypomethylation which activates oncogenes or silences tumor suppressor genes leading to unknown long-term complications (Scaglione & Panzavolta, 2014).

The article emphasizes a vital aspect about the different levels of bioavailability that dietary folate has compared to synthetic folic acid. Blood levels of folic acid exhibit superior stability and absorption efficacy yet its use depends on the variable enzymatic activity of dihydrofolate reductase (DHFR) found in individuals (Czeizel et al., 2013; Scaglione & Panzavolta, 2014). The maximum absorption capacity of DHFR leads to blood circulation of folic acid that has not been metabolized which affects immune system function while possibly causing cancer formation (Christensen et al., 2015).

Multiple clinical consequences emerge from these research results. The evidence supports genetic screening procedures for prenatal care should be established formally in medical care protocols for populations exhibiting high MTHFR mutation frequencies. Supplement manufacturers now propose adopting personified strategy by integrating 5-methyltetrahydrofolate (5-MTHF) into products because it bypasses the DHFR-dependent activation process while maintaining physiological methylation control (Czeizel et al., 2013; Scaglione & Panzavolta, 2014). This research demands a review of food fortification strategies because these policies might produce unpredictable outcomes when dietary patterns meet supplement use and genetic diversity in the post-fortification environment.

Environmental factors such as obesity alongside diabetes and usage of anti-epileptic drugs and smoking behavior worsen folate-related susceptibility (Endalifer & Diress, 2020; Evans et al., 2023; Kondo et al., 2017). NTDs become more likely to occur when these folate-absorbing or metabolizing or demand-related conditions are present because they strengthen the risk regardless of supplementation usage. The anti-epileptic drug valproic acid depletes folate levels and blocks its transport pathways and enzyme activity which requires different supplementation methods for women taking these medications (Endalifer & Diress, 2020). A combined approach in public health needs to address both medication-related factors and lifestyle choices that determine folate status levels. The research emphasizes the ongoing barriers which prevent nutritional equality although supplement provision continues to be a fundamental prevention method for NTDs. Many people cannot access folate-rich foods because of socioeconomic differences even though this natural source provides the most sustainable and safest means of getting vitamin (Ohrvik & Witthoft, 2011; Subandrate et al., 2022). The unequal distribution demands both biochemical therapeutic approaches and structural modifications to food access policies as well as educational strategies.

Conclusion

Folate stands as a vital junction point in human biology because it creates connections between food nutrition sources and genes and child development especially in the prenatal period. DNA synthesis along with cellular replication and epigenetic regulation make folic acid essential for embryonic development as well as neural tube defect prevention. This research demonstrates that folate deficiency remains a proven risk for pregnancy complications yet scientists now

understand how excessive synthetic folic acid consumption affects people with genetic vulnerabilities and need careful evaluation. Folate metabolism requires detailed understanding given its dependence on enzymatic activity together with MTHFR C677T and A1298C gene polymorphisms and vitamin B12 co-factor requirements which support the importance of adopting individualized and genetically based treatment methods for folate supplement determination. At the same time pharmaceutical interactions along with insufficient diets and social-economic gaps create additional hurdles that highlight the dual nature of the challenge where folate management requires a social intervention approach alongside biochemical management needs.

Maternal-fetal medicine research needs to welcome the integration of nutrigenomics together with advanced diagnostics along with individualized clinical strategies for intervention. Knowledge about folate's biochemical nature alongside its epigenetic capabilities enables better protective measures which supports our continued work for fair maternal and neonatal healthcare. The current strategies require smarter supplementation with safer approaches which can provide better response to maternal needs.

References

Bhasker, A., & Veleri, S. (2025). Fundamental origins of neural tube defects with a basis in genetics and nutrition. *Experimental Brain Research*, *243*(4), 79. https://doi.org/10.1007/s00221-025-07016-9

Binia, A., Contreras, A. V., Canizales-Quinteros, S., Alonzo, V. A., Tejero, M. E., & Silva-Zolezzi, I. (2014). Geographical and ethnic distribution of single nucleotide polymorphisms within genes of the folate/homocysteine pathway metabolism. *Genes & Nutrition*, *9*(5), 421. https://doi.org/10.1007/s12263-014-0421-7

Cao, R., Xie, J., & Zhang, L. (2022). Abnormal methylation caused by folic acid deficiency in neural tube defects. *Open Life Sciences*, *17*(1), 1679-1688. https://doi.org/10.1515/biol-2022-0504

Carboni, L. (2022). Active folate versus folic acid: The Role of 5-MTHF (Methylfolate) in Human Health. *Integrative Medicine: A Clinician's Journal*, *21*(3), 36.

Chen, L., Liu, L., Hong, K., Hu, J., & Cheng, X. (2012). Three Genetic Polymorphisms of Homocysteine-Metabolizing Enzymes and Risk of Coronary Heart Disease: A Meta-Analysis Based on 23 Case–Control Studies. *DNA and Cell Biology*, *31*(2), 238–249. https://doi.org/10.1089/dna.2011.1281

Christensen, K. E., Mikael, L. G., Leung, K.-Y., Lévesque, N., Deng, L., Wu, Q., Malysheva, O. V, et al. (2015). High folic acid consumption leads to pseudo-MTHFR deficiency, altered lipid metabolism, and liver injury in mice. *The American Journal of Clinical Nutrition*, *101*(3), 646–658. https://doi.org/10.3945/ajcn.114.086603

Czeizel, A., Dudás, I., Vereczkey, A., & Bánhidy, F. (2013). Folate Deficiency and Folic Acid Supplementation: The Prevention of Neural-Tube Defects and Congenital Heart Defects. *Nutrients*, *5*(11), 4760–4775. https://doi.org/10.3390/nu5114760

De-Regil, L. M., Peña-Rosas, J. P., Fernández-Gaxiola, A. C., & Rayco-Solon, P. (2015). Effects and safety of periconceptional oral folate supplementation for preventing birth defects. *Cochrane Database of Systematic Reviews*, *2015*(12). https://doi.org/10.1002/14651858.cd007950.pub3

Ekundayo, B. E., Adewale, O. B., & Obafemi, T. O. (2025). Neuroprotective Effects of Folic Acid: A Review. *Journal of Dietary Supplements*, *22*(2), 345-363. https://doi.org/10.1080/19390211.2024.2436842

Endalifer, M. L., & Diress, G. (2020). Epidemiology and determinant factors of neural tube defect: Narrative review. *Surgical Neurology International*, *11*, 81. https://doi.org/10.25259/SNI_84_2020

Evans, S. P., Ailes, E. C., Kramer, M. R., Shumate, C. J., Reefhuis, J., Insaf, T. Z., ... & Gilboa, S. M. (2023). Neighborhood deprivation and neural tube defects. *Epidemiology*, *34*(6), 774-785. https://doi.org/10.1097/ede.0000000000001655

Fardous, A. M., & Heydari, A. R. (2023). Uncovering the hidden dangers and molecular mechanisms of excess folate: a narrative review. *Nutrients*, *15*(21), 4699. https://doi.org/10.3390/nu15214699

Félix, T. M., Leistner, S., & Giugliani, R. (2004). Metabolic effects and the methylenetetrahydrofolate reductase (*MTHFR*) polymorphism associated with neural tube defects in southern Brazil. *Birth Defects Research Part A: Clinical and Molecular Teratology*, *70*(7), 459–463. https://doi.org/10.1002/bdra.20011

Goossens, J. F., Thuru, X., & Bailly, C. (2021). Properties and reactivity of the folic acid and folate photoproduct 6-formylpterin. *Free Radical Biology and Medicine*, *171*, 1-10. https://doi.org/10.1016/j.freeradbiomed.2021.05.002

Isaković, J., Šimunić, I., Jagečić, D., Hribljan, V., & Mitrečić, D. (2022). Overview of neural tube defects: gene–environment interactions, preventative approaches and future perspectives. *Biomedicines*, *10*(5), 965. https://doi.org/10.3390/biomedicines10050965

Kancherla, V. (2023). Neural tube defects: a review of global prevalence, causes, and primary prevention. *Child's Nervous System*, *39*(7), 1703–1710. https://doi.org/10.1007/s00381-023-05910-7

Kondo, A., Matsuo, T., Morota, N., Kondo, A. S., Okai, I., & Fukuda, H. (2017). Neural tube defects: Risk factors and preventive measures. *Congenital Anomalies*, *57*(5), 150–156. https://doi.org/10.1111/cga.12227

Lajin, B., Alhaj Sakur, A., Michati, R., & Alachkar, A. (2012). Association between MTHFR C677T and A1298C, and MTRR A66G polymorphisms and susceptibility to schizophrenia in a Syrian study cohort. *Asian Journal of Psychiatry*, *5*(2), 144–149. https://doi.org/10.1016/j.ajp.2012.03.002

Liew, S.-C. (2016). Folic acid and diseases - supplement it or not? *Revista da Associação Médica Brasileira*, *62*(1), 90–100. https://doi.org/10.1590/1806-9282.62.01.90

Ma, R., Wang, L., Jin, L., Li, Z., & Ren, A. (2017). Plasma folate levels and associated factors in women planning to become pregnant in a population with high prevalence of neural tube defects. *Birth Defects Research*, *109*(13), 1039–1047. https://doi.org/10.1002/bdr2.1040

McNulty, H. (2022). Folate. In *Principles of Nutritional Assessment*. Oxford University Press (OUP).

Meng, Q., Zhang, L., Liu, J., Li, Z., Jin, L., Zhang, Y., Wang, L., et al. (2015). Dietary folate intake levels in rural women immediately before pregnancy in Northern China. *Birth Defects Research Part A: Clinical and Molecular Teratology*, *103*(1), 27–36. https://doi.org/10.1002/bdra.23280

Nazki, F. H., Sameer, A. S., & Ganaie, B. A. (2014). Folate: Metabolism, genes, polymorphisms and the associated diseases. *Gene*, *533*(1), 11–20. https://doi.org/10.1016/j.gene.2013.09.063

Ohrvik, V. E., & Witthoft, C. M. (2011). Human Folate Bioavailability. *Nutrients*, *3*(4), 475–490. https://doi.org/10.3390/nu3040475

Paduch-Jakubczyk, W., & Dubińska, M. (2024). The Role of Folic Acid and Its Derivatives in Reproductive Health. *Actual Gynecology & Obstetrics/Aktuální Gynekologie a Porodnictví*, *16*.

Perez, A. B. A., D'Almeida, V., Vergani, N., de Oliveira, A. C., de Lima, F. T., & Brunoni, D. (2003). Methylenetetrahydrofolate reductase (MTHFR): incidence of mutations C677T and A1298C in Brazilian population and its correlation with plasma homocysteine levels in spina bifida. *American Journal of Medical Genetics Part A*, *119A*(1), 20–25. https://doi.org/10.1002/ajmg.a.10059

Pertiwi, H., Nur Mahendra, M. Y., & Kamaludeen, J. (2022). Folic Acid: Sources, Chemistry, Absorption, Metabolism, Beneficial Effects on Poultry Performance and Health. *Veterinary Medicine International*, *2022*, 1–9. https://doi.org/10.1155/2022/2163756

Puspitasari, M. K., Irianti, S., Pramatirta, A. Y., Wirawan, W., Islamy, N., Maelissa, M. M., & Lumentut, A. M. (2025). Folate Metabolism and Its Implications in Pregnancy. *Journal La Medihealtico*, *6*(2), 267-277. https://doi.org/10.37899/journallamedihealtico.v6i2.1885

SACN. (2017). Update on folic acid. *Scientific Advisory Committee on Nutrition*.

Scaglione, F., & Panzavolta, G. (2014). Folate, folic acid and 5-methyltetrahydrofolate are not the same thing. *Xenobiotica*, *44*(5), 480–488. https://doi.org/10.3109/00498254.2013.845705

Shane, B. (2008). Folate and Vitamin B Metabolism: Overview and Interaction with Riboflavin, Vitamin B, and Polymorphisms. *Food and Nutrition Bulletin*, *29*(2_suppl1), S5–S16. https://doi.org/10.1177/15648265080292s103

Shen, Y., Gu, Y., Tang, Y., Shen, H., & Liu, C. (2024). Geographical distribution of MTHFR C677T gene polymorphisms among the reproductive-age women in Chinese Han populations: based on migration. *BMC Women's Health*, *24*(1), 407. https://doi.org/10.1186/s12905-024-03244-3

Shulpekova, Y., Nechaev, V., Kardasheva, S., Sedova, A., Kurbatova, A., Bueverova, E., ... & Ivashkin, V. (2021). The concept of folic acid in health and disease. *Molecules*, *26*(12), 3731. https://doi.org/10.3390/molecules26123731

Subandrate, S., Athiah, M., & Safyudin. (2022). Asam Folat: Peran dalam Metabolisme dan Metode Pemeriksaan. *Majalah Kedokteran Andalas*, *45*(1).

Talaulikar, V., & Arulkumaran, S. (2011). Folic acid in pregnancy. *Obstetrics, Gynaecology & Reproductive Medicine*, *21*(5), 147–148. https://doi.org/10.1097/ogx.0b013e318223614c

Wald, N. J. (2022). Folic acid and neural tube defects: Discovery, debate and the need for policy change. *Journal of medical screening*, *29*(3), 138-146. https://doi.org/10.1177/09691413221102321

Wang, X. W., Luo, Y. L., Wang, W., Zhang, Y., Chen, Q., & Cheng, Y. L. (2012). Association between MTHFR A1298C polymorphism and neural tube defect susceptibility: a metaanalysis. *American Journal of Obstetrics and Gynecology*, *206*(3), 251.e1-251.e7. https://doi.org/10.1016/j.ajog.2011.12.021

World Health Organization. (2015). Serum and Red Blood Cell Folate Concentrations for Assessing Folate Status in Populations. *Vitam Miner Nutr Inf Syst*, 1–7.

Yafei, W., Lijun, P., Jinfeng, W., & Xiaoying, Z. (2012). Is the prevalence of MTHFR C677T polymorphism associated with ultraviolet radiation in Eurasia? *Journal of Human Genetics*, *57*(12), 780–786. https://doi.org/10.1038/jhg.2012.113

Yang, B., Liu, Y., Li, Y., Fan, S., Zhi, X., Lu, X., Wang, D., et al. (2013). Geographical Distribution of MTHFR C677T, A1298C and MTRR A66G Gene Polymorphisms in China: Findings from 15357 Adults of Han Nationality. *PLoS ONE*, *8*(3), e57917. https://doi.org/10.1371/journal.pone.0057917

Zheng, Y., & Cantley, L. C. (2019). Toward a better understanding of folate metabolism in health and disease. *Journal of Experimental Medicine*, *216*(2), 253–266. https://doi.org/10.1084/jem.20181965