Received: 5 December 2023 | Accepted: 3 January 2024

DOI: 10.1002/biof.2044

REVIEW ARTICLE

Biofactors WILEY

The functional roles of S-adenosyl-methionine and S-adenosyl-homocysteine and their involvement in trisomy 21

Maria Caracausi[1] | **Giuseppe Ramacieri**[2,3] | **Francesca Catapano**[2] | **Michela Cicilloni**[1] | **Bassam Lajin**[4] | **Maria Chiara Pelleri**[1] | **Allison Piovesan**[1] | **Lorenza Vitale**[1] | **Chiara Locatelli**[5] | **Gian Luca Pirazzoli**[6] | **Pierluigi Strippoli**[1] | **Francesca Antonaros**[1] | **Beatrice Vione**[1,2]

[1]Unit of Histology, Embryology and Applied Biology, Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna, Bologna, Italy

[2]Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy

[3]Speciality School of Child Neuropsychiatry—Alma Mater Studiorum, University of Bologna, Bologna, Italy

[4]Institute of Chemistry, ChromICP, University of Graz, Graz, Austria

[5]Neonatology Unit, St. Orsola-Malpighi Polyclinic, Bologna, Italy

[6]Medical Department, Maggiore Hospital, Bologna, Italy

Correspondence
Giuseppe Ramacieri, Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy.
Email: giuseppe.ramacieri2@unibo.it

Francesca Antonaros, Unit of Histology, Embryology and Applied Biology, Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna, Bologna, Italy.
Email: francesca.antonaros2@unibo.it

Funding information
National Plan for PNRR Complementary Investments (PNC), Grant/Award Number: B53C22006450001; Fondazione Umano Progresso; Fundraising Initiatives in Cassano Magnago (VA)

Abstract

The one-carbon metabolism pathway is involved in critical human cellular functions such as cell proliferation, mitochondrial respiration, and epigenetic regulation. In the homocysteine-methionine cycle S-adenosyl-methionine (SAM) and S-adenosyl-homocysteine (SAH) are synthetized, and their levels are finely regulated to ensure proper functioning of key enzymes which control cellular growth and differentiation. Here we review the main biological mechanisms involving SAM and SAH and the known related human diseases. It was recently demonstrated that SAM and SAH levels are altered in plasma of subjects with trisomy 21 (T21) but how this metabolic dysregulation influences the clinical manifestation of T21 phenotype has not been previously described. This review aims at providing an overview of the biological mechanisms which are altered in response to changes in the levels of SAM and SAH observed in DS.

KEYWORDS

genetic diseases, one-carbon metabolism pathway, S-adenosyl-homocysteine, S-adenosyl-methionine, trisomy 21

1 | INTRODUCTION

One-carbon metabolism includes the folate cycle, the homocysteine-methionine cycle, and the trans-sulfuration pathway compartmentalized among the cytoplasm, nucleus, and mitochondria.[1] By this process, one-carbon groups at different oxidation states are transferred for biosynthesis of DNA through purine and thymidylate generation and for amino acid homeostasis, antioxidant generation, and epigenetic regulation.[2] These biochemical processes support critical cellular functions such as cell proliferation, mitochondrial respiration, and epigenetic regulation.[3]

Folates are versatile methyl donors that carry and chemically activate one-carbon units.[2] They derive entirely from dietary sources[4] and are absorbed as folic acid in the small intestine.[5] To facilitate absorption, they are hydrolyzed to their monoglutamated forms in the intestinal mucosa by γ-glutamyl hydrolase (GGH) and glutamate carboxypeptidase II (FOLH1). Folates have several transport systems, including the reduced folate carrier, the proton-coupled folate carrier, and folate receptors.[6] Upon reaching target cells, folate monoglutamates are transformed to 5-methyl-tetrahydrofolate (5-methyl-THF), the predominant species in non-hepatic tissues, and are subsequently polyglutamated for cellular retention and one-carbon coenzyme function.[7]

In the homocysteine-methionine cycle, the methyl group from 5-methyl-THF is transferred to homocysteine (Hcy) by the vitamin B12-dependent enzyme methionine synthase (MS), producing methionine and THF.[8,9] Methionine is adenylated by methionine adenosyltransferase (MAT2A and the liver-specific MAT1A) to S-adenosyl-methionine (SAM) the universal methyl donor involved in numerous downstream cellular reactions.[8] During methyl transfer, SAM is converted to S-adenosyl-homocysteine (SAH),[10] which is hydrolyzed back to Hcy and adenosine through a reversible reaction catalyzed by adenosylhomocysteinases (AHCY, AHCYL1, and AHCYL2) to complete the methionine cycle[11] (Figure 1). Starting from SAM, methionine can also be produced through the multienzyme methionine salvage pathway.[12,13] In addition, methionine can also be taken through diet.[14] The role of homocysteine-methionine cycle was already discussed as necessary for the synthesis of cholinergic and adrenergic mediators, for the construction of neurons and of myelin.[15] These mechanisms produce a high consumption of monocarbons, so if their supply is not sufficient brain functions might be impaired.[16]

Trans-sulfuration pathway is mediated by cystathionine ß-synthase (CBS), which catalyzes conjugation of serine with Hcy to yield cystathionine. Cystathionine is converted by cystathionine γ-lyase (CTH) to cysteine, a precursor for glutathionine which is a key redox-regulator in cells.[17]

One-carbon metabolism reactions take place in cytosol and mitochondria and they are linked through exchange of serine and glycine, two non-essential amino acids, which are interconverted by the mitochondrial and cytosolic serine hydroxymethyltransferase (SHMT) through 5,10-methylene-THF.[9] Thereby, the impairment

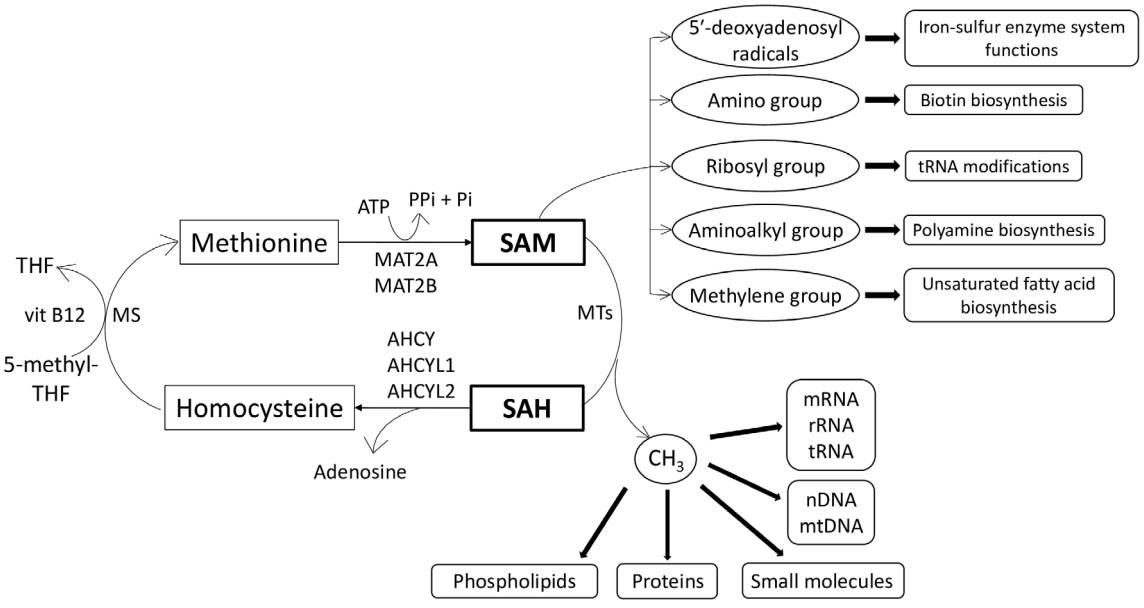

FIGURE 1 Methionine-homocysteine cycle. The functional groups provided by S-adenosyl-methionine (SAM) and their usage are showed. SAH, S-adenosyl-homocysteine.

of global one-carbon pathways might affect both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) methylation.

2 | SAM AND SAH

2.1 | Biochemistry and functions of SAM and SAH in the homocysteine-methionine cycle

In all living cells, SAM is the principal biological methyl donor, the precursor of aminopropyl groups utilized in polyamine biosynthesis and, in the liver, it is also a precursor of glutathione (GSH), the major antioxidant in cellular processes, through its conversion to cysteine via the trans-sulfuration pathway.[18] SAM is synthetized by methionine adenosyltransferase (MAT2A and the liver-specific MAT1A) starting from methionine. It regulates the level of Hcy through two competing pathways: the allosteric activation of CBS and the inhibition of methylenetetrahydrofolate reductase (MTHFR) enzyme, which converts 5,10-methylene-THF to 5-methyl-THF.[12,19]

In transmethylation reactions, SAM methyl groups are added to other compounds from methyltransferase (MT) enzymes, and SAM is converted to SAH.[20] It is also a cofactor of enzymes different from MTs for the transfer of other functional groups derived from SAM. Indeed SAM molecules are a source of methylene groups for the synthesis of unsaturated fatty acids, of amino groups in the biotin biosynthetic pathway, of ribosyl groups for post-transcriptional modification of tRNAs, of aminoalkyl groups for polyamine biosynthesis, and of 5′-deoxyadenosyl radicals for three different iron–sulfur enzyme systems (lysine 2,3-aminomutase, pyruvate-formate lyase, and anaerobic ribonucleotide reductase) in living cells.[21] Each chemical group appended to the sulfur atom of SAM seems to have one or several biological destinations.[22] In addition, it is involved in the formation of monoamine neurotransmitters such as dopamine, serotonin, and norepinephrine. Another essential cofactor of monoamine neurotransmitter biosynthesis is 5-methyl-THF, which also appears to stabilize and enhance production of tetrahydrobiopterin (BH4) involved in cytoprotective pathways.[18,23] Given the large variety of acceptor substrates and the major biological functions, the availability of SAM may have profound effects on cellular growth, differentiation, and functions[24] (Figure 1).

It was determined that 30% of SAM is used by mitochondria, where it is transported by the solute carrier family 25 member 26 (SLC25A26). This transporter is involved in the transport of SAM from cytoplasm to mitochondria and in the antiport transport of SAH using SAM in the inner mitochondrial membrane of the cells.[22] In the mitochondrial compartment, SAM is used for nucleic acid methylation, protein methylation, and cofactor synthesis. A reduced methylating power level was observed in mitochondria of lymphoblastoid cells with trisomy 21 (T21) compared to euploids, probably due to a reduced cellular availability of SAM and its mitochondrial uptake connected to the location of *CBS* and other methyl cycle genes on chromosome 21. Furthermore, the packaging of mtDNA is induced by a transcription factor (transcription factor A, TFAM), which acts like histones on nDNA. It was observed that TFAM is downregulated in cells with T21 determining a change in mitochromosome and exposing mtDNA to the action of DNA methyltransferases (DNMTs). Despite this, the mtDNA resulted hypomethylated in mitochondria of cells with T21.[9]

SAH is formed upon donation of the methyl group of SAM to a methyl acceptor and is subsequently hydrolyzed to adenosine and Hcy in a reversible reaction by adenosylhomocysteinase enzymes or AHCY (in the cytosol), AHCYL1 (in the mitochondria-associated endoplasmic reticulum membrane), and AHCYL2 (in the endoplasmic reticulum and neuron projections).[25] SAH is structurally similar to SAM and binds with a similar range of affinity to the SAM-binding pocket of methyltraferases, thereby inhibiting their activity in a negative feedback loop.[20,26–29] Nevertheless, SAH inhibition is not a uniform process, as it depends on the type of MT and on the type of tissue.[26]

Due to SAM and SAH competition for the SAM-binding pocket of MTs, the SAM/SAH ratio is considered an indicator of the methylation capacity of cells. An increase in SAH or a decrease in the SAM level or a decrease in SAM/SAH ratio can correlate with a reduction of the methylation potential. In addition, SAH is a cytotoxic molecule,[30] thus the removal of SAH excess is essential.[24,31,32] To control the SAM/SAH ratio many enzymes involved in the methionine cycle are controlled at transcriptional and enzymatic levels by the allosteric interaction with SAM. The epigenetic role carried out by SAM and SAH metabolites involves both nuclear and mitochondrial processes such as DNA replication and transcription.[22]

It is interesting to note that lymphocyte SAH concentration was positively correlated with plasma SAH, which in turn was correlated with DNA hypomethylation. No such relationship was observed in the case of SAM concentration. Such results indicate that metabolite modulation of MTs likely occurs primarily through SAH in many cell types.[10]

When dysregulated, the effect of SAH inhibition of methylation reactions potentially gives a wide variety of pathogenic effects, such as a neurotoxic effect responsible for cognitive function impairment.[26] This potential toxic effect was studied in the cobalamin neuropathy in which the hypomethylation of myelin basic protein causes a failure of myelin synthesis.

SAH also inhibits carboxy-O-methyltransferase which results in the activation of the tumor necrosis factor (TNF) induced cell death pathway.[26] Elevated levels of TNF-α are an index of a pro-inflammatory profile which, combined with depletion of insulin growth factor 1 (IGF1), can serve as biomarkers of neurodegeneration and neuroinflammation in subjects with Down syndrome (DS).[33]

2.2 | SAM and SAH measurements

Measurements of SAM and SAH levels are often complicated by the naturally low concentration of these metabolites in plasma (nM range), and particularly the instability of SAM under neutral and alkaline conditions.[34] Stability studies involving serum, plasma, and urine including incubation for 2–80 hours (h) at 4°C and room temperature revealed a progressive increase of SAH levels and a decrease of SAM. Neither the use of heparin or EDTA-plasma nor the times of freezing and thawing significantly influenced these changes of concentrations.[35]

Liquid chromatography (LC) is commonly involved in the analytical methods for the determination of SAH and SAM.[36,37] The stable-isotope-dilution tandem mass spectrometry (MS/MS) is used for chromatographic detection as it provides high sensitivity and selectivity in complex matrices such as urine, plasma, and cerebrospinal fluid (CSF) samples.[38] The determination of the reference intervals for serum, urine, and erythrocytes and the study of the relationships between other metabolites and clinical variables and SAH and SAM levels were performed by a stable-isotope-dilution liquid chromatography–mass spectrometry (LC/MS) method.[35] To overcome the cost limitation, it was replaced by an HPLC method with fluorimetric detection described as an alternative method of detection in blood and tissues.[39] However, the lowest limits of detection were obtained with LC–MS/MS allowing a working concentration range of 8–1024 nM for SAM and 16–1024 nM for SAH.[40] Table 1 summarizes the levels of SAM and SAH commonly reported in plasma using the most commonly employed analytical techniques for these analytes.

Analyses conducted by Bravo et al. showed that when the LC–MS/MS method is used, the concentrations of SAM and SAH are reproducible across different studies,[34] indicating the high sensitivity and affordability of this technique.

In addition, some commercial kits based on an immunoenzymatic approach (enzyme linked immunosorbent assay—ELISA) have been made available and have occasionally been used to measure SAM and SAH levels in human blood.[41] However, it is admittedly difficult to provide affordable detection kits, especially for SAM because this small molecule is degraded in particular alkaline conditions.

3 | SAM-DEPENDENT METHYLATION

3.1 | SAM-dependent methyltransferases

SAM-dependent MTs catalyze the great majority of methylation reactions.[20] These enzymes possess a highly conserved structural fold which binds the common cofactor

TABLE 1 Mean concentrations of S-adenosyl-methionine (SAM) and S-adenosyl-homocysteine (SAH) in healthy biological samples detected by the most widely used experimental methods.

Biological sample	SAM (nM)	SAH (nM)	Method	Reference
Plasma ($n = 12$)	26.5 ± 5.8	28.7 ± 1.8	Fluorescence HPLC chloracetaldehyde	35
Plasma ($n = 7$)	102.7 ± 9.9	22.7 ± 3.1	Fluorescence HPLC chloracetaldehyde	36
Plasma ($n = 15$)	74.7 ± 14.5	26.2 ± 6.1	Stable-isotope dilution tandem mass spectrometry	37
Serum or plasma ($n = 48$)	109 (71–168)	15 (8–26)	Stable-isotope-dilution liquid chromatography–mass spectrometry	34
Blood ($n = 3$)	240 ± 10	206 ± 10	HPLC with fluorimetric detection	38
Plasma ($n = 8$)	95.2 ± 21.6	30.4 ± 6.2	LC–MS/MS	39
Plasma ($n = 33$)	120 ± 36	21.5 ± 6.5	LC–MS/MS	33

Note: Standard deviation or minimum and maximum values are also indicated. n = number of healthy subjects.
Abbreviations: HPLC, high performance liquid chromatography; LC–MS/MS, liquid chromatography–tandem mass spectrometry.

SAM that is localized in the same region of the proteins, but the residues included are very different among the MTs. The core SAM-MT fold incorporates alternating β strands and α helices, which form a seven-stranded β sheet with three helices on each side.[42] The SAM-dependent MT enzymes are involved in the addition of a methyl group to a biologically active molecules and can be distinguished on the basis of the methylation target such as nucleic acids or proteins (e.g., hormones, histones, and transcription factors) or lipids and small molecules (e.g., creatine, phosphatidylcholine, and neurotransmitter).[43,44] The biological functions of methylation include biosynthesis, metabolism, detoxification, signal transduction, protein sorting and repair, and nucleic acid processing.[21]

3.2 | DNA and RNA methylation

DNA MTs and RNA MTs perform fundamental functions in the epigenetic regulation of gene expression and have been implicated in a wide variety of disorders including cancer.[28]

DNA MTs are characterized by two domains: a large domain involved in the catalytic function of the enzyme and the smaller subunit which is involved in the sequence-specific DNA recognition. The smaller domain varies widely both in size and sequence among DNA MTs, it interacts with the major groove of the DNA selecting the target cytosine to be methylated. The larger domain opens the CpG dinucleotide, in order to insert the cytosine in the catalytic pocket that is close to SAM which donates the methyl group. DNA methylation on the CpG dinucleotide induced by SAM is responsible for modulating transcription which alters the regulation of gene activities, somatic inheritance, and cellular differentiation. Sixty percent of the CpG sequences are methylated in the human genome, and their methylation is a signal of inhibition of transcription with a cell type specific pattern. The genomic pattern of cytosine methylation is faithfully inherited and this process, known as "maintenance DNA methylation", is catalyzed by DNA MTs, which are specific for hemimethylated DNA generated during DNA replication. In this way, the original chromatin structure is also re-established including the packaging of the newly synthesized DNA into a nucleosome structure.[45]

DNA methylation is responsible for normal embryonic development, normal brain development, and brain plasticity through the modulation of gene expression. It was also demonstrated that the supplementation or deficiency in the levels of methyl-donor micronutrients during early life can have an impact on offspring brain development. For example, vitamin B12 deficiency correlates with the elevation of Hcy in neurons and astrocytes in specific brain regions such as the striatum, hippocampus, and cerebellum causing altered cognitive functions such as learning and memory deficits during adulthood.[44]

Among the RNA methylation, methylation of adenine in eukaryotic messenger RNA (mRNA) is a postsynthetic modification which occur in 30%–50% of adenine in the mRNAs and that might be considered a necessary modification required for mRNA transport to the cytoplasm, splice site selection or other RNA processing reactions.[46] The methylation of 5′-terminal cap also plays an important role in mRNA export from the nucleus, efficient translation, and protection of the integrity of mRNAs.[47] Apart from guanosine addition to the 5′ end in the most typical cap structure of mRNAs, essentially all cap modifications are due to methylation. The 5′ ends of mRNA and small nuclear RNA are modified further by ribose 2′-O-methylation on the first and second transcribed nucleosides. The U1, U2, U4, and U5 snRNAs are methylated at both the first two positions. These methylations are required for the formation of spliceosomal E-complex and, as consequence, for efficient pre-mRNA splicing.[48]

The methylation of ribosomal RNA (rRNA) occurs after the synthesis of its 47S precursor RNA in the nucleolus before cleavage to smaller fragments. Inhibition of the methylation of rRNA affects its processing to mature 18S and 28S rRNAs.[47]

Methylation of transfer RNA (tRNA) is also another important modification occurring in all cells and tissues. tRNAs methylation is carried out by methylthiotransferases (MTTs), and MTT activity varies with the differentiation state of the cells, and under the influence of many internal and external factors, it is especially elevated in embryonic and cancerous tissues. These enzymes are classified on the basis of the specific target nucleoside.[49] The effects of these modifications represent an important cellular physiological mechanism that can be altered in different diseases.[50] The loss of certain tRNA 2′-O-methylation has been associated with several pathological conditions, including cancers and brain diseases.[51] For example, FTSJ RNA 2′-O-methyltransferase 1 (*FTSJ1*) is a conserved gene encoding for an enzyme that modifies several tRNAs at position 32 and the wobble position 34 in the anticodon loop. Mutations of *FTSJ1* gene are associated to a X-linked recessive inheritance intellectual developmental disorder (XLID9) characterized by moderately to severely impaired intellectual development and eventually delayed motor development, seizures, and/or behavioral problems.[52]

SAM can also bind to certain RNA structures called riboswitches deriving from untranslated mRNA regions

and controlling transcription or translation through the complex of RNA structure with SAM and without proteins. This structure binds mRNA and negatively regulate its expression. Riboswitches evoke a relic of an ancient "RNA world," in which RNA controlled all the processes of life.[53]

3.3 | Protein methylation

Protein methylation is a post-translational modification essential for some proteins to solve their function in many cellular processes like signaling, RNA processing, transcription, and subcellular transport. Protein MTs have been distinguished according to their target, in particular the nitrogen group (arginine or lysine methylation) or carboxyl-group (leucine carboxyl, isoaspartyl methylation, or isoprenyl C-terminal carboxyl methylation). The methylation of the N-terminal is not reversible, while the methylation of the C-terminal can be easily reversed by MTs.[54]

Most protein methylations are carried out by two enzyme families, lysine methyltransferases (KMTs) and protein arginine methyltransferases (PRMTs). In total, over 4000 lysine and arginine methylation sites have been observed in humans. Methylation of other amino acids has also been identified, but the understanding of non-lysine/arginine methylation is currently limited.[55]

Histone proteins are a major and well-known substrate of protein methylation and a crucial part of the epigenetic code playing diverse roles in the establishment of chromatin states that mediate the regulation of gene expression. Histones can be mono-, di-, or trimethylated at lysine and arginine residues by histone methyltransferases (HMTs). There are over 30 characterized HMTs with different methylation capacities and specificities that fall into KMT and PRMT families.[17]

Due to their critical role in cellular physiology, dysregulation of protein methyltransferases is frequently linked to diseases.[55] For example, the set domain-containing 2, histone lysine methyltransferase (*SETD2*) gene encodes for the primary methyltransferase catalyzing H3K36 trimethylation and its function is associated with transcribed regions and functions in transcription fidelity, RNA splicing, and DNA repair.[56] Various mutations of this gene are associated to the autosomal dominant intellectual developmental disorder-70 (MRD70), Luscan-Lumish syndrome, and Rabin-Pappas syndrome, all characterized by intellectual disability (OMIM * 612778).

Central nervous system (CNS) cells possess proteins enabling synthesis and transport of creatine, which plays a modulatory role in neurotransmission, as it acts on GABAA receptor chloride channel complex serving as a competitive antagonist, and on the NMDA receptor with a stimulatory effect.[57,58] It was detected that creatine biosynthesis consumes 40% of methyl groups obtained from SAM[58] starting from the transfer of a guanido group from L-arginine to glycine by glycine-amidinotransferase (GATM), resulting in guanidinoacetic acid. Then guanidinoacetate-N-methyltransferase (GAMT) catalyzes the transfer of a methyl group from SAM to guanidinoacetate producing creatine phosphate necessary for the synthesis of ATP, the main source of cellular energy. The bulk of body creatine is present in skeletal muscle, while lower but substantial concentrations are also recovered in the brain. The body's need of creatine is permanent to allow muscle mass development and to counteract physiological removal in the urine under the form of creatinine.[58]

3.4 | Phospholipid methylation

Phospholipid methylation is another SAM-dependent pathway, particularly the synthesis of phosphatidylcholine (PC), which is the major structural component of cell membrane,[20] through phosphatidyl ethanolamine methyltransferase (PEMT). Altered Hcy metabolism resulting in decreased SAM or increased SAH affects the synthesis of phospholipids (PC from PE). Reduction of PE-methylation decreases very low-density lipoprotein (VLDL) secretion and increases the levels of intracellular triglycerides.[59] Moreover, the SAM-dependent methylation of phospholipids in neuronal cell membrane is reported to modify the signal conduction via neuroreceptors.[60]

3.5 | Methylation of small molecules

There are several MTs that have the function of methylating small molecules. For instance, catechol O-methyltransferase (COMT) O-methylates catecholamines during the deactivation and degradation of catecholamine neurotransmitters. Its role is crucial for the function of the prefrontal cortex of the brain, specifically in organizing and coordinating information from other regions of the brain. When dysregulated, COMT seems to be involved in the onset of different neurological disorders and it has been studied as a major target for the development of drugs used in the treatment of Parkinson's disease. Moreover, because the gene is located on chromosome 22 (Hsa22), people with 22q11.2 deletion have shown increased risk of schizophrenia, depression, anxiety, and bipolar disorder compared with normal individuals.[28,44]

Glycine N-methyltransferase (GNMT) is the major folate binding MT *in vitro* even though it does not utilize folate as a substrate. It transfers a methyl group to glycine to produce sarcosine which has no known essential metabolic function. Sarcosine is converted again to glycine and methylene-THF by a mitochondrial enzyme, sarcosine dehydrogenase. Together, these facts are consistent with and give rise to the interpretation that the importance of GNMT lies not in its ability to form sarcosine but rather in its capacity to regulate the utilization of SAM and thus affect the SAM/SAH ratio.[61]

Hydroxyindole and phenylethanolamine methyltransferases catalyze the transfer of a methyl group from SAM to serotonin and norepinephrine, converting them into melatonin and epinephrine, respectively, whose reduced levels affect the function of the brain.[20]

COQ3-O-methyltransferase and COQ5-C-methyltransferase catalyze the methylation steps in the ubiquinone biosynthetic pathway. Ubiquinone is an essential electron carrier in mitochondrial oxidative phosphorylation, and its loss might impact the ability of the cells to produce energy.[20]

Nicotinamide N-methyltransferase (NNMT) catalyzes the methylation of nicotinamide (NAM) using SAM, directly linking one-carbon metabolism with a cell's methylation balance and nicotinamide adenine dinucleotide (NAD^+) levels. NNMT expression and activity are regulated in a tissue-specific manner, and the protein can act either physiologically or pathologically depending on its distribution.

4 | SAM AND SAH-RELATED HUMAN DISEASES

Methylation is involved in different processes from fetal development to brain function, and its alteration predominantly affect the liver, CNS and muscles. Altered SAM and SAH levels in the cells are the main cause of an altered methylation status of the genome.

High SAM levels might be caused by mutations in MT genes or in SAM transporter genes. Mutations in the *SLC25A26* gene encoding for SAM transporter were reported as the cause of combined oxidative phosphorylation deficiency 28 (COXPD28) and follows an autosomal recessive inheritance pattern. Symptoms range from mild muscle weakness, lactic acidosis, cardiorespiratory insufficiency, and developmental delay to respiratory/multiple organ failure and death.[22] Genetic deficiencies of several methyltransferases illustrate the importance of specific components of transmethylation. For instance, mutations in *GAMT* inherited as autosomal recessive disorder impair creatine synthesis, determining low brain creatinine and causing neurological symptoms which include speech delay, mental retardation, and epilepsy.[58,62] Mutations in the genes encoding for DNMTs methylating both mtDNA and nuclear DNA, such as DNMT1, DNMT3A, and DNMT3B, were associated to several disorders. Mutations in *DNMT1* follow an autosomal dominant inheritance pattern and were reported as the cause of neurological and neurodegenerative pathologies. Mutations in *DNMT3A* gene often were reported to be associated to acute myeloid leukemia (AML) and to autosomal dominant genetic diseases characterized by an impaired intellectual development. Mutations in genes encoding mtRNA MTs, such as *NSUN3*, *TRMT5*, and *TRMT10C*, which are exclusively located in mitochondria, were also reported to be associated to some forms of combined oxidative phosphorylation deficiency whose symptoms include microcephaly, developmental delay, muscle weakness, external ophthalmoplegia, and lactic acidosis.[22] Autosomal recessive mutation inactivating the 18S rRNA methyltransferase gene (*METTL5*) is the cause of intellectual developmental disorder and microcephaly.[63] Autosomal recessive mutation in tRNA MT gene *NSUN2* is the cause of mental retardation-5 (MRT5) phenotype, which is characterized by intellectual disability, facial dysmorphic features, delayed psychomotor and speech development.[64] High SAM levels are typical in plasma of subjects with DS so an altered regulation of the expression of the genes mentioned above should be investigated.[41,65]

Reduced intracellular SAM levels are responsible for folate deficiency characterized by altered DNA synthesis, repair, and methylation. Folic acid deficiency in humans is linked with megaloblastic anemia, neural tube defects, heart disease, and the development of cancer.[66] Low SAM levels were also found in CSF of subjects with neurological disorders such as Alzheimer's dementia or with metabolic disorders such as MTHFR or MAT deficiencies.[67,68] Alzheimer disease (AD) is among the few diseases that may display high Hcy and low vitamin B12 and folate concentrations in blood. It was suggested that amyloid-beta overproduction and accumulation, which may be the cause of the disease, could be due to the loss of epigenetic control in the expression of the genes involved in amyloid-beta protein precursor processing. Indeed, SAM metabolism is strictly related to the methylation of promoters of two genes responsible for amyloid-production (β- and γ-secretases).[69]

It is also known that decreased levels of SAM and neurotransmitters are connected to decreased levels of 5-methyl-THF in CSF. SAM and 5-methyl-THF are responsible for some protein modifications necessary for nerve regeneration, low levels of these one-carbon metabolites were found in the CSF of patients with a reduction in myelination of corticospinal tracts.[70–72] Abnormal one-

carbon metabolism was reported in many diseases, such as cerebral folate deficiencies, a group of disorders frequently characterized by neurological impairments, and neurodevelopment disorders caused by genetic mutations and not.[73,74] The availability of SAM influences the activity of SAM decarboxylase (AMD1) in the synthesis of polyamines. Polyamines, primarily spermidine and spermine, and their precursor putrescine are essential for cellular proliferation and survival. Increased levels of polyamines compete with SAM-dependent methylation and were associated to tumor promotion and many autoimmune diseases.[75] The effect of polyamine increases due to the increase in AMD1 activity in the neonate and throughout adulthood, implying a role for these polyamines in both development and mature brain function.[76]

Another rare genetic disease is caused by autosomal recessive mutations in *COQ5* gene involved in methylation and ubiquinone biosynthetic process, whose protein product is located in mitochondrial matrix. The phenotype of these mutations is characterized by cerebellar ataxia associated with cerebellar atrophy, and often also by intellectual disability and seizures.[77]

The correlation existing between reduced SAM levels and depression[78] is noteworthy, indeed in subjects suffering of depression, SAM has been administrated as an antidepressant.[79–81] Oral administration of a stable salt of SAM has also been shown to restore normal liver function in the presence of various chronic liver diseases, suggesting that increasing circulating levels of SAM, normally involved in the phospholipid synthesis, improves impaired fluidisation of cell membranes.[79,82,83] Molecular studies indicated that deficiency of MAT1A which catalyzes the synthesis of SAM from methionine and ATP in liver is the cause of the abnormal metabolism of methionine and is responsible of human liver cirrhosis.[31,84] Loss of function mutations of *MAT1A* gene are also responsible for severe clinical symptoms, including generalized hypotonia, with death at 8 months,[85] mental retardation,[86] and brain demyelination.[68,87]

In addition, SAM seems to have a proapoptotic effect on liver cancer cells[88] inhibiting the mitogenic effect of tumor inducing factors. When measuring the methylation status of various genes in tumor cells, both hypermethylation as well as hypomethylation can occur. It appears that the methylation pattern of specific genes is altered in tumor cells and normally unmethylated CpG islands may become hypermethylated. These epigenetic alterations can aberrantly silence or activate gene expression during cancer development.

Both SAM and SAH analogs have been recognized and studied as small molecule inhibitors of MTs for studying how the alteration of their function exists and for understanding a plethora of diseases, especially cancer.[28] High SAH levels were found in plasma of subjects with DS and this might be correlated to the low incidence of solid tumors typical of this genetic condition.[89] This unusual pattern of cancer occurrence in DS was considered as a model for understanding carcinogenesis in the general population.[89–91] High SAH levels have important effects on the physiological mechanisms of the cells such as the synthesis of neurotransmitters. An accumulation of SAH in body fluids was associated with vascular disease, tissue damage, and neurological symptoms.[92–96] Among neuropathological disease demyelination is another effect related with altered SAH levels.[20]

The SAH hydrolase deficiency is responsible for high SAH levels and is caused by mutations in the *AHCY* gene. It has an autosomal recessive inheritance and manifests with developmental delay and hypotonia and more variably with cerebral hypomyelination, coagulation abnormalities, and hepatopathy. Baric and coll. observed the inhibition of DNA methyltransferase activities caused by high SAH levels.[97] In addition, they observed that nucleocytoplasmic distribution of AHCY is mediated by the interaction with its paralog AHCYL1 and this cooperation is impaired by the AHCY deficiency.[98]

High SAH and SAM levels were also observed when homozygous mutations in the *ADK* gene encoding for adenosine kinase occur. ADK catalyzes the transfer of the gamma-phosphate from ATP to adenosine, thereby serving as a regulator of extracellular and intracellular adenine nucleotide concentrations. The phenotype caused by these mutations is characterized by global developmental delay, early-onset seizures, mild dysmorphic features, and characteristic biochemical anomalies, including persistent hypermethioninemia.[99]

SAM/SAH ratio is also controlled by GNMT and when inactivated by homozygous gene mutations is the cause of hepatomegaly.[100] High SAH levels and low SAM levels including DNA hypomethylation were also reported in MTHFR deficiency caused by loss of function mutation in the *MTHFR* gene.[101,102] The phenotype of these mutations could be characterized by neural tube defects,[103] thrombophilia due to thrombin defect causing cerebral thrombosis[104] and homocystinuria.[105] Table 2 summarizes the SAM and SAH-related human disease.

5 | ONE-CARBON PATHWAY DYSREGULATION AND METHYLATION ALTERATION IN TRISOMY 21

As mentioned above, SAM and SAH levels and the whole one-carbon cycle can influence neurodevelopment and

TABLE 2 S-adenosyl-methionine (SAM) and S-adenosyl-homocysteine (SAH) related human diseases.

(a)

SAM—Increased level

Related disease	Mutated gene	Reference
Combined oxidative phosphorylation deficiency 28	*SLC25A26*	104
Speech delay, cognitive impairment and epilepsy	*GAMT*	57,61
AD Cerebellar ataxia, deafness and narcolepsy (ADCADN); Neuropathy, hereditary sensory type IE (HSN1E)	*DNMT1*	105,106
Somatic acute myeloid leukemia (AML); Heyn-Sproul-Jackson syndrome (HESJAS); Tatton-Brown-Rahman syndrome (TBRS)	*DNMT3A*	107–109
Intellectual developmental disorder and microcephaly	*METTL5*	62
Mental retardation-5 (MRT5) phenotype	*NSUN2*	63
Combined oxidative phosphorylation deficiencies	*NSUN3, TRMT5, TRMT10C*	22
Cerebellar ataxia, intellectual disability and seizures	*COQ5*	76

(b)

SAM—Decreased level

Related disease	Mutated gene	Reference
Alzheimer's dementia	[a]	68,110
Parkinson's disease	[a]	111
Metabolic defects	*MAT2A, MTHFR*	86,100,112
Generalized hypotonia, cognitive impairment, brain demyelination	*MAT1A*	67,84–86
Reduction in myelination of corticospinal tracts	[a]	69–71
Cerebral folate deficiencies	[a]	72,73
Depression	[a]	77–80

(Continues)

TABLE 2 (Continued)

(c)

SAH—Increased level

Related disease	Mutated gene	Reference
Developmental delay and hypotonia, coagulation abnormalities, and hepatopathy	*AHCY*	96
Hepatomegaly	*GNMT*	98
Neural tube defects, thrombophilia, and homocystinuria	*MTHFR*	101–103

(d)

SAM and SAH—Increased level

Related disease	Mutated gene	Reference
Global developmental delay, early-onset seizures, mild dysmorphic features, and hypermethioninemia	*ADK*	113

Note: (a) High SAM level's related human diseases. AD, Alzheimer Dementia. (b) Low SAM level's related human diseases. (c) High SAH level's related human diseases. (d) High SAM and SAH level's related human diseases.

[a]Not demonstrated genetic bases.

therefore also cognitive development. This aspect must therefore be considered in the presence of conditions with cognitive impairment such as T21 causing DS, currently the human genetic condition most associated with cognitive impairment.

The genetic cause of DS or T21 is the presence of full or partial chromosome 21 (Hsa21) in three copies in all the cells of an individual. It has an incidence of 1 in every 1000–1100 newborn children around the world.[106] The original description of the syndrome was attributed to John Langdon Down in 1866, and the chromosomal cause was discovered for the first time in 1959.[107] The most constant feature and the main clinical phenotypes of DS are intellectual disability, typical *facies* (oblique eyes with epicantic folds and flat nasal bridge) and hypotonia at birth.[108]

A specific cognitive profile has been associated with DS, individuals with DS have more pronounced language and verbal memory challenges and relatively stronger nonverbal abilities and implicit memory skills.[109–111]

To date the biological mechanism determining the impairment of the cognitive functions in DS is unknown. Several genes located on Hsa21 have functions biologically consistent with altered pathways in DS, in particular brain development and function, oxidative metabolism and one-carbon pathway.[112–114] It was proven that the additional Hsa21 copy in T21 cells generates an excess of several Hsa21 gene products as well as

of other human gene products close to 3:2 with respect to the normal cells. The global overexpression of Hsa21 genes and Hsa21 segments in DS samples compared to genes from other chromosomes supports the decisive role of the Hsa21 gene dosage in the pathogenesis of the syndrome.[113,115]

Shifting the focus from what is upstream (gene excess and transcript over- or down-regulation) to what is downstream (enzyme reaction products), a clear discrimination among the metabolomic profile of subjects with DS vs healthy controls was identified. This analysis performed with nuclear magnetic resonance allowed to identify the alteration of metabolite levels related with central carbon metabolism or glycolysis and Krebs cycle, and also one-carbon metabolism.[116] The latest published work about the plasma metabolome in a larger cohort of subjects with DS and healthy controls (almost doubled) confirmed the results of the previous study.[116,117] In this work, a discrimination accuracy of 74.5% between the metabolome profiles of DS subjects with an intelligence quotient (IQ) ≤40 and with an IQ > 40 was found, but the determining correlated metabolites could not be identified.[117] These alterations may be an expression of a shift from an aerobic pathway to an anaerobic one as well as an expression of a hypoxic state of the cell that causes higher mitochondrion stress[118,119] due to alterations in central carbon metabolism.[120]

Comparing DS with several metabolic diseases, Lejeune hypothesized that a disturbance of the one-carbon cycle might occur in DS.[121] Central carbon and one-carbon metabolisms are two pathways included in the carbon metabolism and are interconnected through the serine/glycine biosynthetic pathway by which one-carbon units are generated.[122] Lejeune studied a possible correlation between one-carbon pathway alteration and Hsa21 gene expression levels in DS.[123] Several enzymes involved in the one-carbon metabolism were encoded by genes located on Hsa21, in particular: CBS and phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, and phosphoribosylaminoimidazole synthetase (the three subunits of GART enzyme). Moreover, the gene for the main transporter of folate, solute carrier family 19 member 1 gene (*SLC19A1*), is also located on Hsa21. His hypothesis was confirmed by the observation *in vivo* of an increased toxicity of methotrexate (MTX) in the leukemia therapy in children with DS compared to euploid subjects[124] and by the observation *in vitro* that MTX was twice as toxic in T21 lymphocytes compared to control cells. MTX is a folate analog that inhibits the activity of the dihydrofolate reductase (DHFR) enzyme involved in the conversion of dihydrofolate into THF.[125] It is a cytotoxic drug used as a chemotherapy agent and immune system suppressant.[126,127]

Vitale and coll. recently demonstrated the rescue effect on MTX toxicity mediated by some folate derivatives in T21 and euploid human fibroblast cell lines. In particular, it was demonstrated that 5-methyl-THF and 5-formyl-THF (folinic acid) treatments have significant protective effects on both euploid and T21 cells during MTX treatment.[114]

The increased MTX toxicity in DS was recently related to altered erythrocyte folate concentrations in a recent survey on a cohort of subjects with DS and with juvenile arthritis as euploid control. They reported a lower concentration level of total folate, 5-methyl-THF and 5,10-methenyl-THF in the erythrocytes of subjects with DS. These reductions in erythrocyte folates were also associated with a decrease in short-chain folate polyglutamation 5-methyl-THFGlu3-6 and a corresponding increase in longer chain 5-methyl-THFGlu7-10.[128] These data suggest that in trisomic cells there is a biological adaptation to folate deficiency by an increased level of long-chain folate polyglutamation; indeed, increased expression of folylpolyglutamate synthetase (FPGS) gene was already reported *in vitro* and in animal models.[113,128,129]

In subjects with DS, one-carbon metabolism was considered to be imbalanced by several authors.[121,128,130–132] In particular, subjects with DS commonly present lower blood levels of vitamin B12 (or cobalamin) and folic acid with increasing age, lower erythrocyte folates and Hcy compared to healthy controls, and increased level of cysteine.[65,128,132,133] Moreover, it was demonstrated that the amount of newly formed methionine (from 5-methyl-THF) is decreased in T21 cells compared to normal cells,[134,135] even if recent studies reported that methionine blood level is not altered in subjects with DS compared to control subjects.[65,136]

Finally, several central metabolites of one-carbon pathway were measured in plasma of subjects with DS vs euploid controls. In particular, THF, 5-methyl-THF, SAH, and SAM have altered concentration levels in DS, and the SAM/SAH ratio (the methylation potential)[20,137] is decreased.[41] Based on these findings, 5-methyl-THF has been proposed as the best candidate for a clinical trial that aims to improve cognitive status of subjects with T21.[41] It will also be of interest to check if in such clinical trial with 5-methyl-THF there will be a normalization of SAM/SAH ratio that is altered in children with T21.

It is known that DS presents DNA-methylation alterations. It was demonstrated that many CpG islands, spread in all chromosomes, are mostly hypermethylated, in several tissues and cell lines of subjects with DS compared to controls,[138–141] also in newborns[142] and toddlers with DS.[143] These findings demonstrate that there is an accelerated epigenetic aging in DS and together with

other clinical and molecular evidence, the hypothesis that DS is a progeroid syndrome is advanced.[144,145] These results suggest that the impaired one-carbon metabolism and the epigenetic modifications in DS are associated and the first could be the cause of the second.

Recently, a significant improvement of obstructive sleep apnea (OSA) has been reported in an adult with DS following SAM administration, possibly related to the counteraction by SAM, as a potent methyl donor, of high oxidative stress in the respiratory muscles.[146]

6 | CONCLUSIONS

There is large evidence that altered levels of SAM and SAH are the cause of several diseases and that the SAM/SAH ratio is very finely regulated by the cells.

The connection between one-carbon metabolism in the cytoplasm and that of the mitochondrion shows that an alteration of SAM and SAH levels impacts the functionality of multiple cellular compartments. Altered mitochondrial metabolism has already been widely described in DS, so a link between altered metabolic levels of SAM and SAH and mitochondrial function can also be assumed. The proapoptotic effect of the inhibition of SAM-dependent MTs by SAH could also explain a hypocellularity typical of DS caused by an excess of blood SAH levels.

In the context of a complex disease such as DS in which the phenotype is caused by the presence of all or part of chromosome 21 in three copies, an exact identification of the main altered biological mechanisms has never been obtained and it has never been described how altered levels of SAM and SAH may interfere with the manifestation of the syndrome phenotype. It is already known that plasma levels of SAM and SAH are increased, but their dosage in CSF has never been performed and might provide some more insights into their effects at the brain level.

AUTHOR CONTRIBUTIONS

MaC conceptualized the article and wrote the original draft. BV contributed to writing the original draft, validated data extraction from the literature, and supervised the work. GR contributed to writing the sections related to human diseases and trisomy 21 and the visualization of the data. FA edited the manuscript and revised the original draft. FC, CL, GLP, and PS revised and validated the sections related to human diseases and trisomy 21. MiC, BL, MCP, AP, and LV revised and validated the sections related to the biochemistry and biology of SAM and SAH. All the authors critically reviewed and edited the final draft.

ACKNOWLEDGMENTS

We are especially grateful to the "Fondazione Umano Progresso," Milano, and to "Fondazione Rosa Prístina," Pisa, for their valuable support to our research on trisomy 21, as well as to: the Mele Family; "Gruppo Arzdore di Dozza" and the Costa Family; "Associazione Vola con Martin oltre il 21" ODV, Mandello del Lario (LC); Gabriele's dad and mum; Giovanni Roncoroni; Paolo Ciaroni, main organizer of the car meetings "Top Down 2022," Fifth Edition, devoted to spider cars and ideated with Luciano Perondi; "Uniamoci per la Ricerca" initiative by Jonatan Benvenuti and Alberto Emilio; "Associazione Sette per te ventuno—Non abbiate paura" onlus; Meeting in Trento—Silvia Contino and Giuseppe De Michele; "Associazione Amicorum," Cassano Magnago; "Unità Pastorale di Reda," "Parrocchie di S. Stefano in Corleto e S. Maria in Basiago," "Gruppo Dcuore"; "Il popolo di Elisa Schira"; Sichim Alfa Srl. We are grateful to Kirsten Welter for her kind and expert revision of the manuscript.

FUNDING INFORMATION

The fellowship for G.R. has been funded by donations from the Fondazione Umano Progresso. The fellowship for B.V. has been funded by the Fundraising Initiatives in Cassano Magnago (VA). This study has been realized with the co-financing, in particular regarding the fellowships for M.Ci. and F.A., of the Ministry of University and Research in the framework of PNC "DARE—Digital lifelong prevention project" (PNC0000002—CUP B53C22006450001). The views and opinions expressed are solely those of the authors and do not necessarily reflect those of the European Union, nor can the European Union be held responsible for them.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

ORCID

Bassam Lajin https://orcid.org/0000-0002-5717-1201
Francesca Antonaros https://orcid.org/0000-0001-7668-5833

REFERENCES

1. Clare CE, Brassington AH, Kwong WY, Sinclair KD. One-carbon metabolism: linking nutritional biochemistry to epigenetic programming of long-term development. Annu Rev Anim Biosci. 2019;7:263–87.
2. Lyon P, Strippoli V, Fang B, Cimmino L. B vitamins and one-carbon metabolism: implications in human health and disease. Nutrients. 2020;12(9):2867–90.
3. Mattson MP, Shea TB. Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. Trends Neurosci. 2003;26(3):137–46.

4. Garratt LC, Ortori CA, Tucker GA, Sablitzky F, Bennett MJ, Barrett DA. Comprehensive metabolic profiling of mono- and polyglutamated folates and their precursors in plant and animal tissue using liquid chromatography/negative ion electrospray ionisation tandem mass spectrometry. Rapid Commun Mass Spectrom. 2005;19(17):2390–8.
5. Visentin M, Diop-Bove N, Zhao R, Goldman ID. The intestinal absorption of folates. Annu Rev Physiol. 2014;76: 251–74.
6. Zhao R, Matherly LH, Goldman ID. Membrane transporters and folate homeostasis: intestinal absorption and transport into systemic compartments and tissues. Expert Rev Mol Med. 2009;11:e4.
7. Xu J, Xu B, Shou D, Qin F, Xu Y, Hu Y. Characterization and evaluation of a folic acid receptor-targeted cyclodextrin complex as an anticancer drug delivery system. Eur J Pharm Sci. 2016;83:132–42.
8. Roje S. S-adenosyl-L-methionine: beyond the universal methyl group donor. Phytochemistry. 2006;67(15):1686–98.
9. Iacobazzi V, Castegna A, Infantino V, Andria G. Mitochondrial DNA methylation as a next-generation biomarker and diagnostic tool. Mol Genet Metab. 2013;110(1–2):25–34.
10. Ulrey CL, Liu L, Andrews LG, Tollefsbol TO. The impact of metabolism on DNA methylation. Hum Mol Genet. 2005; 14(1):R139–47.
11. Caudill MA, Wang JC, Melnyk S, Pogribny IP, Jernigan S, Collins MD, et al. Intracellular S-adenosylhomocysteine concentrations predict global DNA hypomethylation in tissues of methyl-deficient cystathionine beta-synthase heterozygous mice. J Nutr. 2001;131(11):2811–8.
12. Bhatia M, Thakur J, Suyal S, Oniel R, Chakraborty R, Pradhan S, et al. Allosteric inhibition of MTHFR prevents futile SAM cycling and maintains nucleotide pools in one-carbon metabolism. J Biol Chem. 2020;295(47):16037–57.
13. Witham KL, Minchin RF, Butcher NJ. Role for human arylamine N-acetyltransferase 1 in the methionine salvage pathway. Biochem Pharmacol. 2017;125:93–100.
14. Bekdash RA. Methyl donors, epigenetic alterations, and brain health: understanding the connection. Int J Mol Sci. 2023; 24(3):2346–60.
15. Lejeune J. On the mechanism of mental deficiency in chromosomal diseases. Hereditas. 1977;86(1):9–14.
16. Lejeune J. Les tables de la Loi de la vie. Acad Natl Dei Lincei. 1985;11:287–93.
17. Mentch SJ, Locasale JW. One-carbon metabolism and epigenetics: understanding the specificity. Ann N Y Acad Sci. 2016; 1363(1):91–8.
18. Miller AL. The methylation, neurotransmitter, and antioxidant connections between folate and depression. Altern Med Rev. 2008;13(3):216–26.
19. Zuhra K, Augsburger F, Majtan T, Szabo C. Cystathionine-β-Synthase: molecular regulation and pharmacological inhibition. Biomolecules. 2020;10(5):697–778.
20. Clarke S, Banfield K. S-adenosylmethionine-dependent methyltransferases. In: Carmel R, Jackobsen DW, editors. Homocysteine in health and disease. Cambridge University Press, Cambridge, UK; 2001.
21. Fontecave M, Atta M, Mulliez E. S-adenosylmethionine: nothing goes to waste. Trends Biochem Sci. 2004;29(5):243–9.
22. Monné M, Marobbio CMT, Agrimi G, Palmieri L, Palmieri F. Mitochondrial transport and metabolism of the major methyl donor and versatile cofactor S-adenosylmethionine, and related diseases: a review. IUBMB Life. 2022;74(7):573–91.
23. Eichwald T, da Silva LB, Staats Pires AC, Niero L, Schnorrenberger E, Filho CC, et al. Tetrahydrobiopterin: beyond its traditional role as a cofactor. Antioxidants. 2023; 12(5):1037–63.
24. Lu SC. S-adenosylmethionine. Int J Biochem Cell Biol. 2000; 32(4):391–5.
25. Bonneau B, Ando H, Kawaai K, Hirose M, Takahashi-Iwanaga H, Mikoshiba K. IRBIT controls apoptosis by interacting with the Bcl-2 homolog, Bcl2l10, and by promoting ER-mitochondria contact. eLife. 2016;5:5.
26. Weir DG, Scott JM. Brain function in the elderly: role of vitamin B12 and folate. Br Med Bull. 1999;55(3):669–82.
27. Richon VM, Johnston D, Sneeringer CJ, Jin L, Majer CR, Elliston K, et al. Chemogenetic analysis of human protein methyltransferases. Chem Biol Drug des. 2011;78(2):199–210.
28. Zhang J, Zheng YG. SAM/SAH analogs as versatile tools for SAM-dependent methyltransferases. ACS Chem Biol. 2016; 11(3):583–97.
29. Vizán P, Di Croce L, Aranda S. Functional and pathological roles of AHCY. Front Cell Dev Biol. 2021;9:654344.
30. Janisiak J, Kopytko P, Tkacz M, Rogińska D, Perużyńska M, Machaliński B, et al. Protein arginine methyltransferase (PRMT) inhibitors-AMI-1 and SAH are effective in attenuating rhabdomyosarcoma growth and proliferation in cell cultures. Int J Mol Sci. 2021;22(15):8023–40.
31. Mato JM, Alvarez L, Ortiz P, Pajares MA. S-adenosylmethionine synthesis: molecular mechanisms and clinical implications. Pharmacol Ther. 1997;73(3):265–80.
32. Finkelstein JD. Methionine metabolism in mammals. J Nutr Biochem. 1990;1(5):228–37.
33. Araya P, Kinning KT, Coughlan C, Smith KP, Granrath RE, Enriquez-Estrada BA, et al. IGF1 deficiency integrates stunted growth and neurodegeneration in down syndrome. Cell Rep. 2022;41(13):111883.
34. Bravo AC, Aguilera MNL, Marziali NR, Moritz L, Wingert V, Klotz K, et al. Analysis of S-adenosylmethionine and S-adenosylhomocysteine: method optimisation and profiling in healthy adults upon short-term dietary intervention. Metabolites. 2022;12(5):373–393.
35. Stabler SP, Allen RH. Quantification of serum and urinary S-adenosylmethionine and S-adenosylhomocysteine by stable-isotope-dilution liquid chromatography-mass spectrometry. Clin Chem. 2004;50(2):365–72.
36. Loehrer FM, Haefeli WE, Angst CP, Browne G, Frick G, Fowler B. Effect of methionine loading on 5-methyltetrahydrofolate, S-adenosylmethionine and S-adenosylhomocysteine in plasma of healthy humans. Clin Sci. 1996;91(1):79–86.
37. Capdevila A, Wagner C. Measurement of plasma S-adenosylmethionine and S-adenosylhomocysteine as their fluorescent isoindoles. Anal Biochem. 1998;264(2):180–4.
38. Struys EA, Jansen EE, de Meer K, Jakobs C. Determination of S-adenosylmethionine and S-adenosylhomocysteine in plasma and cerebrospinal fluid by stable-isotope dilution tandem mass spectrometry. Clin Chem. 2000;46(10):1650–6.

39. Albu C, Litescu SC, Radu GL, Aboul-Enein HY. Validated HPLC-Fl method for the analysis of S-adenosylmethionine and S-adenosylhomocysteine biomarkers in human blood. J Fluoresc. 2013;23(3):381–6.
40. Klepacki J, Brunner N, Schmitz V, Klawitter J, Christians U, Klawitter J. Development and validation of an LC-MS/MS assay for the quantification of the trans-methylation pathway intermediates S-adenosylmethionine and S-adenosylhomocysteine in human plasma. Clin Chim Acta. 2013;421:91–7.
41. Vione B, Ramacieri G, Zavaroni G, Piano A, La Rocca G, Caracausi M, et al. One-carbon pathway metabolites are altered in the plasma of subjects with down syndrome: relation to chromosomal dosage. Front Med. 2022;9:1006891.
42. Martin JL, McMillan FM. SAM (dependent) I AM: the S-adenosylmethionine-dependent methyltransferase fold. Curr Opin Struct Biol. 2002;12(6):783–93.
43. Struck AW, Thompson ML, Wong LS, Micklefield J. S-adenosyl-methionine-dependent methyltransferases: highly versatile enzymes in biocatalysis, biosynthesis and other biotechnological applications. Chembiochem. 2012;13(18):2642–55.
44. Li J, Sun C, Cai W, Li J, Rosen BP, Chen J. Insights into S-adenosyl-l-methionine (SAM)-dependent methyltransferase related diseases and genetic polymorphisms. Mutat Res Rev Mutat Res. 2021;788:108396.
45. Heby O. DNA methylation and polyamines in embryonic development and cancer. Int J Dev Biol. 1995;39(5):737–57.
46. Tuck MT. Partial purification of a 6-methyladenine mRNA methyltransferase which modifies internal adenine residues. Biochem J. 1992;288(1):233–40.
47. Chiang PK, Gordon RK, Tal J, Zeng GC, Doctor BP, Pardhasaradhi K, et al. S-adenosylmethionine and methylation. FASEB J. 1996;10(4):471–80.
48. Byszewska M, Śmietański M, Purta E, Bujnicki JM. RNA methyltransferases involved in 5′ cap biosynthesis. RNA Biol. 2014;11(12):1597–607.
49. Nau F. The methylation of tRNA. Biochimie. 1976;58(6): 629–45.
50. Adami R, Bottai D. S-adenosylmethionine tRNA modification: unexpected/unsuspected implications of former/new players. Int J Biol Sci. 2020;16(15):3018–27.
51. Dimitrova DG, Teysset L, Carré C. RNA 2'-O-methylation (nm) modification in human diseases. Genes. 2019;10(2): 117–139.
52. Brazane M, Dimitrova DG, Pigeon J, Paolantoni C, Ye T, Marchand V, et al. The ribose methylation enzyme FTSJ1 has a conserved role in neuron morphology and learning performance. Life Sci Alliance. 2023;6(4):e202201877.
53. Loenen WA. S-adenosylmethionine: jack of all trades and master of everything? Biochem Soc Trans. 2006;34(Pt 2): 330–3.
54. Grillo MA, Colombatto S. S-adenosylmethionine and protein methylation. Amino Acids. 2005;28(4):357–62.
55. Dilworth D, Barsyte-Lovejoy D. Targeting protein methylation: from chemical tools to precision medicines. Cell Mol Life Sci. 2019;76(15):2967–85.
56. Xu Q, Xiang Y, Wang Q, Wang L, Brind'Amour J, Bogutz AB, et al. SETD2 regulates the maternal epigenome, genomic imprinting and embryonic development. Nat Genet. 2019; 51(5):844–56.
57. Royes LF, Fighera MR, Furian AF, Oliveira MS, Fiorenza NG, Ferreira J, et al. Neuromodulatory effect of creatine on extracellular action potentials in rat hippocampus: role of NMDA receptors. Neurochem Int. 2008;53(1–2):33–7.
58. Joncquel-Chevalier Curt M, Voicu PM, Fontaine M, Dessein AF, Porchet N, Mention-Mulliez K, et al. Creatine biosynthesis and transport in health and disease. Biochimie. 2015;119:146–65.
59. Obeid R, Herrmann W. Homocysteine and lipids: S-adenosyl methionine as a key intermediate. FEBS Lett. 2009;583(8): 1215–25.
60. Bottiglieri T. Folate, vitamin B_{12}, and S-adenosylmethionine. Psychiatr Clin North Am. 2013;36(1):1–13.
61. Luka Z, Mudd SH, Wagner C. Glycine N-methyltransferase and regulation of S-adenosylmethionine levels. J Biol Chem. 2009;284(34):22507–11.
62. Viau KS, Ernst SL, Pasquali M, Botto LD, Hedlund G, Longo N. Evidence-based treatment of guanidinoacetate methyltransferase (GAMT) deficiency. Mol Genet Metab. 2013;110(3):255–62.
63. Richard EM, Polla DL, Assir MZ, Contreras M, Shahzad M, Khan AA, et al. Bi-allelic variants in METTL5 cause autosomal-recessive intellectual disability and microcephaly. Am J Hum Genet. 2019;105(4):869–78.
64. Abbasi-Moheb L, Mertel S, Gonsior M, Nouri-Vahid L, Kahrizi K, Cirak S, et al. Mutations in NSUN2 cause autosomal-recessive intellectual disability. Am J Hum Genet. 2012;90(5):847–55.
65. Obeid R, Hartmuth K, Herrmann W, Gortner L, Rohrer TR, Geisel J, et al. Blood biomarkers of methylation in down syndrome and metabolic simulations using a mathematical model. Mol Nutr Food Res. 2012;56(10):1582–9.
66. Duthie SJ. Folic acid deficiency and cancer: mechanisms of DNA instability. Br Med Bull. 1999;55(3):578–92.
67. Bottiglieri T, Hyland K, Reynolds EH. The clinical potential of ademetionine (S-adenosylmethionine) in neurological disorders. Drugs. 1994;48(2):137–52.
68. Furujo M, Kinoshita M, Nagao M, Kubo T. Methionine adenosyltransferase I/III deficiency: neurological manifestations and relevance of S-adenosylmethionine. Mol Genet Metab. 2012;107(3):253–6.
69. Scarpa S, Cavallaro RA, D'Anselmi F, Fuso A. Gene silencing through methylation: an epigenetic intervention on Alzheimer disease. J Alzheimers Dis. 2006;9(4):407–14.
70. Surtees R, Clelland J, Hann I. Demyelination and single-carbon transfer pathway metabolites during the treatment of acute lymphoblastic leukemia: CSF studies. J Clin Oncol. 1998;16(4):1505–11.
71. Kožich V, Stabler S. Lessons learned from inherited metabolic disorders of sulfur-containing amino acids metabolism. J Nutr. 2020;150(Suppl. 1):2506s–2517s.
72. Cestaro B. Effects of arginine, S-adenosylmethionine and polyamines on nerve regeneration. Acta Neurol Scand Suppl. 1994;154:32–41.
73. Pope S, Artuch R, Heales S, Rahman S. Cerebral folate deficiency: analytical tests and differential diagnosis. J Inherit Metab Dis. 2019;42(4):655–72.
74. Watkins D, Rosenblatt DS. Update and new concepts in vitamin responsive disorders of folate transport and metabolism. J Inherit Metab Dis. 2012;35(4):665–70.

75. Brooks WH. Autoimmune diseases and polyamines. Clin Rev Allergy Immunol. 2012;42(1):58–70.
76. Morrison LD, Becker L, Kish SJ. S-adenosylmethionine decarboxylase in human brain. Regional distribution and influence of aging. Brain Res Dev Brain Res. 1993;73(2):237–41.
77. Nguyen TP, Casarin A, Desbats MA, Doimo M, Trevisson E, Santos-Ocaña C, et al. Molecular characterization of the human COQ5 C-methyltransferase in coenzyme Q10 biosynthesis. Biochim Biophys Acta. 2014;1841(11):1628–38.
78. Cantoni GL, Mudd SH, Andreoli V. Affective disorders and S-adenosylmethionine: a new hypothesis. Trends Neurosci. 1989;12(9):319–24.
79. Friedel HA, Goa KL, Benfield P. S-adenosyl-L-methionine. A review of its pharmacological properties and therapeutic potential in liver dysfunction and affective disorders in relation to its physiological role in cell metabolism. Drugs. 1989; 38(3):389–416.
80. Ortega TM, Andrés P, López-Sobaler A, Ortega A, Redondo R, Jiménez A, et al. The role of folates in the diverse biochemical processes that control mental function. Nutr Hosp. 1994;9(4): 251–6.
81. De Berardis D, Orsolini L, Serroni N, Girinelli G, Iasevoli F, Tomasetti C, et al. A comprehensive review on the efficacy of S-adenosyl-L-methionine in major depressive disorder. CNS Neurol Disord Drug Targets. 2016;15(1):35–44.
82. Kaye GL, Blake JC, Burroughs AK. Metabolism of exogenous S-adenosyl-L-methionine in patients with liver disease. Drugs. 1990;40(Suppl. 3):124–8.
83. Chawla RK, Bonkovsky HL, Galambos JT. Biochemistry and pharmacology of S-adenosyl-L-methionine and rationale for its use in liver disease. Drugs. 1990;40(Suppl 3):98–110.
84. Chou ST, Byrska-Bishop M, Tober JM, Yao Y, Vandorn D, Opalinska JB, et al. Trisomy 21-associated defects in human primitive hematopoiesis revealed through induced pluripotent stem cells. Proc Natl Acad Sci U S A. 2012;109(43):17573–8.
85. Guízar Vázquez J, Sánchez Aguilar G, Velázquez A, Fragoso R, Rostenberg I, Alejandre I. Hypermethioninemia. Apropos of a case in a consanguineous couple. Bol Med Hosp Infant Mex. 1980;37(6):1237–44.
86. Mudd SH, Levy HL, Tangerman A, Boujet C, Buist N, Davidson-Mundt A, et al. Isolated persistent hypermethioninemia. Am J Hum Genet. 1995;57(4):882–92.
87. Surtees R, Leonard J, Austin S. Association of demyelination with deficiency of cerebrospinal-fluid S-adenosylmethionine in inborn errors of methyl-transfer pathway. Lancet. 1991; 338(8782–8783):1550–4.
88. Lu SC, Mato JM. S-Adenosylmethionine in cell growth, apoptosis and liver cancer. J Gastroenterol Hepatol. 2008;23-(Suppl. 1):S73–7.
89. Hasle H, Friedman JM, Olsen JH, Rasmussen SA. Low risk of solid tumors in persons with down syndrome. Genet Med. 2016;18(11):1151–7.
90. Satgé D, Seidel MG. The pattern of malignancies in down syndrome and its potential context with the immune system. Front Immunol. 2018;9:3058.
91. Osuna-Marco MP, López-Barahona M, López-Ibor B, Tejera ÁM. Ten reasons why people with down syndrome are protected from the development of most solid tumors-A review. Front Genet. 2021;12:749480.
92. Loehrer FM, Angst CP, Brunner FP, Haefeli WE, Fowler B. Evidence for disturbed S-adenosylmethionine: S-adenosylhomocysteine ratio in patients with end-stage renal failure: a cause for disturbed methylation reactions? Nephrol Dial Transplant. 1998;13(3):656–61.
93. Kerins DM, Koury MJ, Capdevila A, Rana S, Wagner C. Plasma S-adenosylhomocysteine is a more sensitive indicator of cardiovascular disease than plasma homocysteine. Am J Clin Nutr. 2001;74(6):723–9.
94. Barić I, Erdol S, Saglam H, Lovrić M, Belužić R, Vugrek O, et al. Glycine N-methyltransferase deficiency: a member of dysmethylating liver disorders? JIMD Rep. 2017;31:101–6.
95. Valli A, Carrero JJ, Qureshi AR, Garibotto G, Bárány P, Axelsson J, et al. Elevated serum levels of S-adenosylhomocysteine, but not homocysteine, are associated with cardiovascular disease in stage 5 chronic kidney disease patients. Clin Chim Acta. 2008;395(1–2):106–10.
96. Liu S, Liao R, Dai X, Guo H, Wang D, Xia M, et al. Association between plasma S-adenosylmethionine and risk of mortality in patients with coronary artery disease: a cohort study. Am J Clin Nutr. 2021;114(4):1360–70.
97. Baric I, Fumic K, Glenn B, Cuk M, Schulze A, Finkelstein JD, et al. S-adenosylhomocysteine hydrolase deficiency in a human: a genetic disorder of methionine metabolism. Proc Natl Acad Sci U S A. 2004;101(12):4234–9.
98. Grbeša I, Kalo A, Belužić R, Kovačević L, Lepur A, Rokić F, et al. Mutations in S-adenosylhomocysteine hydrolase (AHCY) affect its nucleocytoplasmic distribution and capability to interact with S-adenosylhomocysteine hydrolase-like 1 protein. Eur J Cell Biol. 2017;96(6):579–90.
99. Bjursell MK, Blom HJ, Cayuela JA, Engvall ML, Lesko N, Balasubramaniam S, et al. Adenosine kinase deficiency disrupts the methionine cycle and causes hypermethioninemia, encephalopathy, and abnormal liver function. Am J Hum Genet. 2011;89(4):507–15.
100. Mudd SH, Cerone R, Schiaffino MC, Fantasia AR, Minniti G, Caruso U, et al. Glycine N-methyltransferase deficiency: a novel inborn error causing persistent isolated hypermethioninaemia. J Inherit Metab Dis. 2001;24(4):448–64.
101. Chen Z, Karaplis AC, Ackerman SL, Pogribny IP, Melnyk S, Lussier-Cacan S, et al. Mice deficient in methylenetetrahydrofolate reductase exhibit hyperhomocysteinemia and decreased methylation capacity, with neuropathology and aortic lipid deposition. Hum Mol Genet. 2001;10(5): 433–43.
102. Strauss KA, Morton DH, Puffenberger EG, Hendrickson C, Robinson DL, Wagner C, et al. Prevention of brain disease from severe 5,10-methylenetetrahydrofolate reductase deficiency. Mol Genet Metab. 2007;91(2):165–75.
103. Motulsky AG. Nutritional ecogenetics: homocysteine-related arteriosclerotic vascular disease, neural tube defects, and folic acid. Am J Hum Genet. 1996;58(1):17–20.
104. Tonetti C, Ruivard M, Rieu V, Zittoun J, Giraudier S. Severe methylenetetrahydrofolate reductase deficiency revealed by a pulmonary embolism in a young adult. Br J Haematol. 2002; 119(2):397–9.
105. Wendel U, Bremer HJ. Betaine in the treatment of homocystinuria due to 5,10-methylenetetrahydrofolate reductase deficiency. Eur J Pediatr. 1984;142(2):147–50.

106. Lukowski AF, Milojevich HM, Eales L. Cognitive functioning in children with down syndrome: current knowledge and future directions. Adv Child Dev Behav. 2019;56:257–89.
107. Lejeune J, Gautier M, Turpin R. Etude des chromosomes somatiques de neuf enfants mongoliens. Comptes rendus hebdomadaires des seances de l'Academie des sciences. 1959; 248(4):1721–2.
108. Pelleri MC, Cicchini E, Petersen MB, Tranebjaerg L, Mattina T, Magini P, et al. Partial trisomy 21 map: ten cases further supporting the highly restricted down syndrome critical region (HR-DSCR) on human chromosome 21. Mol Genet Genomic Med. 2019;7:e797.
109. Silverman W. Down syndrome: cognitive phenotype. Ment Retard Dev Disabil Res Rev. 2007;13(3):228–36.
110. Grieco J, Pulsifer M, Seligsohn K, Skotko B, Schwartz A. Down syndrome: cognitive and behavioral functioning across the lifespan. Am J Med Genet C Semin Med Genet. 2015; 169(2):135–49.
111. Onnivello S, Pulina F, Locatelli C, Marcolin C, Ramacieri G, Antonaros F, et al. Cognitive profiles in children and adolescents with down syndrome. Sci Rep. 2022;12(1):1936.
112. Roizen NJ, Patterson D. Down's syndrome. Lancet. 2003; 361(9365):1281–9.
113. Pelleri MC, Cattani C, Vitale L, Antonaros F, Strippoli P, Locatelli C, et al. Integrated quantitative transcriptome maps of human trisomy 21 tissues and cells. Front Genet. 2018; 9:125.
114. Vitale L, Serpieri V, Lauriola M, Piovesan A, Antonaros F, Cicchini E, et al. Human trisomy 21 fibroblasts rescue methotrexate toxic effect after treatment with 5-methyltetrahydrofolate and 5-formyl-tetrahydrofolate. J Cell Physiol. 2019;234:15010–24.
115. Hunter S, Hendrix J, Freeman J, Dowell RD, Allen MA. Transcription dosage compensation does not occur in down syndrome. BMC Biol. 2023;21(1):228.
116. Caracausi M, Ghini V, Locatelli C, Mericio M, Piovesan A, Antonaros F, et al. Plasma and urinary metabolomic profiles of down syndrome correlate with alteration of mitochondrial metabolism. Sci Rep. 2018;8(1):2977.
117. Antonaros F, Ghini V, Pulina F, Ramacieri G, Cicchini E, Mannini E, et al. Plasma metabolome and cognitive skills in down syndrome. Sci Rep. 2020;10(1):10491.
118. Gross TJ, Doran E, Cheema AK, Head E, Lott IT, Mapstone M. Plasma metabolites related to cellular energy metabolism are altered in adults with down syndrome and Alzheimer's disease. Dev Neurobiol. 2019;79(7):622–38.
119. Pecze L, Randi EB, Szabo C. Meta-analysis of metabolites involved in bioenergetic pathways reveals a pseudohypoxic state in down syndrome. Mol Med. 2020;26(1):102.
120. Anderson CC, Marentette JO, Prutton KM, Rauniyar AK, Reisz JA, D'Alessandro A, et al. Trisomy 21 results in modest impacts on mitochondrial function and central carbon metabolism. Free Radic Biol Med. 2021;172:201–12.
121. Lejeune J. Biochemical investigations and trisomy 21 (author's transl). Ann Genet. 1979;22(2):67–75.
122. Reid MA, Allen AE, Liu S, Liberti MV, Liu P, Liu X, et al. Serine synthesis through PHGDH coordinates nucleotide levels by maintaining central carbon metabolism. Nat Commun. 2018;9(1):5442.
123. Lejeune J. Pathogenesis of mental deficiency in trisomy 21. Am J Med Genet Suppl. 1990;7:20–30.
124. Peeters MA, Poon A, Zipursky A, Lejeune J. Toxicity of leukaemia therapy in children with down syndrome. Lancet. 1986;2(8518):1279.
125. Lejeune J, Rethore MO, de Blois MC, Maunoury-Burolla C, Mir M, Nicolle L, et al. Metabolism of monocarbons and trisomy 21: sensitivity to methotrexate. Ann Genet. 1986;29(1): 16–9.
126. Chabner BA, Allegra CJ, Curt GA, Clendeninn NJ, Baram J, Koizumi S, et al. Polyglutamation of methotrexate. Is methotrexate a prodrug? J Clin Invest. 1985;76(3):907–12.
127. Morgan SL, Baggott JE, Altz-Smith M. Folate status of rheumatoid arthritis patients receiving long-term, low-dose methotrexate therapy. Arthritis Rheum. 1987;30(12): 1348–56.
128. Funk RS, Talib NJ, Zimmerman KO, van Haandel L, Becker ML. Altered folate homeostasis in children with down syndrome: a potential basis for enhanced methotrexate toxicity. J Pediatr. 2020;221:235–9.
129. Ifergan I, Shafran A, Jansen G, Hooijberg JH, Scheffer GL, Assaraf YG. Folate deprivation results in the loss of breast cancer resistance protein (BCRP/ABCG2) expression. A role for BCRP in cellular folate homeostasis. J Biol Chem. 2004; 279(24):25527–34.
130. Rosenblatt DS, Whitehead VM, Matiaszuk NV, Pottier A, Vuchich MJ, Beaulieu D. Differential effects of folinic acid and glycine, adenosine, and thymidine as rescue agents in methotrexate-treated human cells in relation to the accumulation of methotrexate polyglutamates. Mol Pharmacol. 1982; 21(3):718–22.
131. Peeters MA, Rethore MO, Lejeune J. In vivo folic acid supplementation partially corrects in vitro methotrexate toxicity in patients with down syndrome. Br J Haematol. 1995;89(3): 678–80.
132. Song C, He J, Chen J, Liu Y, Xiong F, Wang Y, et al. Effect of the onecarbon unit cycle on overall DNA methylation in children with Down's syndrome. Mol Med Rep. 2015;12(6): 8209–14.
133. Ueland PM, Refsum H, Christensen B. Methotrexate sensitivity in Down's syndrome: a hypothesis. Cancer Chemother Pharmacol. 1990;25(5):384–6.
134. Chadefaux B, Rethore MO, Raoul O, Ceballos I, Poissonnier M, Gilgenkranz S, et al. Cystathionine beta synthase: gene dosage effect in trisomy 21. Biochem Biophys Res Commun. 1985;128(1):40–4.
135. Jérôme H, Chadefaux B, Ceballos I, Allard D. Effects of gene localization and its metabolic significance in trisomy 21. Bull Acad Natl Med. 1985;169(9):1387–95.
136. Orozco JS, Hertz-Picciotto I, Abbeduto L, Slupsky CM. Metabolomics analysis of children with autism, idiopathic-developmental delays, and down syndrome. Transl Psychiatry. 2019;9(1):243.
137. Petrossian TC, Clarke SG. Uncovering the human methyltransferasome. Mol Cell Proteomics. 2011;10(1):M110.000976.
138. Bacalini MG, Gentilini D, Boattini A, Giampieri E, Pirazzini C, Giuliani C, et al. Identification of a DNA methylation signature in blood cells from persons with down syndrome. Aging. 2015;7(2):82–96.

139. Horvath S, Garagnani P, Bacalini MG, Pirazzini C, Salvioli S, Gentilini D, et al. Accelerated epigenetic aging in down syndrome. Aging Cell. 2015;14(3):491–5.
140. Sailani MR, Santoni FA, Letourneau A, Borel C, Makrythanasis P, Hibaoui Y, et al. DNA-methylation patterns in trisomy 21 using cells from monozygotic twins. PLoS ONE. 2015;10(8):e0135555.
141. Laan L, Klar J, Sobol M, Hoeber J, Shahsavani M, Kele M, et al. DNA methylation changes in down syndrome derived neural iPSCs uncover co-dysregulation of ZNF and HOX3 families of transcription factors. Clin Epigenetics. 2020;12(1):9.
142. Xu K, Li S, Muskens IS, Elliott N, Myint SS, Pandey P, et al. Accelerated epigenetic aging in newborns with down syndrome. Aging Cell. 2022;21(7):e13652.
143. Naumova OY, Lipschutz R, Rychkov SY, Zhukova OV, Grigorenko EL. DNA methylation alterations in blood cells of toddlers with down syndrome. Genes. 2021;12(8):1115–31.
144. Martin GM. Genetic syndromes in man with potential relevance to the pathobiology of aging. Birth Defects Orig Artic Ser. 1978;14(1):5–39.
145. Franceschi C, Garagnani P, Gensous N, Bacalini MG, Conte M, Salvioli S. Accelerated bio-cognitive aging in down syndrome: state of the art and possible deceleration strategies. Aging Cell. 2019;18(3):e12903.
146. Dragonieri S, Carpagnano GE, Caccamese S. S-adenosylmethionine may mitigate obstructive sleep apnea in an adult with down syndrome: a case report. Nat Sci Sleep. 2022;14:175–8.

How to cite this article: Caracausi M, Ramacieri G, Catapano F, Cicilloni M, Lajin B, Pelleri MC, et al. The functional roles of S-adenosyl-methionine and S-adenosyl-homocysteine and their involvement in trisomy 21. BioFactors. 2024. https://doi.org/10.1002/biof.2044