

Folate Nutrigenetics: A Convergence of Dietary Folate Metabolism, Folic Acid Supplementation, and Folate Antagonist Pharmacogenetics

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Abstract: Folate (Vitamin B9, Folic acid, folinic acid, folacin, pteroylglutamic acid) is essential for life-sustaining processes of DNA synthesis, replication, and repair which are naturally present in common foods such as peas, oranges, broccoli, and whole-wheat products. Folate levels have been associated with birth defects, cardiovascular disease, and many other important healthcare issues, which has resulted in government-mandated food fortification to deliver minimum levels of intake. Despite this one-size-fits-all recommendation by governmental regulatory bodies, studies suggest that a genetic predisposition may exist within as much as 67% (combining both the CT and TT alleles) of the population that causes a metabolic folate deficiency. Thus, genetic factors may play an important role in folate levels and metabolism. A substantial body of scientific evidence supports the importance of folate, genes associated with folate, genes associated with anti-folate therapeutics, and thereby a convergence in nutritional genetics or nutrigenetics. This review will comment on the substantial body of scientific evidence demonstrating the relevance for nutrigenetic measurements to guide dietary folate intake and nutritional supplementation with folic acid.

INTRODUCTION TO FOLATE

Folate and folic acid (FA) are forms of water-soluble vitamin B9. Folate occurs naturally in food and FA is the synthetic form of this vitamin that is found in supplements and fortified foods. Folate is essential for the life-sustaining processes of DNA synthesis, replication and repair. Folate is also important for protein biosynthesis, another process that is central to cell viability. Folate is present in foods such as dried beans, peas, lentils, oranges, whole-wheat products, liver, asparagus, beets, broccoli, Brussels sprouts, and spinach [1]. Unfortunately, the typical diet does not supply appropriate amounts of folate.

GOVERNMENT EFFORTS TO OVERCOME FOLATE DEFICIENCY

The U.S. government has made numerous strides to overcome folate deficiency through the Recommended Dietary Allowance (RDA) and fortification of foods. Theoretically, the RDA is the average daily dietary intake level that is suggested to be an adequate nutrient requirement for nearly all (97-98%) healthy individuals in each life-stage and gender group. However, a general RDA does not take into consideration the uniqueness of genetic predisposition. The 1998 RDAs for folate are expressed in a term called the Dietary Folate Equivalent. The Dietary Folate Equivalent (DFE) was developed to help account for the differences in absorption of naturally occurring dietary folate and the more bioavailable synthetic FA (1 mcg of food folate equals 0.6 mcg FA from supplements and fortified foods). The 1998 RDAs for folate expressed in micrograms (mcg) of DFE for adult men and women (aged 19 years and older) are 400 mcg. During pregnancy the daily intake should be increased to 500 mcg, and the RDA for breastfeeding women is 500 mcg.

In addition to RDAs, the U.S. government, in 1998, began requiring food manufacturers to add FA to enriched breads, cereals, flours, corn meals, pastas, rice, and other grain products. The National Health and Nutrition Examination Survey (NHANES III 1988-91) and the Continuing Survey of Food Intakes by Individuals (1994-96 CSFII) indicated that most adults did not consume adequate folate. However, the FA fortification program has increased FA content of commonly eaten foods. However, like RDAs, this fortification does not account for genetic differences in the population.

A major motivating factor driving this fortification program related to folate consumption and birth defects. According to the U.S. National Center on Birth Defects and Developmental Disabilities (NCBDDD), [2] folate, as well as FA supplementation in a woman's body before and while she is pregnant results in being less likely to have a baby with a major birth defect of the brain or spine. Folate deficiency in women of child-bearing age who become pregnant can result in birth defects that include neural tube defects (NTDs), spina bifida, and anencephaly which occur in about 3,000 babies every year in the United States. 70 percent of NTDs can be prevented with adequate FA intake every day before becoming and while pregnant. For a family with a child with NTD, the average cost can be well above \$1,000,000, according to the NCBDDD – far beyond the costs of the necessary folate supplementation. In a recent published study by NCBDDD, the annual cost benefit of this FA fortification effort was determined to be between \$312 million to \$425 million dollars over the past ten years. The annual cost savings (net reduction in direct costs) were estimated to be in the range of \$88 million to \$145 million [2].

FOLATE DEFICIENCY AND CHRONIC DISEASE

Generally, while there is no clear causal relationship between folate and cardiovascular disease (CVD), randomized, double-blinded studies suggest that folate supplementation

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reduces both CVD and birth defects [3, 4]. In fact, daily doses of greater than or equal to 0.8 mg FA are typically required to achieve the maximal reduction in plasma homocysteine concentrations produced by FA supplementation. Doses of 0.2 and 0.4 mg are associated with 60% and 90% of this maximal effect [4]. Obesity, classified as a chronic disease in 1995, is often times a precursor to CVD, diabetes, arthritis, and many other chronic conditions [5]. Furthermore, there is an independent homocysteine reducing effect of folate supplementation [6]. In this regard, Russo *et al.* found that hyperhomocysteinemia and the common C677T variant of MTHFR gene are not associated with Metabolic Syndrome in Type 2 diabetic subjects [6]. In one study, FA and a gene polymorphism were found to be significantly related to the overweight/ obese and control groups in logistic regression analysis ($p < 0.05$). The results support the supposition that FA is more important than vitamin B12 and should be studied further related to obesity [7].

In a randomized, double-blinded, placebo-controlled study of obese women, the authors found that folate supplementation of 5,000 mcg led to a weight reduction of 7.7% which did not differ significantly from controls (8.9%), however, there was a 3-fold increase in serum folate, and a trend of lower fasting homocysteine (Hcy) levels (7.6 +/- 0.2 vs. 7.3 +/- 0.3 micromol/L), especially in women with higher baseline Hcy levels [8]. In another study, the authors concluded that adequate oral vitamin-supplementation protects against increased homocysteine production during weight reduction [9]. Volek *et al.* concluded that short-term weight loss resulting from reducing percentage energy from fat, increasing physical activity and vitamin/mineral supplements including FA has a favorable effect on regional body composition and total and LDL cholesterol with minimal effects on HDL cholesterol, triacylglycerols, homocysteine and insulin and the effects are greater in men compared to women [10].

Dixon *et al.* in an obesity study, found levels of folate and B12 together explained 35% of the homocysteine variance in the weight loss group compared with only 9% in controls ($P < 0.001$) [11]. Those taking regular multivitamin supplements had lower homocysteine levels: 9.6 (9.1-10.0) micromol/l vs. 12.3 (11.4-13.3) in those not taking supplements ($P < 0.001$). A low normal plateau of homocysteine levels occurred at levels of folate > 15 ng/l and B12)600 ng/ml. A curvilinear relationship exists between these cofactors and homocysteine levels, with the dose-response relationship shifted to the right in the weight loss group. While this study showed elevated homocysteine levels with weight loss, without lower serum folate or vitamin B (12) levels, there is an altered dose-response relationship with higher serum B (12) and folate levels required to maintain recommended homocysteine levels. The authors further conclude that patients losing weight have significant health benefits; however, they may be at greater risk of vascular events or fetal abnormality in association with raised homocysteine levels. Finally they state that multivitamin supplementation is effective in lowering homocysteine levels, a well established finding.

These studies taken together suggest that FA supplementation in part may be an important part of managing obesity,

reducing BMI and body fat, inversely proportional to insulin levels, promoting healthy body composition, and preventing increases in homocysteine [8-12]. However, more research is required to definitively link folate supplementation and anti-obesity effects.

With credible scientific evidence suggesting that the daily intake of FA provides systemic health benefits, the question arises around individual abilities to metabolize FA. Along with this body of evidence around folate, there has been some important research conducted on FA metabolism.

FOLIC ACID METABOLISM AND GENETIC CONTRIBUTING FACTORS

Folate metabolism begins as folate crosses the cell membrane through the reduced folate carrier (RFC). RFC is an essential folate transporter and functions as a bidirectional anion exchanger, taking up folate cofactors and exporting various organic anions. The reduced folate carrier carries a genetic polymorphism RFC-1 G80A which results in a histidine to arginine substitution at codon 27 [13] and has been associated with elevated RBC folates and increased risk of NTD birth defects and plasma MTX concentrations, respectively [14,15]. Once across the cell membrane, FA is converted by dihydrofolate reductase into tetrahydrofolate. After going through several more stages of metabolism the folate metabolite 5-CH₃-tetrahydrofolate aids in the conversion of homocysteine (Hcy) to methionine. The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) is involved in folate metabolism. The MTHFR gene is located on chromosome 1 (1p36.3), and there are two common alleles, the C677T (thermolabile) allele and the A1298C allele. When this enzyme is reduced due to a genetic variation (MTHFR C677T), homocysteine levels increase and methionine decreases.

By conducting a search in the National Institute of Health's PubMed database on February 24, 2006, an investigator could have found 2,222 studies and/or publications on MTHFR, 796 of which related to MTHFR and folate.

In the Netherlands, in 1997, Verhoef *et al.* found in a study published in 1997 that the MTHFR C677T genotype was associated with plasma total homocysteine levels. Homozygosity for a 677C-->T mutation at the locus that codes for 5,10-methylenetetrahydrofolate reductase (MTHFR), a folate-dependent crucial enzyme in homocysteine metabolism, may render the enzyme thermolabile and less active and has been associated with increased levels of plasma total homocysteine. In this study in the Netherlands with a population primarily in Rotterdam, the study authors found that the homozygosity for this mutation, especially in combination with low folate status, predisposes to high plasma levels of fasting homocysteine [16]. Of 37 male and 112 female obese patients, Thawnashom *et al.* found that FA and the gene variant MTHFR C677T were found to be significantly related to the overweight/obese and control groups in logistic regression analysis ($p < 0.05$). These results support the supposition that FA is important to weight reduction [7]. Another study corroborated these findings demonstrating lower serum folate levels in an obese population [17]. Additionally, the MTHFR C677T genetic variant has also been associated

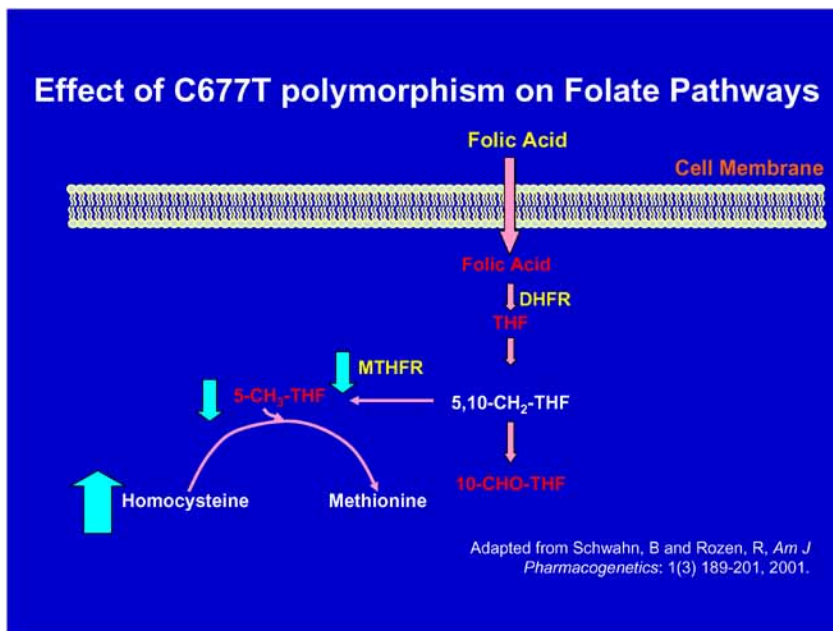


Fig. (1). Effect of MTHFR C677T polymorphism on folate metabolism by reducing the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) enzymatic thereby reducing 5-methyl-tetrahydrofolate's conversion of homocysteine into methionine, increasing homocysteine levels.

as a risk factor in a diabetes type 2 Chinese population [18]. Others studies also support the role of vitamin B's in obesity treatment [19, 20].

One particular study examines a genetically-guided amount of FA supplementation based upon this genotype. In a randomized, double-blinded placebo controlled study studying the impact of the MTHFR C677T genotype and FA supplementation, the study authors found that FA supplementation reduced the plasma homocysteine levels in all three genotypes, but the most severe mutation (homozygous) had a 240% benefit, compared to the normal patient [21]. According to the study authors, this form of testing should be useful when considering DNA-customized prevention of atherosclerosis. Atherosclerosis or coronary artery disease is the most common form of heart disease and the largest cause of death for men and women in the United States [22].

The population frequency of C677T alleles (both CT and TT polymorphic forms) range from as much as 66% of the population to as little as 48% of the population (see Table 1).

Table 1. Prevalence of MTHFR Genotypes

Author	Genotype
Ulrich <i>et al.</i> [66]	TT=16%-variant CT=42%-variant CC=42%-wild
Van Ede <i>et al.</i> [60]	TT=8%-variant CT=40%-variant CC=52%-wild
Urano <i>et al.</i> [69]	TT=17%-variant CT=50%-variant CC=33% wild

Therefore, this scientific evidence suggests that the MTHFR genetic mutation influences folate metabolism, suggesting that dietary intake of a standard dosage of folate may be insufficient for half to two-thirds of the population who have this or a related mutation. With this body of published scientific evidence supporting a genomic influence on folate metabolism, it may be clinically important to provide such information to guide dietary intake and nutritional supplementation. Additionally, there is an analogous body of pharmacogenomic data to support these conclusions as well.

FOLATE-ANTAGONIST PHARMACEUTICALS AS A PRECURSOR TO THE NUTRIGENOMICS OF FOLATE

Over the past thirty years, folate-antagonist pharmaceuticals have been used for a series of chronic diseases including chemotherapy and immunomodulation of rheumatoid arthritis. Folate-analogs, in a nutshell, inhibit several enzymes implicated in folate metabolism (e.g. dihydrofolate reductase), critical for nucleotide and amino-acid synthesis. The science behind folate-analog metabolism provides evidence for FA metabolism, and its overall impact on health and therapeutic decisions. There are several pharmacogenetic proofs of principle demonstrating the importance of genes and anti-folate drug metabolism, and thus reciprocally, genes and folate metabolism [23-26].

The pteridine compound, methotrexate (MTX), is structurally similar to folate and as a result can bind to the active sites of a number of enzymes that normally use folate as a coenzyme for the biosynthesis of the purine and pyrimidine nucleotide precursors of DNA and for the interconversion of amino acids during protein biosynthesis. Despite its structural similarity to FA, MTX cannot be used as a cofactor by enzymes that require folate, and instead competes with the

folate cofactor for enzyme binding sites, thereby inhibiting protein and DNA biosynthesis and, hence, cell division.

The ability of methotrexate to inhibit cell division has been exploited in the treatment of a number of diseases and conditions that are characterized by rapid or aberrant cell growth. As an example, autoimmune diseases are characterized by an inappropriate immune response directed against normal autologous (self) tissues and are mediated by rapidly replicating T-cells or B-cells. Autoimmune diseases that have been treated with MTX include, for example, multiple sclerosis, rheumatoid arthritis, psoriasis, the autoimmune stage of diabetes mellitus (juvenile-onset or Type 1 diabetes), autoimmune uveoretinitis, myasthenia gravis, autoimmune thyroiditis, and systemic lupus erythematosus [27].

Because many malignant cells proliferate more rapidly than normal cells, MTX can also be used to selectively impair cancerous cell growth. As a consequence, methotrexate is a widely used anticancer agent, employed, for example, in the treatment of acute lymphocytic leukemia, breast cancer, epidermoid cancers of the head and neck, advanced mycosis fungoides, lung cancer, non-Hodgkins lymphomas, gestational choriocarcinoma, chorioadenoma destruens, and hydatidiform moles [28].

Despite its therapeutic efficacy for a wide variety of diseases and conditions, treatment with methotrexate can present a risk to the patient. In particular, because MTX interferes with processes required for replication and division of normal as well as diseased cells, inappropriately high levels of the drug can lead to destruction of actively proliferating non-target tissues such as bone marrow and intestinal mucosa. MTX consequently is associated with renal and hepatic toxicity when administered in the "high-dose regimen" that is required for some conditions. In addition, low-dose MTX therapy can lead to toxicity and unwanted side-effects in some patients, where the dosage is not appropriate due to individual variability in pharmacokinetic parameters influencing, for example, drug uptake, targeting and clearance. This situation is especially problematic in the treatment of chronic conditions such as rheumatoid arthritis, where methotrexate can be administered over a period of many years [29].

Low dose weekly methotrexate was first used to treat patients with active rheumatoid arthritis in the 1970s. Over the years, methotrexate has become the most widely used agent among disease modifying anti-rheumatic drugs (DMARD) and has been ranked ahead of such drugs in terms of its efficacy/toxicity ratio [30,31]. In particular, one-third of rheumatoid arthritis patients show major improvement on methotrexate, with this drug generally preferred over azathioprine, sulfasalazine, gold salts and penicillamine because of its relatively favorable ratio of efficacy to toxicity [32-34]. In some cases, methotrexate is combined with other disease modifying anti-rheumatic drugs such as sulfasalazine and hydroxychloroquine or other anti-inflammatory agents, for example, anti-cytokine therapeutics such as anti-tumor necrosis factor- α antibodies [35-38].

Gastrointestinal intolerance such as nausea, abdominal pain, indigestion or diarrhea; asymptomatic elevation of se-

rum hepatic transaminase levels; and stomatitis are the major reasons for dose reduction or premature discontinuation of methotrexate therapy [39-43]. In addition to dose and duration of treatment, other factors such as folate deficiency or FA consumption, advanced age, cumulative dose, renal insufficiency and concomitant use of other anti-folates can influence methotrexate toxicity [44, 45].

Many adverse effects mimic folate deficiency and can be explained by the antifolate properties of methotrexate [46-48]. Depleted intracellular folate levels have been documented in hepatocytes and peripheral blood lymphocytes of methotrexate-treated patients [44, 49-54]. Folate deficiency occurs frequently in patients with rheumatoid arthritis suggesting a nutritional role of folate and inflammatory conditions, and folate stores are further decreased in rheumatoid arthritis patients taking methotrexate [44].

Several studies have shown the advantages of folic or folinic acid supplementation in rheumatoid arthritis and other patients undergoing treatment with methotrexate [41, 44, 48-51, 55-61]. Several studies have been published with folic acid doses ranging from 1,000 mcg per day [60, 61] to 5,000 mcg per day, concomitant with MTX. As an example, in double-blind studies, 5 mg of FA or 2.5 to 5 mg per week of folinic acid, an activated form of FA, substantially reduced side effects of methotrexate without interfering with therapeutic efficacy in rheumatoid arthritis patients [62, 63]. Similarly, 5 mg per day FA was shown to alleviate the side effects from methotrexate observed in patients with severe psoriasis [64]. The folic or folinic acid was generally prescribed to be taken at a different time from methotrexate and, in some cases, was prescribed to be taken only five days per week. In a recent study that involved one of this reviews authors, the study authors found that in subjects taking folic or folinic acid supplementation along with MTX, single nucleotide polymorphisms in folate-dependent enzymes methylene tetrahydrofolate reductase (MTHFR) 677TT, thymidylate synthase (TSER) *2/*2 (variable number of tandem repeats), amino imidazole ribonucleotide transformylase (ATIC) 347GG, and serine hydroxymethyltransferase (SHMT1) 1420CC were associated with a cumulative higher risk of MTX-related toxicities [26, 65]. These studies clearly demonstrate a nutritional role of FA and methotrexate therapy.

The MTHFR genotype has a pharmacogenetic implication on MTX therapy and concomitant use of FA. Individuals with a homozygous mutant TT, or heterozygous CT genotype (>10% and 40% of the population, respectively) present increased risk of side effects following MTX therapy compared to those with the wild type genotype [66-68]. The precise mechanism for this increased toxicity in patients with the 677T variant is not clearly understood but it can be speculated that lower folate levels in those patients may predispose to increase susceptibility to the anti-folate effects of MTX. This is supported by the observation that folate supplementation tends to overcome the effects of decreased MTHFR activity in patients with rheumatoid arthritis [68]. Testing for the C677T mutation in patients undergoing MTX therapy could be important as the dose is often escalated to achieve therapeutic response, and provide valuable insights in FA supplementation.

In these studies, they provide suggestive evidence that when a patient takes folate supplementation, namely through FA consumption over six times the amount of the U.S. Department of Agriculture's standards of Recommended Dietary Allowance, patients can significantly reduce their risk for side effects induced by MTX treatment.

In the Netherlands, researchers at VU Medical Center in Amsterdam have found that folate supplementation can be exploited to enhance chemotherapeutic efficacy of both anti-folate based chemotherapies, such as pemetrexed, methotrexate, and cisplatin, as well as other chemotherapies. These researchers found that folates could modulate the expression and activity of at least two members of the Multi-Drug Resistant transporters: MRP1/ABCC1, and the breast cancer resistance protein BCRP/ABCG2. Thus, folate supplementation may have differential effects on chemotherapy: (1) reduction of toxicity, (2) increase of anti-tumor activity, and (3) induction of MRP1 and BCRP associated cellular drug resistance [70].

In looking at the clinical results of these studies, folate levels correlate with risk for MTX toxicity, as well as MTHFR activity. These studies demonstrate that the MTHFR gene plays an important role of the nutrigenomics of folate supplementation or nutritional supplementation with FA, as it relates to the most commonly used disease modifying anti-rheumatic drug, methotrexate, which is a folate analog [68-71].

CONCLUSION

In this review, we have intended to elucidate the published research around the nutrigenetics of dietary folate and its corresponding FA supplementation. The research involved in folate nutrigenetics begins with clinical evidence and published studies supporting an important role of folate and/or FA intake. There are decades of research establishing the importance of folate on various healthcare concerns. Then, the research builds a scientific case by looking at the role of genetic mutations on enzymes involved in folate metabolism, namely MTHFR, and the published data suggests there is an established genetic influence on folate. Next, there is a strong body of research defining the role of genetic factors on anti-folate therapeutics such as methotrexate and pemetrexed. Despite this evidence, there is still need for further studies to define how these genetic factors specifically should guide dietary intake, but the evidence is clear that genetic factors suggest variable levels of FA intake ranging from 400 mcg to 5,000 mcg. The government has pursued efforts involving a Recommended Dietary Allowance and fortification of foods to overcome apparent folate deficiencies. However, varied published data would suggest that a standard amount of FA supplementation may be insufficient due to genetic predispositions in as much as 50 to 60 percent of the population. By bringing together these genetic influencing factors and nutritional supplementation, there is a need for randomized, double-blind placebo-controlled studies to conclusively demonstrate these associations. In a randomized, double blinded placebo-controlled study conducted recently, this concept was studied by Miyaki *et al.* which demonstrated that DNA-customized folic acid provided a 2.4 fold benefit in reducing incidence of atherosclerosis [75].

Without overstating the issues at hand, even though this science has been established and relevant to chemotherapeutic clinics and rheumatology research institutions where it only benefits a small percentage of the population suffering from cancer and rheumatoid arthritis, the metabolism, intake, and nutrigenetics of folate may even be more relevant to the mass population. Since genetic factors can heavily influence folate metabolism, it may be scientifically appropriate to adjust folate supplementation beyond the 100 percent of US Recommended Dietary Allowance level equaling 400 mcg. In this regard, while it is true that for some patients supplementation in the 400mcg range may be sufficient to reduce homocysteine levels, the converse is equally true that in patients possessing certain polymorphisms and/or mutations in the MTHFR gene, for example, higher dosage of FA supplementation is not only suggestive but is rationale in order to obtain the full benefit of FA intake. However, high dosage FA intake, especially in pregnant females, must be monitored [70].

In conclusion, a significant body of scientific evidence suggests that there is a nutrigenetic component to FA which is relevant to the daily nutritional intake of individuals, the pharmacogenomics of anti-folate therapies, and the nutritional supplementation of individuals suffering from a metabolic folate deficiency due to their genetic predisposition. In light of this scientific evidence, genetics may be a valuable guide for dietary intake of folate and dietary supplementation with FA, however, further research is needed to elucidate how such genetic information should be used most effectively.

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