MTHFR-folate axis as a modulator of the epigenetic landscape in autoimmune diseases (Review)

PABLO MICHAEL NAVARRO-RODRÍGUEZ[1,2*], RAMÓN FRANCISCO BAJECA-SERRANO[2,3*], FRANCISCO JAVIER TURRUBIATES-HERNÁNDEZ[2,4], HAZAEL RAMIRO CEJA-GÁLVEZ[2,5], JORGE HERNÁNDEZ-BELLO[2,5], CRISTIAN OSWALDO HERNÁNDEZ-RAMÍREZ[2,5], SAÚL RAMÍREZ-DE LOS SANTOS[2,6] and JOSÉ FRANCISCO MUÑOZ-VALLE[2,5]

[1]Department of Molecular Biology and Genomics, University Center of Health Sciences, University of Guadalajara, Guadalajara, Jalisco 44340, Mexico; [2]Department of Clinical Medicine, Institute of Research in Biomedical Sciences, University Center of Health Sciences, University of Guadalajara, Guadalajara, Jalisco 44340, Mexico; [3]Department of Food and Nutrition, University Center of Health Sciences, University of Guadalajara, Guadalajara, Jalisco 44340, Mexico; [4]Department of Food and Nutrition, University Center of Health Sciences, University of Guadalajara, Guadalajara, Jalisco 44340, Mexico; [5]Department of Molecular Biology and Genomics, University Center of Health Sciences, University of Guadalajara, Guadalajara, Jalisco 44340, Mexico; [6]Department of Basic Psychology, University Center of Health Sciences, University of Guadalajara, Guadalajara, Jalisco 44340, Mexico

Received November 19, 2025; Accepted December 29, 2025

DOI: 10.3892/ijmm.2026.5741

Abstract. The one-carbon metabolism pathway, regulated by the methylenetetrahydrofolate reductase (MTHFR) enzyme, represents a key nexus where genetic predisposition and nutrient status converge to shape the epigenetic landscape of autoimmune diseases. The objective of the present review is to synthesize evidence of how the *MTHFR*-folate axis drives epigenomic patterns in these conditions. One of the main diseases involved is rheumatoid arthritis, where drug-naïve patients show T-cell and synovial hypomethylation with cytokine-driven DNMT suppression, a process aggravated by reduced folate availability and *MTHFR* polymorphisms that constrain S-adenosylmethionine supply. Similarly, in systemic lupus erythematosus, $CD4^+$ T cells exhibit global hypomethylation with an interferon-skewed signature (such as *IFI44L*), associated with impaired *MTHFR* activity and a folate-dependent SAM:SAH imbalance that further diminishes DNMT function. Finally, in celiac disease, intestinal differential methylation, including LINE-1 hypomethylation, is observed, driven by gluten-induced villous atrophy and folate malabsorption. Overall, impaired one-carbon metabolism and *MTHFR*-dependent methylation capacity may be key determinants of epigenomic dysfunction underlying autoimmune disease and its clinical severity.

Contents

1. Introduction

Autoimmune diseases, once considered rare, now affect a substantial proportion of the global population and arise from a breakdown of immune tolerance, whereby adaptive and innate responses fail to discriminate self from non-self (1). While inherited risk loci explain part of disease susceptibility, mounting evidence implicates epigenetic mechanisms, heritable yet reversible changes in gene regulation, including DNA methylation, histone modifications and non-coding RNAs, as key determinants of pathogenic immune programs (2,3). These mechanisms are metabolically gated: The one-carbon (folate) network supplies methyl groups for DNA and histone methylation, thereby coupling nutrient status to chromatin state and immune function (4). This metabolic-epigenetic axis carries out a central role

Correspondence to: Dr José Francisco Muñoz-Valle, Department of Clinical Medicine, Institute of Research in Biomedical Sciences, University Center of Health Sciences, University of Guadalajara, Building Q, First Floor, 950 Sierra Mojada, Door 7, Guadalajara, Jalisco 44340, Mexico
E-mail: biologiamolecular@hotmail.com

*Contributed equally

Key words: MTHFR, folate, S-adenosylmethionine, DNA methylation, epigenomics, autoimmune diseases

in integrating environmental factors, nutrition and cellular stress responses, thereby shaping immune-system development across the lifespan.

Within this network, methylenetetrahydrofolate reductase (MTHFR) reduces 5,10-methylene-THF to 5-methyl-THF, enabling remethylation of homocysteine to methionine and sustaining S-adenosylmethionine (SAM), the universal methyl donor. Common *MTHFR* variants 677C>T and 1298A>C lower enzymatic flux to varying degrees and, particularly under low folate conditions, are associated with hyperhomocysteinemia and reduced methylation capacity (5-8). Beyond genetics, emerging research indicates that *MTHFR* itself is subject to epigenetic regulation (promoter methylation, chromatin context, microRNAs and lncRNAs) (9-12), positioning the enzyme both as a modulator and as a target within the metabolism-epigenome interface. This dual role underscores why *MTHFR* alterations can propagate broadly across the immune, vascular and neurological systems, especially in environments of chronic inflammation or increased methylation demand.

The research landscape shows convergent epigenetic phenotypes across immune-mediated conditions, for example, synovial and T-cell hypomethylation in rheumatoid arthritis (RA), interferon-driven signatures in systemic lupus erythematosus (SLE), mucosal (and saliva-detectable) alterations in celiac disease (CeD) and therapy-responsive methylomes in multiple sclerosis (MS) (2,3,13,14). These findings are consistent with constraints on methyl-group availability and inflammation-linked suppression of the methylation machinery (2,3). Yet key controversies remain. First, associations between *MTHFR* variants and autoimmunity are heterogeneous across ancestries and clinical phenotypes and appear conditional on environmental factors (dietary folate/B-vitamin status) and medications (such as methotrexate; MTX) (5-8). Second, the direction of causality is debated: Epigenetic abnormalities may be primary drivers, secondary consequences of inflammation and treatment or both and locus-resolved evidence in primary immune cells for *MTHFR* regulation is still limited. Moreover, the majority of existing studies examine isolated components of the pathway (2,15-17), highlighting the need for integrative analyses that jointly evaluate genetic variants, methylation capacity, inflammatory signaling and nutrient availability.

The present review synthesizes current literature on the folate-*MTHFR*-SAM axis as a modulator of epigenetic stability in autoimmune disease. Specifically, the present review i) summarizes epigenetic regulation of *MTHFR* (DNA methylation, histone marks and non-coding RNAs); ii) outlines one-carbon biochemistry and gene-nutrient interactions that tune methylation capacity; iii) integrates disease-specific epigenomic findings across RA, SLE, MS, CeD and fibromyalgia (FM); and iv) discusses translational implications for biomarkers and nutritionally informed or epigenetic adjuncts to immunotherapy. The present review highlights areas of agreement and active debate and delineates priorities for cell type-resolved and mechanism-anchored studies. By doing so, the present review aims to bridge molecular insights relevant to autoimmune diseases, emphasizing how folate-dependent epigenetic regulation can contribute both to disease-risk stratification and to the development of personalized therapeutic strategies.

2. Literature search

For the present review, a literature search was performed in the PubMed (https://pubmed.ncbi.nlm.nih.gov/) and PubMed Central (PMC) (https://pmc.ncbi.nlm.nih.gov/) databases using topics and subtopics associated with the role of one-carbon metabolism (such as MTHFR, folate and homocysteine), epigenetic mechanisms (such as DNA methylation and histone modification) and their impact on autoimmune diseases such as RA, SLE, MS, cEd and FM. The reviewed publications included mechanistic studies, clinical trials and systematic reviews involving human subjects that report relevant findings on direct epigenetic evidence (such as DNA methylation levels), biochemical markers or clinical outcomes associated with disease activity and therapeutic response.

3. Folates

History and discovery. In 1931, studies investigating anemia during pregnancy identified nutritional deficiency as a key etiological factor. Experimental work by Wills and Stewart (18) demonstrated that supplementation with yeast extract (Marmite®) or animal protein could reverse severe anemia in animal models. These findings led to the identification of an unknown nutritional factor distinct from vitamin B_2, called the 'Wills factor', which was later recognized as folate (18-21).

In 1941, Mitchell *et al* (21) published the first study describing the concentration of folic acid, a compound named after the Latin word folium (leaf). Their study revealed that folic acid was capable of promoting the proliferation of *Lactobacillus casei*, *Lactobacillus delbreuckii* and *Streptococcus lactis*. The isolation of the pure, crystalline form of folic acid (pteroglutamic acid) was first achieved by Robert Stokstad and Lederle Laboratories in 1943. This achievement was notable because it enabled researchers to study the characteristics and properties of the compound. The utilization of folic acid-fermenting microorganisms was instrumental in achieving this goal (22).

In 1945, Angier *et al* (23) determined that the chemical synthesis of pteroglutamic acid from liver samples (21). This development represented a pivotal shift in the management of megaloblastic anemias due to the ability to produce folic acid on a large scale for clinical use (23).

Although early research on folic acid focused on treating a specific form of anemia, Kumar (24), was the first to identify key elements of folic acid metabolism associated with acute lymphoblastic leukemia in children. This work and its broader implications have been discussed by Kumar *et al* (24). Despite recognition of the essential roles of folate in DNA synthesis and neural tube defect prevention, its metabolic pathway remained poorly characterized. In 1971, Kutzbach and Stokstad (25) isolated and described the enzyme, MTHFR, for the first time. The enzyme was found to be subject to allosteric regulation by SAM, associating it with methionine metabolism, DNA synthesis, cardiovascular disease and neural tube defects.

In 1973, Tamura and Stokstad (26) evaluated the bioavailability of naturally occurring folates compared with five synthetic derivatives. They observed increased bioavailability for synthetic folates compared with food sources such as liver, yeast, banana, orange juice and lettuce. Based on advancements

in folate metabolism research, in 1980, scientists described the 'folate trap', a phenomenon in which the enzyme methionine synthase (MTR), in the absence of its cofactor (vitamin B_{12}), is unable to convert homocysteine into methionine. This traps folate in its 5-methyltetrahydrofolate form and inhibits purine and thymidine synthesis for DNA, even when folate levels are sufficient (27).

In 1990, Frosst *et al* (28) identified a C677T polymorphism in the *MTHFR* gene that reduced enzyme activity and altered folate concentrations. Epidemiological evidence accumulated since the discovery of folate, its sources and bioavailability has served as a foundation for translating knowledge into preventive and therapeutic public health strategies. Efforts to fortify food products with synthetic folic acid have been implemented in several countries, including the United States, Canada, Argentina, Chile and others across Europe and Latin America (29). These initiatives have led to substantial reductions in the prevalence of neural tube defects in newborns, with decreases ranging from ~25 to >60%, depending on the country and the specifics of program implementation (13,29). Despite these successes, some populations may exceed recommended folate intake, underscoring the importance of careful monitoring. Conversely, countries such as Mexico and Colombia lack comprehensive surveillance and monitoring systems to evaluate the impact of mandatory folic acid fortification on population health (29-31).

Chemical structure. During its discovery, folic acid was assigned multiple names. One of the most widely recognized designations is folic acid, although it is more commonly referred to as vitamin B_9 or folate (32-34). The term folate refers to a family of molecules with similar chemical structures that exert beneficial effects in various health conditions, ranging from anemia to cardiovascular diseases, cancer and inflammatory processes (29,35). The chemical structure of folic acid (C19H19N7O6) serves as the foundation for the diverse chemical forms of folates. The structural components of folates can be categorized as follows: i) A heterocyclic pterin structure in oxidized or reduced form, consisting of a pyrimidine ring, a pyrazine ring and a methyl-group at carbon 6 that serves as a bridge for acid linkage; ii) p-aminobenzoic acid; and iii) a mono or polyglutamate chain of variable length (Fig. 1A).

A carbon unit may be associated with either the pterin or the p-aminobenzoic ring, or with both. The classification of folates depends on the oxidation state of the pterin and carbon unit, as well as the polyglutamylation state. Consequently, folate derivatives such as dihydro, tetrahydro, methyl and formyl forms are expected, invariably conjugated with p-aminobenzoyl-glutamate as mono-, di-, tri- or polyglutamates (36-38).

This group of compounds, associated with water-soluble-B complex vitamins, cannot be synthesized by mammalian cells; therefore, dietary intake is essential, either for natural sources (tetrahydrofolate, THF) or synthetic supplementation (folic acid) (31). In total, ~150 biochemical derivatives with metabolic activity have been described, among which tetrahydrofolates are the most relevant due to their roles in DNA and RNA synthesis, cell division, methylation reactions and as cofactors in multiple metabolic pathways (30,36).

Table I. Natural foods rich in folate and industrialized foods fortified with folic acid.

Food	Folate (μg/100 g)	Scientific source
Cooked beef liver	290	USDA
Raw spinach	194	USDA
Cooked lentils	181	USDA
Cooked white beans	172	BEDCA
Cooked broccoli	108	USDA
Cooked turnip greens	106	FAO/INFOODS
Avocado	81	USDA
Cooked pork kidney	77	BEDCA
Roquefort cheese	50	BEDCA
Cooked egg	25	FAO/INFOODS
Fortified breakfast cereal	150-400	USDA
Enriched wheat pasta	100-150	BEDCA
Enriched white bread	120	USDA
Fortified corn flour	80-120	FAO/INFOODS
Enriched white rice	90-110	USDA

Data were obtained from the following resources: USDA, BEDCA and AnFooD 1.0 of the FAO/INFOODS (42-44). USA, United States Department of Agriculture; BEDCA, the Spanish Food Composition Database; AnFooD 1.0, the Analytical Food Composition Database; FAO/INFOODS, Food and Agriculture Organization/International Network of Food Data Systems.

Folic acid, the most oxidized and stable form of folate, is reduced by dihydrofolate reductase (DHFR) at nitrogen 8 to produce dihydrofolate (DHF). Further reduction at nitrogen 5 yields THF, the active form of the vitamin that functions as a coenzyme (Fig. 1). THF accepts carbon atoms at nitrogen positions 5 and 10, generating cofactor derivatives with specific physiological functions: 5-methyl-THF, 5,10-methyl-THF, 10-formyl-THF and 5-formyl-THF (37).

THF is the principal dietary form of folate in the body, acting as a carrier in one-carbon cycle biosynthesis. Its derivative, 5-methyl-THF, is the predominant active form of folate in blood and supports the conversion of methionine to SAM for methylation processes. Folic acid, a synthetic and fully oxidized compound used in supplementation and food fortification, requires hepatic DHFR for biological activity. By contrast, folinic acid can yield 5,10-methyl-THF or 5-methyl-THF without requiring DHFR, making it particularly useful for counteracting the effects of chemotherapeutic agents (38,39).

The metabolic reactions of folate and its derivatives are closely associated with the one-carbon cycle and subject to feedback regulation, emphasizing the generation of methyl groups that influence epigenetic modifications (36). In addition, folate intermediates participate in the synthesis of purines, pyrimidines and methionine, in the interconversion of serine to glycine and in the catabolism of the latter (40).

Dietary sources. In mammals, the main source of folate comes from dietary intake. Folate is a component of various food groups, including vegetables, cereals, fruits and foods of animal origin (Table I). It can also be produced as a

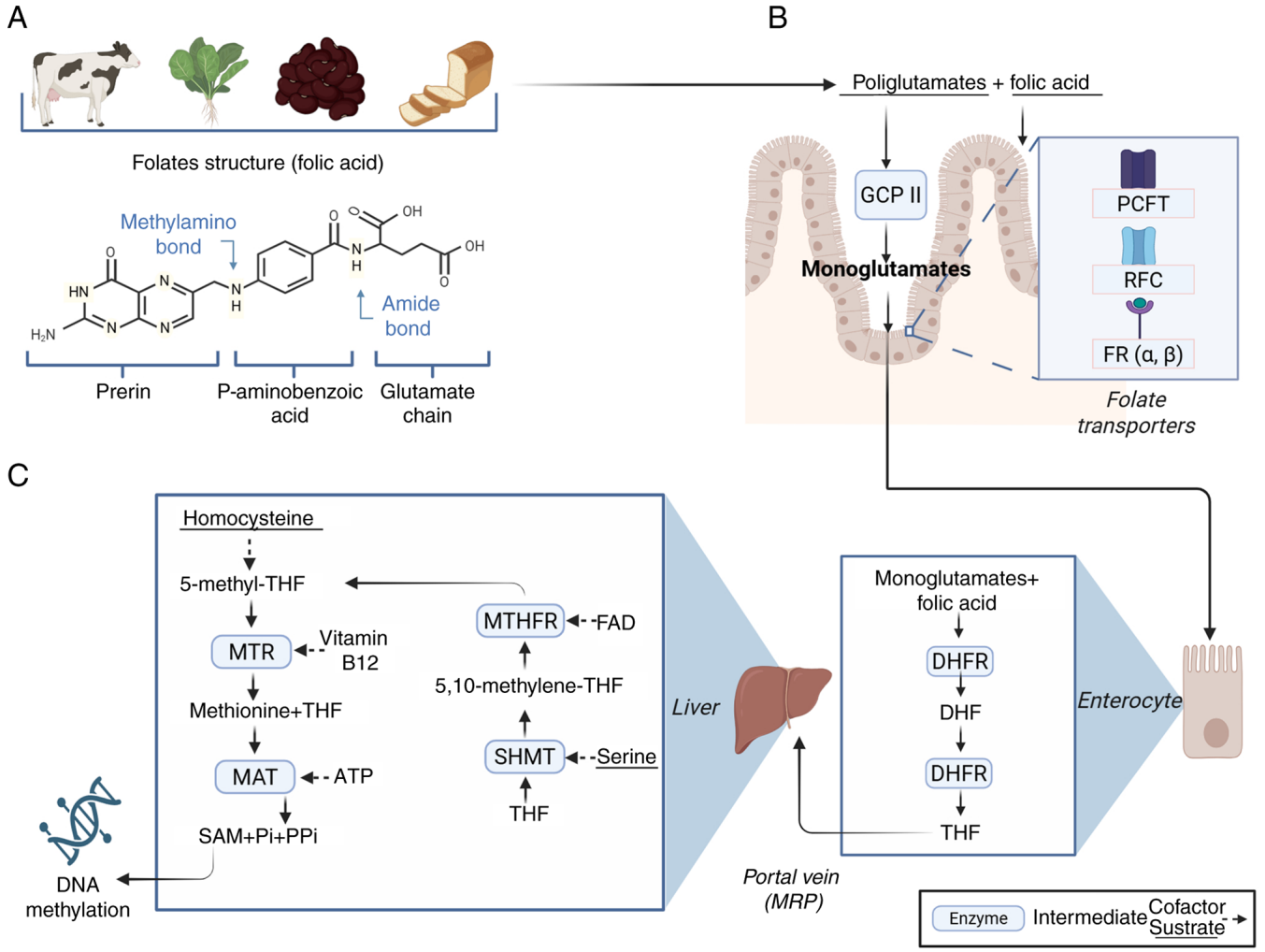

Figure 1. (A) Dietary sources of folate and general structure. Dietary folates, comprising three main components (pterin, *p*-aminobenzoic acid and a glutamate chain), are ingested as polyglutamates and hydrolyzed to monoglutamates by the enzyme GCPII in the intestinal brush border. (B) Intestinal absorption of folate. Folate uptake occurs through transporters located in enterocytes, primarily the PCFT and the RFC, as well as through FRα/FRβ present in other cell types. (C) In folate metabolism, within intestinal cells, folate is sequentially reduced by DHFR to DHF and ultimately to its active form, THF. THF is transported to 5,10-methylenetetrahydrofolate (5,10-MTHF) by serine hydroxymethyltransferase (SHMT) and subsequently reduced by the FAD-dependent enzyme MTHFR to 5-methylenetetrahydrofolate (5-MTHF). The latter donates its methyl group to homocysteine to regenerate methionine, in a reaction catalyzed by MTR that requires vitamin B_{12}. Methionine is then converted by MAT into SAM, the universal methyl donor, in an ATP-dependent process. This sequence associates folate metabolism with methylation reactions that are key for nucleotide synthesis and epigenetic regulation. GCPII, glutamate carboxypeptidase II; PCFT, proton-coupled folate transporter; RFC, reduced folate carrier; FR, folate receptor; DHFR, dihydrofolate reductase; THF, tetrahydrofolate; 5,10-MTFH, 5,10-methylenetetrahydrofolate; SHMT, serine hydroxymethyltransferase; MTR, methionine synthase; MAT, methionine adenosyltransferase; SAM, S-adenosylmethionine.

metabolite by the intestinal microbiota from different phyla such as bacteroidetes, fusobacteria, proteobacteria and actinobacteria (41-44).

Bioavailability and intestinal absorption. In mammals, the main source of folate is dietary intake. Folate is abundant in various food groups, including vegetables, cereals, fruits and foods of animal origin, and it may also be produced as a metabolite by the intestinal microbiota. Once ingested, the efficiency with which folate becomes available for metabolic reactions depends not only on its chemical form but also on how it is processed and absorbed in the body. Dietary folates are typically present as polyglutamates, which are chemically labile and can be lost during food processing and cooking. Depending on the food type and preparation, losses can range from 20-60% in vegetables and 30-70% in cereals (45). The bioavailability of folate refers to the proportion that is absorbed and available for metabolic reactions or storage. Thus, foods may be rich in folate yet exhibit low bioavailability (46,47).

The bioavailability of dietary folates can be compromised by several factors: i) Incomplete release of the molecule from the original food matrix; ii) degradation of the molecule within the gastrointestinal tract; and iii) incomplete hydrolysis of the molecule due to the presence of other dietary components, such as fatty acids. Additionally, individual characteristics (such as sex and genetic variations), folate stores and the availability of other nutrients (vitamin C, vitamin B_{12}, vitamin B_6, niacin, riboflavin or choline) influence the bioavailability of both dietary and synthetic folate (46).

Folate absorption primarily occurs in the proximal segments of the small intestine, the duodenum and jejunum,

where an acidic environment facilitates folate transport (48-50). Dietary polyglutamates undergo hydrolysis at the intestinal brush border through the action of glutamate carboxypeptidase II (GCPII), converting them into monoglutamates that can be absorbed in a manner similar to synthetic folic acid (48,49). Due to the charge and hydrophilic nature of the molecule, passive diffusion across cell membranes is inefficient (50). Reduced folate carriers (RFCs) serve as the main transporters mediating systemic folate metabolism (50,51). In addition, dietary folates are absorbed primarily via proton-coupled folate transporters (PCFTs), which are located mainly in the upper gastrointestinal tract and in certain tumors (Fig. 1B) (48,50,52). Folate receptors (FRα and FRβ) located in the cell membrane possess a glycosylphosphoinositol anchor and mediate endocytosis of folate at neutral or slightly acidic pH (50,51). Across the basolateral membrane, folates are subsequently transported into the vascular system via ABCC proteins, particularly ABCC3 (50).

Folate metabolism. The transformation of dietary folate into its active monoglutamate form requires the action of the enzyme GCPII, located on the intestinal brush border. GCPII catalyzes the hydrolysis of folate, generating monoglutamate, which is then internalized by PCFT within enterocytes. After absorption, DHFR sequentially reduces monoglutamate to DHF and then to 5-methyl-THF. The resulting metabolite is exported via the portal vein through interacting with multidrug resistance proteins until it enters the bloodstream and reaches tissues, where it is taken up by the RFC system or by folate receptors. Once inside cells, folate is converted to polyglutamate forms within tissues, while its active form is primarily metabolized in the liver (53,54).

5-methyl-THF enters cells via transporters or receptors. Inside the cell, it participates in the remethylation of homocysteine to methionine through the activity of MTR and its cofactor vitamin B_{12}. Methionine is subsequently converted by methionine adenosyltransferase (MAT) into SAM, the universal methyl donor for DNA methylation (Fig. 1C). Following methyl group donation, SAM is converted to S-adenosylhomocysteine (SAH), which is then hydrolyzed by SAH hydrolase into homocysteine and adenosine (55). To maintain balanced production of bioactive folate metabolites, interconversion occurs at both the mitochondrial and cytosolic levels. This process involves the cytosolic enzyme serine hydroxymethyltransferase (SHMT1), which catalyzes the conversion of serine to glycine and transfers a one-carbon unit to THF to form 5,10-methylenetetrahydrofolate. The mitochondrial isoform (SHMT2) catalyzes the reverse reaction, synthesizing serine from glycine. The newly formed serine serves as a feedback substrate in the metabolic pathways for thymidylate and methionine synthesis and supports DNA methylation (56).

These reactions underscore the close interconnection between folate metabolism, the one-carbon cycle and methionine metabolism. The capacity of folate to accept and donate methyl groups is fundamental to epigenetic regulation, influencing gene expression and protein synthesis through methylation processes (54). MTX, the primary treatment for inflammatory autoimmune diseases such as RA and MS, acts as a folate antagonist. It directly inhibits folate metabolism and indirectly disrupts associated pathways, including purine and pyrimidine synthesis. Within these pathways, folate-derived metabolites such as 5,10-methylenetetrahydrofolate (5,10-THF) and 10-formyl-THF carry out essential roles. MTX inhibits key enzymes of folate metabolism, including DHFR, thymidylate synthase, MTHFR and SHMT (57,58).

In addition to these pharmacological effects, MTX interacts with genetic variants in the *MTHFR* gene (C677T and A1298C), which independently reduce biologically active folate levels and elevate homocysteine concentrations. These alterations contribute to gastrointestinal and hematologic toxicity, inflammation, oxidative stress and increased cardiovascular risk. Therefore, maintaining adequate levels of biologically active folate is essential to preserve cellular homeostasis, support methylation-dependent processes and minimize systemic dysfunction across multiple organ systems (59).

Normal values and clinical evaluation of folate status. Alterations in folate concentration have been associated with various health complications due to the essential role of folate in DNA replication, cell proliferation and growth. This has led to widespread implementation of supplementation and fortification programs in staple foods across several countries (60). The prevailing scientific consensus indicates that high folate intake does not adversely affect healthy individuals. However, in individuals with preexisting neoplastic conditions, excessive intake, particularly of synthetic folic acid, may increase cancer risk, although this relationship remains to be fully elucidated (61). The erythrocyte folate concentrations (RBC folate) are considered a stable and reliable long-term indicator of folate status, as it reflects intracellular stores, whereas plasma folate concentrations are more transient and influenced by recent dietary intake. Similarly, analysis of plasma homocysteine concentrations also facilitates the identification of disturbances in the methylation cycle (Table II) (62-64).

Recommended daily intake (RDI). Although the Food and Drug Administration (FDA) established the mandatory fortification of all enriched cereal grain products with folic acid in 1996, full implementation, providing 140 μg per 100 g of product, was achieved in 1998 (65). Subsequently, in 2016, the FDA issued a voluntary recommendation for the fortification of cornmeal. In the United States, the primary dietary sources of folate include enriched grain products, fortified cornmeal, ready-to-eat cereals containing 100-400 μg per serving and adult supplements providing 400-800 μg of folic acid (66).

Folate requirements depend on age and physiological status, particularly in women and adolescents of reproductive age. The RDI is defined as the amount of nutrients necessary to meet the nutritional needs of 97-98% of the healthy population. For folate, the average recommended intake is expressed as micrograms (μg) of dietary folate equivalents (DFE). For adults, the recommended amount is 400 μg per day (Table III) (67,68).

Table II. Reference concentrations of folate in serum and erythrocytes (62).

A, Serum or plasma folate concentration	
Concentration, ng/ml (nmol/l)	Interpretation
>20 (45.3)	Elevated
6-20 (13.5-45.3)	Normal range
3-5.9 (6.8-13.4)	Possible deficiency
<3 (<6.8)	Deficiency
B, Erythrocyte folate concentrations	
Concentration ng/ml (nmol/l)	Interpretation
>140 (>317.5)	Elevated
100-140 (226.5-317.5)	Normal range
<100 (<226.5)	Possible deficiency

Folate deficiency in all age groups can also be inferred from homocysteine concentrations, corresponding to serum or plasma folate <4 ng/ml (<10 nmol/l) or erythrocyte folate <151 ng/ml (<340 nmol/l).

Table III. Recommended daily intake of folate by age group and physiological condition.

Age group/condition	Daily recommendation (μg DFE per day)[a]
Infants 0-6 months	65
Infants 7-12 months	80
Children 1-3 years	150
Children 4-8 years	200
Children 9-13 years	300
Adolescents 14-18 years (men)	400
Adolescents 14-18 years (women)	400
Adults (≥19 years)	400
Pregnancy	600
Lactation	500

[a]1 DFE=1 μg food folate=0.6 μg folic acid from fortified food or supplements consumed with food; or 0.5 μg folic acid from supplements taken on an empty stomach (68).

4. The *MTHFR*: Genetics and regulation

Genomic location and structure. MTHFR is located on chromosome 1p36.22 and spans ~20.3 kb, comprising 12 exons in the human genome. Its promoter is GC-rich and TATA-less, containing Sp1, AP-1, AP-2 and CAAT elements consistent with housekeeping-type regulation. Multiple transcription start sites and alternative splicing events generate two protein isoforms (70 and 77 kDa) and heterogeneous mRNA 5' and 3'untranslated regions (UTRs), reflecting complex transcriptional control. Predicted and observed protein lengths across transcripts range from 656 to 700 amino acids (69-71). The gene encodes MTHFR, a cytosolic flavoprotein that catalyzes the reduction of 5,10-methylene-THF to 5-methyl-THF, thereby associating the folate and methionine cycles. Human MTHFR contains an N-terminal catalytic domain that binds FAD and the folate substrate, and a C-terminal regulatory domain that binds SAM to mediate allosteric inhibition. Phosphorylation of N-terminal residues further sensitizes the enzyme to SAM-dependent feedback (72-74). At the genetic level, two common functional polymorphisms, C677T (rs1801133) and A1298C (rs1801131), are widely studied for their effects on enzyme thermolability and folate/homocysteine status. By contrast, rare truncating or severe missense variants across catalytic or regulatory domains underlie classical MTHFR deficiency (75-77).

Epigenetic regulation of MTHFR (DNA methylation, histone marks and non-coding RNAs). Expression of the *MTHFR* gene is finely controlled by multiple epigenetic mechanisms, including DNA methylation, histone modifications and non-coding RNAs. Functionally, MTHFR bridges one-carbon metabolism with the epigenome by generating 5-methyl-THF for methionine remethylation and SAM synthesis, thereby determining the methyl-group supply available for DNA and histone methyltransferases. Evidence shows that *MTHFR* acts both as a modulator and a target of epigenetic regulation: Promoter DNA methylation, chromatin state and non-coding RNAs can alter its expression, while reduced MTHFR activity, whether genetic or epigenetic, reduces SAM levels, limiting methyltransferase capacity and favoring hypomethylation at methylation-sensitive loci. The result is a metabolic-epigenetic feedback loop in which folate flux and chromatin control co-regulate each other (78,79).

The first regulatory layer involves DNA methylation, the addition of a methyl group to cytosine bases (typically in CpG promoter regions), which generally represses gene transcription. In *MTHFR*, promoter methylation modulates expression in a tissue and context-dependent manner. For example, in sperm DNA from men with idiopathic infertility, a case-control study reported *MTHFR* promoter hypermethylation in 45% (41/94) of cases vs. 15% (8/54) of fertile controls, with higher methylation levels in the oligozoospermic subgroup (80). These findings illustrate that absolute percentages vary widely by CpG site, tissue and methodology, and that direct data from autoimmune cohorts remain scarce (81-83).

A second regulatory layer involves histone post-translational modifications, such as acetylation (for example H3K9ac) or methylation (for example H3K9me3 and H3K27me3), which remodel chromatin to activate or repress transcription. This process is highly sensitive to the metabolic state. During 2-acetylaminofluorene-induced hepatocarcinogenesis in rats, *MTHFR* expression is downregulated early; concomitant promoter-level increases in repressive marks (H3K27me3 gain and H3K18ac loss) were also observed in the associated methylation gene *Mat1a*, while *MTHFR* repression was mechanistically associated with miR-22 and miR-29b (84). Models of folate stress likewise show global reductions in H3K27 and H3K9 methylation, consistent with SAM limitation (85). In acute myeloid leukemia cells, reduced MTHFR function, whether due to polymorphisms or pharmacologic inhibition,

decreases intracellular SAM, leading to loss of the repressive histone marks H3K27me3 and H3K9me3 and de-repression of transcription factors such as *SPI1* (85). In neuronal (SH-SY5Y) cell models, MTHFR acts as a metabolic buffer: Excessive folate disturbs histone-modifying enzyme expression and shifts the H3K4me3/H3K9me2 balance (86,87). These effects are markedly amplified when MTHFR is deficient, emphasizing its role in safeguarding the epigenome against nutrient fluctuations (86-87).

A third regulatory layer involves non-coding RNAs. MicroRNAs (miRNAs) bind mRNA to inhibit translation and can modulate *MTHFR* expression. For instance, miR-22-3p and miR-149-5p bind the 3'UTR of *MTHFR* mRNA, and under folate deficiency, their upregulation suppresses MTHFR protein, further restricting one-carbon flux. Long non-coding RNAs (lncRNAs) can also guide chromatin modifiers: The lncRNA HOTAIR, for example, recruits protein complexes to the *MTHFR* promoter in esophageal cancer cells, depositing repressive H3K27me3 marks and silencing transcription (88-91).

While the majority of direct evidence of *MTHFR* epigenetic regulation derives from reproductive and cancer models, autoimmune contexts offer a compelling rationale for analogous studies in immune cells. Both RA and SLE exhibit pronounced DNA-methylation defects in T cells (such as hypomethylation of type-I-interferon-stimulated genes such as *IFI44L*) and show responsiveness to SAM-linked chromatin mechanisms. These parallels make *MTHFR*-centered regulation a plausible contributor in autoimmune pathogenesis and a promising target for future cell-specific investigations (92-94).

5. The biochemical axis: One-carbon metabolism

One-carbon metabolism, SAM and homocysteine. The one-carbon network integrates the folate and methionine cycles to supply methyl groups for biosynthesis and epigenetic regulation. Folate coenzymes carry and transform one-carbon units primarily derived from serine and glycine. MTHFR catalyzes the reduction of 5,10-methylene-THF to 5-methyl-THF, which donates a methyl group to homocysteine via MTR (a vitamin B_{12}-dependent enzyme) to regenerate methionine. Methionine is subsequently adenylated by MAT to form SAM, the universal methyl donor utilized by DNA, RNA and histone methyltransferases.

After methyl transfer, SAH is formed and hydrolyzed by adenosylhomocysteinase (AHCY) into homocysteine and adenosine, thereby removing a potent inhibitor of methyltransferases. Consequently, the SAM:SAH ratio serves as a proximate index of cellular methylation capacity. In the liver and kidney, an alternative remethylation pathway involves betaine-homocysteine methyltransferase (BHMT), while homocysteine can also leave the cycle through transsulfuration, catalyzed by cystathionine β-synthase and cystathionine γ-lyase, to generate cysteine and glutathione. Collectively, these reactions couple nutrient status to epigenetic regulation via SAM production and SAH clearance (Fig. 2) (95-99).

A fundamental biochemical principle is that SAH inhibits the majority of methyltransferases with low-micromolar inhibition constants (Ki). Accumulation of SAH, or a reduction in SAM, thus constrains DNA and histone methylation even when substrate (cytosine or lysine) is available. Manipulating AHCY activity or methionine flux can therefore alter global methylation states. This inhibitory 'SAH brake' explains why the SAM:SAH ratio, rather than SAM alone, more accurately reflects methylation capacity in cells and tissues (98,100,101).

In humans, the *MTHFR* C677T variant decreases enzymatic activity and interacts with folate status to influence genomic DNA methylation and homocysteine levels, with the lowest methylation observed in TT homozygotes under low-folate conditions. In mice, MTHFR deficiency decreases SAM, increases SAH and reduces global DNA methylation, directly associating impaired MTHFR flux to a hypomethylated genome (102,103). Dietary interventions further demonstrate causal control of leukocyte methylation by one-carbon nutrients. Randomized and longitudinal studies have shown that folic acid and vitamin B_{12} supplementation modify DNA methylation profiles, both globally and at specific loci, consistent with enhanced methyl-group availability for DNMT-mediated reactions (104-106). Epigenome-wide analyses of habitual folate and B_{12} intake corroborate these associations. Although the magnitude and direction of methylation change are locus-specific, the collective evidence supports nutrient-sensitive modulation of the blood methylome through the folate-MTHFR-SAM axis (104-106).

Finally, plasma homocysteine serves as a clinically accessible marker of one-carbon imbalance. Elevated concentrations often indicate insufficient folate or vitamin B_{12} intake, or reduced MTHFR or MTR activity, and are typically accompanied by a low SAM:SAH ratio and reduced methylation potential. In hepatic and renal tissues, BHMT provides a compensatory remethylation pathway that becomes particularly relevant when folate-dependent remethylation is limited, underscoring tissue-specific buffering within the one-carbon network. These biochemical relationships demonstrate that folate availability and MTHFR activity define the upper limit for DNA and histone methylation, providing a mechanistic bridge to the epigenetic phenotypes described in autoimmune diseases (95-97,99).

Crosstalk with inflammatory and immunological pathways. Epigenetic regulation intersects immune function through the one-carbon network that maintains cellular methylation potential in leukocytes. DNA methylation at promoters and enhancers, together with SAM-dependent histone methylation, regulates cytokine programs and lineage stability. Because both processes depend on the folate-MTHFR-SAM axis and are inhibited by SAH, immune activation is tightly associated with one-carbon flux (107,108).

In RA, inflammatory signaling actively suppresses the methylation machinery. IL-1 rapidly downregulates DNMT1 and DNMT3A in synovial fibroblasts at picogram concentrations, a change associated with DNA hypomethylation and sustained inflammatory gene expression. In SLE, oxidative stress inhibits ERK signaling in $CD4^+$ T cells, decreases DNMT1 expression and induces promoter demethylation with aberrant overexpression of normally silenced genes, thus mechanistically associating inflammatory stress to erosion of the T-cell methylome (109-111).

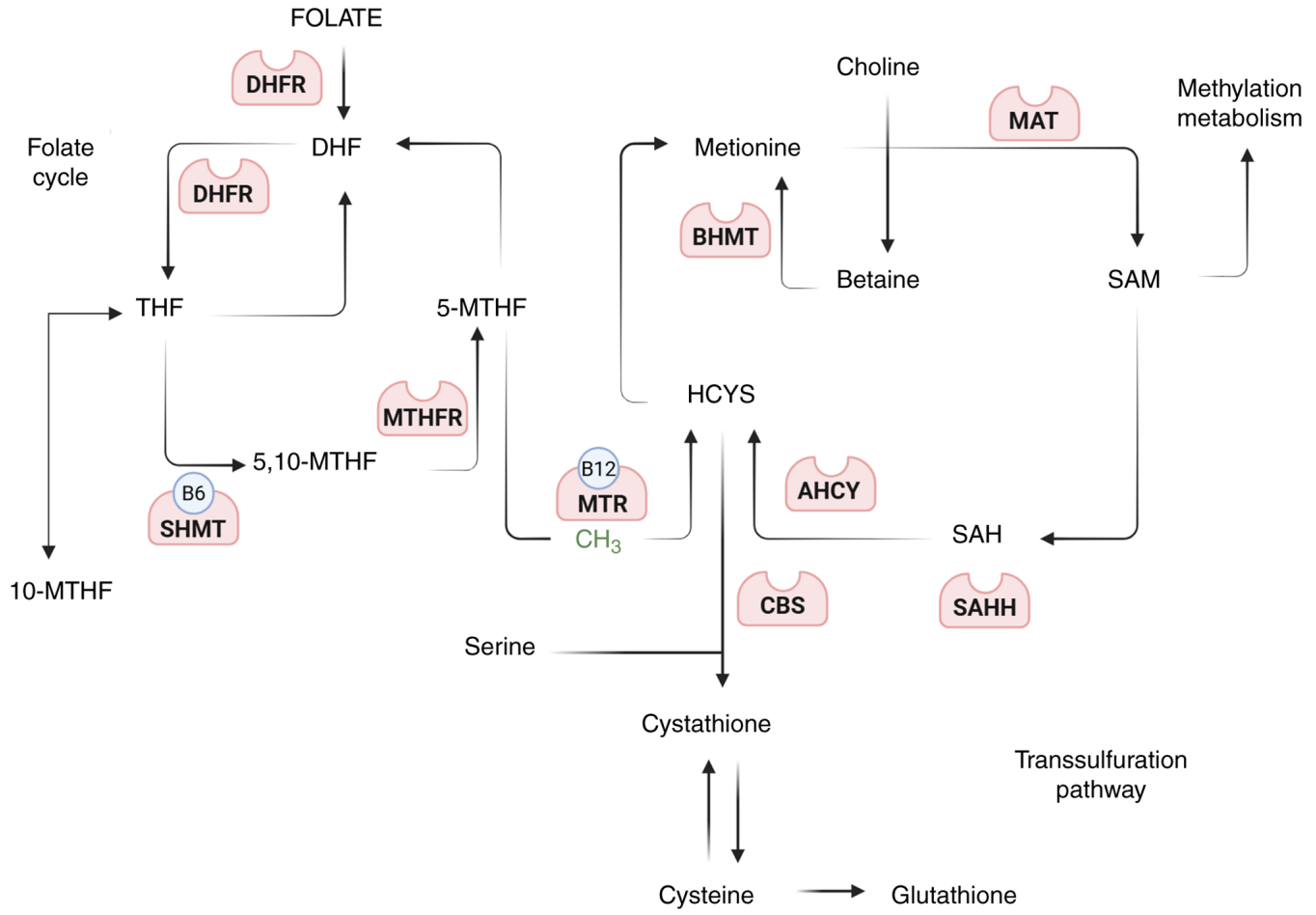

Figure 2. Schematic representation of the folate and methionine cycles illustrating their interconnection with one-carbon metabolism, methylation reactions and transsulfuration. Dietary folate is initially reduced to DHF and THF by DHFR. THF is converted to 5,10-MTHF by SHMT (a vitamin B_6-dependent enzyme) and subsequently reduced to 5-MTHF by MTHFR. 5-MTHF donates a methyl group to Hcy via MTR (a vitamin B_{12}-dependent enzyme), thereby regenerating methionine. Methionine is then converted to SAM by MAT. SAM serves as the universal methyl donor for DNA, RNA, protein and lipid methylation. Following methyl donation, SAM is converted to SAH, which is hydrolyzed by AHCY to Hcy. Homocysteine can be remethylated by BHMT using betaine as a methyl donor or diverted into the transsulfuration pathway via CBS to generate cystathionine, cysteine and ultimately glutathione. This pathway highlights the biochemical integration of folate status, methylation potential and redox homeostasis (97-99). DHR, dihydrofolate; THF, tetrahydrofolate; DHFR, dihydrofolate reductase; 5,10-MTHF; 5,10-methylene-THF, SHMT, serine hydroxymethyltransferase; 5-MTHF, 5-methyl-THF; 5-MTHF, 5-methyl-THF; MTHFR, methylenetetrahydrofolate reductase; Hcy, homocysteine; MTR, methionine synthase; SAM, S-adenosylmethionine; MAT, methionine adenosyltransferase; SAH; S-adenosylhomocysteine; AHCY, adenosylhomocysteinase; BHMT, betaine-homocysteine methyltransferase; CBS, cystathionine β-synthase; SAHH, S-adenosylhomocysteine hydrolase.

Beyond T and stromal compartments, innate immune cells can also rely on one-carbon-supported SAM to mount proinflammatory responses. Upon LPS stimulation, macrophages upregulate serine synthesis and one-carbon metabolism, fueling epigenetic reprogramming that licenses *IL-1β* expression. Inhibition of serine metabolism, in turn, blunts IL-1β production both *in vitro* and *in vivo*. Complementing these disease-specific examples, epigenome-wide studies in type 1 diabetes (T1D) have demonstrated methylation abnormalities in immune effector cells ($CD4^+$ T cells, B cells and monocytes), underscoring that immune epigenomes are broadly sensitive to both inflammatory context and metabolic state (112,113).

Converging evidence associates folate status and homocysteine to inflammatory tone. Folate deficiency increases oxidative and nitrosative stress, activating NF-κB, while homocysteine triggers NF-κB activation and IL-6/IL-1β production in vascular and myeloid cells, biochemical routes through which impaired remethylation amplifies inflammation. In macrophage-lineage cells, experimental folate restriction enhances proinflammatory responses, consistent with a model in which limited methyl-donor availability constrains methyltransferase activity and shifts signaling toward activation (114,115).

Collectively, these findings support a bidirectional feedback loop: Inflammatory cues (such as IL-1 and oxidative stress) suppress DNMT expression and activity, eroding genomic methylation, while low folate or MTHFR-limited flux and the resulting homocysteine/SAM:SAH imbalance promote oxidative and NF-κB signaling. Together, these mechanisms stabilize a proinflammatory, hypomethylated epigenetic state within immune tissues. This mechanistic crosstalk provides the biochemical bridge associating nutrient status and MTHFR activity to the immune-epigenetic phenotypes observed across RA, SLE, T1D and other immune-mediated diseases (107,108,111,116-118).

6. *MTHFR*-mediated epigenetic dysregulation in autoimmune diseases

RA. RA is a chronic, systemic autoimmune disorder characterized by persistent synovitis, pannus formation and extra-articular manifestations. Superimposed on this pathogenetic framework is a robust epigenetic component. Drug-naïve patients already exhibit disease-associated DNA-methylation differences in circulating T-cell subsets and synovial tissue, including global hypomethylation in early disease and cell-type-specific changes across naïve and memory $CD4^+$ lineages (119,120). In synovial fibroblasts, proinflammatory cues directly impair methylation capacity: IL-1 rapidly downregulates DNMT1 and DNMT3A/3B expression and activity, promoting demethylation and stable activation of inflammatory gene programs (116).

At the gene level, methylation alterations converge on RA-relevant loci. Hypomethylation at the *TNF* locus associates with increased expression and, importantly, predicts response to biological therapies in clinical cohorts (121). An additional chromatin study in synovial fibroblasts reveal histone-methylation/STAT3 crosstalk controlling *IL-6*-driven effector genes, underscoring how methyl-donor availability and chromatin state co-regulate cytokine programs (122). Regulatory-T-cell instability also arises through epigenetic mechanisms: Patients with RA exhibit reduced *FOXP3* expression and insufficient demethylation of the *FOXP3* TSDR, while MTX therapy can restore Treg function by demethylating a *FOXP3* enhancer, associating pharmacologic intervention to methylome repair (123,124).

Biochemically, these patterns align with one-carbon control. The folate-MTHFR-SAM axis determines the methyl-group supply for DNMTs. Common *MTHFR* variants (C677T and A1298C) and low folate levels reduce SAM and increase SAH, limiting methyltransferase capacity. Consistently, baseline leukocyte DNA methylation (including global indices) predicts MTX non-response in early RA, while multiple cohorts reveal methylation signatures associated with MTX response, pointing to a pharmaco-epigenetic interface between folate metabolism and RA therapy (125-127). Preliminary evidence further suggests *MTHFR* variants may modulate anti-TNF responses in an allele-dose manner, although results remain population-specific (128). Overall, folate status, *MTHFR* genotype and DNMT activity appear to co-determine the epigenetic tone of RA tissues and the likelihood of therapeutic control (129).

SLE. SLE is a chronic autoimmune disease that causes widespread inflammation and tissue damage affecting the skin, joints, kidneys, brain, lungs and heart. A defining molecular hallmark is global DNA hypomethylation in lymphocytes, especially $CD4^+$ T cells, which associates with disease activity and stabilizes a type-I-interferon-driven program. Among interferon-stimulated genes, *IFI44L* promoter hypomethylation is exceptionally consistent and has emerged as a diagnostic biomarker across tissues (130). Mechanistically, SLE T cells exhibit reduced DNMT1 (maintenance methyltransferase) and dysregulated DNMT3A/3B, partly due to oxidative-stress mediated inhibition of ERK signaling, directly associating inflammatory stress to methylome erosion (131,132).

This enzymatic deficit is compounded by a one-carbon bottleneck. MTHFR activity is essential for generating SAM, the universal methyl donor for all DNMTs. Polymorphic depression of *MTHFR* activity (such as C677T) and folate/B_{12} insufficiency favor hyperhomocysteinemia and a low SAM:SAH ratio, both of which inhibit methyltransferases. Meta-analyses confirm associations between *MTHFR* C677T and SLE susceptibility; in SLE cohorts, the 677TT genotype and elevated homocysteine levels associate with subclinical atherosclerosis, emphasizing the clinical consequences of impaired remethylation (133,134).

Functionally, SLE exhibits promoter hypomethylation and overexpression of proinflammatory cytokines (such as IL-6, particularly in T cells and affected tissues) alongside IL-17 axis activation. Conversely, tolerance-maintaining pathways become epigenetically repressed, such as IL-2 transcriptional silencing and *FOXP3* locus instability, where insufficient TSDR demethylation undermines Treg stability (135,136). Altogether, this evidence positions the MTHFR-SAM-DNMT axis at the core of SLE immunoepigenetics: Metabolic constraint yields methylation defects that hardwire the interferon-skewed, proinflammatory state. The reversible nature of DNA methylation underscores its diagnostic and therapeutic potential, motivating interventions that restore one-carbon flux or target epigenetic writers and erasers (130).

MS. MS is a chronic, immune-mediated neurodegenerative disorder of the central nervous system, characterized by demyelination, axonal injury and progressive neurological impairment (137). Epigenetic deregulation contributes to its pathogenesis by promoting a proinflammatory phenotype in peripheral immune cells. Cell-sorted studies have revealed widespread methylation shifts in T cells and monocytes, including reproducible changes in $CD8^+$ T cells and distinct profiles relative to $CD4^+$ subsets, indicative of compartment-specific immune methylome remodeling (138-141). These patterns are clinically dynamic: Both global and locus-specific methylation signals associate with disability scores and can be modified by disease-modifying therapies, highlighting the plasticity of the MS methylome (138,139). In line with immune polarization, proinflammatory pathways (Th1/Th17) and regulatory circuits (Treg networks) represent methylation-sensitive axes in MS (140,141). Central nervous system tissue analyses further reveal lesion-associated methylation changes affecting myelin biology and glial programs, associating epigenetic remodeling to demyelination and repair potential (137).

These epigenetic alterations are biochemically associated with one-carbon metabolism. The folate-MTHFR-SAM axis supplies the methyl donor required by DNMTs. Across cohorts, plasma homocysteine levels are elevated in MS (with folate and B_{12} largely unchanged), consistent with impaired methyl-group homeostasis and a reduced SAM:SAH ratio. Associations between *MTHFR* C677T and MS vary across populations, some case-control studies identify risk signals, while others do not, supporting a 'substrate-limitation' model where genetic predisposition interacts with B-vitamin status to constrain DNMT activity and stabilize a proinflammatory methylation landscape (7,142,143).

Several agents modulate immune cell methylomes. Dimethyl fumarate induces coordinated DNA methylation changes in

circulating leukocytes (including $CD4^+$ T cells and monocytes), while interferon-β produces targeted, cell-type-specific methylation shifts, demonstrating that MS-relevant epigenetic programs are reversible and may be corrected in tandem with the restoration of one-carbon flux (138,144-146).

CeD. CeD is an autoimmune enteropathy triggered by dietary gluten in genetically susceptible individuals carrying *HLA-DQ2* or *HLA-DQ8* haplotypes (147). Beyond its genetic predisposition, CeD exhibits distinctive DNA methylation alterations in intestinal mucosa and saliva, revealing the importance of gene-environment interactions mediated by epigenetic mechanisms (147-149). Genome-wide studies demonstrate differential methylation within the HLA region, partly independent of genotype, and tissue-specific analyses confirm mucosal remodeling (148). Remarkably, saliva methylation profiles associate with intestinal patterns, supporting their potential as non-invasive biomarkers (150).

A compelling mechanistic explanation arises from the interplay between intestinal pathology and one-carbon metabolism. Untreated CeD leads to villous atrophy, causing malabsorption of key nutrients, including folate, thereby disrupting the MTHFR-dependent remethylation cycle. Common *MTHFR* variants may further impair this pathway. Consistent with this model, hyperhomocysteinemia is frequently observed at diagnosis and typically improves with a gluten-free diet; however, in patients with *MTHFR* variants, elevated homocysteine may persist despite supplementation. At the tissue level, global hypomethylation signals, such as LINE-1 hypomethylation, have been identified in CeD-associated intestinal mucosa, consistent with substrate limitation of SAM-dependent methylation (151).

This convergence of factors constrains SAM synthesis, limiting DNMT activity and thereby compromising maintenance of the methylome. It provides a biochemical explanation for the epigenetic alterations observed in CeD (152-154). The resulting model establishes a mechanistic cascade associating dietary triggers (gluten), intestinal injury (malabsorption), metabolic disruption (folate/MTHFR deficiency) and epigenetic dysregulation (SAM/DNMTs imbalance). Clinically, residual folate insufficiency has been documented even in treated cohorts, emphasizing the need to ensure methyl-donor adequacy. Methylation profiles, including saliva-based assays, may assist in diagnosis, monitoring and assessment of dietary adherence (148,153,154).

FM. Although FM is not a classical autoimmune disease, it consistently presents with epigenetic abnormalities intersecting immune and neuroendocrine pathways. Genome-wide and candidate-region studies reveal global DNA hypomethylation and locus-specific changes in peripheral blood, enriched for stress-response, immune-inflammatory and central-sensitization pathways. These alterations, replicated across independent cohorts, involve disease-relevant loci such as *COMT* and *BDNF* (155-160).

Mechanistically, these epigenetic findings align with one-carbon metabolism. A 'substrate-limitation' model in FM is supported by elevated homocysteine levels observed in FM/chronic fatigue syndrome cohorts, by associations between symptoms and the *MTHFR* C677T (rs1801133) variant, and by gene-environment interactions showing that rs1801133 modifies the effect of physical activity on fatigue. Together, these findings indicate that genetic variation and B-vitamin status can limit SAM availability and consequently DNMT activity (161-163).

The translational implications are direct. Blood methylation panels already demonstrate diagnostic and prognostic potential. Clinical trials further provide proof-of-concept: Vitamin B_{12} supplementation markedly improves symptom severity and anxiety in patients with FM, consistent with restoration of one-carbon flux and normalization of methylation-dependent pathways. Similarly, folate and B_{12} supplementation have been shown to enhance leukocyte and mucosal DNA methylation in colorectal adenoma cohorts, confirming that methyl-donor interventions can modulate the human methylome. Despite heterogeneity and modest sample sizes, these findings establish a functional association between nutrient availability, *MTHFR*-dependent SAM synthesis and epigenetic control, underscoring the rationale for larger, mechanistically informed intervention studies (106,164,165).

These disease-specific epigenetic and metabolic patterns, encompassing RA, SLE, MS, CeD and FM, are summarized in a comparative framework (Table IV), providing an integrated overview of autoimmune diseases and related disorders.

7. Clinical implications and therapeutic potential

Dietary interventions and folate supplementation. A systematic review and meta-analysis evaluated the dose-response relationship between folate intake and changes in blood biomarkers. The review included 120 clinical trials in healthy participants with study durations ranging from 3 to 144 weeks. Doses of 375-570 μg/day produced a 1.7-fold increase in erythrocyte folate concentrations relative to baseline (95% CI, 1.66-1.93), reaching normalization by week 36. The analysis reported moderate heterogeneity among studies (posterior predictive interval=1.37-2.34) (166).

Individuals carrying the *MTHFR* C677T TT genotype (homozygous mutant) exhibit lower folate and higher homocysteine concentrations, often presenting with fatigue, irritability and megaloblastic anemia. By contrast, those with the *MTHFR* A1298C CC genotype (homozygous mutant) tend to have higher folate concentrations without overt clinical symptoms, although vitamin B_{12} deficiency may be masked (63).

In RA, methotrexate, a folate antagonist with gastrointestinal toxicity, is a cornerstone therapy. Meta-analytic data indicate that folic or folinic acid supplementation <5 mg/day reduces gastrointestinal adverse effects by 79% (odds ratio=0.21; 95% CI, 0.10-0.44) but does not notably modify disease activity as measured by tender-joint count (167).

Conversely, in SLE, methyl-donor-rich diets or folic acid supplementation have been proposed to modulate epigenetic mechanisms underlying proinflammatory gene expression. Experimental evidence has demonstrated that folic acid can epigenetically silence the *IRF5* gene, a key driver of TNF-α synthesis and suppress type I and III interferon pathways, both commonly overexpressed in SLE (168).

In MS, a recent systematic review and meta-analysis assessed folate metabolism and epigenetic implications. No notable differences were found in folate levels between patients and controls (weighted mean difference [WMD]=0.00 μg/l;

Table IV. Epigenetic and metabolic comparison of autoimmune and associated disorders, a summary of converging evidence associating one-carbon metabolism dysregulation to epigenetic alterations, particularly DNA hypomethylation, across autoimmune and associated disorders.

Feature	RA	SLE	MS	CeD	FM
Primary pathophysiology	Chronic systemic autoimmune disorder characterized by persistent joint inflammation (synovitis).	Systemic autoimmune disease-causing widespread inflammation and multi-organ damage.	Immune-mediated neurodegenerative disorder of the CNS causing demyelination.	Autoimmune enteropathy triggered by gluten, resulting in villous intestinal malabsorption.	Neuro-sensory disorder with chronic pain and fatigue; not a classical autoimmune disease but involves immune dysregulation.
Key epigenetic signature	Global hypomethylation in early diseases and cell-type specific changes.	Global DNA hypomethylation in lymphocytes (especially $CD4^+$ T cells) as a defining molecular hallmark.	Widespread, dynamic methylation shifts in T cells and monocytes.	Altered DNA methylation in intestinal mucosa and saliva; hypomethylation in the HLA region.	Global DNA hypomethylation in peripheral blood, involving stress and pain-regulation pathways.
Affected cells/tissues	$CD4^+$ T cells, synovial fibroblasts, circulating leukocytes.	$CD4^+$ T cells, lymphocytes.	$CD4^+$/$CD8^+$ T cells, monocytes, CNS tissue.	Intestinal epithelium/mucosa, saliva.	Peripheral blood leukocytes.
Role of one-carbon metabolism	*MTHFR* variants and low folate reduce SAM levels, impairing methylation. Methylation status predicts response to MTX.	*MTHFR* variants (C677T) increase susceptibility. Low folate/B_{12} leads to a reduced SAM:SAH ratio, inhibiting methylation.	Elevated homocysteine indicates impaired methyl-group metabolism. A 'substrate-limitation' model has been proposed.	Gluten-induced malabsorption causes folate deficiency, leading to hyperhomocysteinemia and limited SAM availability for methylation.	Elevated homocysteine and symptom association with the *MTHFR* C677T support a 'substrate-limitation' model.
Key genes and pathways	*TNF*, *IL*-6, *FOXP3* (Treg stability).	*IFI44L* (biomarker), *IL*-6, *IL*-17, *IL*-2, *FOXP3* (Treg stability).	Th1/Th17 proinflammatory programs, Treg networks, myelin-associated genes.	*HLA* region, *RNF5*.	Stress-response genes (*COMT*, *BDNF*), immune-inflammatory pathways.
Clinical implications	*TNF* methylation predicts biological response; methylation profiles predict MTX response.	*IFI44L* hypomethylation serves as a diagnostic biomarker; restoration of one-carbon flux is a potential therapy.	Methylation patterns associate with disability and are modified by treatments (for example dimethyl fumarate), suggesting therapeutic targets.	Saliva methylation profiles may serve as non-invasive biomarkers; diet and folate status are key for management.	Blood methylation panels show diagnostic potential; B-vitamin supplementation has demonstrated clinical benefits.

BDNF, brain-derived neurotrophic factor; CeD, celiac disease; CNS, central nervous system; COMT, catechol-O-methyltransferase; FM, fibromyalgia; HLA, human leukocyte antigen; MS, multiple sclerosis; MTX, methotrexate; MTHFR, methylenetetrahydrofolate reductase; RA, rheumatoid arthritis; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SLE, systemic lupus erythematosus; Treg, regulatory T cell.

95% CI, -0.01 to 0.01; I^2=0%). However, homocysteine levels were notably increased in MS (WMD=2.47 μmol/; 95% CI, 0.40 to 4.55; I^2=92%), suggesting a potential association between altered one-carbon metabolism, inflammation and disease activity (169).

Patients with CeD frequently exhibit low folate concentrations due to persistent enteropathy, inadequate adherence to a gluten-free diet or consumption of non-fortified gluten-free products. Consequently, management strategies should emphasize a well-planned gluten-free diet, folic acid supplementation, nutritional education and the fortification of gluten-free foods (170).

In FM, low dietary folate intake has been inversely associated with disease severity. A study using the Fibromyalgia Impact Questionnaire-Revised reported a negative association between dietary folate intake and symptom burden (r=-0.250; P=0.017) (171). This relationship likely reflects the role of folate in neurotransmitter synthesis, epigenetic regulation, DNA methylation and the modulation of inflammation and oxidative stress (172).

Therapeutic strategies based on epigenetic modulation. A meta-analysis of intervention studies (folic acid supplementation, fortified foods and natural folate sources) in individuals stratified by *MTHFR* C677T genotype included six randomized controlled trials and four quasi-experimental studies, each lasting ≥4 weeks and using doses of 400-1,670 μg DFE. Homozygous TT carriers displayed higher baseline homocysteine and lower folate concentrations compared with CT and CC genotypes. Following supplementation, homocysteine levels decreased across all genotypes; however, serum folate increases were smaller among TT carriers. These data highlight a persistent biochemical vulnerability in individuals with reduced MTHFR activity. Populations of Asian and Latin American ancestry carrying the TT genotype may require higher daily folate intakes, direct supplementation with 5-methyltetrahydrofolate (5-MTHF), prolonged interventions and/or combined B-vitamin therapy (173).

Genotype-specific differences in folate and homocysteine metabolism have implications for methylation-dependent regulatory pathways. Given the central role of folate in one-carbon metabolism, optimizing folate status in TT carriers could enhance epigenetic stability, particularly in tissues sensitive to methylation imbalance (174).

8. Limitations and future perspectives

The main strength of the present review is its comprehensive integration of the biological mechanisms associating one-carbon metabolism and epigenetic regulation in autoimmune diseases, which contributes to the literature, as, to the best of our knowledge, few studies have synthesized these complex interactions. However, it is important to note that the analysis of the present review focused primarily on the folate-dependent pathway, which constitutes a limitation because it excludes other nutrients involved in epigenetic regulation, such as choline, serine and vitamins B_{12} and B_6, all of which participate in SAM availability and overall methylation capacity (175,176).

Current evidence also poses challenges due to the heterogeneity of study designs and methodologies. Several studies rely on small cohorts that do not stratify participants by folate level or *MTHFR* genotype and employ disparate methods to quantify DNA methylation (for example, global, LINE-1 or locus-specific promoter assays) (177-182). These methodological differences hinder the ability to compare findings and to draw firm conclusions regarding the mechanisms associating methylation changes to autoimmune diseases. In addition, the predominance of cross-sectional designs limits the capacity to determine causality between folate deficiency and disease progression.

Future research must move beyond associations toward establishing causality. This requires longitudinal, multi-omics studies that integrate genomics, epigenomics and metabolomics in disease-specific cohorts. Such approaches will enable the identification of more precise molecular markers of one-carbon metabolism dysfunction and its relationship to immune regulation. Once improved definition of these mechanisms are established, the next step should involve targeted interventions. Clinical trials are needed to determine whether restoring methylation capacity, through folate supplementation or strategies that increase SAM availability, actually reduces inflammatory phenotypes. Ultimately, the goal is to validate sensitive biomarkers that support the development of personalized prevention and treatment strategies tailored to the genotype of each patient.

9. Conclusions

The *MTHFR*-folate axis represents a pivotal intersection between metabolism, immune homeostasis and epigenetic regulation in autoimmune diseases. Genetic and environmental perturbations affecting this axis have been consistently associated with epigenetic alterations underlying the pathophysiology of RA and SLE. By contrast, evidence regarding MS, CeD and FM remains heterogeneous and warrants further clarification. Importantly, this axis not only influences DNA and histone methylation but also modulates inflammatory signaling and cellular stress responses, highlighting its potential as both a biomarker and a therapeutic target.

Future research should prioritize clinical trials that integrate genetic background, nutritional status, inflammatory load and emerging biomarkers, such as homocysteine levels and the SAM:SAH ratio, while evaluating the effects of targeted nutritional interventions involving folic acid and vitamin B_{12}. Complementary studies using primary immune cells and tissue-specific models are essential to elucidate how *MTHFR*-related methylation changes translate into functional dysregulation of the immune system. This integrative approach will facilitate the characterization of disease-specific epigenetic and metabolomic profiles, supporting the development of personalized, mechanism-based therapeutic strategies in autoimmune conditions.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

Not applicable.

Authors' contributions

PMNR, RFBS, FJTH and HRCG contribute to conceptualization; PMNR and RFBS contributed to investigation; JFMV contributed to project administration; FJTH, HRCG, JHB and JFV contributed to supervision; FJTH, HRCG, JHB, COHR, SRDLS and JFMV contributed to validation; PPMNR, RFBS and FJTH contributed to visualization; PMNR and RFBS contributed to writing of the original draft; FJTH, HRCG, JHB, COHR, SRDLS and JFMV contributed to review and editing. All authors read and approved the final manuscript. Data authentication not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Song Y, Li J and Wu Y: Evolving understanding of autoimmune mechanisms and new therapeutic strategies of autoimmune disorders. Signal Transduct Target Ther 9: 263, 2024.
2. Danieli MG, Casciaro M, Paladini A, Bartolucci M, Sordoni M, Shoenfeld Y and Gangemi S: Exposome: Epigenetics and autoimmune diseases. Autoimmun Rev 23: 103584, 2024.
3. Gurugubelli KR and Ballambattu VB: Perspectives on folate with special reference to epigenetics and neural tube defects. Reprod Toxicol 125: 108576, 2024.
4. Souza LL, da Mota JCNL, Carvalho LM, Ribeiro AA, Caponi CA, Pinhel MAS, Costa-Fraga N, Diaz-Lagares A, Izquierdo AG, Nonino CB, *et al*: Genome-wide impact of folic acid on DNA methylation and gene expression in lupus adipocytes: An in vitro study on obesity. Nutrients 17: 1086, 2025.
5. Lu M, Peng K, Song L, Luo L, Liang P and Liang Y: Association between Genetic polymorphisms in Methylenetetrahydrofolate reductase and risk of autoimmune diseases: A systematic review and meta-analysis. Dis Markers 2022: 4568145, 2022.
6. Tsai TY, Lee TH, Wang HH, Yang TH, Chang IJ and Huang YC: Serum Homocysteine, Folate, and vitamin B_{12} levels in patients with systemic lupus Erythematosus: A meta-analysis and meta-regression. J Am Coll Nutr 40: 443-453, 2021.
7. Dardiotis E, Arseniou S, Sokratous M, Tsouris Z, Siokas V, Mentis AA, Michalopoulou A, Andravizou A, Dastamani M, Paterakis K, *et al*: Vitamin B12, folate, and homocysteine levels and multiple sclerosis: A meta-analysis. Mult Scler Relat Disord 17: 190-197, 2017.
8. Nomair AM, Abdelati A, Dwedar FI, Elnemr R, Kamel YN and Nomeir HM: The impact of folate pathway variants on the outcome of methotrexate therapy in rheumatoid arthritis patients. Clin Rheumatol 43: 971-983, 2024.
9. Crider KS, Yang TP, Berry RJ and Bailey LB: Folate and DNA methylation: A review of molecular mechanisms and the evidence for Folate's role. Adv Nutr 3: 21-38, 2012.
10. Coppedè F, Denaro M, Tannorella P and Migliore L: Increased MTHFR promoter methylation in mothers of Down syndrome individuals. Mutat Res 787: 1-6, 2016.
11. Sun H, Song K, Zhou Y, Ding JF, Tu B, Yang JJ, Sha JM, Zhao JY, Zhang Y and Tao H: MTHFR epigenetic derepression protects against diabetes cardiac fibrosis. Free Radic Biol Med 193: 330-341, 2022.
12. Prasad S, Adivikolanu H, Banerjee A, Mittal M, Lemos JRN, Mittal R and Hirani K: The role of microRNAs and long non-coding RNAs in epigenetic regulation of T cells: Implications for autoimmunity. Front Immunol 16: 1695894, 2025.
13. Nuermaimaiti K, Li T, Li N, Shi T, Liu W, Abulaiti P, Abulaihaiti K and Gao F: Vitamin and trace elements imbalance are very common in adult patients with newly diagnosed Celiac disease. Sci Rep 15: 28315, 2025.
14. Fusco R, Siracusa R, D'Amico R, Peritore AF, Cordaro M, Gugliandolo E, Crupi R, Impellizzeri D, Cuzzocrea S and Di Paola R: Melatonin plus folic acid treatment ameliorates reserpine-induced fibromyalgia: An evaluation of pain, oxidative stress, and inflammation. Antioxidants (Basel) 8: 628, 2019.
15. Nielsen HM and Tost J: Epigenetic Changes in Inflammatory and Autoimmune Diseases. In: Epigenetics: Development and Disease. Kundu TK (ed). Vol 61. Springer Netherlands, Dordrecht, pp455-478, 2013.
16. Surace AEA and Hedrich CM: The role of epigenetics in autoimmune/inflammatory disease. Front Immunol 10: 1525, 2019.
17. Funes SC, Fernández-Fierro A, Rebolledo-Zelada D, Mackern-Oberti JP and Kalergis AM: Contribution of Dysregulated DNA methylation to autoimmunity. Int J Mol Sci 22: 11892, 2021.
18. Wills L and Stewart A: Experimental anæmia in monkeys, with special reference to macrocytic nutritional anæmia. Br J Exp Pathol 16: 444-453, 1935.
19. Bastian H: Lucy Wills (1888-1964): The life and research of an adventurous independent woman. J R Coll Phys Edinb 38: 89-91, 2008.
20. Viswanathan M, Urrutia RP, Hudson KN, Middleton JC and Kahwati LC: Folic acid supplementation to prevent neural tube defects: Updated evidence report and systematic review for the US Preventive Services Task Force. JAMA 330: 460-466, 2023.
21. Mitchell HK, Snell EE and Williams RJ: Journal of the American Chemical Society, Vol. 63, 1941: The concentration of 'folic acid' by Herschel K. Mitchell, Esmond E. Snell, and Roger J. Williams. Nutr Rev 46: 324-325, 1988.
22. Rosenberg IH: A history of the isolation and identification of folic acid (folate). Ann Nutr Metab 61: 231-235, 2012.
23. Angier RB, Boothe JH, Hutchings BL, Mowat JH, Semb J, Stokstad EL, Subbarow Y, Waller CW, Cosulich DB, Fahrenbach MJ, *et al*: Synthesis of a compound identical with the *L. casei* factor isolated from liver. Science 102: 227-228, 1945.
24. Kumar Upadhyay A, Prakash A, Kumar A, Jena S, Sinha N and Sharma S: Dr. Sidney Farber (1903-1973): Founder of pediatric pathology and the father of modern chemotherapy. Cureus 16: e68286, 2024.
25. Kutzbach C and Stokstad ELR: Mammalian methylenetetrahydrofolate reductase. Partial purification, properties, and inhibition by S-adenosylmethionine. Biochim Biophys Acta 250: 459-477, 1971.
26. Tamura T and Stokstad ELR: The availability of food folate in man. Br J Haematol 25: 513-532, 1973.
27. Castillo LF, Pelletier CM, Heyden KE and Field MS: New insights into folate-vitamin B12 interactions. Annu Rev Nutr 45: 23-39, 2025.
28. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, *et al*: A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. Nat Genet 10: 111-113, 1995.
29. Loperfido F, Sottotetti F, Bianco I, El Masri D, Maccarini B, Ferrara C, Limitone A, Cena H and De Giuseppe R: Folic acid supplementation in European women of reproductive age and during pregnancy with excessive weight: A systematic review. Reprod Health 22: 13, 2025.
30. Arynchyna-Smith A, Arynchyn AN, Kancherla V, Anselmi K, Aban I, Hoogeveen RC, Steffen LM, Becker DJ, Kulczycki A, Carlo WA and Blount JP: Improvement of serum folate status in the US women of reproductive age with fortified iodised salt with folic acid (FISFA study). Public Health Nutr 27: e218, 2024.

31. Quinn M, Halsey J, Sherliker P, Pan H, Chen Z, Bennett DA and Clarke R: Global heterogeneity in folic acid fortification policies and implications for prevention of neural tube defects and stroke: A systematic review. EClinicalMedicine 67: 102366, 2023.
32. He Q and Li J: The evolution of folate supplementation-from one size for all to personalized, precision, poly-paths. J Transl Int Med 11: 128-137, 2023.
33. Scaglione F and Panzavolta G: Folate, folic acid and 5-methyltetrahydrofolate are not the same thing. Xenobiotica 44: 480-488, 2014.
34. Hoffbrand AV and Weir DG: The history of folic acid. Br J Haematol 113: 579-589, 2001.
35. Mishra VK, Rodriguez-Lecompte JC and Ahmed M: Nanoparticles mediated folic acid enrichment. Food Chem 456: 139964, 2024.
36. Wusigale and Liang L: Folates: Stability and interaction with biological molecules. J Agric Food Res 2: 100039, 2020.
37. Siatka T, Mát'uš M, Moravcová M, Harčárová P, Lomozová Z, Matoušová K, Suwanvecho C, Kujovská Krčmová L and Mladěnka P: Biological, dietetic and pharmacological properties of vitamin B_9. NPJ Sci Food 9: 30, 2025.
38. Erşan S, Chen Y and Park JO: Comprehensive profiling of folates across polyglutamylation and one-carbon states. Metabolomics 21: 71, 2025.
39. Yang M, Wang D, Wang X, Mei J and Gong Q: Role of folate in liver diseases. Nutrients 16: 1872, 2024.
40. Revuelta JL, Serrano-Amatriain C, Ledesma-Amaro R and Jiménez A: Formation of folates by microorganisms: Towards the biotechnological production of this vitamin. Appl Microbiol Biotechnol 102: 8613-8620, 2018.
41. Shulpekova Y, Nechaev V, Kardasheva S, Sedova A, Kurbatova A, Bueverova E, Kopylov A, Malsagova K, Dlamini JC and Ivashkin V: The concept of folic acid in health and disease. Molecules 26: 3731, 2021.
42. U.S. Department of Agriculture, National Agricultural Library: Nutrient Lists from Standard Reference Legacy (2018). https://www.nal.usda.gov/human-nutrition-and-food-safety/nutrient-lists-standard-reference-legacy-2018. Accessed Dec 17, 2025.
43. Spanish Agency for Food Safety and Nutrition: Composition of Foods, 2025. https://www.aesan.gob.es/en/AECOSAN/web/seguridad_alimentaria/subseccion/composicion_alimentos_BD.htm. Accessed Dec 17, 2025.
44. Food and Agriculture Organization of the United Nations: FAO/INFOODS Food Composition Database. https://www.fao.org/infoods/infoods/tables-and-databases/faoinfoods-databases/en/. Accessed Dec 17, 2025.
45. Li J, Duan H, Ramaswamy H and Wang C: A comprehensive review of fortification, bioavailability, and health benefits of folate. Int J Mol Sci 26: 7703, 2025.
46. Beltramo B, Urlings M, Padilla-Díaz CM, Bast A, Diliën H and De Boer A: Bioavailability of vitamins C, B2 and B9 (Folate) in nutrition and health claims: A critical appraisal. Food Prod Process Nutr 7: 55, 2025.
47. Liu F, Kariluoto S, Edelmann M and Piironen V: Bioaccessibility of folate in faba bean, oat, rye and wheat matrices. Food Chem 350: 129259, 2021.
48. Visentin M, Diop-Bove N, Zhao R and Goldman ID: The intestinal absorption of folates. Annu Rev Physiol 76: 251-274, 2014.
49. Pietrzik K, Bailey L and Shane B: Folic acid and L-5-methyltetrahydrofolate: Comparison of clinical pharmacokinetics and pharmacodynamics. Clin Pharmacokinet 49: 535-548, 2010.
50. Zhao R, Matherly LH and Goldman ID: Membrane transporters and folate homeostasis: Intestinal absorption and transport into systemic compartments and tissues. Expert Rev Mol Med 11: e4, 2009.
51. O'Connor C, Wallace-Povirk A, Ning C, Frühauf J, Tong N, Gangjee A, Matherly LH and Hou Z: Folate transporter dynamics and therapy with classic and tumor-targeted antifolates. Sci Rep 11: 6389, 2021.
52. Matherly LH, Schneider M, Gangjee A and Hou Z: Biology and therapeutic applications of the proton-coupled folate transporter. Expert Opin Drug Metab Toxicol 18: 695-706, 2022.
53. Alpers DH: Absorption and blood/cellular transport of folate and cobalamin: Pharmacokinetic and physiological considerations. Biochimie 126: 52-56, 2016.
54. Ebara S: Nutritional role of folate. Congenit Anom (Kyoto) 57: 138-141, 2017.
55. Froese DS, Fowler B and Baumgartner MR: Vitamin B_{12}, folate, and the methionine remethylation cycle-biochemistry, pathways, and regulation. J Inherit Metab Dis 42: 673-685, 2019.
56. Zarou MM, Vazquez A and Vignir Helgason G: Folate metabolism: A re-emerging therapeutic target in haematological cancers. Leukemia 35: 1539-1551, 2021.
57. Bedoui Y, Guillot X, Sélambarom J, Guiraud P, Giry C, Jaffar-Bandjee MC, Ralandison S and Gasque P: Methotrexate an old drug with new tricks. Int J Mol Sci 20: 5023, 2019.
58. Cronstein BN and Aune TM: Methotrexate and its mechanisms of action in inflammatory arthritis. Nat Rev Rheumatol 16: 145-154, 2020.
59. Zhao X, Wu P, Yang Z and Miao RR: Relationship between the efficacy and adverse effects of methotrexate and gene polymorphism. Egypt J Med Hum Genet 25: 89, 2024.
60. Rogers LM, Cordero AM, Pfeiffer CM, Hausman DB, Tsang BL, De-Regil LM, Rosenthal J, Razzaghi H, Wong EC, Weakland AP and Bailey LB: Global folate status in women of reproductive age: A systematic review with emphasis on methodological issues. Ann N Y Acad Sci 1431: 35-57, 2018.
61. Colapinto CK, O'Connor DL, Sampson M, Williams B and Tremblay MS: Systematic review of adverse health outcomes associated with high serum or red blood cell folate concentrations. J Public Health (Oxf) 38: e84-e97, 2016.
62. Cordero AM, Crider KS, Rogers LM, Cannon MJ and Berry RJ: Optimal serum and red blood cell folate concentrations in women of reproductive age for prevention of neural tube defects: World Health Organization guidelines. MMWR Morb Mortal Wkly Rep 64: 421-423, 2015.
63. Novaković R, Geelen A, Ristić-Medić D, Nikolić M, Souverein OW, McNulty H, Duffy M, Hoey L, Dullemeijer C, Renkema JMS, *et al*: Systematic review of observational studies with Dose-response meta-analysis between folate intake and status biomarkers in adults and the elderly. Ann Nutr Metab 73: 30-43, 2018.
64. Zhou Y, Sinnathamby V, Yu Y, Sikora L, Johnson CY, Mossey P and Little J: Folate intake, markers of folate status and oral clefts: An updated set of systematic reviews and meta-analyses. Birth Defects Res 112: 1699-1719, 2020.
65. Choumenkovitch SF, Selhub J, Wilson PW, Rader JI, Rosenberg IH and Jacques PF: Folic acid intake from fortification in United States exceeds predictions. J Nutr 132: 2792-2798, 2002.
66. Zhou Y, Wang A, Yeung LF, Qi YP, Pfeiffer CM and Crider KS: Folate and vitamin B12 usual intake and biomarker status by intake source in United States adults aged ≥19 y: NHANES 2007-2018. Am J Clin Nutr 118: 241-254, 2023.
67. National Institutes of Health: Office of Dietary Supplements: Folate-Consumer, 2022.
68. National Academies Press: Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC, 2011.
69. Zarembska E, Ślusarczyk K and Wrzosek M: The implication of a polymorphism in the methylenetetrahydrofolate reductase gene in homocysteine metabolism and related civilisation diseases. Int J Mol Sci 25: 193, 2023.
70. Tran P, Leclerc D, Chan M, Pai A, Hiou-Tim F, Wu Q, Goyette P, Artigas C, Milos R and Rozen R: Multiple transcription start sites and alternative splicing in the methylenetetrahydrofolate reductase gene result in two enzyme isoforms. Mamm Genome 13: 483-492, 2002.
71. Homberger A, Linnebank M, Winter C, Willenbring H, Marquardt T, Harms E and Koch HG: Genomic structure and transcript variants of the human methylenetetrahydrofolate reductase gene. Eur J Hum Genet 8: 725-729, 2000.
72. Araszkiewicz AF, Jańczak K, Wójcik P, Białecki B, Kubiak S, Szczechowski M and Januszkiewicz-Lewandowska D: MTHFR gene polymorphisms: A single gene with wide-ranging clinical implications-a review. Genes (Basel) 16: 441, 2025.
73. Wrzosek M and Ślusarczyk K: Methylenetetrahydrofolate reductase C677T gene variant in relation to body mass index and folate concentration in a polish population. Biomedicines 10: 3140, 2022.
74. Froese DS, Kopec J, Rembeza E, Bezerra GA, Oberholzer AE, Suormala T, Lutz S, Chalk R, Borkowska O, Baumgartner MR and Yue WW: Structural basis for the regulation of human 5,10-methylenetetrahydrofolate reductase by phosphorylation and S-adenosylmethionine inhibition. Nat Commun 9: 2261, 2018.
75. Sibani S, Christensen B, O'Ferrall E, Saadi I, Hiou-Tim F, Rosenblatt DS and Rozen R: Characterization of six novel mutations in the methylenetetrahydrofolate reductase (MTHFR) gene in patients with homocystinuria. Hum Mutat 15: 280-287, 2000.

76. Weisberg I, Tran P, Christensen B, Sibani S and Rozen R: A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 64: 169-172, 1998.
77. Liew SC and Gupta ED: Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: Epidemiology, metabolism and the associated diseases. Eur J Med Genet 58: 1-10, 2015.
78. Bhatia M, Thakur J, Suyal S, Oniel R, Chakraborty R, Pradhan S, Sharma M, Sengupta S, Laxman S, Masakapalli SK and Bachhawat AK: Allosteric inhibition of MTHFR prevents futile SAM cycling and maintains nucleotide pools in one-carbon metabolism. J Biol Chem 295: 16037-16057, 2020.
79. Wang G and Han JJ: Connections between metabolism and epigenetic modifications in cancer. Med Rev (Berl) 1: 199-221, 2022.
80. Wu W, Shen O, Qin Y, Niu X, Lu C, Xia Y, Song L, Wang S and Wang X: Idiopathic male infertility is strongly associated with aberrant promoter methylation of methylenetetrahydrofolate reductase (MTHFR). PLoS One 5: e13884, 2010.
81. Kulac T, Hekim N, Kocamanoglu F, Beyaz C, Gunes S and Asci R: Methylation patterns of methylenetetrahydrofolate reductase gene promoter in infertile males. Andrologia 53: e13942, 2021.
82. Shaker MM, Shalabi TA and Amr KS: Correlation of methylation status in MTHFR promoter region with recurrent pregnancy loss. J Genet Eng Biotechnol 19: 44, 2021.
83. Saraswathy KN, Kaur L, Talwar S, Mishra J, Huidrom S, Sachdeva MP and Puri M: Methylenetetrahydrofolate reductase gene-specific methylation and recurrent miscarriages: A case-control study from North India. J Hum Reprod Sci 11: 142-147, 2018.
84. Koturbash I, Melnyk S, James SJ, Beland FA and Pogribny IP: Role of epigenetic and miR-22 and miR-29b alterations in the downregulation of Mat1a and Mthfr genes in early preneoplastic livers in rats induced by 2-acetylaminofluorene. Mol Carcinog 52: 318-327, 2013.
85. Su A, Ling F, Vaganay C, Sodaro G, Benaksas C, Dal Bello R, Forget A, Pardieu B, Lin KH, Rutter JC, *et al*: The folate cycle enzyme MTHFR is a critical regulator of cell response to MYC-targeting therapies. Cancer Discov 10: 1894-1911, 2020.
86. Clark DF, Schmelz R, Rogers N, Smith NE and Shorter KR: Acute high folic acid treatment in SH-SY5Y cells with and without MTHFR function leads to gene expression changes in epigenetic modifying enzymes, changes in epigenetic marks, and changes in dendritic spine densities PLoS One 16: e0245005, 2021.
87. Al Sayed R, Smith W, Rogers N, Smith N, Clark D, Castillo G, McLeod H, Glenister S and Shorter KR: A 2x folic acid treatment affects epigenetics and dendritic spine densities in SHSY5Y cells. Biochem Biophys Rep 20: 100681, 2019.
88. Wu C, Gong Y, Sun A, Zhang Y, Zhang C, Zhang W, Zhao G, Zou Y and Ge J: The human MTHFR rs4846049 polymorphism increases coronary heart disease risk through modifying miRNA binding. Nutr Metab Cardiovasc Dis 23: 693-698, 2013.
89. Li C, Ni J, Liu YX, Wang H, Liang ZQ and Wang X: Response of MiRNA-22-3p and MiRNA-149-5p to folate deficiency and the differential regulation of MTHFR expression in normal and cancerous human hepatocytes. PLoS One 12: e0168049, 2017.
90. Zhang S, Zheng F, Zhang L, Huang Z, Huang X, Pan Z, Chen S, Xu C, Jiang Y, Gu S, *et al*: LncRNA HOTAIR-mediated MTHFR methylation inhibits 5-fluorouracil sensitivity in esophageal cancer cells. J Exp Clin Cancer Res 39: 131, 2020.
91. Li C, Li X, Wang H, Guo X, Xue J, Wang X and Ni J: MicroRNA-22-3p and MicroRNA-149-5p inhibit human hepatocellular carcinoma cell growth and metastasis properties by regulating methylenetetrahydrofolate reductase. Curr Issues Mol Biol 44: 952-962, 2022.
92. Glossop JR, Emes RD, Nixon NB, Haworth KE, Packham JC, Dawes PT, Fryer AA, Mattey DL and Farrell WE: Genome-wide DNA methylation profiling in rheumatoid arthritis identifies disease-associated methylation changes that are distinct to individual T- and B-lymphocyte populations. Epigenetics 9: 1228-1237, 2014.
93. Zhao M, Zhou Y, Zhu B, Wan M, Jiang T, Tan Q, Liu Y, Jiang J, Luo S, Tan Y, *et al*: IFI44L promoter methylation as a blood biomarker for systemic lupus erythematosus. Ann Rheum Dis 75: 1998-2006, 2016.
94. Wang J, Dang X, Wu X, Xiang Z, Li Y, Fu Y and Shen T: DNA methylation of IFI44L as a potential blood biomarker for childhood-onset systemic lupus erythematosus. Pediatr Res 96: 494-501, 2024.
95. Li F, Feng Q, Lee C, Wang S, Pelleymounter LL, Moon I, Eckloff BW, Wieben ED, Schaid DJ, Yee V and Weinshilboum RM: Human betaine-homocysteine methyltransferase (BHMT) and BHMT2: Common gene sequence variation and functional characterization. Mol Genet Metab 94: 326-335, 2008.
96. Delgado-Reyes CV, Wallig MA and Garrow TA: Immunohistochemical detection of betaine-homocysteine S-methyltransferase in human, pig, and rat liver and kidney. Arch Biochem Biophys 393: 184-186, 2001.
97. Sunden SLF, Renduchintala MS, Park EI, Miklasz SD and Garrow TA: Betaine-homocysteine methyltransferase expression in porcine and human tissues and chromosomal localization of the human gene. Arch Biochem Biophys 345: 171-174, 1997.
98. Tehlivets O, Malanovic N, Visram M, Pavkov-Keller T and Keller W: S-adenosyl-L-homocysteine hydrolase and methylation disorders: Yeast as a model system. Biochim Biophys Acta 1832: 204-215, 2013.
99. Stipanuk MH: Sulfur amino acid metabolism: Pathways for production and removal of homocysteine and cysteine. Annu Rev Nutr 24: 539-577, 2004.
100. Chiang PK: Biological effects of inhibitors of S-adenosylhomocysteine hydrolase. Pharmacol Ther 77: 115-134, 1998.
101. Mentch SJ, Mehrmohamadi M, Huang L, Liu X, Gupta D, Mattocks D, Gómez Padilla P, Ables G, Bamman MM, Thalacker-Mercer AE, *et al*: Histone methylation dynamics and gene regulation occur through the sensing of one-carbon metabolism. Cell Metab 22: 861-873, 2015.
102. Chen Z, Karaplis AC, Ackerman SL, Pogribny IP, Melnyk S, Lussier-Cacan S, Chen MF, Pai A, John SW, Smith RS, *et al*: Mice deficient in methylenetetrahydrofolate reductase exhibit hyperhomocysteinemia and decreased methylation capacity, with neuropathology and aortic lipid deposition. Hum Mol Genet 10: 433-443, 2001.
103. Friso S, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, Olivieri O, Jacques PF, Rosenberg IH, Corrocher R, *et al*: A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci USA 99: 5606-5611, 2002.
104. Mandaviya PR, Joehanes R, Brody J, Castillo-Fernandez JE, Dekkers KF, Do AN, Graff M, Hänninen IK, Tanaka T, de Jonge EAL, *et al*: Association of dietary folate and vitamin B-12 intake with genome-wide DNA methylation in blood: A large-scale epigenome-wide association analysis in 5841 individuals. Am J Clin Nutr 110: 437-450, 2019.
105. Kok DEG, Dhonukshe-Rutten RAM, Lute C, Heil SG, Uitterlinden AG, van der Velde N, van Meurs JB, van Schoor NM, Hooiveld GJ, de Groot LC, *et al*: The effects of long-term daily folic acid and vitamin B12 supplementation on genome-wide DNA methylation in elderly subjects. Clin Epigenetics 7: 121, 2015.
106. Pufulete M, Al-Ghnaniem R, Khushal A, Appleby P, Harris N, Gout S, Emery PW and Sanders TA: Effect of folic acid supplementation on genomic DNA methylation in patients with colorectal adenoma. Gut 54: 648-653, 2005.
107. Lingappan K: NF-κB in oxidative stress. Curr Opin Toxicol 7: 81-86, 2018.
108. Yu W, Wang Z, Zhang K, Chi Z, Xu T, Jiang D, Chen S, Li W, Yang X, Zhang X, *et al*: One-carbon metabolism supports S-Adenosylmethionine and histone methylation to drive inflammatory macrophages. Mol Cell 75: 1147-1160.e5, 2019.
109. Strickland FM, Li Y, Johnson K, Sun Z and Richardson BC: CD4(+) T cells epigenetically modified by oxidative stress cause lupus-like autoimmunity in mice. J Autoimmun 62: 75-80, 2015.
110. Gorelik GJ, Yarlagadda S, Patel DR and Richardson BC: Protein kinase Cδ oxidation contributes to ERK inactivation in lupus T cells. Arthritis Rheum 64: 2964-2974, 2012.
111. Li Y, Gorelik G, Strickland FM and Richardson BC: Oxidative stress, T cell DNA methylation, and lupus. Arthritis Rheumatol 66: 1574-1582, 2014.
112. Paul DS, Teschendorff AE, Dang MAN, Lowe R, Hawa MI, Ecker S, Beyan H, Cunningham S, Fouts AR, Ramelius A, *et al*: Increased DNA methylation variability in type 1 diabetes across three immune effector cell types. Nat Commun 7: 13555, 2016.
113. Cerna M: Epigenetic regulation in etiology of type 1 diabetes mellitus. Int J Mol Sci 21: 36, 2019.
114. Cheng M, Yang L, Dong Z, Wang M, Sun Y, Liu H, Wang X, Sai N, Huang G and Zhang X: Folic acid deficiency enhanced microglial immune response via the Notch1/nuclear factor kappa B p65 pathway in hippocampus following rat brain I/R injury and BV2 cells. J Cell Mol Med 23: 4795-4807, 2019.

115. Kolb AF and Petrie L: Folate deficiency enhances the inflammatory response of macrophages. Mol Immunol 54: 164-172, 2013.
116. Nakano K, Boyle DL and Firestein GS: Regulation of DNA methylation in rheumatoid arthritis synoviocytes. J Immunol 190: 1297-1303, 2013.
117. Wang G, Siow YL and O K: Homocysteine stimulates nuclear factor kappaB activity and monocyte chemoattractant protein-1 expression in vascular smooth-muscle cells: A possible role for protein kinase C. Biochem J 352: 817-826, 2000.
118. Au-Yeung KKW, Woo CWH, Sung FL, Yip JCW, Siow YL and O K: Hyperhomocysteinemia activates nuclear factor-kappaB in endothelial cells via oxidative stress. Circ Res 94: 28-36, 2004.
119. Webster AP, Plant D, Ecker S, Zufferey F, Bell JT, Feber A, Paul DS, Beck S, Barton A, Williams FMK, *et al*: Increased DNA methylation variability in rheumatoid arthritis-discordant monozygotic twins. Genome Med 10: 64, 2018.
120. Guderud K, Sunde LH, Flåm ST, Mæhlen MT, Mjaavatten MD, Lillegraven S, Aga AB, Evenrød IM, Norli ES, Andreassen BK, *et al*: Rheumatoid arthritis patients, both newly diagnosed and methotrexate treated, show more DNA methylation differences in $CD4^+$ memory than in $CD4^+$ Naïve T cells. Front Immunol 11: 194, 2020.
121. Pitaksalee R, Parmar R, Hodgett R, Emery P and Ponchel F: DNA Hypomethylation in the TNF-alpha gene predicts rheumatoid arthritis classification in patients with early inflammatory symptoms. Cells 12: 2376, 2023.
122. Zafari P, Yari K, Mostafaei S, Iranshahi N, Assar S, Fekri A and Taghadosi M: Analysis of Helios gene expression and Foxp3 TSDR methylation in the newly diagnosed Rheumatoid Arthritis patients. Immunol Invest 47: 632-642, 2018.
123. Cribbs AP, Kennedy A, Penn H, Amjadi P, Green P, Read JE, Brennan F, Gregory B and Williams RO: Methotrexate restores regulatory T cell function through demethylation of the FoxP3 upstream enhancer in patients with rheumatoid arthritis. Arthritis Rheumatol 67: 1182-1192, 2015.
124. Adams C, Nair N, Plant D, Verstappen SMM, Quach HL, Quach DL, Carvidi A, Nititham J, Nakamura M, Graf J, *et al*: Identification of cell-specific differential DNA methylation associated with methotrexate treatment response in rheumatoid arthritis. Arthritis Rheumatol 75: 1088-1097, 2023.
125. Nair N, Plant D, Verstappen SM, Isaacs JD, Morgan AW, Hyrich KL, Barton A and Wilson AG; MATURA investigators: Differential DNA methylation correlates with response to methotrexate in rheumatoid arthritis. Rheumatology (Oxford) 59: 1364-1371, 2020.
126. Gosselt HR, van Zelst BD, de Rotte MCFJ, Hazes JMW, de Jonge R and Heil SG: Higher baseline global leukocyte DNA methylation is associated with MTX non-response in early RA patients. Arthritis Res Ther 21: 157, 2019.
127. Ravaei A, Pulsatelli L, Assirelli E, Ciaffi J, Meliconi R, Salvarani C, Govoni M and Rubini M: MTHFR c.665C>T and c.1298A>C polymorphisms in tailoring personalized Anti-TNF-α therapy for rheumatoid arthritis. Int J Mol Sci 24: 4110, 2023.
128. Nemtsova MV, Zaletaev DV, Bure IV, Mikhaylenko DS, Kuznetsova EB, Alekseeva EA, Beloukhova MI, Deviatkin AA, Lukashev AN and Zamyatnin AA Jr: Epigenetic changes in the pathogenesis of rheumatoid arthritis. Front Genet 10: 570, 2019.
129. Yang C, Li D, Teng D, Zhou Y, Zhang L, Zhong Z and Yang GJ: Epigenetic regulation in the pathogenesis of rheumatoid arthritis. Front Immunol 13: 859400, 2022.
130. Jeffries MA, Dozmorov M, Tang Y, Merrill JT, Wren JD and Sawalha AH: Genome-wide DNA methylation patterns in CD4+ T cells from patients with systemic lupus erythematosus. Epigenetics 6: 593-601, 2011.
131. Hanaei S, Sanati G, Zoghi S, Gharibzadeh S, Ziaee V and Rezaei N: The status of FOXP3 gene methylation in pediatric systemic lupus erythematosus. Allergol Immunopathol (Madr) 48: 332-338, 2020.
132. Ribeiro AA, Carvalho LM, Da Mota JCNL, Nonino CB, Gualano B, Nunes JAV, Martinez JA and Nicoletti CF: Diet, DNA methylation and systemic lupus erythematosus: Evidence and perspectives focused on personalized nutrition. Lifestyle Genomics 17: 31-40, 2024.
133. Liu X, Zhou S, Huang M, Zhao M, Zhang W, Liu Q, Song K, Wang X, Liu J, OuYang Q, *et al*: DNA methylation and whole-genome transcription analysis in $CD4^+$ T cells from systemic lupus erythematosus patients with or without renal damage. Clin Epigenetics 16: 98, 2024.
134. da Mota JCNL, Carvalho LM, Ribeiro AA, Souza LL, Borba EF, Roschel H, Gualano B and Nicoletti CF: Methyl-donor supplementation in women with systemic lupus erythematosus with different nutritional status: The protocol for a randomised, double-blind, placebo-controlled trial. Lupus Sci Med 11: e001279, 2024.
135. Ferreira RC, Simons HZ, Thompson WS, Rainbow DB, Yang X, Cutler AJ, Oliveira J, Castro Dopico X, Smyth DJ, Savinykh N, *et al*: Cells with Treg-specific FOXP3 demethylation but low CD25 are prevalent in autoimmunity. J Autoimmun 84: 75-86, 2017.
136. Coit P, Jeffries M, Altorok N, Dozmorov MG, Koelsch KA, Wren JD, Merrill JT, McCune WJ and Sawalha AH: Genome-wide DNA methylation study suggests epigenetic accessibility and transcriptional poising of interferon-regulated genes in naïve CD4+ T cells from lupus patients. J Autoimmun 43: 78-84, 2013.
137. Tiane A, Schepers M, Reijnders RA, van Veggel L, Chenine S, Rombaut B, Dempster E, Verfaillie C, Wasner K, Grünewald A, *et al*: From methylation to myelination: Epigenomic and transcriptomic profiling of chronic inactive demyelinated multiple sclerosis lesions. Acta Neuropathol 146: 283-299, 2023.
138. Carlström KE, Ewing E, Granqvist M, Gyllenberg A, Aeinehband S, Enoksson SL, Checa A, Badam TVS, Huang J, Gomez-Cabrero D, *et al*: Therapeutic efficacy of dimethyl fumarate in relapsing-remitting multiple sclerosis associates with ROS pathway in monocytes. Nat Commun 10: 3081, 2019.
139. Pinto-Medel MJ, Oliver-Martos B, Urbaneja-Romero P, Hurtado-Guerrero I, Ortega-Pinazo J, Serrano-Castro P, Fernández Ó and Leyva L: Global methylation correlates with clinical status in multiple sclerosis patients in the first year of IFNbeta treatment. Sci Rep 7: 8727, 2017.
140. Maltby VE, Graves MC, Lea RA, Benton MC, Sanders KA, Tajouri L, Scott RJ and Lechner-Scott J: Genome-wide DNA methylation profiling of CD8+ T cells shows a distinct epigenetic signature to CD4+ T cells in multiple sclerosis patients. Clin Epigenetics 7: 118, 2015.
141. Bos SD, Page CM, Andreassen BK, Elboudwarej E, Gustavsen MW, Briggs F, Quach H, Leikfoss IS, Bjølgerud A, Berge T, *et al*: Genome-wide DNA methylation profiles indicate CD8+ T cell hypermethylation in multiple sclerosis. PLoS One 10: e0117403, 2015.
142. Azizi S, Shamshirian A, Alizadeh-Navaei R, Jafarpour H, Asemi Z, Tamtaji OR, Vaziri MS, Homayounfar R, Rezaei Shahmirzadi A and Alipoor R: A genetic association study of MTHFR C677T polymorphism with risk of metabolic syndrome: A systematic review and meta-analysis. Galen Med J 8: e1472, 2019.
143. Cevik B, Yigit S, Karakus N, Aksoy D, Kurt S and Ates O: Association of methylenetetrahydrofolate reductase gene C677T polymorphism with multiple sclerosis in Turkish patients. J Investig Med 62: 980-984, 2014.
144. Xavier A, Campagna MP, Maltby VE, Kilpatrick T, Taylor BV, Butzkueven H, Ponsonby AL, Scott RJ, Jokubaitis VG, Lea RA, *et al*: Interferon beta treatment is a potent and targeted epigenetic modifier in multiple sclerosis. Front Immunol 14: 1162796, 2023.
145. Holm Hansen R, Højsgaard Chow H, Christensen JR, Sellebjerg F and von Essen MR: Dimethyl fumarate therapy reduces memory T cells and the CNS migration potential in patients with multiple sclerosis. Mult Scler Relat Disord 37: 101451, 2020.
146. Maltby VE, Lea RA, Ribbons KA, Sanders KA, Kennedy D, Min M, Scott RJ and Lechner-Scott J: DNA methylation changes in $CD4^+$ T cells isolated from multiple sclerosis patients on dimethyl fumarate. Mult Scler J Exp Transl Clin 4: 2055217318787826, 2018.
147. Cielo D, Galatola M, Fernandez-Jimenez N, De Leo L, Garcia-Etxebarria K, Loganes C, Tommasini A, Not T, Auricchio R, Greco L, *et al*: Combined analysis of methylation and gene expression profiles in separate compartments of small bowel mucosa identified celiac disease patients' signatures. Sci Rep 9: 10020, 2019.
148. Fernandez-Jimenez N, Garcia-Etxebarria K, Plaza-Izurieta L, Romero-Garmendia I, Jauregi-Miguel A, Legarda M, Ecsedi S, Castellanos-Rubio A, Cahais V, Cuenin C, *et al*: The methylome of the celiac intestinal epithelium harbours genotype-independent alterations in the HLA region. Sci Rep 9: 1298, 2019.
149. Fernandez-Jimenez N, Castellanos-Rubio A, Plaza-Izurieta L, Irastorza I, Elcoroaristizabal X, Jauregi-Miguel A, Lopez-Euba T, Tutau C, de Pancorbo MM, Vitoria JC, *et al*: Coregulation and modulation of NFκB-related genes in celiac disease: Uncovered aspects of gut mucosal inflammation. Hum Mol Genet 23: 1298-1310, 2014.

150. Hearn NL, Coleman AS, Ho V, Chiu CL and Lind JM: Comparing DNA methylation profiles in saliva and intestinal mucosa. BMC Genomics 20: 163, 2019.
151. Libera L, Vanoli A, Sahnane N, Adnan M, Guerini C, Arpa G, Bianchi PI, Lenti MV, Corazza GR, La Rosa S, *et al*: LINE-1 hypomethylation characterizes the inflammatory response in coeliac disease associated-intestinal mucosa and small bowel adenocarcinomas. J Pathol 265: 99-109, 2025.
152. Cardo A, Churruca I, Lasa A, Navarro V, Vázquez-Polo M, Perez-Junkera G and Larretxi I: Nutritional imbalances in adult celiac patients following a gluten-free diet. Nutrients 13: 2877, 2021.
153. Valente FX, Campos TN, Moraes LFDS, Hermsdorff HH, Cardoso LM, Pinheiro-Sant'Ana HM, Gilberti FA and Peluzio MC: B vitamins related to homocysteine metabolism in adults celiac disease patients: A cross-sectional study. Nutr J 14: 110, 2015.
154. Wierdsma NJ, van Bokhorst-de van der Schueren MA, Berkenpas M, Mulder CJ and van Bodegraven AA: Vitamin and mineral deficiencies are highly prevalent in newly diagnosed celiac disease patients. Nutrients 5: 3975-3992, 2013.
155. Polli A, Ghosh M, Bakusic J, Ickmans K, Monteyne D, Velkeniers B, Bekaert B, Godderis L and Nijs J: DNA methylation and brain-derived neurotrophic factor expression account for symptoms and widespread hyperalgesia in patients with chronic fatigue syndrome and comorbid fibromyalgia. Arthritis Rheumatol 72: 1936-1944, 2020.
156. Lee YH, Kim JH and Song GG: Association between the COMT Val158Met polymorphism and fibromyalgia susceptibility and fibromyalgia impact questionnaire score: A meta-analysis. Rheumatol Int 35: 159-166, 2015.
157. Ovrom EA, Mostert KA, Khakhkhar S, McKee DP, Yang P and Her YF: A comprehensive review of the genetic and epigenetic contributions to the development of fibromyalgia. Biomedicines 11: 1119, 2023.
158. Gerra MC, Carnevali D, Ossola P, González-Villar A, Pedersen IS, Triñanes Y, Donnini C, Manfredini M, Arendt-Nielsen L and Carrillo-de-la-Peña MT: DNA methylation changes in fibromyalgia suggest the role of the immune-inflammatory response and central sensitization. J Clin Med 10: 4992, 2021.
159. Przybylowicz PK, Sokolowska KE, Rola H and Wojdacz TK: DNA methylation changes in blood cells of fibromyalgia and chronic fatigue syndrome patients. J Pain Res 16: 4025-4036, 2023.
160. Ciampi de Andrade D, Maschietto M, Galhardoni R, Gouveia G, Chile T, Victorino Krepischi AC, Dale CS, Brunoni AR, Parravano DC, Cueva Moscoso AS, *et al*: Epigenetics insights into chronic pain: DNA hypomethylation in fibromyalgia-a controlled pilot-study. Pain 158: 1473-1480, 2017.
161. Estévez-López F, Salazar-Tortosa DF, Camiletti-Moirón D, Gavilán-Carrera B, Aparicio VA, Acosta-Manzano P, Segura-Jiménez V, Álvarez-Gallardo IC, Carbonell-Baeza A, Munguía-Izquierdo D, *et al*: Fatigue in women with fibromyalgia: A gene-physical activity interaction study. J Clin Med 10: 1902, 2021.
162. Inanir A, Yigit S, Tekcan A, Pinarli FA, Inanir S and Karakus N: Angiotensin converting enzyme and methylenetetrahydrofolate reductase gene variations in fibromyalgia syndrome. Gene 564: 188-192, 2015.
163. Regland B, Forsmark S, Halaouate L, Matousek M, Peilot B, Zachrisson O and Gottfries CG: Response to vitamin B12 and folic acid in myalgic encephalomyelitis and fibromyalgia. PLoS One 10: e0124648, 2015.
164. Gharibpoor F, Ghavidel-Parsa B, Sattari N, Bidari A, Nejatifar F and Montazeri A: Effect of vitamin B12 on the symptom severity and psychological profile of fibromyalgia patients; a prospective pre-post study. BMC Rheumatol 6: 51, 2022.
165. Huang C, Zhang N, Wei M, Pan Q, Cheng C, Lu KE, Mo J and Chen Y: Methylation factors as biomarkers of fibromyalgia. Ann Transl Med 11: 169-169, 2023.
166. Crider KS, Devine O, Qi YP, Yeung LF, Sekkarie A, Zaganjor I, Wong E, Rose CE and Berry RJ: Systematic review and Bayesian Meta-analysis of the Dose-response relationship between folic acid intake and changes in blood folate concentrations. Nutrients 11: 71, 2019.
167. Rennie KL, Hughes J, Lang R and Jebb SA: Nutritional management of rheumatoid arthritis: A review of the evidence. J Hum Nutr Diet 16: 97-109, 2003.
168. Nikolova-Ganeva K and Tchorbanov A: Folic acid in systemic lupus erythematosus-a new aspect. Clin Rheumatol 42: 1729-1730, 2023.
169. Kirsty CW, Mary H and Sumner J: The relationship of cobalamin and/or folate to the patient-centred outcomes in multiple sclerosis: A systematic review and meta-analysis. Nutr Health 28: 527-542, 2022.
170. Lamjadli S, Oujamaa I, Souli I, Eddehbi FE, Lakhouaja N, M'raouni B, Salami A, Guennouni M, Belghali MY, Hazime R and Admou B: Micronutrient deficiencies in patients with celiac disease: A systematic review and meta-analysis. Int J Immunopathol Pharmacol 39: 3946320241313426, 2025.
171. Bennett RM, Friend R, Jones KD, Ward R, Han BK and Ross RL: The revised fibromyalgia impact questionnaire (FIQR): Validation and psychometric properties. Arthritis Res Ther 11: R120, 2009.
172. Correa-Rodríguez M, Rueda-Medina B, Casas-Barragán A, Tapia-Haro RM, Molina F and Aguilar-Ferrándiz ME: Dietary intake assessment, severity of symptoms, and pain in women with fibromyalgia. Clin Nurs Res 30: 1164-1173, 2021.
173. Colson NJ, Naug HL, Nikbakht E, Zhang P and McCormack J: The impact of MTHFR 677 C/T genotypes on folate status markers: A meta-analysis of folic acid intervention studies. Eur J Nutr 56: 247-260, 2017.
174. Kumar A, Palfrey HA, Pathak R, Kadowitz PJ, Gettys TW and Murthy SN: The metabolism and significance of homocysteine in nutrition and health. Nutr Metab (Lond) 14: 78, 2017.
175. Łoboś P and Regulska-Ilow B: Link between methyl nutrients and the DNA methylation process in the course of selected diseases in adults. Rocz Panstw Zakl Hig 72: 123-136, 2021.
176. van Vliet MM, Schoenmakers S, Gribnau J and Steegers-Theunissen RPM: The one-carbon metabolism as an underlying pathway for placental DNA methylation-a systematic review. Epigenetics 19: 2318516, 2024.
177. Wernimont SM, Clark AG, Stover PJ, Wells MT, Litonjua AA, Weiss ST, Gaziano JM, Tucker KL, Baccarelli A, Schwartz J, *et al*: Folate network genetic variation, plasma homocysteine, and global genomic methylation content: A genetic association study. BMC Med Genet 12: 150, 2011.
178. Bakulski KM, Dou JF, Feinberg JI, Brieger KK, Croen LA, Hertz-Picciotto I, Newschaffer CJ, Schmidt RJ and Fallin MD: Prenatal multivitamin use and MTHFR genotype are associated with newborn cord blood DNA methylation. Int J Environ Res Public Health 17: 9190, 2020.
179. de Oliveira NFP, Persuhn DC and Dos Santos MCLG: Can global DNA methylation be influenced by polymorphisms in genes involved in epigenetic mechanisms? A review. Genes (Basel) 15: 1504, 2024.
180. Dević Pavlić S, Šverko R, Barišić A, Mladenić T, Vraneković J, Stanković A, Peterlin A, Peterlin B, Ostojić S and Pereza N: MTHFR gene polymorphisms and DNA methylation in idiopathic spontaneous preterm birth. Medicina (Kaunas) 60: 2028, 2024.
181. Majstorović D, Stoccoro A, Barišić A, Buretić Tomljanović A, Giangreco M, Nicolì V, Coppedè F and Vraneković J: Increased methylation levels of the MTHFR gene promoter in Down syndrome. Epigenomics 17: 1141-1151, 2025.
182. Tsymbalova EA, Chernyavskaya EA, Bisaga GN, Polushin AY, Lopatina EI, Abdurasulova IN and Lioudyno VI: LINE-1 methylation status in multiple sclerosis patients is associated with changes in folate metabolism. Acta Nat 17: 94-103, 2025.

Copyright © 2026 Navarro-Rodríguez et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.