

## MTHFR and LC, CFS, POTS, MCAS, SIBO, EDS: Methylating the Alphabet

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### Abstract

Long Covid (LC), Chronic Fatigue Syndrome (CFS), Postural Orthostatic Tachycardia Syndrome (POTS), Mast Cell Activation Syndrome (MCAS), Small Intestine Bacterial Overgrowth (SIBO), and Ehlers-Danlos Syndrome (EDS) are all loosely connected, some poorly defined, some with overlapping symptoms. The female preponderance, the prominence of fatigue and chronic inflammation, and methylenetetrahydrofolate reductase (MTHFR) abnormalities may connect them all. Indeed differential methylation may lie at the root. Two-EDS and MTHFR are genetic. But epigenetic factors may ultimately determine their phenotypic expression. Oxidative stress, overloaded mitochondria, an antioxidant and nutrient shortfall, and suboptimal gut microbiome appear to be the primary determinants. A deep dive into the folate and methionine cycles is undertaken in an attempt to connect these syndromes. The active forms of vitamin D and vitamins B2,3,6,9,12 are shown to be biochemically integral to optimal methylation and control of the epigenome. Their status largely determines the symptoms of abnormal MTHFR in all its phenotypes. The wider implications for aging, cancer, cardiovascular disease, neurodegenerative disease, and autoimmune disease are briefly explored.

**Keywords:** SAME, Methylation, Methionine, Folate, Riboflavin, Magnesium, Tryptophan

### Introduction

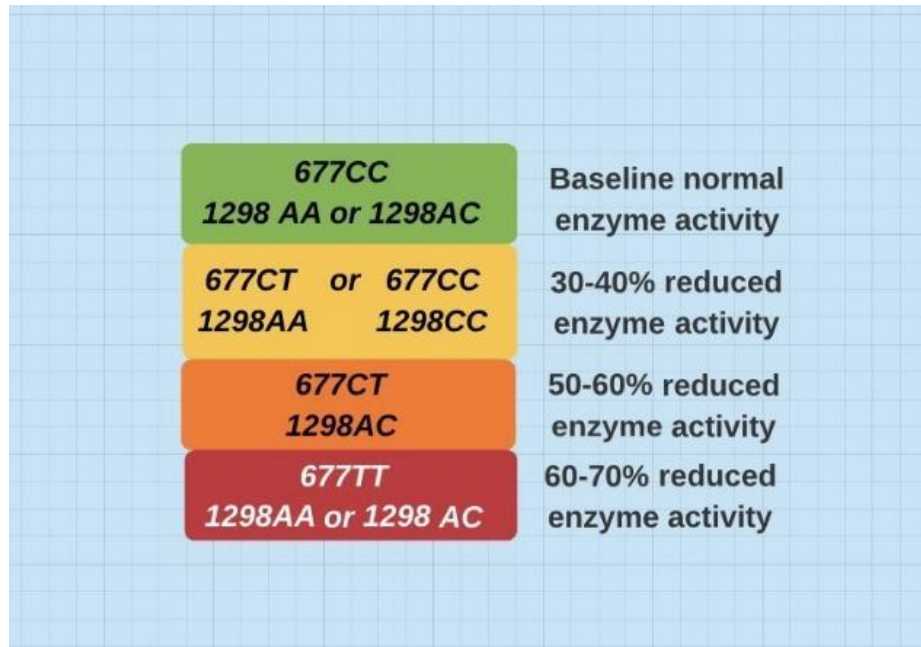
S-Adenosylmethionine (SAME) aka adenosylmethionine (AdoMet) is the second-most used enzyme substrate after ATP. It is used by over 200 methyltransferase enzymes to methylate histone and non-histone proteins, nucleic acids (DNA and RNA), phospholipids, hormones, and small molecules [1].

SAME is important because recent research has demonstrated that differential methylation of DNA is the primary driver of the epigenome, which determines which genes are silenced (DNA promoter region is hypermethylated) and which are activated (DNA promoter region is hypomethylated). The folate and methionine cycles are the primary determinants of SAME status and control of the epigenome via hypomethylation or hypermethylation. MTHFR is the primary enzyme determining the status of these intertwined cycles. MTHFR mutations, common in the general public, play a vital role in the phenotypic expression of many diseases, including LC, CFS, POTS, MCAS, SIBO, and EDS.

### Discussion

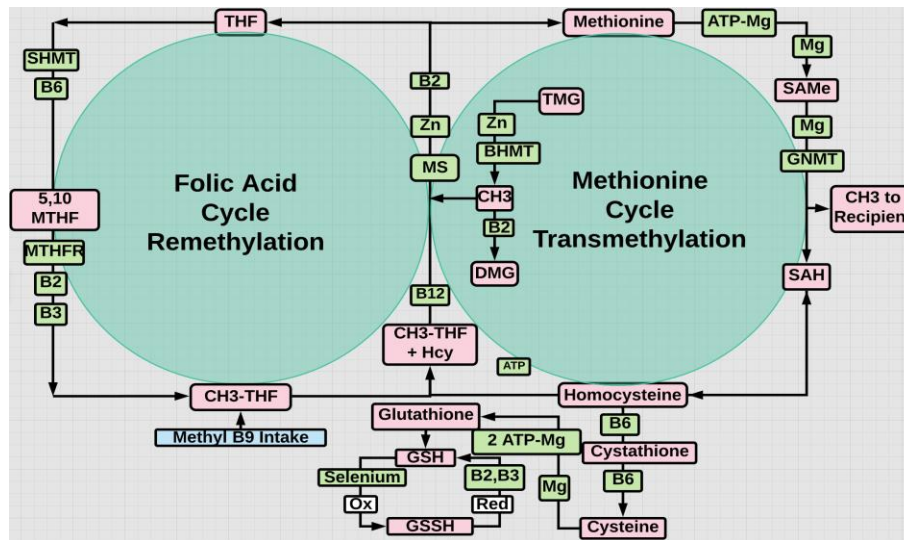
#### MTHFR and Methylation

The healthy wild type MTHFR gene is 1298AA/677CC. There are at least 30 variants of MTHFR, but 1298C and/or 677T represent over 90% of the abnormal MTHFR single nucleotide polymorphisms in the general population. Abnormal MTHFRs plague many. According to the NIH [2], about 1/3 of Caucasians/Asians and about 10% of Africans are heterozygous for 677T. About 1/3 of Europeans, 1/4 of Asians, and 1/6 of Africans are heterozygous for 1298C [3]. Genotype roughly predicts activity of the enzyme (see Figure 1). The defective gene creates a state of hypomethylation and differential DNA methylation controls the epigenome. A single gene can be promoted or suppressed, depending on the state of methylation.



**Figure 1:** Various combinations of these two SNPs reflect predictable enzyme activity. 677T (heterozygous) and 677TT (homozygous) are the prime determinants of reduced activity [4].

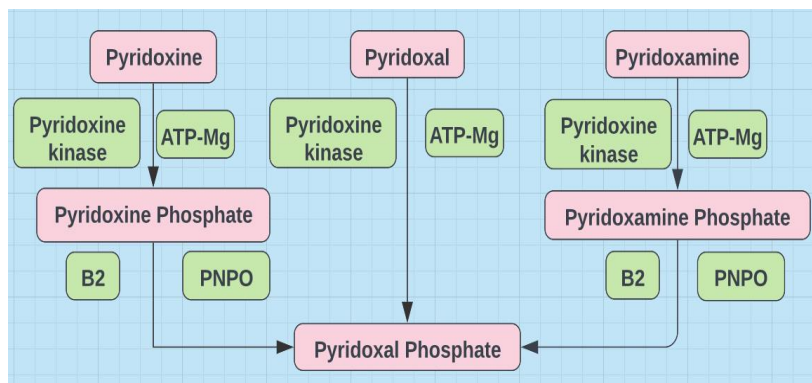
Furthermore, phenotypic expression is greatly influenced by presence or not of required cofactors. Riboflavin (B2), as flavin adenine dinucleotide (FAD) is a required cofactor for MTHFR and methionine synthase [5]. But B3,6,9 (folate),12 have significant impact on other enzymes in the two cycles [6] (see figure 2).



**Figure 2:** THF:Tetrahydrofolate; TMG:Trimethylglycine Aka Betaine; MS:Methionine Synthase; DMG:Dimethylglycine; BHMT:Betaine Homocysteine Methyltransferase; GNMT:Glycine-N-Methyltransferase; SHMT:Serine Hydroxymethyltransferase; SAH:S-Adenosylhomocysteine; enzymes and cofactors are green; substrates are pink.

Those with abnormal MTHFR experience mild to moderate symptoms from Covid-19 and are hypomethylated, whereas those with LC tend to be hypermethylated<sup>7</sup>. Although hypomethylation is less likely in African-Americans on a genetic MTHFR basis, it is more likely on a vitamin D deficient basis, which increases the risk of hypomethylation [8].

Activation of B6 requires not only ATP-Mg dependent phosphorylation but also riboflavin dependent pyridoxine phosphate oxidase (PNPO), the rate limiting step [9] (see Figure 3).

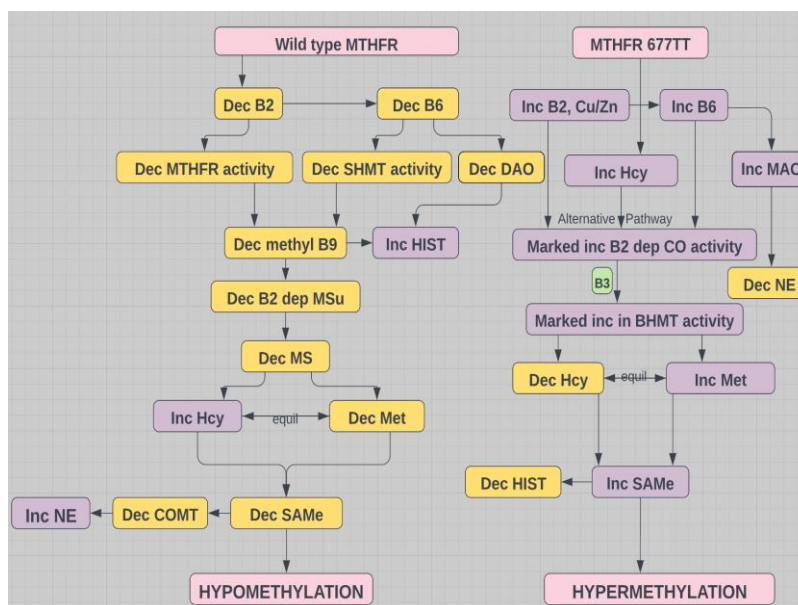


**Figure 3:** Most B vitamin supplements contain B6 as pyridoxine that ironically can compete with pyridoxal-5-phosphate (P5P) aka pyridoxal phosphate (PLP) for receptor sites and lead to symptoms of B6 deficiency. B2 sufficiency may be the most important indicator of B6 sufficiency.

SAMe is the universal methyl donor. To produce one molecule of SAMe requires methionine, an ATP-Mg<sup>++</sup> complex, methionine adenosyltransferase and an additional Mg<sup>++</sup> as cofactor. Furthermore, the enzyme that frees the CH<sub>3</sub> group from SAMe, glycine-N-methyltransferase, also requires Mg<sup>++</sup> as a cofactor. In fact all methyltransferase activities require magnesium [10].

Methylation or transfer of a CH<sub>3</sub> group is a complex topic. There

mustn't be too much or too little and the inputs for either are myriad, e.g., diet, lifestyle, genes. Maintaining a balance between hypomethylation and hypermethylation is precarious [11]. The balance is impacted by magnesium status. If deficient, hypomethylation predominates (see Figure 4). However, 1,25(OH)<sub>2</sub>D<sub>3</sub> (active form of D) is critical to preventing hypermethylation, as it increases the expression of DNA demethylases that prevent hypermethylation of multiple gene promoter regions [12,13].



**Figure 4:** Comparison of methylation states for wild type MTHFR with low B2 versus abnormal MTHFR with elevated B2, MTHFR: Methylenetetrahydrofolate reductase; HIST:Histamine; NE:Norepinephrine; COMT:Catechol-O-Methyltransferase; SHMT:Serine Hydroxymethyl Transferase; Hcy:Homocysteine; CO:Choline Oxidase; BHMT:Betaine Hydroxymethyl Transferase; MSu:Methionine Sulfoxidase; MS:Methionine Synthase; SAMe:S-Adenosyl Methionine.

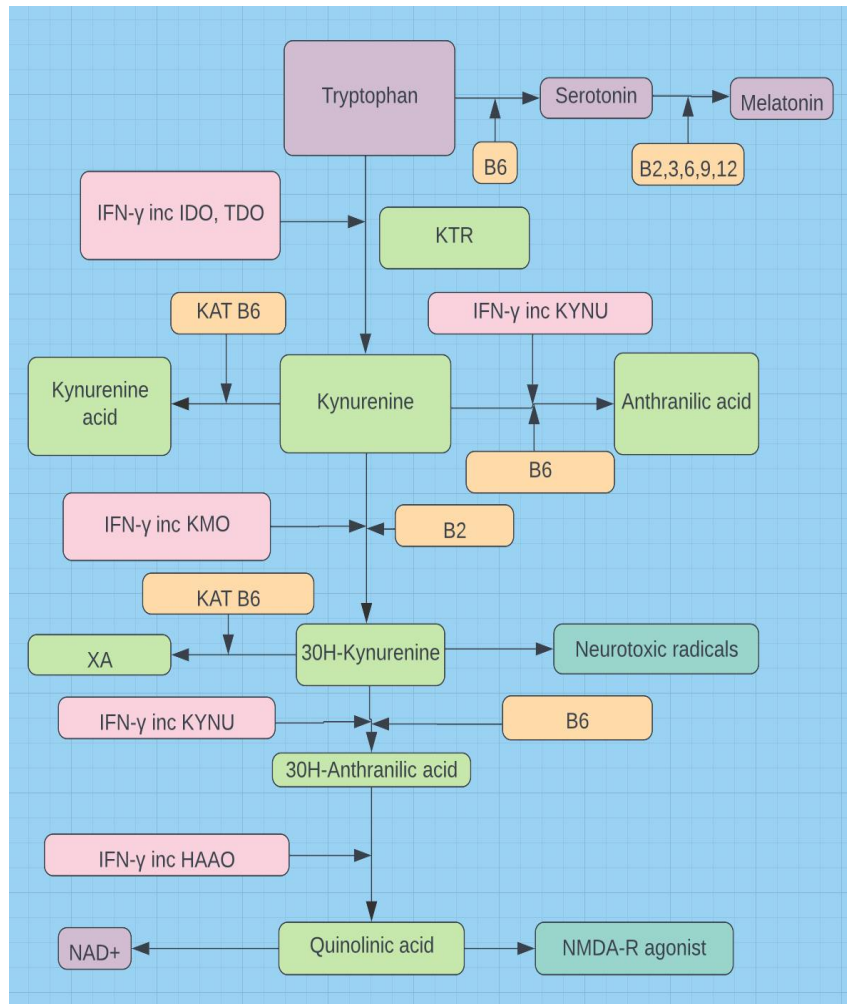
### Vitamins D and B, Magnesium and Zinc

Covid-19 is worse and LC is more frequent in the vitamin D deficient [14,15]. Vitamin D deficiency is widespread even at the serum level of 30 ng/mL, recommended by the National Academy of Medicine. A much more realistic target is 50 ng/mL, underscored

by numerous clinical studies and physiologic reviews demonstrating this via biochemical analysis of the interactions between PTH, calcium, and 25(OH)D<sub>3</sub>. The latter is the storage form of D and the analyte used to assess adequacy. On the other hand, riboflavin (B2) deficiency is never mentioned in any top ten list of nutrient

shortfalls. But its deficiency is only likely to occur after prolonged chronic inflammation. It is probably the first vitamin to be consumed during inflammation and mitochondrial overload, as tryptophan,

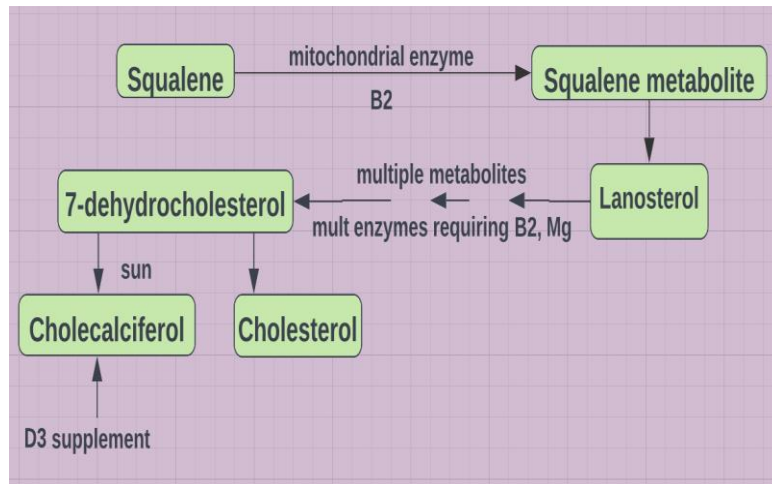
driven by IFN- $\gamma$ , is stolen from synthesis of serotonin and melatonin and redirected via the kynurenine pathway to produce more NAD<sup>+</sup> (see Figure 5 and section on Tryptophan).



**Figure 5:** Demonstrates the goal (NAD) behind the tryptophan steal. IDO: indoleamine-2,3-dioxygenase-1; TDO: Tryptophan 2,3-Dioxygenase; KTR: Kynurenine Tryptophan Ratio; KAT:Kynurenine Aminotransferases; KYNU:Kynureninase (Mitochondrial); KMO:Kynurenine 3-Monooxygenase; XA:Xanthurenic Acid; HAAO:Hydroxyanthranilic Acid Dioxygenase [16].

Active forms of both B2 and B6 are easily consumed with detrimental effects on MTHFR. Under such conditions B2 and D become tightly linked. The storage form of D must be hydroxylated to attain active status. CYP27B1 is the only enzyme that can do this, mitochondria are the only organelles that are suitable for the reaction [17,18], and B2 as FAD (in addition to Mg<sup>++</sup>) is a required cofactor. Furthermore, one of the most pronounced effects of the active form (1,25(OH)<sub>2</sub>D<sub>3</sub>) is increased synthesis of CYP24A1, which degrades both the active form and the storage form [19]. Oxidative stress, compromised mitochondria, and riboflavin/mag-

nesium deficiency translate to less of the active form. Accordingly, the storage form of D may overestimate the status of its active form. This overestimate is directly proportional to the degree of mitochondrial dysfunction. Under such conditions perhaps a 50 ng/mL target is insufficient. Mitochondrial dysfunction compromises not only synthesis of the active form of vitamin D, but also synthesis of the precursors to D3. Formation of 7-dehydrocholesterol, the precursor to D3 of solar origin, requires intra-mitochondrial flavin dependent enzymes (see Figure 6).



**Figure 6:** Solar exposure in the face of a B2 shortfall or mitochondrial compromise promises little benefit.

Stress induces release of norepinephrine and glucocorticoids. Both increase oxidative stress [20,21]. Aldosterone and cortisol are synthesized in mitochondria. They are both magnesuric. Exercise is good, but cationic sodium lost in sweat is replaced by excreting  $Mg^{++}$  and  $K^+$ . Dehydration exacerbates this.

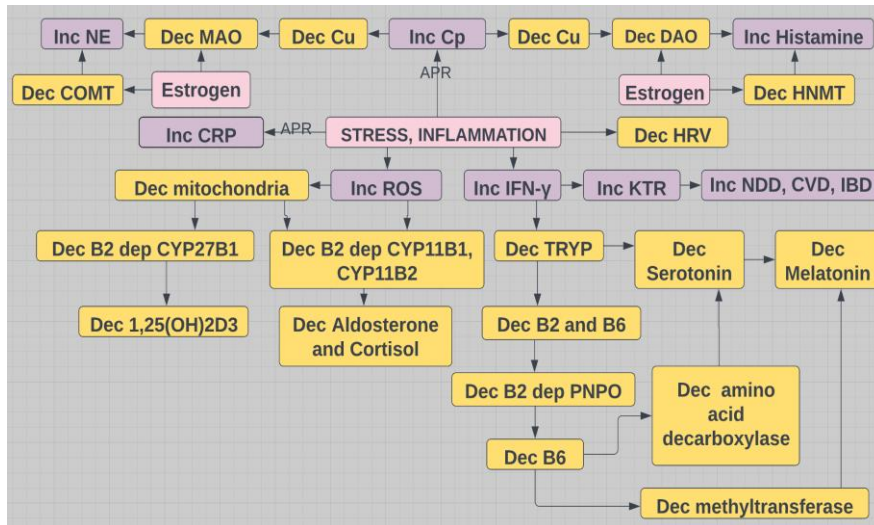
B2 is not only a required cofactor for the synthesis of the active form of vitamin D, but also a required cofactor for the synthesis of B6. Therefore all B6 dependent enzymes (see figure 3) are B2 dependent, unless B6 is taken as P5P aka PLP. Indeed B2 may be the limiting nutrient for maintaining vitamin B6 status, particularly in individuals with the MTHFR 677TT genotype. MTHFR also requires B3 as cofactor [22]. B2 and B6 have an oversized impact on the synthesis of glutathione, the master antioxidant (see Figure 2). However, the active forms of other B vitamins, especially B6,9,12, figure prominently in the folate and methionine cycles (see Figure 2). In view of the prevalence of MTHFR problems, they deserve specific attention. Both B9 and B12 must be methylated for activation. Ironically, abnormal MTHFRs only yield hypomethylation,

unless Hcy significantly increases and triggers the alternative pathway (see Figure 2) and hypermethylation (see Figure 4).

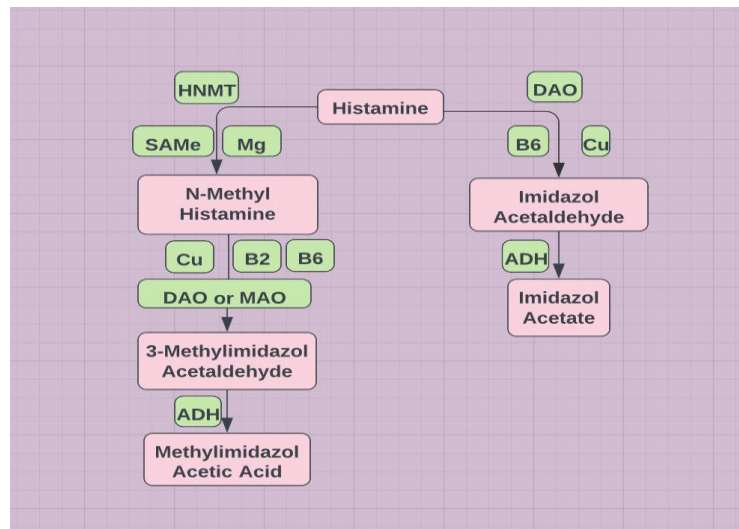
Synthesis of FAD from riboflavin and NAD from niacin requires phosphorylation to reach their activated states. Phosphorylation requires  $ATP-Mg^{++}$ . If the supply of ATP from mitochondria or magnesium from diet is marginal, then the activated forms (B1,2,3,6) are compromised.

The Cu/Zn ratio is also important with regard to the alternative pathway. Because Western plumbing is primarily copper piping, copper is probably more readily ingested than zinc. When elevated, copper is pro-inflammatory. Zinc is generally both an antioxidant and anti-inflammatory [23]. These divalent cations compete for absorption. An elevated Cu/Zn is directly proportional to Covid severity [24]. But the heavy emphasis on Zn during the pandemic may have sparked a Cu shortfall in some [25] (see Figure 7,8). It has also been shown that zinc deficiency and zinc excess cause cellular oxidative stress [26].





**Figure 7:** The interdependencies of vitamins B2, B6, D and the imbalance caused by inflammation. Both CYP enzymes are mitochondria based. All CYP enzymes require  $Mg^{++}$ . All methyltransferases require  $Mg^{++}$ . APR:Acute Phase Reactant; MAO:Monoamine Oxidase; DAO:Diamine Oxidase; Cp:Ceruloplasmin; CRP:C-Reactive Protein; HRV:Heart Rate Variability; KTR:Kynurenine Tryptophan ratio; NDD:Neurodegenerative Disease; CVD:Cardiovascular Disease; IBD:Inflammatory Bowel Disease; TRYP:Tryptophan; PNPO:Pyridoxine Phosphate Oxidase; Yellow Indicates Decrease; Purple Indicates Increase.



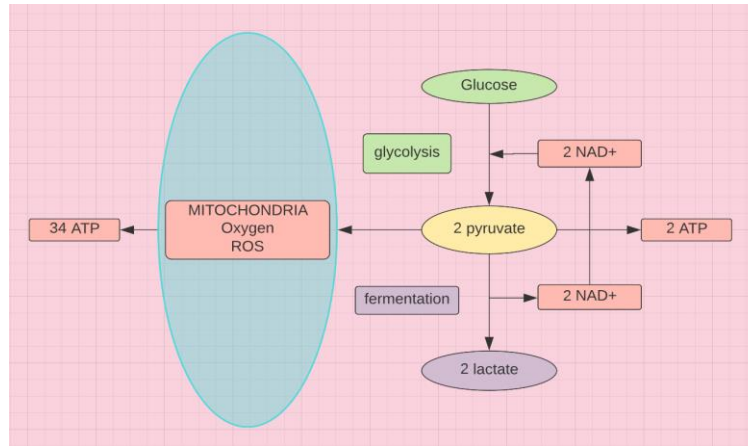
**Figure 8:** DAO:Diamond Oxidase; HNMT:Histamine-N-Methyltransferase; ADH:Aldehyde Dehydrogenase (Mitochondrial).

### The Tryptophan Connection

Tryptophan is an essential amino acid and is the only precursor for the neurotransmitter serotonin, which is vital to mood, cognition, sleep, gastrointestinal function, and the gut-brain axis. Tryptophan is also the precursor for melatonin (serotonin pathway) and niacin (kynurenine pathway). The kynurenine pathway dominates 95% of tryptophan metabolism (see Figure 5) [27].

Altered tryptophan metabolism characterizes LC [28] and is a

marker for inflammaging [29]. Acute or chronic inflammation induced by invading pathogens or other foreign toxins increases demand for energy. Mitochondria go into overdrive. They have the capacity to produce 34 ATPs from each molecule of glucose (see Figure 9). But this requires oxidative phosphorylation that can generate excessive ROS, if onboard antioxidants are insufficient. Accordingly, to avoid cell lysis due to oxidative stress, mitochondrial activity decreases and the cell pivots to glycolysis and fermentation (see Figure 9).



**Figure 9:** Demonstrates the switch from glycolysis to fermentation for energy thru NAD<sup>+</sup> during mitochondrial dysfunction.

During elevated interferon-gamma (IFN- $\gamma$ ) activity, riboflavin and vitamin B6 may be depleted, given the role of these nutrients as cofactors for tryptophan catabolism, a metabolic process that is increased during IFN- $\gamma$ -mediated immune activity (see Figure 5) [30]. Because FAD serves as a cofactor for MTHFR, riboflavin deficiency secondary to inflammation induced consumption can also contribute to increased homocysteine [31].

In pivoting from oxidative phosphorylation to fermentation to prevent oxidative stress induced cell lysis, ATP production is severely curtailed. Fermentation supplies the increased need for NAD<sup>+</sup>, which initiates the tryptophan steal, driven by IFN- $\gamma$  (see Figure 5). Tryptophan, induced by IFN- $\gamma$  from T cells and NK cells, is slowly depleted and metabolites increase during chronic immune activation [32]. Depletion of tryptophan with increased metabolites (3-hydroxykynurenine and quinolinic acid) (see Figure 2), increased tryptophan/kynurenine ratio (KTR), and reduced serotonin production contributes to the neurological sequelae of LC.

Females have higher levels of type I IFN ( $\alpha$  and  $\beta$ ) [33]. T cell production of IFN- $\gamma$  (IFN type II) is triggered by IFN- $\alpha$  and - $\beta$ , primary release IFNs. IFN- $\gamma$  is a STAT dependent secondary release IFN [34]. T cell population decreases with age in males but not females. T cell production of IFN- $\gamma$  persists in females [35]. This may in part explain greater longevity, increased frequency of autoimmune disease, and more frequent encounters with chronic fatigue syndromes in females.

### LC, CFS, POTS, MCAS, SIBO, EDS

**LC, CFS:** The symptoms for this alphabet soup of syndromes appear to be linked by oxidative stress. LC and CFS are the poster children for this. An antioxidant shortfall leads to an excess of mitochondrial reactive oxygen species. If unquenched, cell lysis is

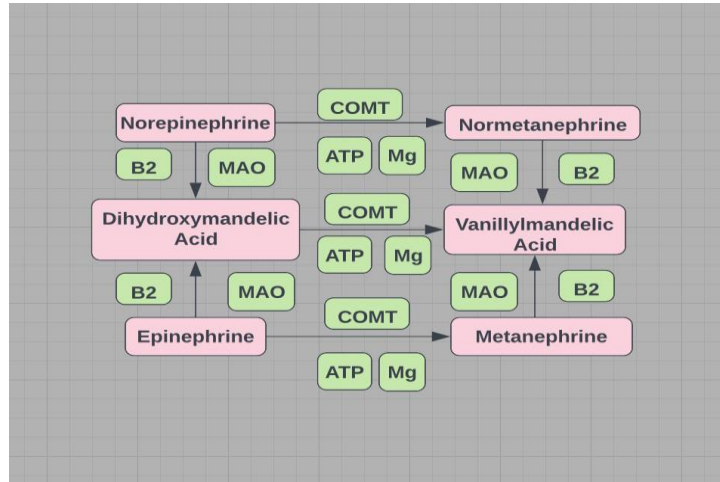
threatened. In order to avoid this mitochondria are down regulated and tryptophan is stolen from that earmarked for serotonin/melatonin synthesis and redirected to the synthesis of NAD (B3). Fatigue and brain fog in LC and CFS may in part be due to mitochondrial dysfunction and the neurotoxic metabolites of the kynurenine pathway respectively (see Figure 5).

The very common MTHFR allele 677T generally results in hypomethylation and may activate genes expressed in EDS, MCAS, and SIBO. Hypermethylation generally characterizes LC [7] and MTHFR 677 TT [36] and hypomethylation generally characterizes mild to moderate Covid-19 [7] and MTHFR 677T [36].

Elevated free Cu is also characteristic of 677TT and hypomethylation [36]. Zinc deficiency is typically associated with hypomethylation, but may be seen in hypermethylation [37], when copper is increased [36]. This suggests that the hypermethylation in LC may represent those homozygous for the 677T allele. Zn supplements in LC may prove helpful.

Hypermethylation and hypomethylation are demonstrated in CFS [38]. Although the connection between MTHFR variants and CFS is debated, both LC and CFS are linked by redox imbalance and elevated homocysteine [39]. Abnormal MTHFR is the most frequent cause of elevated homocysteine. Perhaps 677TT is linked to CFS with hypermethylation and the heterozygous 677T allele is linked to CFS with hypomethylation.

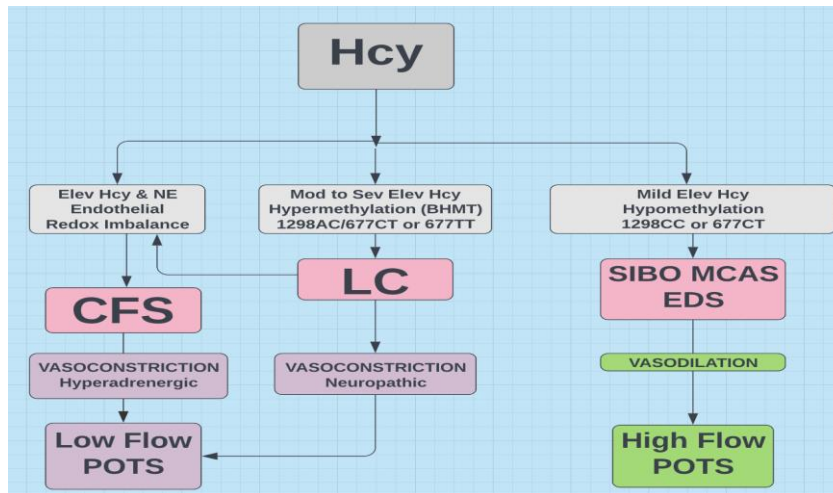
**POTS:** The vast majority of POTS exhibits low blood volume and is classified as either low flow or high flow type. POTS (low flow)-vasoconstriction due to high norepinephrine; catecholamines are primarily degraded by B2,6 dependent MAO (mitochondrial) and Mg<sup>++</sup> dependent COMT (see Figure 10).



**Figure 10:** COMT: Catecholamines-O-Methyltransferase. MAO: Monoamine Oxidase (Mitochondrial).

A shortfall in the required ATP-Mg<sup>++</sup> compromises MAO/DAO (mitochondrial) and a shortfall in the required cofactor Mg<sup>++</sup> compromises HNMT (see Figure 8). Estrogen down-regulates COMT/HNMT and MAO/DAO, increasing NE and low flow POTS or increasing histamine and high flow POTS (see Figure

11). Gut dysbiosis is often associated with MCAS, SIBO, and EDS and impairs DAO activity [40]. Dysbiosis and histamine secreting bacteria and/or impaired DAO may represent an additional incentive for those with MCAS, SIBO, and EDS to favor high flow POTS over low flow POTS (see Figure 11).



**Figure 11:** Proposed flow chart indicating hypothetical relationships for LC, CFS, SIBO, MCAS, EDS, POTS, and MTHFR with Hcy as common denominator. BHMT:Betaine-Homocysteine Methyltransferase and Represents the Alternative Pathway (see Figure 2). NE:Norepinephrine.

POTS (high flow)-vasodilation due to histamine; histamine is degraded by methylation and involves several mitochondrial based enzymes-DAO and ADH (see Figure 8). MTHFR variants primarily exhibit hypomethylation and low SAME.

MTHFR 677T and 1298C work in concert to create hypomethylation. However, if Hcy is sufficiently elevated by an abnormal MTHFR, the alternative pathway (BHMT) can be initiated to lower Hcy (see Figures 2, 11). This pathway redirects to hypermethylation, enabling SAME for MAO/DAO (mitochondrial), and COMT/HNMT to degrade monoamines and histamine respectively.

In summary

1. Catecholamines are degraded by MAO oxidation or COMT methylation.
2. Histamine is degraded by DAO oxidation or HNMT methylation
3. MAO/DAO are the primary determinants of catecholamine and histamine degradation, as both COMT and HNMT require subsequent mitochondrial based metabolism by MAO/DAO and aldehyde dehydrogenase (ADH) (see Figures 8,10)
4. Estrogen down regulates COMT/HNMT and MAO/DAO.
5. Hypomethylation and low Hcy favor high flow POTS, while high Hcy favors low flow POTS (see Figure 11).



**SIBO, MCAS:** SIBO is characterized by gut dysbiosis and malabsorption of critical nutrients. In general gut microbiota are net consumers of B12 and net producers of folate [41]. Both B9 and B12 are critical to optimal function of the folate and methionine cycles.

Sulfur reducing bacteria detectable by breath test produce H<sub>2</sub>S. This compromises the availability of methionine, one of the nine essential amino acids and the only amino acid of the official 21 that contains sulfur. It is critical to the methionine cycle (see Figure 2) and methylation.

Histamine intolerance is characterized by gut dysbiosis and a compromised intestinal barrier. This suggests a possible link to high flow POTS often associated with MCAS. POTS [42], EDS [42], and MCAS [43] are all linked to low magnesium. POTS [44], EDS [45], and MCAS [46] are also linked to low vitamin D. The gut microbiome of those with histamine intolerance lacks biodiversity [47].

Elevated tryptase has been linked to MCAS, EDS, and POTS. Tryptase primarily reflects mast cell function. Up-regulated mast cells produce symptoms that are seen in many conditions that overlap with Long Covid (and magnesium deficiency), including CFS, FM, MCAS, and POTS [48]. The brain fog of Long Covid resembles that of MCAS [49] and may be due to inadequate magnesium [50]. Histamine is upregulated in magnesium deficiency. Magnesium deficiency not only increases histamine release but also increases mast cells [43].

**EDS:** EDS is clearly genetic, but is just as clearly epigenetically modifiable. For example, in Covid-19 magnesium intervention in those with Ca:Mg>2.6, transmembrane serine protease 2 (TMPRSS2) restricted cellular entry by enabling methylation of its gene. This assisted in the prevention of Covid-19 [51].

It is well known that hypermethylation leads to overall gene silencing and hypomethylation leads to overall gene activation. DNA methylation typically acts to repress gene transcription, i.e., serine protease production was suppressed by Mg<sup>++</sup> intervention.

An elevated mixed metalloproteinase-2 (MMP-2) has been implicated in EDS and a connection to elevated folate and MTHFR polymorphisms proposed [52]. In EDS unmetabolized folate accumulates in the blood, i.e., MMP-2 is hypomethylated and its promoter gene activated in EDS. An abnormal MTHFR is linked to hypomethylation, which tends to activate promoter genes. The authors speculate that methyl folate therapy might improve symptoms by methylating the MMP-2 promoter region. Support for this speculation can be found in ischemic stroke. Hypomethylation of the MMP-2 gene is associated with ischemic stroke [53], which is increased in EDS type IV [54]. Not surprisingly EDS appears to be associated with cognitive decline [55]. A nutritional shortfall has been implicated in the dysautonomia seen in EDS. Intramuscular magnesium sulfate lessened pain, improved emotional state, and increased energy [42]. EDS is linked to magnesium deficiency and

POTS. Magnesium deficiency in rats leads to a blunted baroreflex and enhanced HR response to stress, e.g., standing from lying [56].

The abundance of gastrointestinal issues in EDS suggests possible concomitant histamine intolerance and high flow POTS [57]. MTHFR and EDS are frequently viewed as genetic and beyond host control. But epigenetics has revealed that there is much that can be controlled. For another example, the APOE epsilon or ε4 allele, when homozygous, predicts about 15 times increase in AD. However, Native Americans have an abundance of this allele, but AD is largely absent [58].

Their Paleolithic diet provides an excellent Ca:Mg balance. Perhaps this dementia is not primarily genetic but is primarily epigenetic, due to an elevated Ca:Mg and decreased Mg<sup>++</sup> absorption. These two cations share the same receptor - CaSR (calcium sensing receptor). DNA methylation occurs via S<sub>AM</sub>e and requires Mg<sup>++</sup>. DNA in AD exhibits a characteristic differential methylation pattern.

### Autoimmunity and Cancer

Hypermethylation can help drive carcinogenesis, autoimmune disease, and neurodegenerative disease [59]. Hypomethylation can also drive cancer, autoimmune disease [60], and perhaps neurodegenerative disease as well [61].

The discussed syndromes all exhibit a distinct female preponderance. Autoimmune disease is also more common in females. Many believe that autoimmunity is at the heart of POTS, LC, CFS, and MCAS [62].

Possible explanations for the female preponderance include

1. Estrogen downregulates COMT [63] and DAO/MAO [64] => increased NE (low flow POTS), histamine (high flow POTS)
2. Females produce more IFN-γ which drives the tryptophan steal
3. Mast cells secrete not only histamine but also bradykinin; estrogen degrades ACE, which would otherwise degrade bradykinin
4. Magnesium deficiency is greater in females during their reproductive years
5. Chronic inflammation, migraines, and pain are prominent features in all these syndromes, even EDS [65].

Gut dysbiosis and abnormal tryptophan metabolism have been associated with autoimmunity [67]. Pleiotropic IFN-γ, prominent in the tryptophan steal, is tightly linked to autoimmune disease [68].

The constant flow of IFN in autoimmune disease depletes both B6 and tryptophan. Perhaps the constant flow of IFN and subsequent demand for B2, the B2-requiring B6, and the B2-requiring MTHFR (required to methylate B9 (folate) and B12) might connect POTS, LC, CFS, and MCAS to reports of autoantibodies to Ang II and beta adrenergic receptors [28].

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This then poses another link between LC and abnormal MTHFR.

An increased KTR induced by IFN- $\gamma$  provides additional support for the tryptophan connection to autoimmune disease [69], neurodegenerative disease, cancer, CVD, and aging. An elevated KTR responds to ARBs [70]. Indeed, a post mortem study of brains demonstrated more neuropathology in non-hypertensives, than in hypertensives on ARBs [71].

IFN- $\gamma$  is an anti-inflammatory pleiotropic cytokine and in an environment of chronic inflammation IFN- $\gamma$  may switch to a pro-inflammatory (autoimmune) role [68]. Hypomethylation of the IFN- $\gamma$  promoter gene may mediate this transition in autoimmune disease [72]. Hcy [73] and B2 deficiency [74] are associated with increased risk of MS. The latter is also linked to SLE [75]. MTHFR 677T is associated with MS [76] and RA [77].

Methylation operates similarly with respect to carcinogenesis. Some cancers are potentiated by hypomethylation, others by hypermethylation. Some, including breast and lung, can be seen under both conditions [78]. Hypomethylation is linked to hypertension [79] and B2 given to hypertensives with 677TT is more effective than anti-hypertensives alone [80].

### Therapeutic Considerations

The first goal is to avoid the extremes of hypomethylation and hypermethylation. Optimal levels of vitamin D (at least 50 ng/mL) have the capacity to demethylate and may assist in the search for the Goldilocks state of methylation [12,13]. Clearly sufficient intake of antioxidants is also in the equation, but many require methylation for activation. Those requiring methylation include melatonin, betaine, choline, cysteine, taurine, CoQ10, carnitine, creatine, creatinine, and lysine.

Knowing one's MTHFR status is not necessary, but knowing one's Hcy level might help motivate.

To avoid hypomethylation, maintain an optimal Ca:Mg of around 2.6. Otherwise calcium crowds out magnesium in the competition for the CaSR sites. Water soluble B vitamins, especially B2,3,6,9,12, must be in their active forms. This means methyl folate, methylcobalamin, and phosphorylated B6. Most supplements contain B6 as pyridoxine. Magnesium absorption is enhanced, possibly doubled when accompanied by P5P during a meal [81]. The inactive pyridoxine form of B6 competitively inhibits the P5P aka pyridoxal phosphate (PLP). So B6 (pyridoxine) supplementation can produce symptoms of vitamin B6 deficiency [82].

Beware of blindly increasing methylating agents to address an elevated Hcy. For example, intake of trimethylglycine aka betaine or methyl folate can trigger cold sores [83,84]. Choline is often recommended as a methylating agent, as it is the precursor to betaine. But this metabolic process is intramitochondrial. Eating more fruits and vegetables is a healthful dietary change, but food today is less nutritious and supplements are rightfully becoming more popular. Probiotics for improving the gut microbiome and antioxi-

dants to protect mitochondria add security.

It is not necessary to understand all the biochemical intricacies. Common characteristics of the 677T phenotype have been described. An acceptable glimpse of your own status can be gained from self examination and some rudimentary tests. Are you pre-hypertensive? Are you mildly anemic with macrocytic indices? A concomitant iron or B6 deficiency with microcytic indices could cloak this. Are you slightly myopic? Were you a good student? Do you tend toward "hay fever" or migraines? Do you have a family history of neuropsychiatric illness? If an Hcy level within normal limits is revealed, a value near the upper limit of normal should give pause for minor concern, as the MTHFR variants are quite prevalent in the healthy population from which the normal limits are determined. Serum histamine may provide further insight, as histamine is inversely proportional to methylation. In addition a complete blood count (CBC) with a minimally elevated mean corpuscular volume (MCV) or a low normal hematocrit, hemoglobin, and/or red cell count suggest a possible B9 or B12 shortage (active forms require methylation). Stress and/or chronic inflammation compromise mitochondrial function, exacerbate hypomethylation, and increase Hcy. What is your CRP or resting HRV? What is your 25(OH)D3 level? Keep a balanced Cu:Zn. A diet rich in nuts and seeds should help. Try an antihistamine. ARBs may offer relief if all else has failed.

### Conclusion

Two MTHFR variant alleles (1298C and 677T) are present in a majority of Americans. They directly impact the folic acid and methionine cycles, the primary determinants of the methylation process. The six listed syndromes may represent related phenotypes triggered by hypomethylation of epigenes due to abnormal MTHFRs.

The extremely common MTHFR variants are the most frequent cause of an elevated Hcy. Hcy is the predominant marker for abnormal methylation. Abnormal methylation plays vital epigenetic and biochemical roles in not only LC, CFS, POTS, MCAS/SIBO, and EDS but also aging, cancer, CVD, IBD, neurodegenerative disease, autoimmune disease, and quality of life.

The preventative benefits of vitamin D are legion and well known, but the intra-mitochondrial site for synthesis of its active form compromises efficacy for the inflamed. This underscores the prophylactic value of a diet rich in antioxidants, judiciously supplemented. Synthesis of vitamin D is heavily dependent on magnesium. Methyltransferases all require magnesium as cofactor. Vitamin D and magnesium deficiencies are common in these six syndromes. MTHFR and EDS are clearly genetic. We may not be in direct control of the genome, but the epigenome is beckoning.

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