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

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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

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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

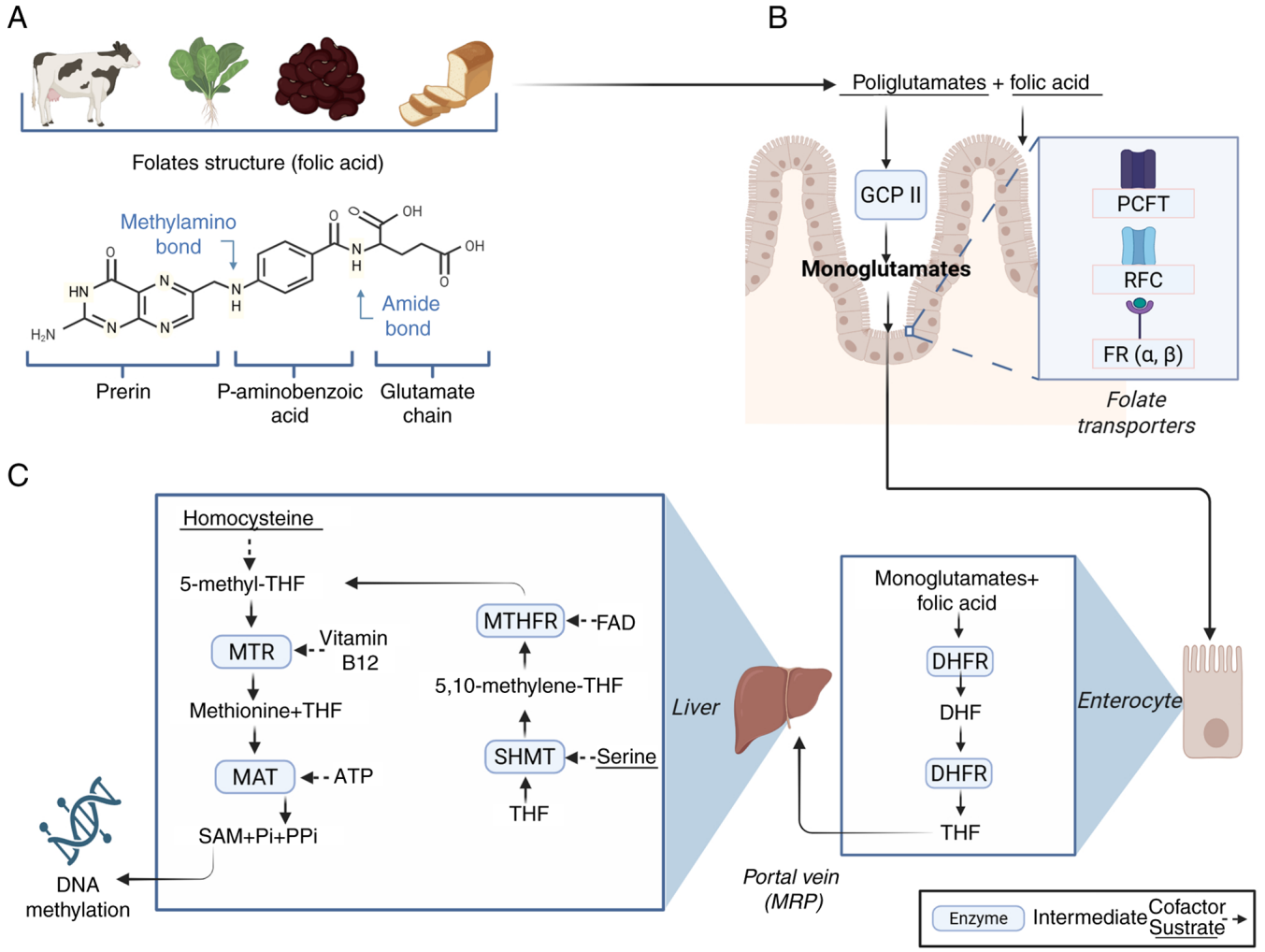

Figure 1. (A) Dietary sources of folate and general structure. Dietary folates, comprising three main components (pterin, *p*-aminobenzoic acid and a glutamate chain), are ingested as polyglutamates and hydrolyzed to monoglutamates by the enzyme GCPII in the intestinal brush border. (B) Intestinal absorption 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).

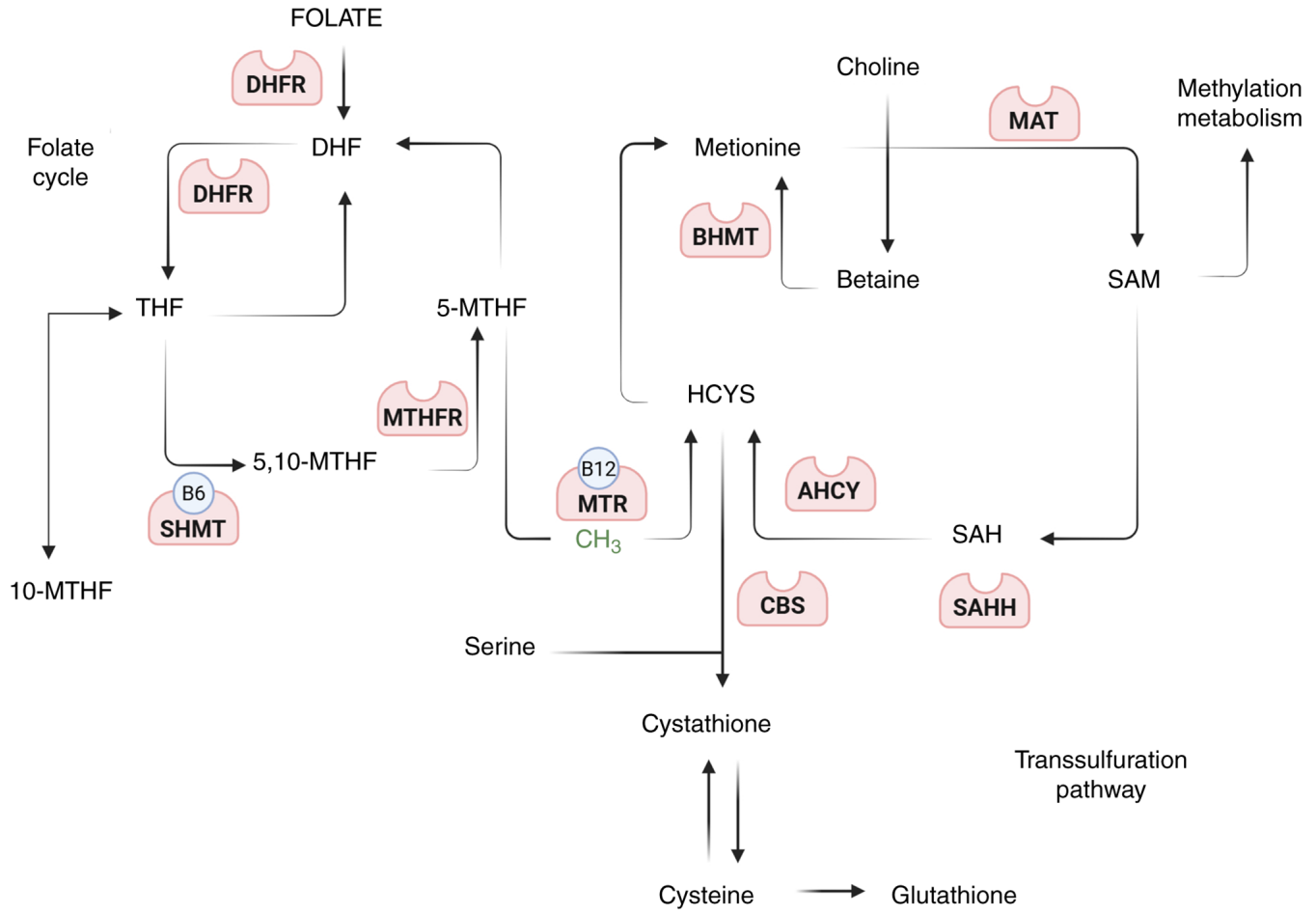

Figure 2. Schematic representation of the folate and methionine cycles illustrating their interconnection with one-carbon metabolism, methylation reactions and transsulfuration. Dietary folate is initially reduced to DHF and THF by DHFR. THF is converted to 5,10-MTHF by SHMT (a vitamin B_6-dependent 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.

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Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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