



S-Adenosyl Methionine and Transmethylation Pathways in Neuropsychiatric Diseases Throughout Life

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Abstract

S-Adenosyl methionine (SAME), as a major methyl donor, exerts its influence on central nervous system function through cellular transmethylation pathways, including the methylation of DNA, histones, protein phosphatase 2A, and several catecholamine moieties. Based on available evidence, this review focuses on the lifelong range of severe neuropsychiatric and neurodegenerative diseases and their associated neuropathologies, which have been linked to the deficiency/load of SAME production or/and the disturbance in transmethylation pathways. Also included in this review are the present-day applications of SAME in the treatment in these diseases in each age group.

Keywords S-Adenosyl-methionine · Transmethylation · Pathway · Psychiatric disease · Neurodegenerative disease

Background

Since its discovery in 1951, S-adenosyl-L-methionine (SAME, also known as AdoMet), has been shown to be second only to adenosine triphosphate when serving as a cofactor [1], and it is involved in a great number of reactions. SAME is found in every living cell and has been commonly used as a dietary supplement since 1952. The liver plays a central role in the homeostasis of SAME through both its synthesis and degradation [2].

Three principal pathways are related to SAME metabolism: transmethylation, trans-sulfuration, and transaminopropylation. The transmethylation pathway is thought to play an especially important role in brain development, reflecting its dominant and ubiquitous effects on cell biology [3].

Most cells contain numerous SAME-dependent methyltransferases that can transfer the methyl group ($-CH_3$) to all varieties of key substrates, for example nucleic acid, protein, phospholipids, and monoamine neurotransmitters [4]. These molecular targets not only support normal biological development, but can also contribute to pathological states in the central nervous system (CNS) when epigenetically methylated at inappropriate times in the human life span.

The notion that a combination of environment and gene-environment interactions modulates brain development has become an accepted tenet of modern neuroscience, which has led to the definition of a new term, “neuroepigenetics”, for example DNA methylation and histone modification (methylation and acetylation) [5]. SAME, as the principal methyl donor in cells, takes part in these critical epigenetic mechanisms, thus connecting nurture-based metabolism with brain development, in addition to other direct targets of methyl group-dependent biological functions in cells.

It is generally recognized that brain development is a long-term equilibrium dynamically vibrating between neurogenesis and neurodegeneration (neural cell deletion) throughout the whole mammalian life span. Emerging studies have linked deficient SAME production and disruption in transmethylation pathways with delayed brain development and severe neuropsychiatric diseases [6].

Besides a broad application in the treatment of hepatic diseases, numerous preclinical and clinical studies over the last 2

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decades demonstrated that S-Adenosyl Methionine (SAME) showed promise in the treatment of critical neuropsychiatric diseases, including Alzheimer's disease (AD) and major depression disorder (MDD) [7, 8]. However, despite its increasing popularity as a supplement, SAME has not been as well studied as more conventional neural psychotherapeutic agents, especially with regards of underlying mechanisms [9]. The purpose of this review is to focus on the mechanistic role of SAME in transmethylation pathways and its relation to neuropsychiatric diseases, as well as to discuss its efficacy.

SAME processing and 1-carbon cycle

The process of generating SAME involves a bicyclic cellular pathway consisting of folate and methionine cycles, which both belong to the 1-carbon cycle, a metabolic network that integrates nutrients from the environment to yield multiple epigenetic regulatory and biological functions [10]. A simplified version of this 1-carbon cycle is illustrated in Figure 1.

SAME is a metabolic product of methionine generated by methionine-adenosyltransferase (MAT), which catalyzes the reaction at the expense of the complete dephosphorylation of 1 molecule of adenosine triphosphate in the presence of potassium and magnesium ions [11]. SAME is then utilized as a donor of its methyl group to methylate many substrates to fulfill diverse biological functions. S-Adenosylhomocysteine (SAH or AdoHC) is produced in this process and then is hydrolyzed to homocysteine (Hcy). This reaction is reversible, during which process increased Hcy levels can lead to the accumulation of SAH, an inhibitor of the transmethylation events related to SAME. Lower levels of SAME and higher levels of SAH may result in a reduced methylation capacity, which is represented by the SAM/SAH ratio [3].

Of note, Hcy can be re-methylated to regenerate methionine by donation of a methyl group from 5-methyltetrahydrofolate (5-MTHF) via methionine synthase (MTR). Then the regenerated methionine goes into next cycle to produce SAME (Fig. 1). B vitamins play important roles in this process, involving vitamin B₁₂ (cobalamin, Cbl) as an essential cofactor and 5-MTHF as an indispensable substrate.

Vitamin B₁₂ is used to mediate the MTR catalyzing process via 2 methyl transfer reactions [12]. Methylcobalamin is the metabolically active form of vitamin B₁₂, and serves as an essential cofactor for the methylation of Hcy to methionine, affecting hundreds of SAME-dependent methylation reactions [13]. In addition, adenosylcobalamin is another metabolically active form of vitamin B₁₂ to be used as a cofactor for the enzyme methylmalonyl CoA mutase, which converts methylmalonylCoA to succinylCoA [14]. Of note, a deficiency in vitamin B₁₂ leads to a functional loss of folate, which results from folate being trapped in the form of methyltetrahydrofolate [15].

5-MTHF is an important product of folate metabolism cycle and this is also the primary circulating form of folate [16]. Dietary folate or dihydrofolate can be reduced to tetrahydrofolate by dihydrofolate reductase. Then tetrahydrofolate is catalyzed by serine hydroxy methyltransferase to produce 5,10-methylenetetrahydrofolate (5,10-MTHF). 5,10-MTHF reductase (5,10-MTHFR) carries out a central reaction in folate metabolism with the production of 5-MTHF, which is required for the biosynthesis of methionine, purine nucleotides, and thymidylate [17].

A failure or abnormality of either of these 2 cycles has been implicated in the pathogenesis of neuropsychiatric diseases. The most common example comes from the deficiency of folate and/or vitamin B₁₂, which would disturb the

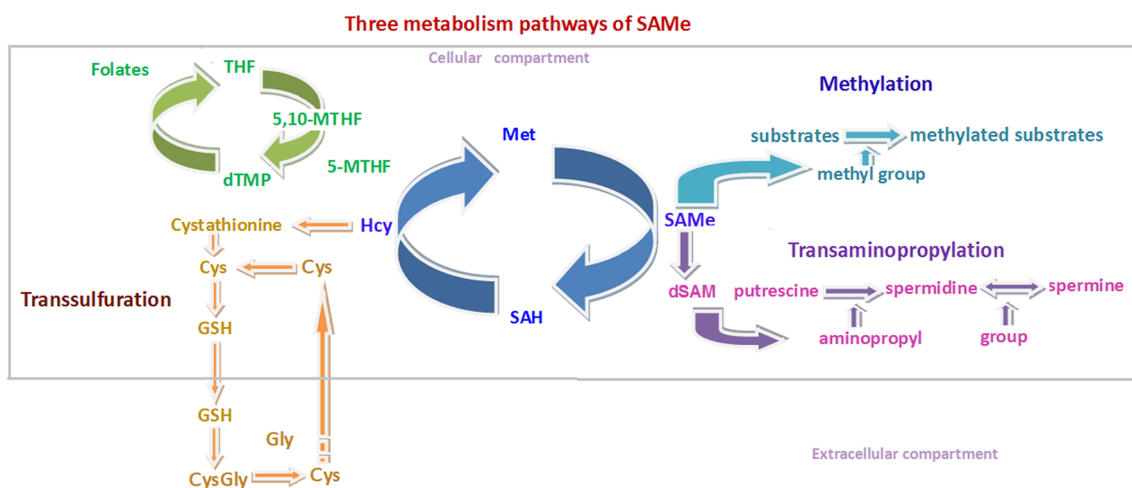


Fig. 1 Three pathways of S-adenosyl-L-methionine (SAME) metabolism and the 1-carbon cycle. The transmethylation pathway (light blue), trans-sulfuration pathway (brown), and transaminopropylation pathway (purple); 1-carbon cycle: the folate cycle (green) and methionine cycle (dark blue). THF = tetrahydrofolate;

5-MTHF = 5-methyltetrahydrofolate; 5, 10 - methylenetetrahydrofolate; Hcy = homocysteine; Cys = cysteine; GSH = glutathione; Gly - Glycine; Met = Methionine; SAH = Sadenosylhomocysteine; dSAM = decarboxylated S-adenosyl-L-methionine

methylation-dependent pathways by incapacitating a source of methyl groups, a phenomenon that has been linked to mental retardation, psychiatric disorders, myelopathy and neurological diseases [9, 18].

The most common condition associated with folate deficiency is affective disorders, such as MDD. Depressed patients with low folate appeared to have greater severity of depression and poorer response to various antidepressants and augmentation strategies [19, 20]. Furthermore, folate deficiency also correlates with dementia and cognitive impairment [21]. The mechanisms underlying this activity include a reduction in purine/pyrimidine synthesis and genomic and/or specific methylation reactions in brain, leading to decreased DNA stability and altered gene expression resulting in the mental dysfunction [22].

Vitamin B₁₂ deficiency is often manifested in the form of neurological symptoms [22]. During vitamin B₁₂ deficiency, Hcy cannot be converted to methionine, leading to Hcy accumulation; methylmalonic acid (MMA) cannot be converted to succinyl-CoA, resulting in MMA accumulation [23]. Both of these consequences contribute to the damage of myelin, resulting in a condition known as subacute combined degeneration of the spinal cord [24]. In addition, SAME production is reduced and methylation reactions are impaired, which are subsequently associated with a reduced methylation capacity [25]. Fernández-Roig et al. [26] reported that vitamin B₁₂ deficiency in the brain led to DNA hypomethylation in the TCb1R/CD320 knockout mouse, which supported that DNA hypomethylation could be a link between vitamin B₁₂ deficiency and neurological pathology [26]. Vitamin B₁₂ can influence cell proliferation and differentiation in brain through protein phosphatase 2 (PP2A), nerve growth factor, and TACE [tumor necrosis factor (TNF)- α ADAM metalloprotease converting enzyme] pathways [13]. Methylcobalamin can facilitate neurite outgrowth and inhibit neural apoptosis through the Erk1/2 and Akt signaling pathways [27], which were implicated in neuropsychiatric diseases. For instance, Zhang et al. [28] found decreased brain levels of vitamin B₁₂ across the lifespan and in autism and schizophrenia. Fortunately, high vitamin B₁₂ levels can help maintain low blood levels of Hcy and MMA, and enhance the synthesis of SAME [9].

SAME is a major end product of the “1-carbon cycle”. The dysregulation of SAME leads to alterations in > 100 methyltransferase reactions, which modulate metabolic pathways [29]. The aberrant methylation has been implicated as a pathogenic mechanism in psychiatric disorders, such as MDD, as outlined in the section of the review (below), and also in neurological diseases, such as AD, as outlined in later sections. Consequently, this methyl-donor therapy has become a therapeutic strategy to deal with disease progression and/or enhance clinical outcome [30]. The detailed interpretation is described below.

Cellular targets of SAME transmethylation pathways pertinent to mental/neurological diseases

SAME and DNA Methylation Status

DNA methylation, as an epigenetic mechanism, plays a pivotal role in the regulation of gene transcription and the establishment and maintenance of cellular identity [31], which is disrupted in many diseases. It allows for sustained adaptability of gene expression in response to developmental or environmental factors [31]. Several DNA methylation marks have been implicated in the dynamics of gene regulation in the CNS. There are 2 kinds of DNA methylation dynamics that include 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC), involving 2 kinds of enzymes as DNA methyltransferases (DNMTs; Dnmt1, Dnmt3a and Dnmt3b) and the demethylases ten-eleven translocation (TET) methylcytosine dioxygenase (Tet1–3), and the methyl-CpG-binding protein 2 (MeCP2). We will interpret these events in the following sections. Tables (1, 2 and 3).

5-mC, derived from the addition of a methyl group from SAME to the 5' position of cytosine, has generally been viewed as a stable covalent modification to DNA [32]. There are 2 types of it in brain: 5mCG and 5mCH (H = A, T, or C), respectively according to whether the cytosine methylated predominantly occurs at cytosine-phosphate-guanine dinucleotides (CpGs) or non-CpGs [33, 34].

In fact, in the mammalian brain ~62% of CpGs are found to be methylated [33]. 5mCG is mainly located at intergenic regions, repetitive DNA regions, and silenced genes, directly interfering with the binding of certain transcriptional regulators to their cognate DNA sequences or recruiting repressive methyl-CpG binding domain (MBD) proteins [33, 35]. This results in transcriptional repression in differentiated neurons, whereas 5mCG levels remain unchanged during neurodevelopment [36].

However, 5mCH is the predominant form of DNA methylation in adult neurons [33, 37]. 5mCH methylation differences occur around neuronal transcription factor binding sites and around transcription start sites, suggesting that it is sufficient to cause transcriptional repression in neurons, as effectively as, but independently of, 5-mCG [38]. Furthermore, neuronal 5mCH is actively maintained by DNMT3a *in vivo* [38], supporting the accumulation of 5mCH in neurons during postnatal neuronal maturation, which makes it an even better signal than 5mCG in this regard [35, 38].

5-hmC is enzymatically modified from 5-mC by TET [39]. Mouse brain tissues contain high levels of 5-hmC [39]. 5hmC levels in neurons are up to 10-fold higher than other cell types, accounting for ~40% of modified cytosine in the brain [38]. 5-hmC was reported to present at many highly methylated CG sites in mouse adult and fetal frontal cortex [40]. 5hmC is the

main modification status for a many CpGs situated at transcriptionally active regions, such as DNaseI-hypersensitive regions and enhancers [35], suggesting its positive correlation with gene expression [41]. Moreover, 5-hmC is also enriched within gene bodies and at intron–exon boundaries of synaptic plasticity-related genes [42]. 5-hmC increases markedly from the fetal to the adult stage and in the adult brain, which implies the importance of 5hmC in neuronal maturation and function [35]. Besides acting as an intermediate in an active DNA demethylation pathway, 5hmC may also serve as a stable neuronal epigenetic mark in its own right [43].

Three DNMTs include 2 *de novo* methyltransferases, Dnmt3a and Dnmt3b [44], and 1 classical maintenance methyltransferase, Dnmt1 [45]. The former establish methylation patterns on unmethylated DNA [44] and the latter preserves existing methylation patterns by methylating hemi-methylated DNA [46]. Three enzymes were confirmed to catalyze the chemical reaction:

SAMe + DNA → SAH + DNA containing 5-methylcytosine.

Suetake et al. [47] reported that the methyl transferases Dnmt3a or Dnmt3b showed similar *K_m* values towards poly(dIdC)-poly(dIdC) and poly(dGdC)-poly(dGdC), suggesting little room for regulator activity dependent on the relative abundance of at least these 2 enzymes between tissues. The *K_m* values for SAMe were not affected by the methyl-group acceptors, poly(dIdC)-poly(dIdC) and poly(dG-dC)-poly(dGdC) [47]. The *K_m* values of Dnmt3a for dIdC and dGdC, respectively, were $1.2 \pm 0.1 \mu\text{M}$ and $1.6 \pm 0.2 \mu\text{M}$; those of Dnmt3b for dIdC and dGdC, respectively, were $0.4 \pm 0.1 \mu\text{M}$ and $0.5 \pm 0.1 \mu\text{M}$; the *K_m* values of Dnmt3b for SAMe were $0.3 \pm 0.1 \mu\text{M}$ and $0.7 \pm 0.3 \mu\text{M}$ [47]. Gros et al. [48] reported that the *K_m* values of Dnmt1 were $4.4 \pm 0.5 \mu\text{M}$ and $0.27 \pm 0.03 \mu\text{M}$ for SAMe and DNA, respectively.

In support of developmental selectivity, Dnmt3a and Dnmt1 are expressed in both the embryonic and adult stages of the brain, whereas Dnmt3b is detectable only during early neurogenesis [49]. Furthermore, there is a transition of mouse *de novo* methyltransferase expression from Dnmt3b to Dnmt3a during neural progenitor cell development [50], suggesting that particular DNMTs play a role in neuronal function at specific times over neuronal development and maturation.

The 3 prominent demethylases are the TETs, which are expressed in the brain, respectively, as Tet1 [39], Tet2, and Tet3 [51]. Tet2 and Tet3 levels are considerably higher than Tet1 postnatally [51]. Tet1 is thought to have a role in neuronal activity-induced, region-specific, active DNA demethylation with concomitant gene expression changes [43]. In addition to its catalytic activity, Tet1 controls DNA methylation levels and thus regulates memory formation [52]. Tet3, the most highly expressed TET in the brain, is activity-dependent in primary cortical neurons [53]. Tet3 activity within the

infralimbic prefrontal cortex is essential for the learning-dependent accumulation of 5-hmC and related chromatin modifications, which underpins rapid behavioral adaptations based on epigenetic activation [53]. Moreover, Tet3-KD neurons or Tetrodotoxin-induced decreases in Tet3 expression exhibited increased CpG methylation at the *BDNF* promoter IV region, whereas bicuculline-induced increases in Tet3 expression were shown to be associated with decreased methylation at the same region, supporting a pivotal role of Tet3 in regulating gene expression in response to global synaptic activity changes [54].

MeCP2 belongs to the MBD protein family, known as “readers” of DNA methylation, which bind methylated DNA to mediate the molecular consequences of this epigenetic mark [55]. MeCP2 is the highest expressed nuclear protein in the brain among various tissues [56], and 7 times higher in neurons than glia [57]. Several studies showed that MeCP2 is important for synapse maturation [57]. MeCP2 has 2 functional domains, transcriptional repression domain, which exhibits a long-range repression and MBD [58]. MBD confers it to bind DNA at CG methylation-dense regions with high levels [55], which also interacts with the nuclear receptor co-repressor/histone deacetylase 3 and Sin3a transcriptional co-repressor complexes, suggesting a role for MeCP2 as a transcriptional repressor [59]. In addition to 5mCG, several studies showed that MeCP2 can also bind to 5mCH in neurons with high affinity [60]. Later, Gabel et al. [61] reported that MeCP2 repressed gene expression by binding to methylated Cornu Ammonis (CA) sites within long genes. Chen et al. [62] also reported that MeCP2 binds to mCH in the mature brain to regulate transcription and the timing of onset for Rett’s syndrome (see below).

The development of brain is a spatial temporally orchestrated process that partly requires appropriate gene regulation through the control of DNA methylation and demethylation [63]. During the differentiation and maturation of CNS, a dynamic process, involving both *de novo* DNA methylation and demethylation, is required. Several studies have established that Dnmt1 plays a critical role in cellular differentiation as well as in dividing cells [64]. Some other findings support that Dnmt3b is required during early development, whereas Dnmt3a is required for normal cellular differentiation [49]. In the dividing cells, passive DNA demethylation occurs as the depletion of Dnmt1 results in newly incorporated cytosine to remain unmethylated and consequent hypomethylation [65]. The coincidence of the differentiation of the astrocytic lineage with DNA methylation and demethylation occurs on the *Gfap* gene promoter region [64]. Thus, the coordinated expression of *Dnmts*, key to the dynamic methylation and demethylation pattern, plays an important role in regulating neuronal development.

Moreover, in several recent studies, Chen et al. [66] found that the mammalian DNMTs (*Dnmt3a* and *Dnmt3b*) can also

act as Ca^{2+} ion- and redox state-dependent active DNA demethylases. In the context of extremely low levels of SAME, *Dnmt3a* and *Dnmt3b* might demethylate 5mC via deamination to thymine, which confirmed the dynamic activity of DNMTs with methyltransferases and demethylases according to the levels of SAME [67].

In addition to acting as the body's principal methyl donor, SAME can play an important role in regulating the methylation status in any given cell type. In a recent study, Varela-Rey et al. [68] found that peripheral nerves with enforced elevated levels of SAME, were specially characterized by DNA hypermethylation at promoter and enhancer regions of several lipid synthesis genes, which contributed to the peripheral myelin defects. Reduced SAME levels correlate with DNA demethylation in peripheral nerves, which also can play a part in the peripheral myelin defects [68]. In fact, this group demonstrated that Schwann cell myelination was characterized by methylation dynamics, which were dependent on a tight control of SAME levels [68]. Thus, the levels of SAME likely also play a key gene regulatory role during development of neuropathies.

In the adult brain, dynamic DNA modifications (active demethylation or *de novo* methylation), is a general mechanism for rapid modifications in response to external stimuli *in vivo* [69]. As one of the active demethylation forms, TET enzymes and their corresponding product, 5hmC, partly act as intermediates in DNA demethylation. Several studies have shown that specific promoters of genes, related to neural plasticity and learning and memory, could be modified by neuronal activity through methylation and demethylation [70].

In a recent study, Saunderson et al. [71] reported an intricate balance between DNA methylation, DNA demethylation, and availability of SAME, which governed the induction of the immediate-early gene (IEG) products in dentate gyrus neurons, as well as to determine behavioral responses after stress. In the context of normal SAME levels, forced swim (FS) testing resulted in DNA demethylation at the IEG loci; conversely, with increased SAME levels, DNA methylation at these loci was induced in response to FS [71]. The underlying mechanism may relate to the activity changes of *Dnmt3a* from DNA demethylase to methyltransferase after raising SAME levels [71]. Therefore, even though little is known about the control of SAME levels in the brain, the neuronal concentration of SAME can be further investigated as a key factor in the molecular and behavioral responses to the stressors [71].

In a study in yeast cells, Sutter et al. [72] proposed an intriguing mechanism for sensing SAME levels. They found the methylation modification of the catalytic subunit of PP2A, determining metabolic processes underlying growth, was responsive to methionine and SAME levels [72]. They modeled that PP2A acted as a "sensor" of methionine and SAME levels in yeast cells [72]. Current investigation seeks to elucidate the underlying mechanisms that are available to monitor SAME

levels in the mammalian brains and to determine the neuronal responses to environmental stressors. Overall, the DNA methylation status of brain neurons may be determined, not only by expression levels of DNA methylation/demethylation enzymes, but also by levels of SAME.

SAME, DNA Methylation, and Related Disease

Marked disturbances of DNA methylation in neurodevelopment can occur through the loss of the key enzymes—DNMTs and TETs—thereby leading to deficits in neuronal function. In addition to the examples listed in the last section, embryonic deletion of *Dnmt3a* results in neuronal dysfunction, such as hypoactivity, motor abnormalities, decreased neuromuscular function, fewer motor neurons, and a shortened lifespan [73]. The deletion of *Dnmt1* in excitatory neurons of the dorsal forebrain in mice, although these mice are viable into adulthood, results in hypomethylation, dysregulation of gene expression, defects in dendritic branching, and progressive apoptotic degeneration of dorsal forebrain [74]. Depletion of *Tet1* and *Tet2*, causes a bias towards trophoblast differentiation in embryonic stem cells [75].

Notably, in a recent study, Shojaei Saadi et al. [76] treated bovine early embryos with SAME *in vitro* and found that SAME caused genome-wide hypermethylation mainly in exonic regions and in CpG islands, imprinting that significantly improved embryo hatching [76]. This study demonstrated that SAME, as a dietary treatment, may operate in some circumstances to therapeutically boost methylation patterns to improve neurodevelopment. We will list numerous studies that are associated with specific neuropsychiatric diseases to demonstrate that, except for the loss of key enzymes in DNA methylation, nutritional deficiency and/or abnormal levels in the 1-carbon cycle or mutations in genes that control DNA methylation patterns have been linked to hypomethylated CNS neurons with impaired function [77].

Neural Tube Defects, Rett's Syndrome, and Down's Syndrome

SAME metabolism has a critical role in the pathway of nutritional deprivation or mutations in genes is associated with neural tube defects (NTDs) and several developmental disorders, such as Rett's syndrome and Down's syndrome (DS).

NTDs are severe birth defects that result from a failure of the neural tube to close during the fourth week of embryogenesis. A group of studies support that an imbalance of SAME metabolism, caused by the deficiency of B vitamins (folate, vitamin B₁₂, and vitamin B₆), leads to decreased availability of donor methyl groups as associated with NTDs [78, 79]. Two studies support that B vitamin supplements can increase the metabolic capacity of SAME to provide methyl units and ameliorate NTDs [80, 81]. In fact, the literature provides

explicit recommendations about pre- and postconception use of oral folic acid with or without a multivitamin/micronutrient supplement for the prevention of NTDs and other congenital anomalies [82]. Furthermore, a dietary supplement of methyl groups—methionine or betaine—plays a unique role in SAME metabolism. For instance, previous studies showed the importance of methionine in neural tube closure [83], and the degree to which Hcy is re-methylated appears to be dependent on the ingestion of dietary methionine [83]. Different levels of methionine supplementation provide different functionalities in SAME metabolism. Consistent with this, Dunlevy et al. [84] reported excess methionine supplement (≥ 5 mM) suppressed the methylation cycle with a decreased ratio of SAM/SAH, and unexpectedly caused NTDs in cultured mouse embryos [84]. To further explain this phenomenon, SAH might be increased to a greater extent than SAME; thus, the ratio of SAM/SAH would be altered consistently with NTDs [84]. In fact, reduction in the SAM/SAH ratio is predicted to inhibit transmethylation reactions and is associated with NTDs [85]. Another study supported the use of betaine, a specific methyl donor, which resulted in a lowering of Hcy and the production of methionine or SAME in conversion of Hcy to methionine [86]. Regarding drug-induced nutritional changes, methotrexate, an inhibitor of folate metabolism, can cause DNA hypomethylation and NTDs [87]. In a further therapeutic study, folate was found to serve as a supplement to restore the SAM/SAH ratio to benefit patients. For instance, periconceptional supplemental maternal folic acid increased methylation of *IGF2*, improving intrauterine programming of growth and development [88]. In these cases, dietary supplements, as outlined in Figure 1, help to increase the production of SAME and restore the SAM/SAH ratio to benefit patients.

Rett's syndrome, which is caused by a mutation in the gene for MeCP2 [89], is characterized by normal development for 6 to 12 months of life followed by regression and symptomatic presentation of intellectual disability in females [90]. The mouse models of MeCP2-null or MeCP2-mutant display features found in patients with Rett's syndrome [91]. This mutation influences the interplay of the MeCP2 protein with methylated DNA and consequently changes the repression of transcription associated with a mental retardation [92]. Several studies supported an association between Rett's syndrome and disturbances of cerebral methyl-group pools, including folate, SAME, and the SAM/SAH ratio, and speculated on a compromised DNA-methylation [93–96]. However, there were no objective signs of clinical improvement or upregulation of SAME and SAH levels in cerebrospinal fluid (CSF) following folic acid supplement [93–96]; thus, the authors did not recommend folic acid therapy for individuals with Rett's syndrome. Interestingly, emerging literature has shown that prenatal folic acid supplements reduced risk for autism [97]. In sum, we have outlined evidence for an apparent lack of

capacity of patients with Rett's syndrome to regulate SAME, SAH levels, and SAM/SAH ratio in the CSF, which leads to disturbances in the donation of methyl groups in defined cell types.

DS is a complex metabolic and genetic disorder that results from nondisjunction of chromosome 21 [98]. Premature aging and disturbed methylation are components of DS [99]. Linking these 2 facts, the gene for cystathionine β -synthase is located on chromosome 21 and is overexpressed in children with DS [91]. The 1-carbon metabolism pathway is greatly affected by overexpression of cystathionine β -synthase, for instance, decreasing levels of Hcy and thus methionine and SAME, to thereby reduce the ratio of SAM/SAH [98, 100]. Consistent with this observation, Fountoulakis et al. [101] found an overexpression of tetrahydrofolate synthase, a key element in folate metabolism, in the fetal DS brain during the early second trimester to suggest abnormal folate metabolism and folate deficiency. In recent studies, Song et al. [102] showed that folic acid and vitamin B₁₂ levels were decreased with increasing age in patients with DS [102]. Obeid et al. [99] found that methylation of 3 CpGs combined with plasma amyloid- β (A β) showed a 3-year accelerated aging in subjects with DS at the age of 12 years. DNA hyper- and hypomethylation were, respectively, observed in patients with DS with vitamin B₁₂ deficiency and hyperhomocysteinemia [102]. Infantino et al. [103] found that the mitochondrial levels of SAM were reduced in DS, suggesting that impairment of the methyl cycle affected mitochondrial methyl availability in DS. We conclude that it will be worth investigating the role of the abnormalities in the 1-carbon cycle and methylation cycle to explain pathoepigenetic modifications in subjects with DS. Perturbation of the 1-carbon cycle result in folate deficiency and abnormal plasma Hcy concentrations [104, 105], which may contribute to the risk that any given mother will or will not have a child with DS. Specific enzymes, MTR, methionine synthase reductase, and MTHFR, can express polymorphisms that result in elevated homocysteinemia, a risk factor for having a child with DS [106].

In a key study, Pogribna et al. [98] supplied specific nutrients, (folinic acid, methyl-B₁₂, methionine) to DS lymphoblastoid cells, resulting in modulation of the metabolites in 1-carbon cycle. It is premature to suggest that the use of 1-carbon cycle supplements (folate acid, vitamin B, SAME) could ameliorate the progression of DS. However, it will be useful to determine whether such supplements could be of benefit for women in order to reduce their risks of giving birth to a child with DS.

Schizophrenia

The hypothesis that there might be a relationship between the disorders of 1-carbon metabolism (and transmethylation) with the genesis of psychiatric disorders was first forwarded by

Table 1 Association of SAM (metabolism) and transmethylation pathway with several neuropsychiatric diseases in early life stages

Disease	Condition	Target	Methylation effect	Read-out	Cell/tissue	Reference
NTDs	Excessive methionine (≥ 5 mM)	Genomic DNA	↓	SAM/SAH↓; cranial mesenchyme density↓	Mouse embryos	[84]
	Periconceptual maternal folic acid	<i>IGF2</i>	↑	A significant correlation between the maternal SAMe concentration and IGF-2 methylation of the child	Venous blood	[88]
	Folic acid deficiency	H3K4	↓		Human K562 cells	[162]
	methionine deficiency	Global DNA	↓		NTD mice model	[86, 87]
Rett's syndrome	MTX	<i>Siah1b</i>	↓	5-MeTHF, 5-FoTHF, SAMe↓	Venous blood	
	Betaine addition	<i>Prkx</i>	↓	Hcy, SAH↑		
	Folate-betaine addition		↓	Hcy↓, methionine↑, SAMe↑		
	Folic acid addition		↓	Mutant MeCP2; binding affinities for methylated DNA↓	Venous blood	[92, 93, 95, 96]
DS	Folic acid addition			Dimethylglycine↑, betaine↑, Hcy↓	Venous blood	
	Folic acid addition			Objective signs of clinical improvement (N)	CSF	
	Folic acid addition			CSF 5-MTHF↑	CSF	
				Objective signs of clinical improvement (N)	Venous blood	
				CSF 5-MTHF↑, SAMe-, SAH-, SAM/SAH-plasma SAMe↑, SAH↑, SAM/SAH-		
	CBS overexpression	<i>ASPA</i> <i>ITGA2B</i>	↓	Plasma SAMe↑, Aβ↑, SAM/SAH↓	Venous blood	[98–100, 103]
	Children with DS	lymphocyte DNA	↓	Plasma Hcy↓, SAMe↓, SAH↓	Venous blood	
	Methionine addition	mtDNA	↑	Cystathionine↑, cysteine↑	Lymphoblastoid cells	
	folic acid addition		↓	SAM↑, SAH↑, adenosine↑	Venous blood	
	Children with DS			tHcy↑, methionine↑, SAM↑, SAH↑	Lymphoblastoid cells	
Vitamin B ₁₂ deficiency				cystathionine↑, cysteine↑, betaine↑, choline↑, SAH↑, SAM↑, SAM/SAH↓		
				Mitochondrial SAMe↓; SAMC↑; GSH↓		
				total glutathione↓		
Hyperhomocysteinemia		Overall DNA	↑	Vitamin B ₁₂ ↓; Hcy↑;	Venous blood	[102]
			↓	FA, vitamin B ₁₂ ↓with increasing age		

SAH = S-adenosylhomocysteine; NTD = neural tube defect; SAMe = S-adenosyl-L-methionine; IGF-2 = insulin-like growth factor 2; MTX = methotrexate; 5-MeTHF = 5-methyltetrahydrofolate; 5-FoTHF = 5-formyltetrahydrofolate; Hcy = homocysteine; MeCP2 protein 2; CSF = cerebrospinal fluid; 5-MTHF = 5-methyltetrahydrofolate; DS = Down's cystathionine β-synthase; Aβ = amyloid-β; tHcy = total homocysteine; GSH = Glutathione; FA = folic acid

Smythies as early as 1963 [107]. Several studies demonstrated that MAT, the critical enzyme of the 1-carbon cycle to produce SAME, was significantly underactive in patients with schizophrenia (SZ) [108]. However, other studies showed that the disturbance in folate and/or Hcy metabolism was a risk factor for SZ, such that low plasma folate might be a potential independent factor for SZ [109]. This is also true for high Hcy levels [110]. In addition, several reports showed that low maternal folate linked with high Hcy levels were related to the teratogenic effect at the fetal stage [111] with late-stage risk of the development of SZ [112]. These studies implied that the disorders of 1-carbon cycle, including that of epigenetic DNA methylation pathways, played a role both in the etiology and progression of SZ and also in the predisposition toward SZ in offspring. This idea is also supported by Picker and Coyle [113], and has received additional traction through replicated studies demonstrating a transient doubling of SZ risk 2 decades following fetal exposure to famine conditions [114].

With regard to the disruption of 1-carbon cycle leading to the development of SZ, the abnormality in DNA methylation in many loci has been found to relate to brain development, stress response, and mitochondrial function [115]. Several reports show increased expression of DNA methyltransferases in telencephalic γ -aminobutyric acidergic neurons, for instance increased expression of DNMT1 and DNMT3a [116, 117], which can methylate several SZ-specific candidate genes including reelin, glutamic acid decarboxylase-67, and catechol-O-methyltransferase (COMT). Some examples include the hypermethylation of the reelin gene promoter with its related hypoactivity in SZ [118]; also observed were higher amounts of methylation in the human reelin promoter in post-mortem SZ cortices compared with controls [119, 120]. To ascribe the mechanism, the overexpression of Dnmt 1 mRNA presented in the same γ -aminobutyric acidergic neurons in which the levels of the reelin and glutamic acid decarboxylase-67 genes were reduced, suggesting a common negative regulatory mechanism that likely involves the coordinated hypermethylation of these promoters [116, 121]. The DNA promoter for the membrane-bound protein for COMT (MB-COMT) was found frequently to be hypomethylated in patients with SZ and bipolar disorder *versus* controls, thus contributing to MB-COMT overexpression and, consequently, increased dopamine degradation in the frontal lobe [122, 123]. Problems with attention, cognitive processes, and working memory have been linked to frontal lobe dopamine deficiency in patients with SZ [124].

Our MEDLINE searches have revealed little evidence of clinical studies for SAME as a treatment for patients with SZ (PubMed, PubMed Central, Google Scholar; keywords: S-adenosylmethionine, schizophrenia). However, earlier studies for this purpose concerned the use of L-methionine, a precursor in the biosynthesis of SAME. These clinical trials found that excessive oral methionine actually exacerbated the

symptoms of SZ (reviewed in Grayson et al. [125]). Tremolizzo et al. [126] fed a protracted methionine diet to heterozygous reeler mice and found that their behavioral startle response worsened, whereas there was a marked decrease of reelin mRNA associated with an increase in the methylation of the reelin promoter. The startle response can be seen as a model of SZ vulnerability [126]. Of note, there is always a higher conversion rate of SAME into SAH during such protracted L-methionine treatment [126]. Therefore, it seems that there may be a methionine-induced hypermethylation of CpG island-containing promoters in patients with SZ treated with methionine.

Moreover, there have been several randomized controlled trials of supplementation of folate or methylfolate in patients with SZ [127–131]. Levine et al. [127] reported that the symptoms of patients with SZ with hyperhomocysteinemia were improved after addition of folate and vitamin B₁₂ [127]. However, Hill et al. [128] did not report benefits of folate supplementation in patients with SZ with prominent negative symptoms, but did report a significant interaction between treatment and *MTHFR* genotype. Consistent with this, Roffman et al. [129] reported that folate plus vitamin B₁₂ supplementation can improve negative symptoms of SZ but that the treatment response is influenced by the *MTHFR* and *FOLH1* genotype. Methylfolate is the fully reduced and bioactive form of folate. Several previous studies reported symptom improvement in patients with SZ with low red blood cell folate levels when treated with methylfolate [130, 132]. In a recent study, Roffman et al. [131] reported that methylfolate supplementation was associated with salutary physiological changes and negative symptomatic improvement in patients with SZ, regardless of genotype.

In conclusion, the phenomenon of a higher conversion rate of SAME into SAH may be related to hypermethylation of reelin promoter and symptoms exacerbation in patients with SZ treated with late-stage dietary intake (methionine). However, folate or methylfolate supplementation in certain SZ genotype may ameliorate the negative symptoms, which may be reflect reverse changes of hypomethylation of the MB-COMT promoter that resulted from decreased SAME levels or a reduced ratio of SAM/SAH in variants in folate-related enzymes. The normative expression of MB-COMT may help sustain dopamine levels in the frontal lobe associated with negative symptoms in patients with SZ.

AD

Perturbations of DNA methylation pathways can be, at least in part, included as an epigenetic basis of AD pathology. This was supported by several reports of an AD-associated reduction of SAME levels in various neocortical areas, such as the hippocampus and putamen [133], and this reduction sometimes was found concurrent with an increase in brain SAH

levels. As stated above, SAH is a strong DNA methyltransferase inhibitor contributing to DNA hypomethylation [134].

Although it is clear that 5mC is altered in AD, there remains no clear conclusion about global DNA methylation changes in the AD brain. Previous studies referred to both hypo- and hypermethylation, which may arise from the types of techniques used to investigate the DNA modifications [135, 136]. Emerging studies focused on changes in 5mC at specific promoter regions of genes related to AD pathology, such as the amyloid precursor protein (APP); presenilin1 (PS1), an enzyme involved in the processing of APP; β -site amyloid precursor protein-cleaving enzyme (BACE); microtubule-associated protein tau (MAPT); and apolipoprotein 4 in AD, as are described below.

It is well documented and accepted that the A β peptide production resulting from APP cleavage is of great relevance in the amyloidogenic etiology of AD [137]. A previous study showed that APP expression is subject to DNA methylation regulation (hypomethylation of the APP gene) [138] and the demethylation of the APP gene with age may have a significant influence on the development of A β deposition [139].

The group of Fuso has studied the impact of 1-carbon cycle and DNA methylation pathway on the genes encoding APP processing enzymes underlying AD pathology. SAME was shown to inhibit PS1 promoter demethylation and thereby downregulated the PS1 mRNA expression in SK-N-SH human neuroblastoma cells, which resulted in a lowered secretion of A β [140]. In order to correlate changes in the SAM/SAH cycle and DNA methylation with AD pathology, Fuso et al. [6] studied the consequences of growing SK-N-SH and SK-N-BE neuroblastoma cells in a medium deprived of folate and vitamin B₁₂. They found that an imbalance of SAME and SAH resulting from these vitamin deficiencies was associated with changes in methylation pattern of *PSEN1* and *BACE* with consequent increases in PS1 and BACE levels, contributing to neurotoxic A β deposition [6]. Hypomethylation of the promoter of *PSEN1* induced an overexpression of this protein, which is consistent with another report of Scarpa et al. [140]. It is particularly noteworthy that they proposed the regulatory effect of A β production is gene-specific based on the model of SAM/SAH cycle and therapeutic intervention to reduce, but not totally inhibit, the slight overproduction of A β . Subsequently, the same team also used glioblastoma cells to further confirm that vitamin B deprivation could impair the DNA methylation potential of *PSEN1* and *BACE*, providing the basis for A β overproduction [141]. Using *in vivo* studies, they demonstrated that a B vitamin deprivation diet led to hyperhomocysteinemia, an imbalance of SAM/SAH, PS1, and BACE upregulation, each of which increased A β deposition in TgCRND8 mice [142, 143].

Of note, some studies have indicated that A β itself could play a role in the pathological mechanisms associated with AD. Hodgson et al. [144] investigated the effects of soluble

A β oligomers (oA β) on intracellular thiol metabolite levels, DNA methylation, and the transcription status of redox and methylation-associated genes. They found a reduced ratio of SAM/SAH and decreased global DNA methylation with conversely increased Hcy levels. These authors concluded that the capacity of oA β to modulate gene expression could generate redox and methylation-dependent epigenetic effects that contributed to the pathology of AD [144]. This prospect has been supported by a recent study [145]. Using primary hippocampal neuronal cells and HT-22 cells, as well as AD transgenic mice, Liu et al. [145] investigated the influence of oA β on DNA methylation pathway in these cells, including DNMT activity, methylation potential of cells, APP and PS1 expression, cell viability, and the methylation of respective promoters. They found that oA β caused neuronal toxicity with lowered DNMT activity, increased APP and PS1 expression, and decreased cell viability, each consequence of which could be prevented by folic acid via methylation-dependent pathways [145].

Other than those genes participating in A β processing (i.e., *APP*, *PSEN1* and *BACE*), several target genes show significant interindividual epigenetic variability in late-onset AD [146]. For instance, the methylation pattern of *APOE* was found to be of a bimodal structure, ranging from a hypomethylated CpG-poor promoter to a fully methylated 3'-CpG-island [146]. By comparison, *MTHFR*, crucial for SAME synthesis, was found to be hypermethylated [146]. These authors emphasized that an associated epigenetic drift may affect AD predisposition and course of disease [146].

The most proximal hallmark of AD is intraneuronal neurofibrillary tangles, which are composed of abnormally hyperphosphorylated tau protein. Tau phosphorylation expression is subject to epigenetic regulation mechanisms. For instance, Mastroeni et al. [147] reported that detriments in the DNA methylation pathways, including 2 markers of DNA methylation and 8 methylation maintenance factors, particularly those marked in PHF1/PS396 immunoreactivity, caused an increase in AD-specific neurofibrillary tangle-bearing neurons. In a vitamin B deficiency study, Nicolia et al. [148] reported that B vitamin deficiency promoted tau phosphorylation through upregulation of glycogen synthase kinase 3 β (GSK3 β) and protein phosphatase 2A (PP2A) genes by inhibiting methylation [148].

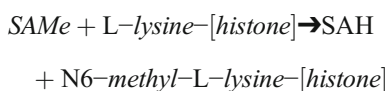
Further work by Iwata et al. [138] found aberrant CpG methylation of *APP*, *MAPT*, and *GSK3B* in AD brain. The increased expression of APP and MAPT in both neuronal and non-neuronal cells, and the decreased expression of GSK3B in non-neuronal cells, may be directly linked to AD pathogenesis [138]. The cells that overproduce APP and MAPT are located among normal cells in AD brains, which could serve as seed clones for aggregated protein production, which supports the aggregation propagation hypothesis [138].

In general, there is an age-related increase of 5hmC levels in the brain, pointing towards a role for 5hmC in the development

of AD [136, 149, 150]. However, some results differed between these studies, which could be explained by differences in tissue processing and immunostaining. Any dysregulation of 5hmC in AD brain could be due to aging or environmental stressors [136]. Bradley-Whitman and Lovell [135] reported that global levels of 5mC and 5hmC were significantly increased, whereas 2 intermediates (5-formylcytosine and 5-carboxylcytosine), in the demethylation steps, were significantly decreased. They proposed that the absence of a mechanism to restore alterations in methylation/demethylation pattern after an environmental insult could result in an accumulation of 5mC and 5hmC during the course of AD [135]. In fact, 5mC and 5hmC both have been linked to neuronal cell death [151, 152]. In a recent study, Bernstein et al. [153] identified 325 genes containing differentially hydroxymethylated loci, which are involved in neuron projection development and neurogenesis. Some of these 5hmC-associated genes modulated tau-induced neurotoxicity in AD [153]. Ellison et al. [154] found significant alterations in 5-mC and 5-hmC in early AD stages across multiple regions of the brain. They suggested that the alterations in 5-mC and 5-hmC may play an early role in the progression of AD [154]. In conclusion, DNA methylation/demethylation patterns play a central role in the connection between amyloidogenic, fibrillogenic pathways in AD, both as a cause and as a consequence of the disease.

SAME and Histone Methylation Patterns During Related Neuropsychiatric Diseases

Histone methylation refers to the fact that histones can be mono-, di-, or trimethylated at lysines and arginines by histone methyltransferases (HMTs) [155]. It was reported that transcriptionally silent genes correlate with di- and trimethylation of histone H3 at lysine-9 (H3K9) [156], whereas active genes are associated with di- and trimethylation of histone H3 at lysine4 (H3K4) [157]. Of note, several studies reported that these specific histone methylations have been implicated in the regulation of chromatin structure in the nervous system [158]. Two kinds of histone lysine methyltransferase, histone-lysine *N*-methyltransferase 2A (corresponding gene: *MLL1*) [159], and histone-lysine *N*-methyltransferase EHMT2 (corresponding gene: *G9a*) [160], respectively, catalyze the chemical reaction as below with different products, H3K4 and H3K9:



One recent study reported the kinetic constants for histone-lysine *N*-methyltransferase EHMT2 (*G9a*): the *K_m* value of *G9a* for SAME was $0.53 \pm 0.043 \mu\text{M}$; the *K_m* value of *G9a* for H3K9 was $0.6 \pm 0.096 \mu\text{M}$ [161].

Emerging evidence demonstrates that the dynamic modification of histones through methylation/demethylation can link metabolism with transcription, acting as molecular transducers of metabolic signals to chromatin. For example, Sadhu et al. [162] demonstrated that folate and methionine deficiency, which are both important for the maintenance of the SAME pool, led to reduced methylation of lysine 4 of histone H3 (H3K4) in *Saccharomyces cerevisiae* and human cells in culture, suggesting histone methylation marks are susceptible to nutritional limitation [162]. In this report, results with K562 erythroleukemia cells led the authors to hypothesize that the abnormalities of histone methylation, as generated with SAME, could be tested to impact on the development of NTDs secondary to folate deficiency [162].

This mechanism is also supported by other studies. Mentch et al. [163] showed that modulation of methionine metabolism, via altered nutrient availability, regulated SAME and SAH levels to drive specific histone methylation events that affect disease-specific gene expression. Using the *Drosophila melanogaster* model, Liu et al. [164] reported that disruption of methionine metabolism can directly affect histone methylation levels, consistent with the viewpoint of Mench et al. [163], leading to lethality, as well as wing development and cell proliferation defects. In naïve human embryonic stem cells, Sperber et al. [165] recently showed that the knockdown of nicotinamide-*N*-methyl transferase led to higher SAME levels and upregulation of H3K27me3. nicotinamide-*N*-methyl transferase is conferred to catalyze the chemical reaction:



This path therefore appears to make SAME unavailable for the histone methylation that represses Wnt and activates HIF pathway in primed human embryonic stem cells, thus supporting the hypothesis that the metabolome regulates the epigenetic landscape of the earliest steps in human development [165]. In sum, the specificities of HMTs, and the availability of the cofactor SAME, can be found in detailed reviews on methionine metabolism as an essential regulator of histone methylation and aforementioned possibility for specific gene regulation and related to biochemical events [10].

Ara et al. [166] found that the consequence of upregulation of TNF- α due to lipopolysaccharide (LPS) stimulation could be blocked by SAME, MAT, or SAH pretreatment, through increasing the binding of H3K4 to the *TNFA* promoter. In this case, SAME displayed no methyl donor role but served as an inhibitor to the trimethylation of H3K4 binding to the promoter region of *TNFA*. In the future, SAME can be tested for its capacity to alter the histone methylation in studies when it is to be applied as a therapeutic agent to ameliorate neurodegenerative diseases, such as AD.

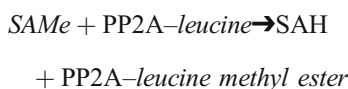
Table 2 Association of SAM (metabolism) and transmethylation pathway with psychiatric diseases in middle life stages

Disease	Condition	Target	Methylation effect	Read-out	Cell/tissue	Reference
SZ				Density of DNMT1 mRNA-positive neurons↑; density of GAD67 mRNA-positive neurons↓	BA9	[116]
		RELN promoter	↑	Activity of the RELN↓; DNMT1 ↑; GAD67↓ mRNA and protein levels of reelin↓	BA9/10 BA9/10; occipital cortex	[119, 120]
				The number of DNMT1 mRNA-positive neurons↑; the number of reelin mRNA-positive neurons↓	PFC; BA10; BA17; caudate nucleus; cerebellum	[121]
		MB-COMT promoter	↓	MB-COMT expression↑	Frontal cortical area; saliva samples from SZ	[122, 123]
MDD	SAM	Norepinephrine		NA concentration↑	Hippocampus, frontal cortex in rat brain	[177]
	SAM			5-HT levels↑	Corpus striatum, hippocampus, frontal cortex in rat brain	[179]
				Cofactor of COMT		[183]
	SAM	Plasma phospholipids	↑	The fluidity of the neuronal membrane↑		[4]
	Acute imipramine administration			Prevent 5-HT _{1A} receptor up-regulation	Frontal cortex	[184]

SZ = schizophrenia; RELN = reelin; DNMT = DNA methyltransferase; PFC = prefrontal cortex; MB-COMT = membrane-bound catechol-O-methyltransferase; NA = noradrenaline; 5-HT = 5-hydroxytryptophan; MDD = major depressive disorder; SAM = S-adenosylmethionine

SAME, PP2A Methylation, and Related Diseases

PP2A is a heterotrimeric protein composed of a catalytic subunit, scaffolding subunit, and regulatory subunit [167]. PP2A plays an important role in the biological function of tau protein, which stimulates microtubule assembly and stabilizes microtubules. In the AD brain, tau is abnormally hyperphosphorylated. PP2A has been shown to be methylated by methyltransferase, leucine carboxyl methyltransferases 1 (LCMT-1) [168] and demethylated by methylesterase, a potent phosphatase methylesterase 1 [169]. LCMT-1 is conferred to catalyze the chemical reaction:



The kinetic constants for LCMT-1 were reported in 1 experiment: the K_m value of LCMT-1 for SAME was 1.3 μM ; the K_m value of LCMT-1 for PP2AD was 0.1 μM [170]. Alterations in PP2A-dependent (de)methylation patterns certainly play an important role in the pathogenesis of AD. For instance, Sontag et al. [171] reported that PP2A methyltransferase protein expression and PP2A_C

methylation levels were quantitatively decreased in AD-affected brain regions, and there was a regional loss of PP2A methyltransferase immunoreactivity that closely paralleled the severity of phosphorylative tau pathology. Nicholls et al. [172] reported that LCMT-1 overexpression protected animals from A β -induced impairments, whereas overexpression of phosphatase methylesterase 1 worsened A β neurotoxicity [172]. Therefore, PP2A methylation may control sensitivity and resistance to A β -induced cognitive and electrophysiological impairments, whereas PP2A demethylation functions in reverse.

The degree of PP2A methylation is subject to changes according to metabolism. For example, incubation of Neuro-2a cells with SAH resulted in reduced methylation of PP2A and detrimentally increased tau phosphorylation [173]. By contrast, incubation of Neuro-2a cells with SAME enhanced PP2A methylation, led to the beneficial accumulation of dephosphorylated tau, and increased secretion of neuroprotective α -secretase-cleaved APP fragments [173]. This finding provides a new link between metabolism, PP2A methylation, and key CNS proteins involved in AD pathogenesis. In a follow-up study, the same team reported that, in neuroblastoma cells, folic acid deficiency contributed to decreased SAME levels, and induced downregulation of LCMT-1 expression

Table 3 Association of SAM (metabolism) and transmethylation pathway with neurodegenerative disease in late stage

Disease	Condition	Target	Methylation effect	Read-out	Cell/tissue	Reference
	Folate and Vitamin B ₁₂ deficiency	<i>PSEN1</i>	↓	SAM/SAH↓; PSEN-1 and BACE↑; Aβ↑	SK-N-BE neuroblastoma cells	[6, 139]
	B vitamin deprivation	<i>PSEN1</i>	↓	SAM/SAH↓; PSEN-1 and BACE↑; Aβ↑	TgCRND8 mice	[141]
	B vitamin deficiency	<i>BACE</i>				
		<i>GSK3β</i>	↓	GSK3β↑; PP2A activity↓; phosphorylated tau↑	SK-N-BE cells	[142]
	SAH (SAM and PPMT)	<i>PP2Ac</i>	↓	Tau phosphorylation↑; APP phosphorylation at Thr-668↑; β-Secretase-cleaved APP fragments↑ Aβ peptides↑ (tau dephosphorylation↑; α-secretase-cleaved APP fragment↑)	TgCRND8 mice Neuro-2a cells	[148]
	High-methionine, low-folate diet	<i>PP2A</i>	↓	PPMT↓; LCMT-1↓; demethylated PP2Aβ↓	Cystathionine-β-synthase +/- mice	[173]
	Folate deficiency, folate-deficient diets	<i>PP2A</i>	↓	Phosphorylated tau↑	Neuro-2a cells C57BL/6J mice	[174]
	Methotrexate			Phosphorylated tau↑	Rat primary neuron cells	[175]

SAH = S-adenosylhomocysteine; PSEN-1 = presenilin 1; BACE = β-site amyloid precursor protein-cleaving enzyme; Aβ = amyloid β; SK-N-BE = ??; PP2A = protein phosphatase 2A; APP = amyloid precursor protein; PPMT = PP2A methyltransferases; LCMT-1 = leucine carboxyl methyltransferases 1

[174]. This resulted in accumulation of demethylated PP2A and ultimately tau mediated cell death [174]. In another study, Yoon et al. [175] reported that after treatment of rat primary neurons with methotrexate, a folate antagonist, the levels of PP2A methylation decreased and phosphorylated tau, APP, and β-secretase were increased. These findings supported that PP2A methylation correlated with metabolism, which played a corresponding role in pathological progression of AD [175]. In these cases, SAME, acting as an adaptive responder to nutrition deficiency and also as a methyl group donor, contributed to the dynamic regulation of PP2A by methylation/demethylation pathways.

SAME, Monoamine Neurotransmitters, and Depression

SAME is required for the synthesis of monoamine neurotransmitters, such as norepinephrine (NE), dopamine (DA), and serotonin [5-hydroxytryptophan (5-HT)], which play important roles in maintaining normal mood [176]. The neurotransmitters DA and NE are synthesized from the amino acid tyrosine in a series of chemical reactions dependent on tyrosine hydroxylase [177]. Serotonin is synthesized from the amino acid tryptophan in a series of chemical reactions, of which the rate-limiting step is catalyzed by the enzyme tryptophan hydroxylase [178, 179], wherein SAME functions as a methyl-donating cofactor in the rate-limiting step in these synthetic reactions to increase the levels of this neurotransmitter. In addition, the folate cycle is essential for the synthesis and regeneration of tetrahydrobiopterin [180]. The latter acts as an

important cofactor for the enzymes that convert amino acids to monoamine neurotransmitters [181].

We will address SAME as a treatment option for depression that operates by increasing neurotransmitter levels. Bottiglieri et al. [182] reported that elevated Hcy levels in MDD have been associated with low SAME levels, and low CSF levels of 5-HT, DA, and NE metabolites. Enhancement of SAME levels permit it to act as the cofactor of COMT, which prevents catalysis in the first step in a degradation pathway of the catecholamine neurotransmitters, and can therapeutically decrease COMT enzyme activity [183].

In another mode, SAME has shown efficacy in accelerating the onset of the antidepressant effect of imipramine in depressed patients. Bellido et al. [184] found that SAME prevented 5-HT_{1A} receptor up-regulation induced by acute imipramine in the frontal cortex, which may contribute to the augmentation effect of SAME [184]. Mechanistically, there is a structural similarity of serotonin indole ring to the adenosine motif of SAME. Selective serotonin reuptake inhibitor (SSRIs) can bind to the COMT catalytic site, as a COMT inhibitor, and inhibit SAME access, thus preventing the methylation of COMT substrates [185]. In these cases, SAME, either acting as a methyl group donor, or a mediator of specific enzyme activity, has augmentative effects on MMD treatments.

SAME, Phospholipids, and Depression

Phospholipids play essential roles in the composition of the cellular membrane and membrane dynamic functions, which

are pertinent to maintaining healthy neurons and adequate neurotransmission, thus preventing depression. Specifically, a major SAME-consuming pathway is methylation of phospholipids in mammals [186]. Normal membrane fluidity enhances receptor function and enhances the role of receptor coupling. To promote this function by methylating plasma phospholipids, SAME may alter the fluidity of the neuronal membrane, affecting the function of proteins that traverse the membrane. This beneficial induction includes that of defined monoamine receptors, monoamine transporters, and other elements of the second messenger system [4].

SAME Treatment

Numerous preclinical and clinical studies over the past 2 decades have shown that SAME is effective in the treatment of psychiatric/neurological diseases, with the consequences of inhibiting progression and improving prognosis of these diseases. The mechanism underlying its therapeutic effects partly correlated to its influence on dynamic methylation/demethylation pathways. It has been difficult for clinicians to employ an easy measure the levels of SAME and SAH in blood or tissue samples. However, research has recently generated antigens and antibodies that will pave the way for immunoassays for SAME and related molecules [187]. This development of Immunoassays of SAME and SAH should in the future provide a methylation index as a biomarker for disease and health status and tissue specific and brain region prevalence abundance of SAME levels. Experimental and clinical studies about the applications of SAME to specific neuropsychiatric diseases will be discussed below.

SAME Treatment in Psychiatric Disorders

The FS test monitors inappropriate responses that have been linked with psychiatric disorders, such as major depression and anxiety [188]. Saunderson et al. [71] found that administration of SAME before the FS challenge resulted in enhanced CpG methylation at IEG loci. The suppression of IEG induction, specifically in the dentate gyrus, was associated with significant changes to behavioral immobility responses 24 h later, suggesting that SAME may enhance behavioral responses after stress by suppressing the induction of IEG. This finding is consistent with a previous study conducted by Weaver et al. [189]. Those investigators centrally infused the adult offspring with the essential amino acid L-methionine, a precursor to SAME, to examine whether the epigenetic programming of stress responses established early in life is reversible. They found that the inherent stability of the epigenomic marks is potentially reversible through methyl supplementation [189]. According to these results, we propose a potential role of earlier administration of SAME to improve

depression and also to alter epigenetic markers, which supports its potential capability for inhibiting and reversing neuropathogenesis secondary to stress early in life.

In mood disorders, recent clinical studies supported the use of SAME as a supplement to standard medications, which effectively ameliorates depressive symptom and cognitive dysfunction in MDD [190]. This clinical trial convincingly demonstrated that the abundance and bioavailability of SAME increased in the serum of treated depressed patients as associated with clinical improvement in depression [190]. Another systematic review assessed the effects of SAME in comparison with placebo or antidepressants for the treatment of depression in adults with MDD, with less support for firm conclusions [191]. Both high intracellular levels of SAME (due to SAME administration) and low intracellular levels of serotonin (due to SSRI action) prevent binding of serotonin to COMT and its consequent inhibition. Therefore, SAME has been used as a supplement to treat patients with MDD [8], even in SSRI-resistant patients [192]. Furthermore, despite its good safety profile, SAME has been contraindicated in patients with bipolar disorder owing to its induction of mania or hypomania [191].

Strous et al. [193] investigated the efficacy of SAME in managing SZ symptoms in patients with the low-activity COMT polymorphism and found an improvement of aggressive behavior and quality of life following SAME augmentation in SZ [193]. SAME can increase COMT enzyme activity, which may help to ameliorate aggressive symptoms in specific patients with SZ.

SAME Treatment in Neurological Diseases

SAME was proposed to tilt the epigenetic marking of DNA towards increased methylation and reduced demethylation as a treatment in neurological diseases. In demyelinating diseases, after the dietary restoration of SAME, substantial clinical improvement has long been associated with apparent remyelination [194].

The deficiency of SAME may underlie the gradual hypomethylation of DNA that accompanies aging, and this hypomethylation can be alleviated by supplementation with SAME [195]. The lower availability of SAME may be related to the altered expression of genes involved in APP metabolism, finally producing the accumulation of A β peptide, contributing to the pathological processing of AD [6, 140]. DNA demethylation, when reduced by aging or nutritional deficiencies, may at least be partially avoided or restored by SAME administration [6, 140]. In addition, *BACE* expression is modulated by methylation, and SAME can restore its normal expression pattern [6]. Therefore, there have been several attempts to use SAME nutritional supplementation in clinical trials in AD [7]. As an example of its mechanism of action, SAME supplementation was shown to inhibit DNA demethylase, DNMT1

activity, and demethylation of PS1, a prime gene product involved in amyloidogenesis [143], as discovered in transgenic AD mice.

A further study examined whether SAME could delay neuropathology in 3×Tg-AD mice, which harbor mutant genes for human A β PP, PS-1, and tau, here showing that SAME supplementation in the diet did, in fact, reduce extracellular A β deposits [196]. SAME treatment should therefore start early to effectively delay onset of AD symptoms. Fuso et al. [197] reported that SAME reduced amyloid production and increased spatial memory in TgCRND8 mice. Here, SAME inhibited the effects of B vitamin deficiency to induce *PSEN1* and *BACE1* expression and tau phosphorylation in TgCRND8 and wild-type mice. Certainly, SAME treatment also reduced plaque spreading in pathways independent of B vitamin deficiency [197]. Persichilli et al. [198] reported a significant decrease of thiol levels when the B vitamin-deficient diet was supplemented with SAME + superoxide dismutase and superoxide dismutase alone [198]. In a recent study on the APP transgenic mouse model of AD-like amyloid pathology, Do Carmo et al. [199] found that the early BACE-1 and global DNA demethylation were rescued by chronic administration of SAME, which contributed to the reduction of amyloid pathology and the improvement in cognition function. In conclusion, these studies in AD support the rationale that it is possible to use SAME to treat brain disorders with a plausible epigenetic mechanism. The studies are consistent with the hypothesis that SAME administered orally is bioavailable in an active form or as an active metabolite in the brain.

SAME Treatment in Chronic Inflammation Associated With Depression, SZ, and AD

It has been reported that inflammation plays a critical role in chronic conditions in the brain, such as depression [200], SZ [201], and AD [202]. Previous studies showed that the anti-inflammatory effects of SAME in LPS-stimulated monocytes/macrophages were mediated by reducing the expression of the [203], and increasing the expression of the anti-inflammatory cytokine interleukin 10 [204]. Later, Ara et al. [166] reported that the mechanism of the pharmacologic inhibitory effects of SAME on proinflammatory mediators, are, in part, mediated by altering the methylation capacity and blocking the binding of trimethylated H3K4 to the TNF- α promoter through increasing MTA and SAH levels [166]. In addition, Gobejishvili et al. [205] reported that SAME pretreatment led to decreased *PDE4B* expression by increasing the binding of transcriptionally repressive trimethylated H3K9 to its promoter region, thus inhibiting cyclic adenosine monophosphate-mediated TNF- α expression. Pfalzer et al. [206] evaluated the effects of SAME on global and site-specific DNA methylation and gene expression in the context of LPS-stimulated inflammation in human macrophages, and

found a decrease in DNA methylation status of inflammatory genes after SAME treatment. So it is proposed that the anti-inflammation feature of SAME through methylation regulation supports its therapeutic effects in treatment of chronic inflammatory conditions.

Prospects

SAME, as a universal methyl donor, clearly shows promise in the treatment of neuropsychiatric diseases throughout the human life span. The malfunction of methylation in the developing brain due to inherited mutations, compounded by epigenetic stresses, underlies the pathological process of a variety of neurodevelopmental and/or psychiatric disorders, including diseases that manifest at different stages of life, such as MDD and SZ. The critical period for use of SAME or related agents has been proposed to be as early as possible, thus enhancing therapeutic effects and/or potentially preventing the illness from manifesting in the first place. Disease reversal and the harmless nature of SAME are projected to counteract the latent risk of pathological diseases in brain.

Relevant to its efficacy in individuals with existing psychiatric disorders, there has been considerable evidence to support the use of SAME in the treatment of MDD. SAME is recommended as a second-line treatment option following inadequate treatment response to conventional antidepressants [207]. Owing to its favorable safety profile, SAME may be specifically suitable for the treatment of MDD in children, adolescents, and pregnant women. However, further studies are required because there is little scientific support for its use in these potentially vulnerable populations. However, as a cautionary note, in patients with SZ, SAME may at times exacerbate the symptoms of this disease. One study showed that the treatment of heterozygous reeler mice with dietary L-methionine led to a decreased PPI [126] as a model of SZ. However, the treatment of SAME resulted in an improved neurotransmission and thus attenuated aggressive behavior and ameliorated the quality of life of patients with SZ with a low-activity COMT polymorphism [193]. Therefore, further studies are required to assess the use of SAME in patients with SZ. Owing to concerns about neurotransmitter hyperactivity, SAME is not recommended in patients with bipolar disorder because of its history of leading to manic and hypomanic states [191].

Emerging studies have focused on the function of SAME as a potential disease-modifying agent to treat the pathological process of AD. AD is a progressive and irreversible disease without effective treatments. A number of risk factors exacerbate AD, including folate and vitamin B₁₂ deficiency. SAME showed a positive effect by preventing the progression of the hallmarks of AD resulting from this dietary deficiency. SAME reduced amyloid production and prevented phospho-tau accumulation in animal models. At the same time, the clinical

manifestations of AD were also ameliorated by SAME. Thus, recent clinical efficacy trials have shown that SAME can be used as a part of a nutritional formulation [208], to maintain or improve cognitive performance and mood/behavior in patients with AD [209].

Furthermore, the dosage of SAME recommended in different situations needs to be carefully studied with additional clinical trials. The usual starting dose of SAME is 400 mg/day and the maximum reported in the literature is 3200 mg/day (given in 2 doses) [210]. It is clear that this dosing should be increased gradually every 5 to 7-day period [9].

Although SAME has a favorable safety profile, it is still worth paying attention to the risk that SAME might cause increased Hcy levels, which is associated with higher risk of cardiovascular disease [211]. Despite evidence that SAME augmentation of antidepressants did not result in elevation of total Hcy [190], caution with vulnerable populations seems appropriate until additional data emerge. For example, patients lacking the efficient capability of eliminating excessive Hcy might not be advised to use SAME as a long-term treatment, and if they choose to use it, they should do so under physician supervision.

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