

# METHYLATION PANEL

## Support Guide

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# Overview of Methylation

Methylation is a biochemical process in which methyl groups (CH<sub>3</sub>) are transferred or donated between molecules, thereby changing their structure and function. This happens billions of times per second in every cell throughout the body.<sup>1</sup> The methylation cycle is dependent on amino acids, vitamin cofactors, and minerals obtained from the diet to ensure adequate function of this biochemical pathway.<sup>2</sup>

The incredibly vast processes in the body that depend upon methylation are what ultimately make functional testing for methylation impairment a valuable clinical tool. Some of these processes include, but are not limited to:

- creatine production for skeletal muscle contraction
- DNA and RNA synthesis
- gene regulation (epigenetics)
- hormone regulation and detoxification
- energy production
- cell membrane repair
- fat metabolism
- myelination
- immune function
- neurotransmitter production and metabolism<sup>3-7</sup>
- vascular endothelial function and nitric oxide production<sup>8</sup>

To keep these processes functioning optimally, there is a necessary balance between many different biochemical pathways. What is termed the “methylation cycle” involves an interplay between folate metabolism, methionine metabolism, and homocysteine transsulfuration.<sup>8</sup> The body continually adapts these interconnected pathways in order to maintain homeostasis.<sup>9</sup> However, key amino acid deficiencies, a lack of vitamin and mineral cofactors, genetic enzymatic predispositions, and a wide array of oxidative stressors can impact multiple enzymes leading to a disruption in a patient’s overall methylation status.<sup>10-12</sup>

## GENOVA’S APPROACH TO METHYLATION TESTING

Genova Diagnostics’ **Methylation Panel** is an innovative assessment designed to offer insight into the methylation pathway, also known as 1-carbon metabolism. By measuring the functional analytes involved in the methylation cycle, as well as genetic predispositions for altered enzymatic activity, this profile can help clinicians design more targeted treatment strategies to optimize patient outcomes.

**Genova’s Methylation Panel is both a genotypic and phenotypic assessment.** Methylation metabolites are measured in plasma, and genetic single nucleotide polymorphisms (SNPs) are analyzed using a buccal swab. Using this method, clinicians are able to go beyond only looking at genetic predisposition (SNPs) or only measuring single biomarkers. There is no gold standard test available to assess a patient’s overall methylation status. However, the markers chosen for Genova’s Methylation Panel are integral to these pathways and are well represented in literature.<sup>2,8,13-15</sup>

### Methylation Panel Biomarkers

Betaine
Choline
Cystathionine
Cysteine
Dimethylglycine (DMG)
Glutathione (GSH)
Glycine
Homocysteine
Methionine
S-adenosylhomocysteine (SAH)
S-adenosylmethionine (SAM)
Sarcosine
Serine
Taurine

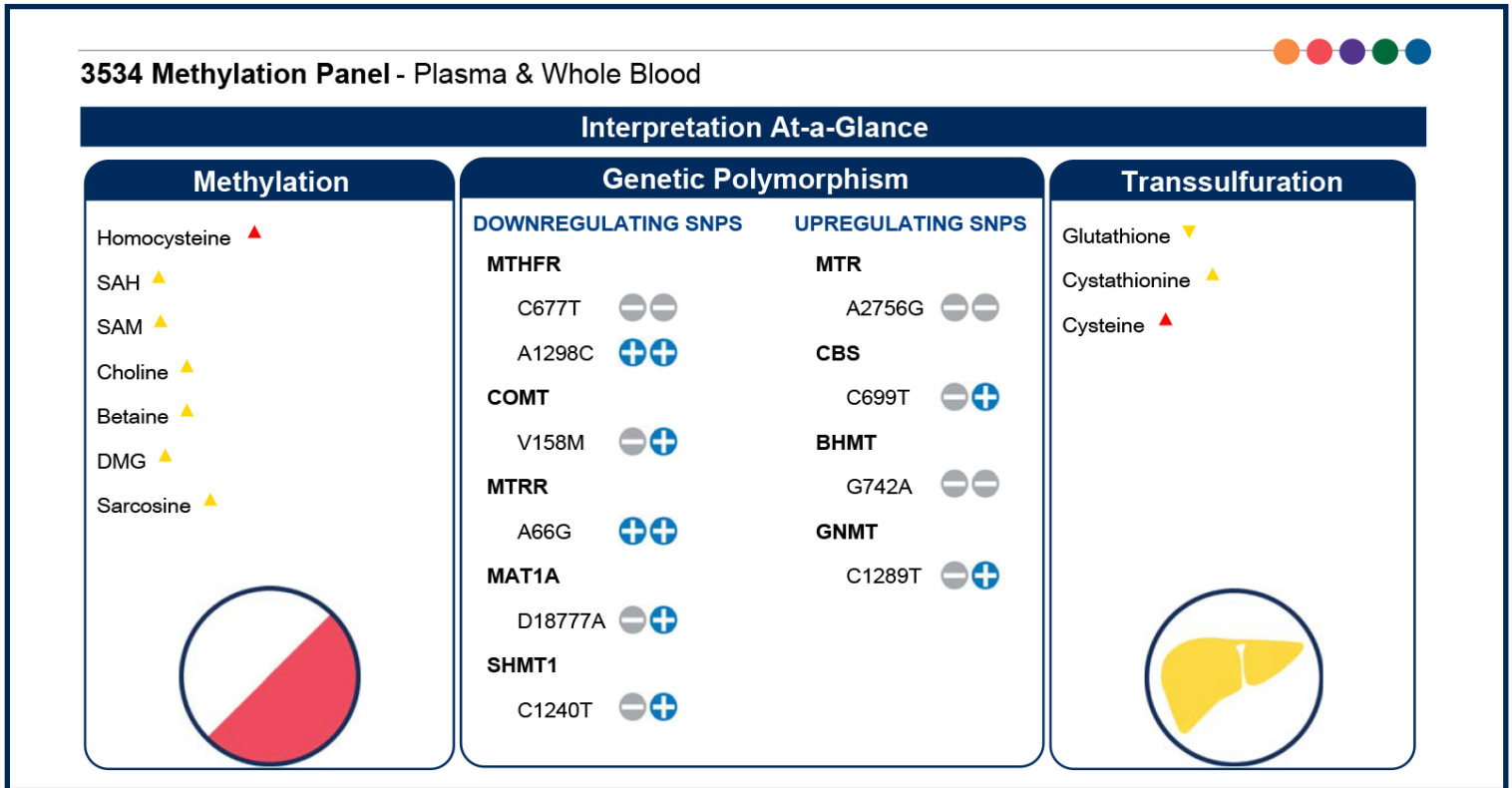
### Add-on Genomics

BHMT G724A
CBS C699T
COMT V158M
GNMT C1289T
MAT1A D18777A
MTHFR A1298C
MTHFR C677T
MTR A2756G
MTRR A66G
SHMT1 C1240T

# Interpretation-At-A-Glance Summary

Similar to other comprehensive Genova laboratory reports, Methylation Panel results are displayed on the front page as a convenient report-of-findings. This report synthesis provides clinicians with a quick overall methylation status assessment and separates the various aspects of the report into key clinical areas.

The page is divided into two main sections: **1) Functional Pillars, and 2) Methylation Status Ratios.**



## FUNCTIONAL PILLARS

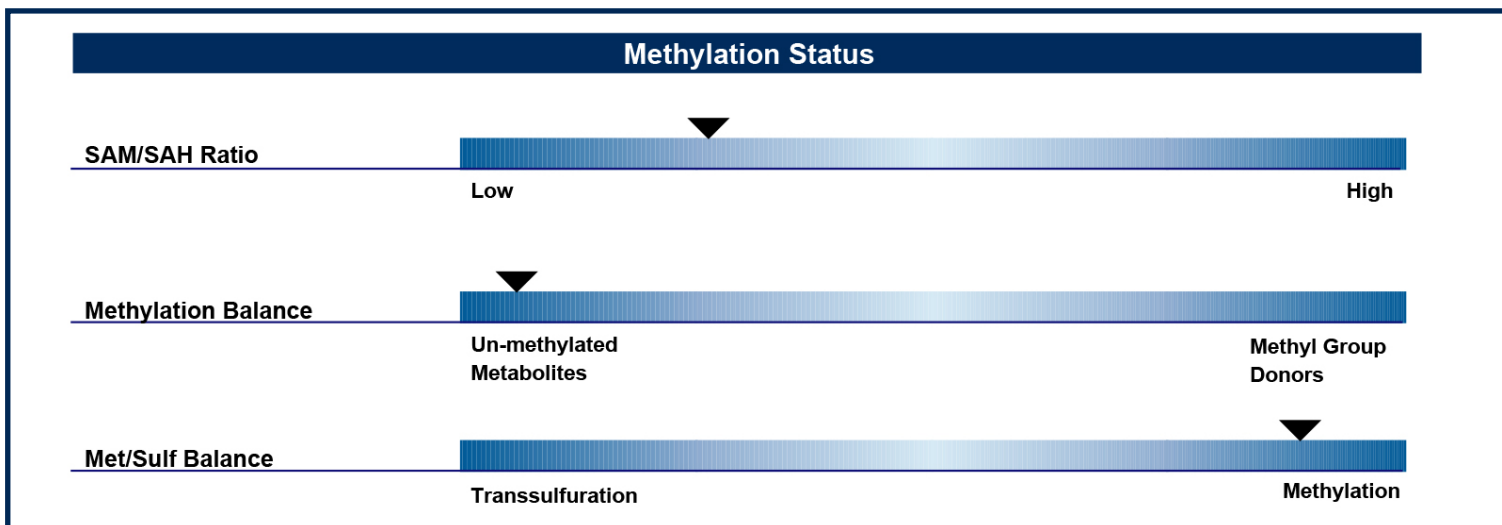
The functional pillars help separate the biomarkers into distinct categories: **Methylation Biomarkers, Genetic Polymorphisms, and Transsulfuration Biomarkers.** The methylation and transsulfuration pillars capture abnormal biomarkers from page 2 and use an algorithm to provide a suspected degree of clinical impact. The algorithm takes into account the severity of each biomarker alongside each biomarker's clinical relevance to generate an overall pillar score. This is then displayed as color-coded icons depicted as either green, yellow, or red. These colors signify either a low, medium, or high degree of suspected clinical impact, respectively.

The **Genomic Polymorphism** pillar provides the results of genetic SNPs, which are an add-on to the Methylation Panel. The enzymes are categorized based on whether polymorphisms (SNPs) result in either an upregulation or downregulation in that enzyme's activity. Each enzyme evaluated will have two results representing each inherited allele (one from each parent). A [+] indicates that a genetic polymorphism was detected and a [-] indicates that no polymorphism was detected (also known as a "wild-type"). Having two mutations is referred to as a "homozygous positive" genotype, one mutation is referred to as "heterozygous," and no mutations is referred to as "homozygous negative" genotype.



## METHYLATION STATUS RATIOS

Underneath the functional pillars on the first page is an overview assessment of Methylation Status. The three indicators in this section include the SAM/SAH Ratio, the Methylation Balance Ratio, and the Met/Sulf Balance Ratio. Unlike the SAM/SAH ratio, the Methylation Balance Ratio and Met/Sulf Ratio are completely unique to Genova's Methylation Panel. These novel algorithms represent a broader attempt to capture functional disturbances within the methylation and transsulfuration pathways by comparing multiple analytes.



### SAM/SAH Ratio

The SAM/SAH ratio is commonly referred to as the "Methylation Index" in the literature and has well-documented clinical associations.<sup>14</sup> Global methylation is dependent on two key factors: adequate SAM supply and SAH removal. The SAM/SAH ratio has been proposed to indicate the likelihood of hyper- or hypo-methylation.<sup>16</sup> Overall, the SAM/SAH ratio is under tight homeostatic control.<sup>17</sup> SAM levels remain fairly stable due to de novo synthesis and feedback mechanisms.<sup>18</sup> Given this, alterations in the methylation index are more likely a result of SAH fluctuations.

As will be discussed later, SAH is a potent inhibitor of methyltransferase reactions, which is likely why SAH elevations and a low methylation index are correlated with global hypo-methylation.<sup>14</sup> Furthermore, poor homocysteine clearance contributes significantly to SAH elevations which provides insight into the relationship between these two analytes and cardiovascular disease.<sup>19-21</sup>

It is important to note that while the Methylation Panel does not directly measure DNA methylation, the SAM/SAH ratio has been shown to correlate with DNA methylation.<sup>22</sup>

### Methylation Balance Ratio

The Methylation Balance Ratio compares four methylated metabolites (including SAM) to four un-methylated metabolites (including SAH). Therefore, the Methylation Balance Ratio can be thought of as an expanded SAM/SAH ratio.

The four methyl group donors that make up the numerator of the equation are SAM, methionine, betaine, and serine. The four un-methylated metabolites are SAH, homocysteine, DMG, and sarcosine.

#### Methylation Balance Ratio

Methylated Metabolites	Un-Methylated Metabolites
SAM	SAH
Methionine	Homocysteine
Betaine	DMG
Serine	Sarcosine

The clinical utility of the Methylation Balance Ratio is that it represents a potential way to detect subtle methylation imbalance prior to alterations in the SAM/SAH ratio. This approach is still novel and is based on biochemical pathway analysis. However, early Genova data analysis of this biomarker has demonstrated its ability to distinguish a healthy cohort from an unqualified cohort. Genova will continue to conduct research on this novel biomarker's clinical application.

## Met/Sulf Balance (Methylation/Transsulfuration Balance) Ratio

The second calculated ratio is called the 'Met/Sulf Balance' and it compares analytes between the methylation pathway and transsulfuration pathway. Biomarker levels are compared proportionately allowing potential insight into which of the pathways is being favored.

### Met/Sulf Balance Ratio

Methylation Metabolites	Transsulfuration Metabolites
SAM	Cystathionine
SAH	Cysteine
Methionine	Taurine
Homocysteine	Glutathione

The four analytes from the main methylation pathway that are used in the Met/Sulf Balance are SAM, SAH, methionine, and homocysteine. The four analytes from the transsulfuration pathway are cystathionine, cysteine, taurine, and glutathione.

There is a relative balance that exists between the methylation and transsulfuration pathways. This balance ensures that adequate levels of glutathione are produced to counteract oxidative stress and that an adequate amount of SAM is made for methylation reactions.

As with the Methylation Balance ratio, the Met/Sulf ratio is a novel biochemical pathway analysis. Early Genova data analysis of this biomarker has demonstrated its ability to distinguish a healthy cohort from an unqualified cohort. Genova will continue to conduct research on this novel biomarker's clinical application.

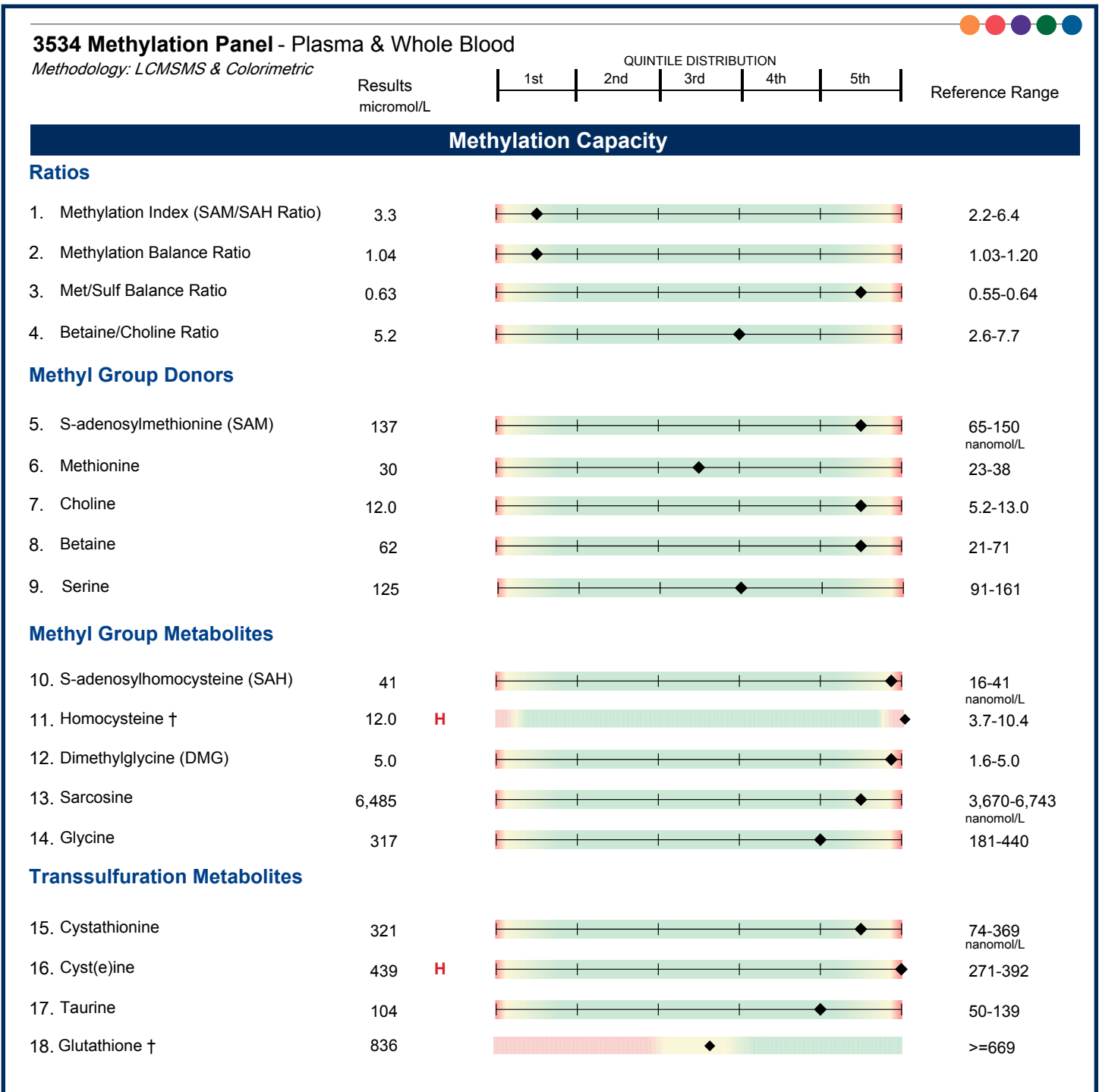
# Biomarker Layout

The second page of the report consists of the biomarker results. Biomarkers are groups based on their significance to the Methylation pathway:

- Ratios
- Methyl Group Donors
- Methyl Group Metabolites
- Transsulfuration Metabolites

Biomarkers that are outside the reference range will appear under its corresponding pillar on the first page with a red triangle pointed either upward or downward, depending on whether the biomarker is low or high. Biomarkers that are high-normal or low-normal (as determined by falling outside 1 standard deviation) will appear on the pillar with a yellow triangle rather than a red triangle.

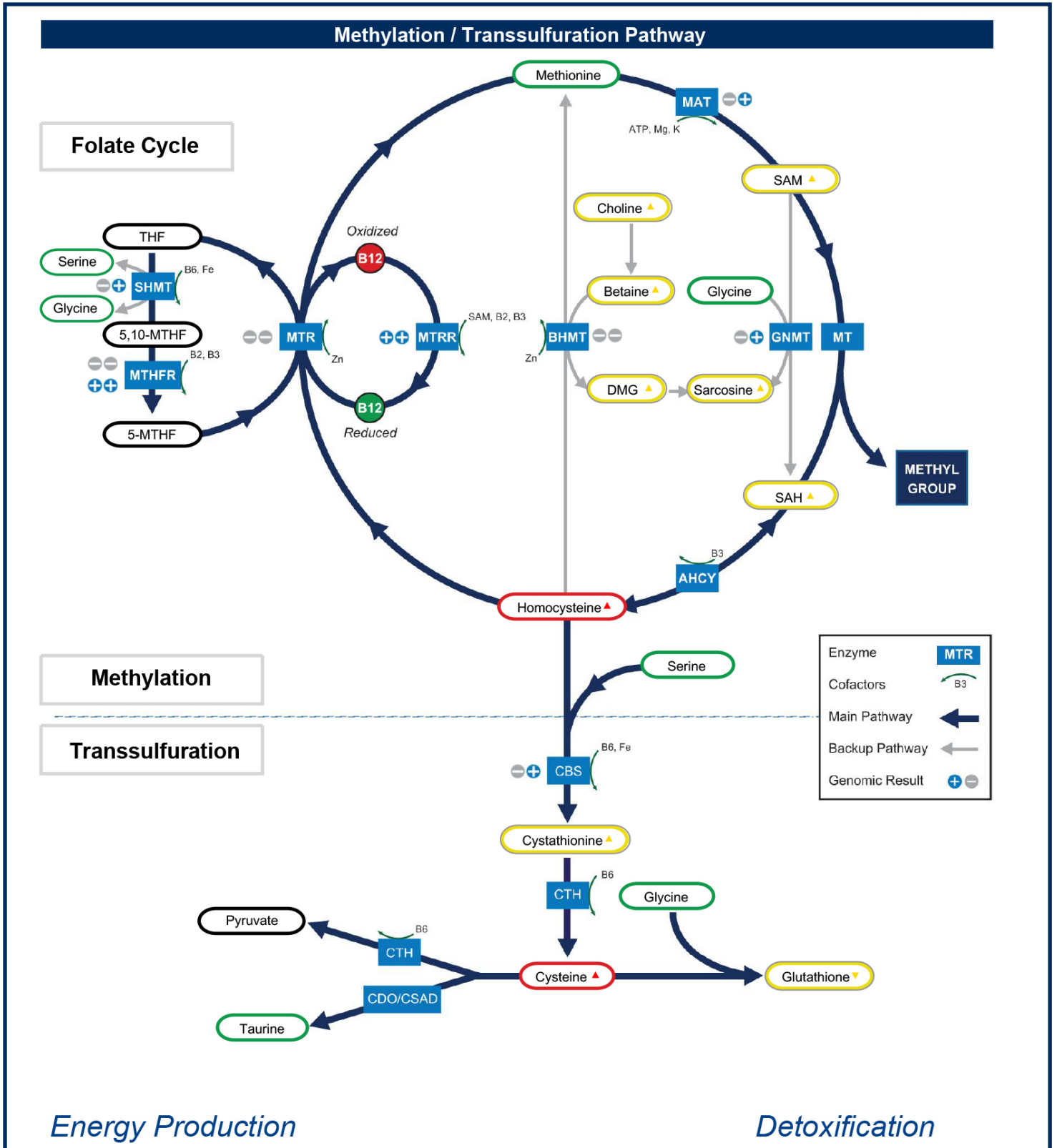
Patient results are listed in the first column and are accompanied by an "L" or "H" flag if the results fall outside of the reference range.



# Pathway Layout

On page three of the report, the patient's biomarker results and genomic results are displayed together in a pathway format. This allows clinicians to visualize a patient's unique biochemistry based on their individual genomic and phenotypic results.

Genomic results appear next to the enzymes that are evaluated and utilize the same positive/negative designations as on the first page. Any abnormal biomarkers appear with either a yellow or red border indicating either a borderline or abnormal finding, respectively.

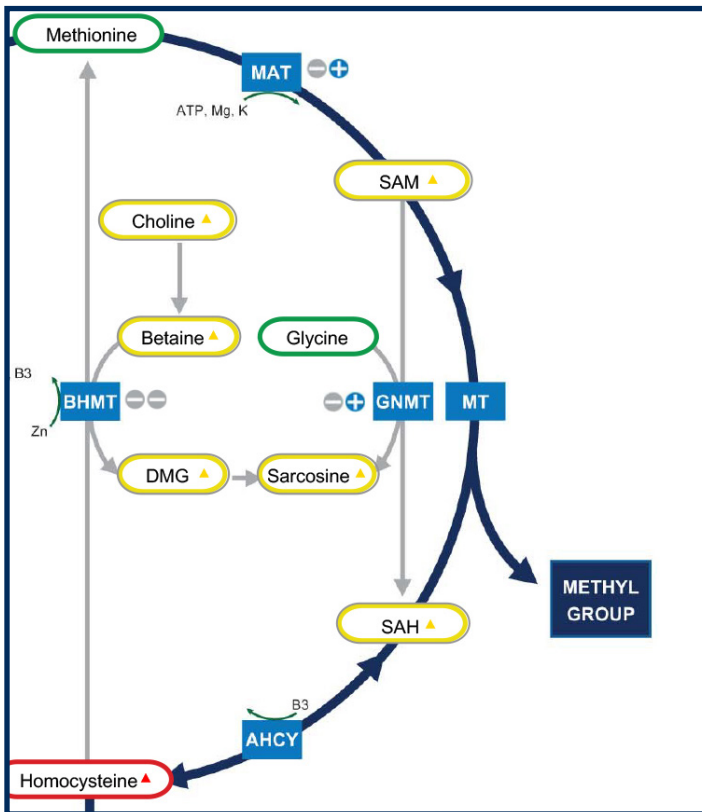




# Biomarker Layout

## METHYL GROUP DONORS

### S-adenosylmethionine (SAM)



SAM is found in every cell of the human body and serves an important biologic function as the sole methyl donor in more than 100 different reactions catalyzed by methyltransferase enzymes. It also plays a key role in activating the transsulfuration pathway, resulting in glutathione synthesis and energy production.<sup>23</sup>

**S-adenosylmethionine (SAM)** is formed from the essential amino acid methionine and adenosine triphosphate (ATP) using the enzyme methionine adenosyltransferase (MAT). In literature, SAM is sometimes referred to as AdoMet.

SAM levels are an important feedback mechanism throughout the body. For example, SAM inhibits methylenetetrahydrofolate (MTHFR), thereby reducing the availability of 5-MTHF as a methyl donor.<sup>17</sup> SAM also has been shown to downregulate the BHMT enzyme leading to another form of feedback inhibition while upregulating the CBS transsulfuration enzyme.

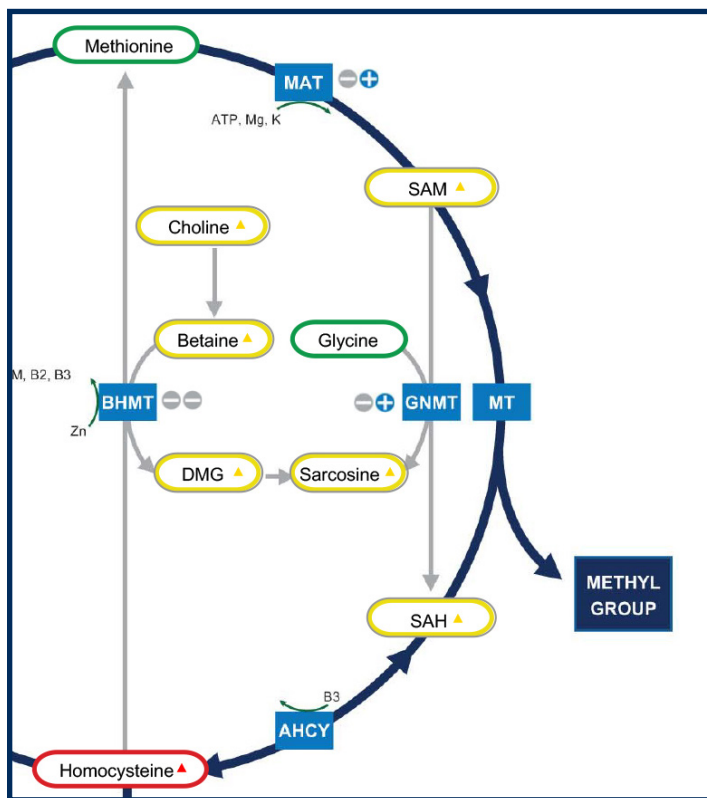
SAM levels in the body depend on the availability of the essential amino acid methionine and the enzyme MAT. Diet alone cannot provide adequate SAM; therefore, the body must also rely on de novo synthesis. The liver tightly regulates SAM levels.<sup>18</sup> However, it has been shown, that plasma SAM levels increase in proportion to adiposity and obesity. Methionine and SAH levels are not associated with fat mass, suggesting increased conversion of methionine to SAM in obese individuals. The mechanism of this increased conversion is still being studied.<sup>24-27</sup>

Since March 1999, SAM-e has been available in the US as an over-the-counter nutraceutical supplement.<sup>23</sup> SAM-e supplementation may be considered when methionine is low, or when the enzymatic conversion from methionine is dysfunctional. SAM-e has been shown to improve patient outcomes in conditions such as cancer, cognitive decline, arthritis, depression, and liver disease.<sup>28-33</sup> Treatment with SAM-e has been shown to be as effective as prescription tricyclic antidepressants. SAM-e was also as effective as non-steroidal anti-inflammatory drugs to treat osteoarthritis, and has been successful in treating some liver conditions.<sup>34</sup> SAM-e is generally well tolerated, and no serious side effects have been observed.<sup>35</sup> However, there have been rare case reports of bipolar patients developing manic episodes due to SAM-e supplementation.<sup>36</sup>

#### S-ADENOSYLMETHIONINE (SAM)

LOW	HIGH
<ul style="list-style-type: none"> <li>• Low methionine due to decreased protein intake, or malabsorption/ maldigestion</li> <li>• MAT SNP or lack of cofactors (ATP, Mg, K)</li> </ul>	<ul style="list-style-type: none"> <li>• Supplementation with SAM-e</li> <li>• High caloric intake, high BMI<sup>24</sup></li> </ul>

# Methionine



**Methionine** is an essential amino acid that plays an important role in the methylation cycle. Methionine is obtained from dietary intake or through homocysteine remethylation. Methionine’s dietary sources include eggs, fish, meats, Brazil nuts, and other plant seeds.<sup>37</sup>

As discussed previously, methionine is converted to the body’s main methyl donor, S-adenosylmethionine (SAM). This conversion requires the enzyme methionine adenosyltransferase (MAT).

Methionine elevations are most commonly caused by increased dietary intake.<sup>37</sup> However, increases can also be due to abnormalities within the methylation cycle itself producing a passive methionine elevation.

Genetic SNPs for several methylation and transsulfuration enzymes, or the lack of necessary vitamin and mineral cofactors, can alter methionine’s metabolism. For example, a nutritional cofactor deficiency (magnesium/potassium), ATP depletion, or a SNP in the MAT enzyme, can downregulate the conversion to SAM and may lead to elevated methionine.<sup>38</sup> Vitamin B<sub>6</sub> deficiency, a cofactor for the downstream enzyme responsible for homocysteine transsulfuration (cystathionine beta-synthase-CBS), can result in excess homocysteine re-methylation back to methionine, thus increasing methionine.<sup>1</sup>

Mild elevations in methionine do not cause serious adverse clinical effects. There is literature regarding CNS abnormalities seen with excessive elevations, but this is rare and more commonly seen with inborn errors of metabolism (MATI/III deficiency also known as Mudd’s disease).<sup>39</sup>

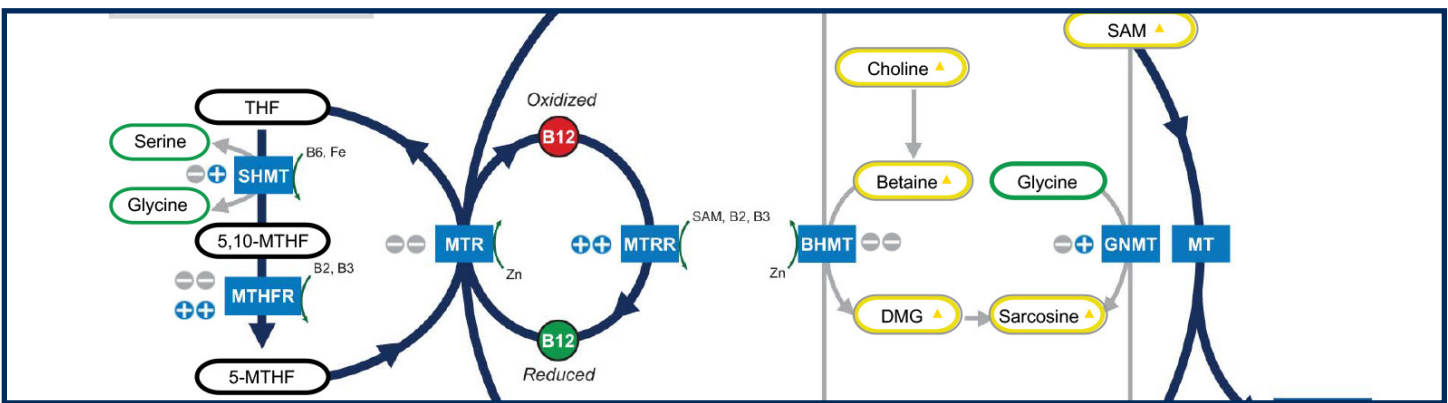
A dietary methionine deficiency (low intake or malabsorption/maldigestion) can affect the methylation cycle, given its critical role. Increasing methionine dietary sources, methionine supplementation, or methylated product supplementation can mitigate the adverse impact.<sup>40</sup>

## METHIONINE

LOW	HIGH
<ul style="list-style-type: none"> <li>Decreased protein intake</li> <li>Protein malabsorption/maldigestion</li> </ul>	<ul style="list-style-type: none"> <li>Increased dietary intake or supplementation (i.e. eggs, fish, meat, Brazil nuts, and other plant seeds)<sup>37</sup></li> <li>MAT SNP or lack of cofactors (ATP, Mg, K)</li> <li>Alcohol, oxidative stress affecting MAT<sup>41,42</sup></li> </ul>

## Choline

## Betaine



**Choline** is an essential dietary nutrient found in many foods such as meat, eggs, soybeans, and wheat germ. Choline can be made endogenously, but dietary intake is needed to prevent deficiency.<sup>43</sup>

Choline is a critical cell membrane component. It also helps to ensure the structural integrity and signaling functions within the cell. Choline is a precursor for the important neurotransmitter acetylcholine and the membrane phospholipids phosphatidylcholine and sphingomyelin.<sup>43</sup>

In the methylation cycle, choline is oxidized to form betaine, which can then be used as a methyl donor. Because choline and betaine are involved in the re-methylation of Hcy back to methionine, they form a backup-pathway that is particularly favored in folate deficiency.<sup>44</sup>

Elevated choline levels are associated with key components of metabolic syndrome (dysglycemia, dyslipidemia, and BMI). Betaine showed an opposite relationship. This may suggest a disruption of mitochondrial choline oxidation to betaine as part of the mitochondrial dysfunction seen in metabolic syndrome.<sup>44</sup>

**Betaine**, also known as **trimethylglycine**, is an amino acid - so named because it was first discovered as a beet byproduct. It is also found in wheat, shellfish, and spinach. As stated previously, it can be produced via choline oxidation. Betaine is a major methyl donor and acts as an osmolyte in the cell to regulate cell volume.<sup>17</sup>

Betaine donates a methyl group, using the enzyme betaine homocysteine methyltransferase (BHMT), to re-methylate homocysteine back to methionine.<sup>45</sup> The product of this methyl transfer is dimethylglycine (DMG), which is then available for further methyl donation.

In folate deficiency, this betaine pathway compensates to maintain Hcy re-methylation. Folate-deficient patients have elevated serum DMG levels, as would be expected with the increased betaine use as a methyl source.<sup>45</sup>

Because the betaine pathway is a salvage pathway to remethylate Hcy, betaine is a significant determinant of plasma Hcy levels, particularly in folate deficiency.<sup>17</sup> Betaine supplementation lowers Hcy after methionine load.<sup>17</sup>

Betaine deficiencies have many clinical implications, including metabolic syndrome, diabetes, vascular disease, liver diseases, cancer, and fetal abnormalities.<sup>45</sup> Not surprisingly, supplementation with betaine has been shown to be beneficial in many conditions associated with poor methylation status and higher homocysteine, such as metabolic syndrome.

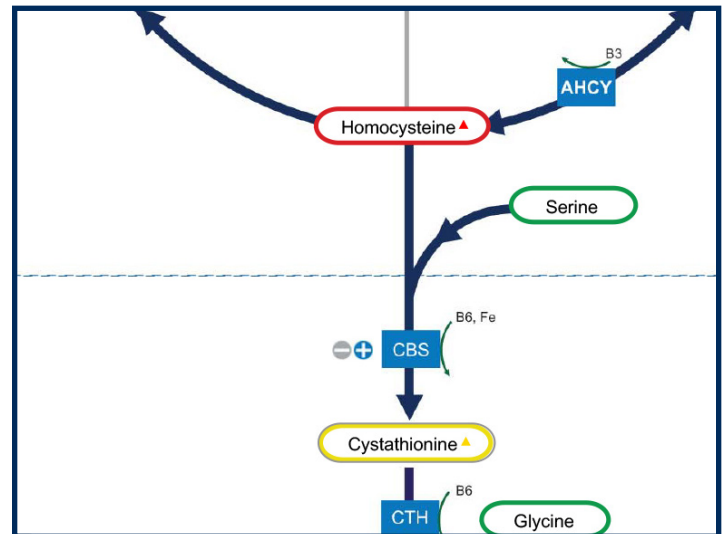
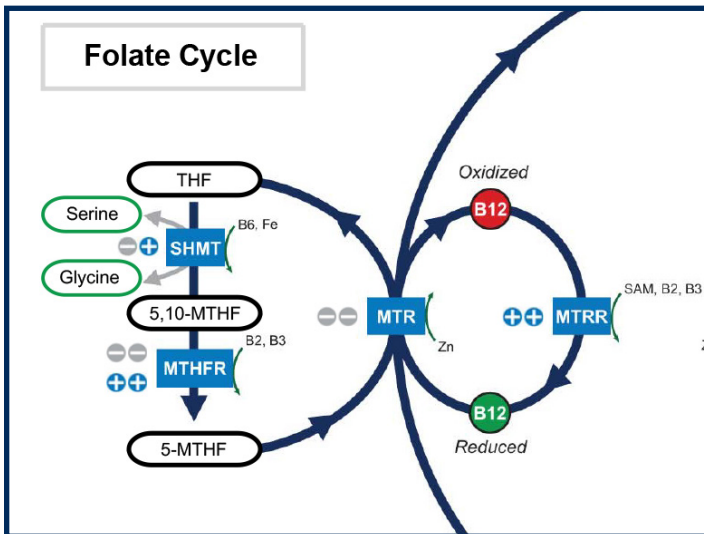
### CHOLINE

LOW	HIGH
<ul style="list-style-type: none"> <li>Low intake</li> <li>Malabsorption/maldigestion</li> </ul>	<ul style="list-style-type: none"> <li>Dietary intake (i.e. meat, eggs, soybeans, and wheat germ)<sup>43</sup></li> <li>Upregulation of the betaine/choline backup pathway</li> </ul>

### BETAINE

LOW	HIGH
<ul style="list-style-type: none"> <li>Decreased dietary choline intake</li> </ul>	<ul style="list-style-type: none"> <li>Supplementation</li> <li>High dietary betaine (i.e. beets, wheat, shellfish, and spinach) or choline intake (see above)<sup>43</sup></li> </ul>

## Serine



**Serine** is a nonessential amino acid used in protein biosynthesis. In the folate cycle, glycine and serine are interconverted by the enzyme serine hydroxymethyltransferase (SHMT). Glycine accepts a methyl donor from 5-10 MTHF and becomes serine; therefore, serine is methylated glycine.<sup>46</sup> These methyltransferase reactions and interconversions are readily reversible depending on the needs of the folate cycle.<sup>47</sup>

Glycine and serine's interconversion is important in mitochondrial glycolysis. Glycolysis provides ATP and energy in most cell types. Serine-glycine biosynthesis is a component in glycolysis-diverting pathways and nucleotide biosynthesis. This is clinically important, and specifically evident, in cancer. Cancer cells use glycolysis to sustain anabolism for tumor growth. Genetic and functional evidence suggests that abnormalities in the glycine-serine pathway represent an essential process in cancer pathogenesis by promoting energy production and promoting defective purine synthesis.<sup>47</sup> Serine is also a cofactor for the enzyme cystathionine- $\beta$ -synthase making its availability important for glutathione production.

Serine can be supplied from foods or synthesized by the body from glycine and other cofactors. Serine is found in soybeans, nuts, eggs, lentils, shellfish, and meats. Dietary serine is not fully converted to glycine; therefore, supplementation has little value, though is not harmful.<sup>48</sup>

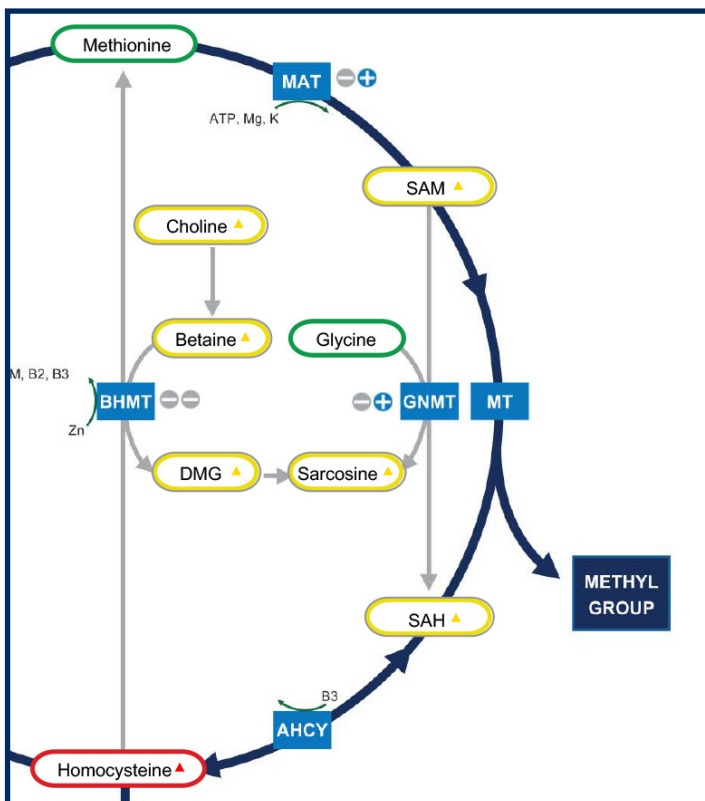
The clinical relevance of abnormal plasma serine is still the subject of ongoing research. However, it plays a role in the methylation balance ratio owing to the fact that it is a major methyl donor relevant to the folate pathway. Therefore, it assists in the flux of methyl groups between folate and methylation cycles.

### SERINE

LOW	HIGH
<ul style="list-style-type: none"> <li>Decreased dietary intake</li> <li>Malabsorption/maldigestion</li> </ul>	<ul style="list-style-type: none"> <li>High dietary intake (i.e. soybeans, nuts, eggs, lentils, shellfish, and meats)</li> </ul>

# METHYL GROUP METABOLITES

## S-adenosylhomocysteine (SAH)



**S-adenosylhomocysteine (SAH)** is the end-product of methylation reactions in the body. SAM ultimately donates a methyl group for methylation (DNA, detoxification, etc.) resulting in SAH formation. SAH is also the metabolic precursor of all the homocysteine (Hcy) produced in the body. In literature, SAH is sometimes referred to as AdoHcy.

SAH is hydrolyzed to homocysteine; however, this reaction is readily reversible, and the dynamics strongly favor SAH synthesis. Therefore, any elevation in homocysteine will lead to an increase in SAH.<sup>49</sup> In spite of the reversible nature of this reaction, efficient cellular removal of homocysteine allows sustained SAH metabolism and homocysteine production.<sup>50</sup>

SAH accumulation is implicated in many chronic clinical conditions.<sup>20,21,51</sup> SAH is a potent feedback inhibitor in methyltransferase reactions. SAH's pathogenicity lies in its binding affinity for, and inhibition of, methyltransferase enzymes within many tissue components, including DNA, RNA, phospholipids, and others. One example of SAH accumulation is in vascular cell phenotypic changes and atherosclerotic disease development.<sup>52</sup> Therefore, plasma SAH levels have been shown to be a more sensitive marker for clinical cardiovascular disease, renal disease, and Alzheimer's disease than plasma homocysteine.<sup>20,21,52-54</sup>

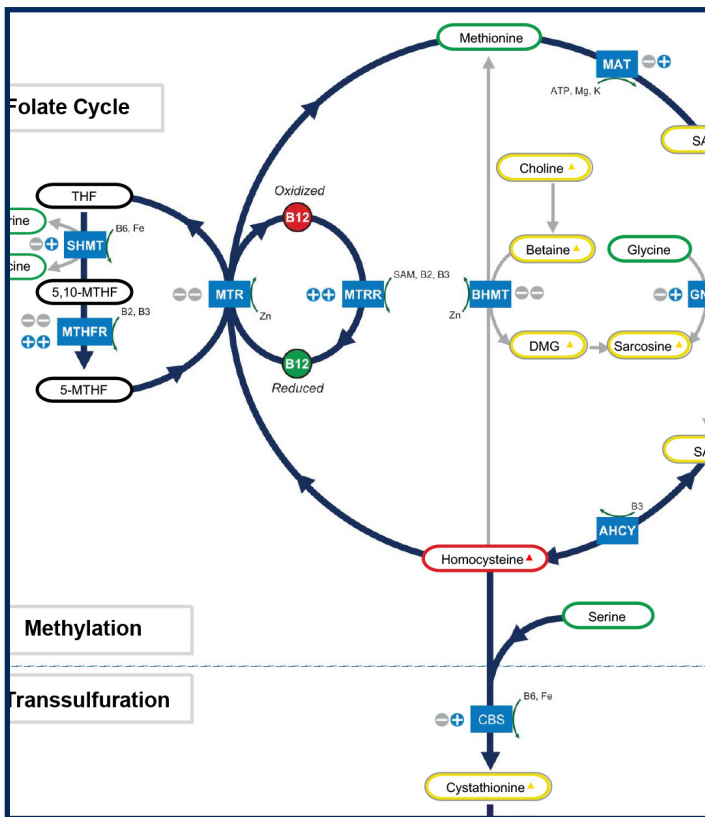
Methylation reactions are ultimately dependent on SAH removal.<sup>49,55</sup> Genetic SNPs, or nutritional deficiencies that hinder Hcy or adenosine metabolism, can induce SAH accumulation and subsequent hypomethylation defects, which are implicated in a variety of diseases, as previously outlined.<sup>50,56</sup> Nutritional therapies that encourage Hcy metabolism (i.e. folate, B<sub>12</sub>, betaine, choline) may passively lower SAH levels.<sup>57-59</sup>

### S-ADENOSYLHOMOCYSTEINE (SAH)

LOW	HIGH
<ul style="list-style-type: none"> <li>Unknown clinical significance</li> </ul>	<ul style="list-style-type: none"> <li>AHCY deficiency or need for Vitamin B<sub>3</sub> cofactor<sup>60</sup></li> </ul>
<ul style="list-style-type: none"> <li>Lack of SAM/ methyl donors</li> </ul>	<ul style="list-style-type: none"> <li>Elevated Hcy<sup>49</sup></li> </ul>



## Homocysteine



**Homocysteine (Hcy)** is not a classic amino acid found in dietary protein. Homocysteine's only source in humans is the demethylation of s-adenosylmethionine (SAM).

Homocysteine is a major branch point in the methylation pathway. It can be metabolized via two pathways: degraded irreversibly through the transsulfuration pathway or re-methylated back to methionine. These two pathways are greatly affected by vitamin and mineral cofactor availability and enzymatic SNPs.<sup>8</sup>

Transsulfuration is the main route for irreversible Hcy disposal. Transsulfuration begins when Hcy is converted to cystathionine, using the cystathionine  $\beta$ -synthase enzyme (CBS). This reaction requires nutrient cofactors, such as vitamin B<sub>6</sub> and iron.

Alternatively, Hcy can be re-methylated back to methionine.<sup>61</sup> Two distinct routes exist for Hcy remethylation. The first reaction is dependent on folate and vitamin B<sub>12</sub>. The second route for Hcy remethylation is independent of folate, but requires betaine. The betaine pathway for Hcy remethylation is a salvage pathway when folate metabolism abnormalities are present or in folate deficiency.<sup>8</sup> Under normal conditions, the body will remethylate Hcy several times before allowing irreversible transsulfuration.<sup>62</sup>

Whereas SAM-dependent methylation occurs in nearly all tissues, the transsulfuration pathway and Hcy remethylation occur primarily in the liver and kidneys.<sup>63</sup>

Hcy intracellular concentration is under tight control. As mentioned above, SAH accumulation must be avoided as it can inhibit all methylation reactions. Because of AHCY's reversible nature, it is mandatory that intracellular Hcy concentrations are kept within strict limits. Optimal Hcy concentrations in cells are maintained or re-established through folate-dependent remethylation. Whenever the cellular capacity to metabolize Hcy is exceeded, this amino acid will be exported to the extracellular space until intracellular levels are normalized. This results in elevated plasma Hcy levels. Exceptions are liver and kidney cells, where Hcy can enter the transsulfuration pathway.

As alluded to above, several factors can affect Hcy metabolism causing hyperhomocysteinemia. These include B-vitamin deficiencies, impaired renal excretion, advanced age, sex (male), smoking, alcohol, and genetic enzyme deficiencies.<sup>61</sup>

Elevated homocysteine levels have many clinical implications.

- Hyperhomocysteinemia is regarded as a risk factor for non-coronary atherosclerosis and coronary artery disease. Elevated homocysteine enhances vascular smooth-muscle cell proliferation, increases platelet aggregation, and acts on the coagulation cascade and fibrinolysis, causing normal endothelium to become more thrombotic. The mechanism may be related to elevations in SAH, due to the reversible nature of Hcy formation.<sup>61</sup> SAH has been shown to be a more sensitive marker in many diseases as previously outlined.<sup>20,21</sup>
- Diabetes, both type 1 and type 2, initially causes hypohomocysteinemia, due to renal hyperperfusion early in the diabetic nephropathy disease process. This progresses to hyperhomocysteinemia as renal function becomes compromised.<sup>61,64</sup>
- Elevated homocysteine levels have also been implicated in gastrointestinal disorders such as inflammatory bowel disease and colon cancer.<sup>61,65</sup> Hyperhomocysteinemia may be partially due to nutrient malabsorption (methyl donor and B-vitamin deficiency). Subsequently, elevated Hcy has been shown to induce inflammatory cytokines and contribute to disease progression.<sup>65</sup>

- Homocysteine can impair bone health by interfering with osteoclast activity. The increased Hcy impairs the cellular and molecular mechanism of bone marrow-derived osteoclasts by causing imbalance between phosphorylation and de-phosphorylation of various protein kinases that modulate bone cell remodeling.<sup>66</sup>
- Homocysteinemia contributes to neurodegenerative diseases (Alzheimer's and Parkinson's diseases) and mood disorders.<sup>67-69</sup> Elevated Hcy increases CNS phosphorylated tau leading to increased neurofibrillary tangle formation, seen in Alzheimer's dementia.<sup>70</sup> Hyperhomocysteinemia related to mood disorders may be multifactorial. Elevated Hcy causes elevations in SAH, which interferes with many methyltransferase reactions involved in neurotransmitter synthesis and metabolism. Hcy may also have direct neurotoxic effects. Research is ongoing regarding the exact mechanisms regarding Hcy and psychiatric disorders.<sup>71,72</sup>

Dietary supplementation with folate, vitamin B<sub>12</sub>, and SAM has been shown to effectively lower plasma homocysteine levels and improve outcomes.<sup>61,67,73</sup> The clinical implications associated with low homocysteine levels are not well represented in literature. Furthermore, there is no consensus on what constitutes a 'low level' or if it is something that needs correcting.

However, because Hcy is used to make glutathione and is remethylated to maintain methionine levels, the theoretical importance of low Hcy exists. Without Hcy, glutathione production is compromised. Excessive oxidative stress may accelerate the transsulfuration pathway toward glutathione production, which can lower Hcy. A SNP in the CBS enzyme accelerates homocysteine transsulfuration, which may result in a low Hcy.

Many 'methylation experts' and key opinion leaders teach that low plasma homocysteine leads to disease and can be cancer-producing; therefore it should be corrected. Many recommend protein and sulfur-containing foods, as well as evaluating for excessive oxidative stress and decreasing methyl support. There is currently no literature that has looked at correcting low plasma homocysteine.

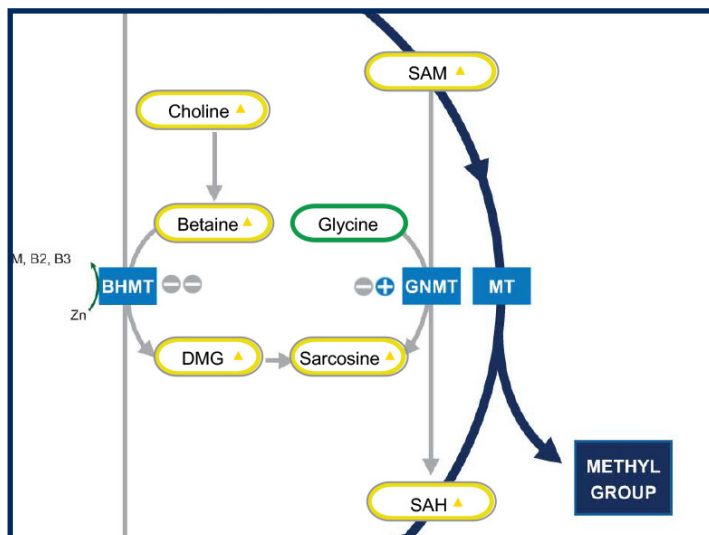
Literature is evolving to include low Hcy implications; however, the only literature-based clinical correlation currently available is an association with peripheral neuropathy.<sup>74</sup> There are a few animal studies looking for implications, physiologic impacts, and treatment strategies to correct hypohomocysteinemia, but currently no human studies exist.<sup>75,76</sup>

As a final point, when referring to Hcy, the terms 'homocysteine' and 'homocystine' are used interchangeably. However, the reduced sulfhydryl form is 'homocysteine,' while the oxidized disulfide form is 'homocystine.' The composite of both forms are routinely described by the term 'homocysteine.'<sup>77</sup> Most conventional laboratories that offer homocysteine measurement are actually measuring a total of homocystine, homocysteine, and SAH. To note, Genova's Methylation Panel measures SAH and homocysteine as separate clinically significant entities. Homocystine (an oxidized form of homocysteine) is not measured by Genova. SAH and homocystine levels are negligible as compared to homocysteine, though direct comparisons have not yet been evaluated by Genova.

## HOMOCYSTEINE

LOW	HIGH
<ul style="list-style-type: none"> <li>• Unknown clinical significance</li> <li>• May be a sign of over-methylation, though literature not available</li> <li>• CBS SNP in the presence of oxidative stress or inflammation<sup>78,79</sup></li> <li>• AHCY deficiency (lack of vitamin B<sub>3</sub>)<sup>60</sup></li> </ul>	<ul style="list-style-type: none"> <li>• Vitamin B<sub>6</sub> or iron deficiency (CBS enzyme cofactors)<sup>80</sup></li> <li>• Enzymatic deficiency in MTR/MTRR/BHMT<sup>11,17,81</sup></li> <li>• Folate deficiency with low choline intake<sup>44</sup></li> <li>• Alcohol<sup>82</sup></li> <li>• Tobacco<sup>83</sup></li> </ul>

## Dimethylglycine (DMG)



DMG has been marketed safely as a nutritional supplement since 1974. It was used in the Soviet Union in the 1960's as part of a formula to benefit athletic performance.<sup>84</sup> Dietary sources include cereal grains, seeds, beans and liver. Intake and supplementation with DMG plays an important role in keeping Hcy levels stable and providing methyl donors.<sup>84</sup>

Because it contains two methyl groups for donation, therapeutic interventions with DMG have been successful in many conditions including autism, hyperlipidemia, chronic fatigue syndrome, systemic lupus erythematosus, and cardiovascular disease.<sup>84</sup>

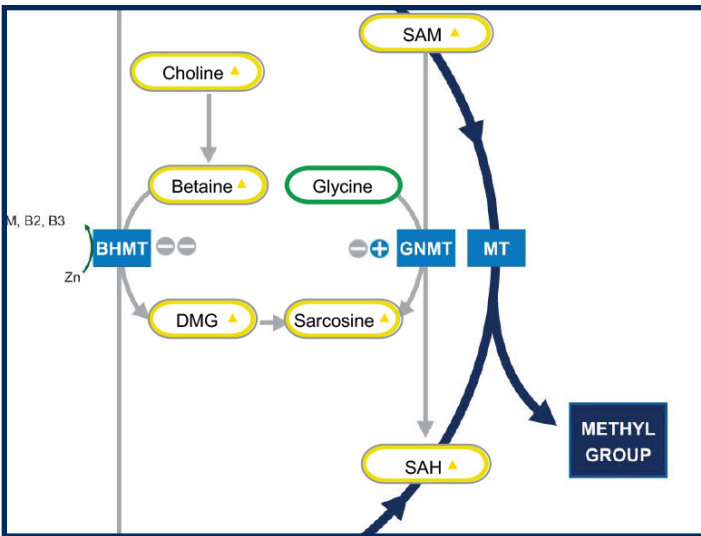
The amino acid derivative **dimethylglycine (DMG)** is produced when betaine (trimethylglycine) donates a methyl group to homocysteine for re-methylation back to methionine. This methyl donation is mediated by the enzyme betaine homocysteine methyltransferase (BHMT). Elevations in DMG act as a negative feedback by inhibiting this enzymatic conversion.<sup>17</sup>

Clinically, DMG elevations may indicate an increase in activity of the BHMT "salvage-pathway." Therefore, high DMG may reflect the need for additional folate, vitamin B<sub>12</sub>, and zinc.

### DIMETHYLGLYCINE (DMG)

LOW	HIGH
<ul style="list-style-type: none"> <li>Decreased choline/betaine intake</li> <li>Malabsorption/maldigestion</li> <li>Zinc deficiency (cofactor BHMT)<sup>85</sup></li> </ul>	<ul style="list-style-type: none"> <li>Supplementation with DMG or Betaine</li> <li>BHMT SNP<sup>17,86,87</sup></li> </ul>

## Sarcosine



**Sarcosine** is an amino acid made when SAM is conjugated with glycine by the glycine-N-methyltransferase (GNMT) enzyme. It can also be made by catabolism of DMG (see previous section). There are many dietary sources of sarcosine including eggs, legumes, nuts, and meats.<sup>88</sup> Sarcosine is also available as an over-the-counter supplement, and it is widely used in cosmetic formulations (toothpaste, creams, and soaps) and detergents.<sup>88</sup>

In the methylation cycle, sarcosine is created by the GNMT enzyme, which functions to control SAM excess. Disposal of excess SAM is seen in excess methyl donor supplementation, or SAM elevation due to adiposity/obesity. Some clinicians use sarcosine elevation as a marker of 'excess methyl supplementation' or 'over-methylation.' Currently, there is no literature to support this hypothesis, but rather it is based on physiology.<sup>89</sup>

Sarcosine can also be produced through the breakdown of DMG. Both sarcosine and dimethylglycine have pharmacological actions in the central nervous system.<sup>90</sup>

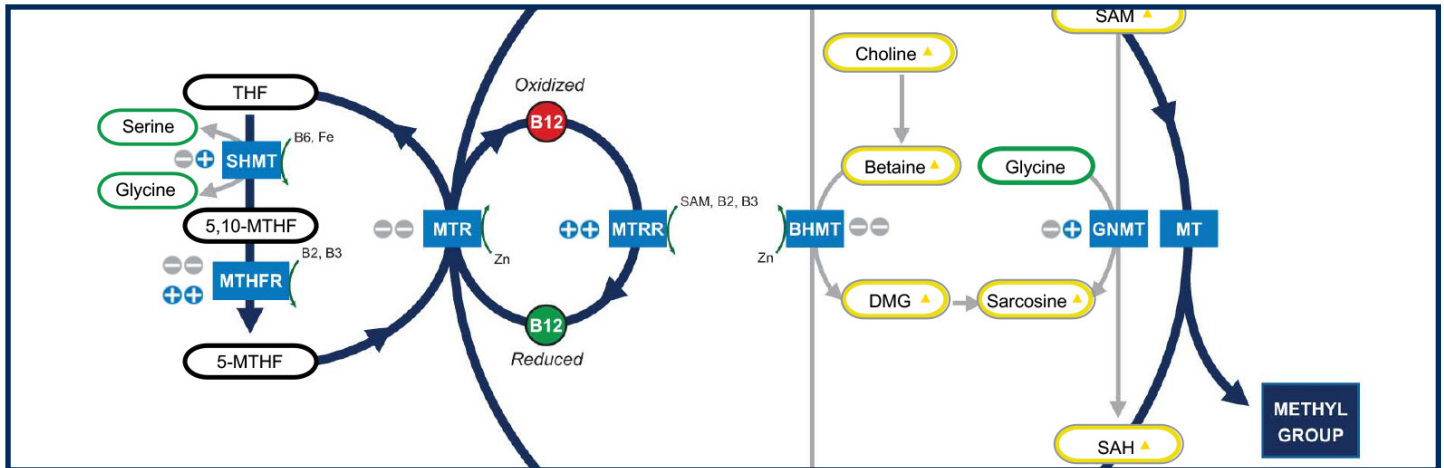
Sarcosine is a natural glycine transport inhibitor in the CNS, enhancing N-methyl-D-aspartate (NMDA) receptors. NMDA synaptic receptors are not only important for basic CNS functions (breathing, motor function), but also learning, memory, and neuroplasticity. Decreased NMDA function results in cognitive defects, and overstimulation causes excitotoxicity.<sup>91</sup> Abnormalities in these receptors are implicated in many diseases and targeted for pharmacologic therapy.<sup>92</sup> Sarcosine has been shown to be a co-agonist for NMDA receptors. For this reason, there are many studies evaluating sarcosine as an adjunct treatment for psychiatric diseases, such as schizophrenia, which is characterized by decreased NMDA function. In addition, using sarcosine to enhance NMDA function can improve depression-like behaviors.<sup>93</sup> Since DMG is essentially sarcosine with an extra methyl group, research shows that they have similar effects.<sup>90</sup>

Some studies have evaluated urinary and serum sarcosine's use as a prostate cancer progression marker; however, the data is mixed.<sup>94-96</sup> These studies are based on nonspecific metabolomic profiling, which followed random metabolite elevation patterns. But, as noted, literature is mixed regarding whether this translates to clinical practice.

Sarcosine has no known toxicity, as evidenced by the lack of phenotypic expression of inborn errors of sarcosine metabolism.<sup>97</sup>

### SARCOSINE

LOW	HIGH
<ul style="list-style-type: none"> <li>Unknown clinical significance</li> </ul>	<ul style="list-style-type: none"> <li>Betaine, DMG supplementation<sup>89,98</sup></li> <li>Dietary intake (i.e. eggs, legumes, nuts, and meats) and environmental sources (i.e. toothpaste, creams, and soaps)<sup>88</sup></li> <li>Folate deficiency (increases use of betaine backup pathway and needed as cofactor in the mitochondrial enzyme sarcosine dehydrogenase used to convert sarcosine to glycine)<sup>89</sup></li> <li>SAM-e supplementation<sup>89</sup></li> <li>Consider over-methylation</li> </ul>



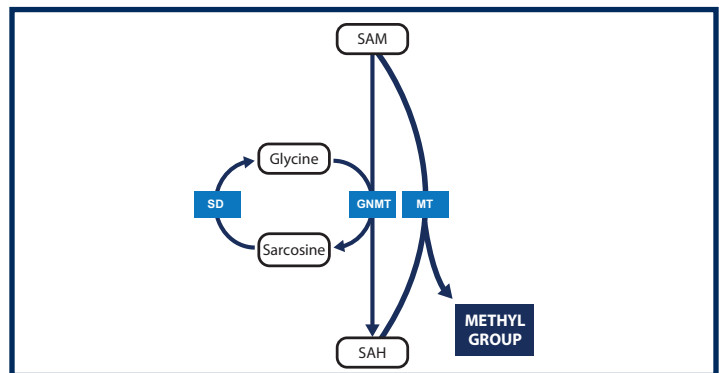
**Glycine** is a nonessential amino acid with many important physiologic functions. It is one of three amino acids that make up glutathione. Glycine’s dietary sources include meat, fish, legumes, and gelatins.

Glycine is a major collagen and elastin component, which are the most abundant proteins in the body. Like taurine, it is an amino acid necessary for bile acid conjugation; therefore, it plays a key role in lipid digestion and absorption.<sup>99</sup> Glycine is the precursor to various important metabolites such as porphyrins, purines, heme, and creatine. It acts both as an inhibitory neurotransmitter in the CNS (via its interaction with strychnine-sensitive glycine receptors), and as an excitatory neurotransmitter on N-methyl-D-aspartate (NMDA) receptors.<sup>93</sup> Glycine has anti-oxidant, anti-inflammatory, immunomodulatory and cytoprotective roles in all tissues.<sup>99</sup>

As mentioned previously, glycine accepts a methyl group from SAM to form sarcosine, using the enzyme glycine-N-methyltransferase (GNMT); therefore, sarcosine is methylated glycine. This conversion functions to control SAM excess, as is seen in excess methyl donor supplementation, SAM elevations due to adiposity/obesity and general over-methylation.<sup>100</sup>

In the folate cycle, glycine and serine are interconverted by the enzyme serine hydroxymethyltransferase (SHMT). Glycine accepts a methyl donor from 5-10 MTHF and becomes serine; therefore, serine is methylated glycine. These methyltransferase reactions and interconversions are readily reversible depending on the needs of the folate cycle to synthesize purines.<sup>47</sup>

Glycine can also be generated from choline, betaine, dimethylglycine, and sarcosine using the sarcosine dehydrogenase enzyme.<sup>2</sup> This reaction is not outlined on the methylation pathway chart.



ameliorate metabolic disorders in patients with obesity, diabetes, cardiovascular disease, ischemia-reperfusion injuries, inflammatory diseases, and cancers.<sup>99</sup> Because of glycine’s excitatory effects on CNS NMDA receptors, research regarding the treatment of psychiatric disorders, such as schizophrenia, using glycine transport antagonists have shown great promise.<sup>93</sup>

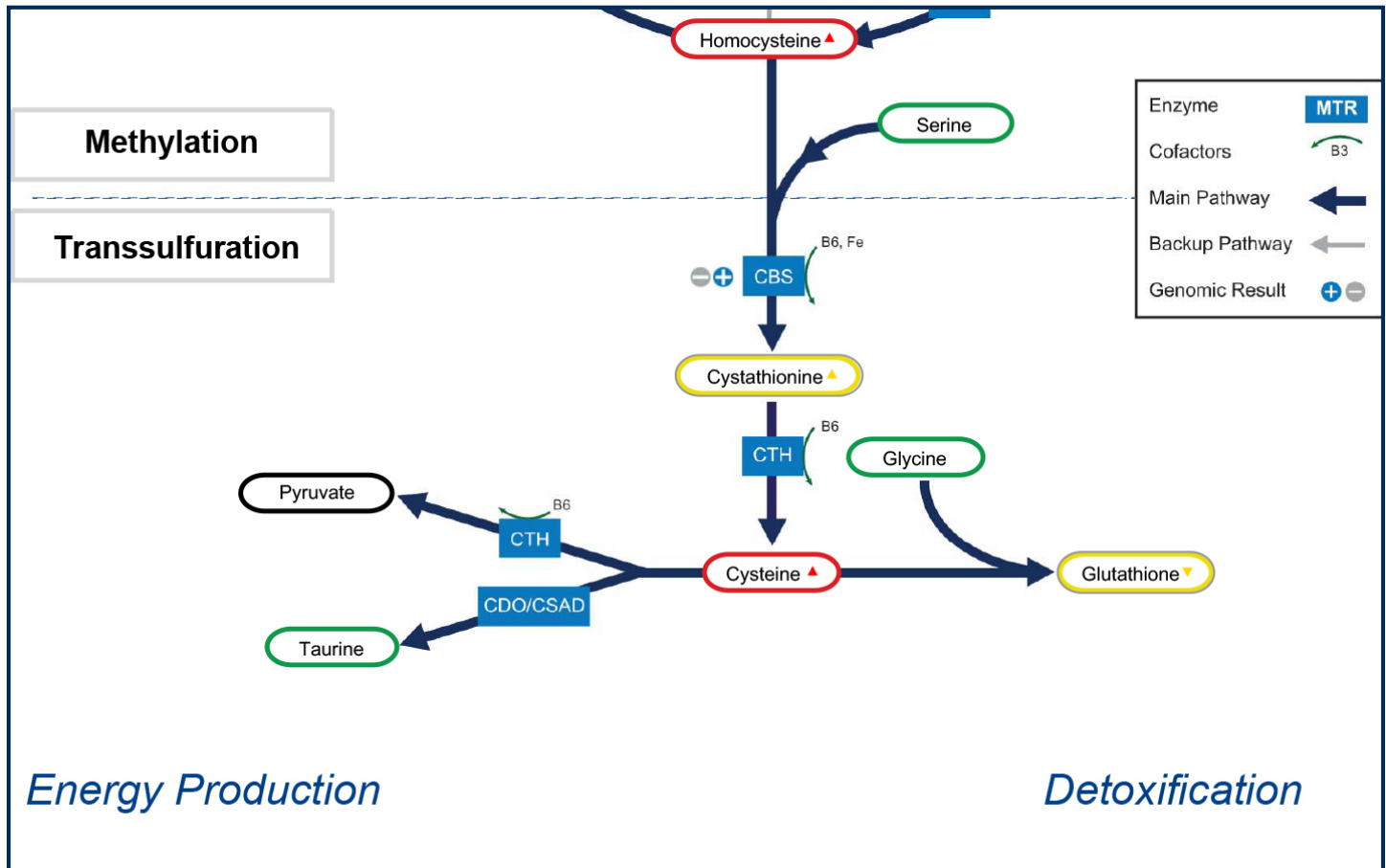
Oral glycine can boost tissue levels of glutathione, especially with concurrent NAC and/or lipoic acid. Because glutathione levels decline during the aging process, supplementing with glycine can impact elderly patients with low protein intake.<sup>101</sup>

GLYCINE LOW	HIGH
<ul style="list-style-type: none"> <li>Decreased intake</li> <li>Potentially over-methylation, excessive methyl supplementation</li> </ul>	<ul style="list-style-type: none"> <li>Dietary intake (i.e. meat, fish, legumes, and gelatins)<sup>99</sup></li> <li>Supplementation</li> <li>GNMT SNP or cofactor deficiency<sup>102</sup></li> <li>SHMT SNP or cofactor deficiency (vitamin B<sub>6</sub>, iron)<sup>47</sup></li> </ul>



## TRANSULFURATION METABOLITES

Transsulfuration is a cellular biochemical pathway which connects methylation, energy production, and glutathione biosynthesis. Transsulfuration occurs when a sulfur group is transferred to various molecules which ultimately ends with glutathione synthesis. This process is upregulated when needed such as when the body is under increased oxidative stress. Alternatively, transsulfuration will contribute to energy production when glutathione is not in high demand.



## Cystathionine

Transsulfuration is the main route for irreversible homocysteine disposal, and begins by replacing a serine hydroxyl group with a sulfhydryl group to form cystathionine, using the cystathionine  $\beta$ -synthase enzyme (CBS). This reaction requires nutrient cofactors such as vitamin B<sub>6</sub> and iron.

**Cystathionine** is a dipeptide which is then converted to cysteine using the enzyme cystathionine gamma-lyase (CTH). Currently, there is no known source or physiologic function for cystathionine other than serving as a transsulfuration intermediate. Some literature suggests that cystathionine may exert protection against endoplasmic reticulum stress-induced tissue damage and cell death, but studies are sparse.<sup>103</sup>

Because cystathionine is an intermediate of the transsulfuration pathway, elevation of this biomarker may indicate a backup of the transsulfuration pathway. Conversion of cystathionine to glutathione requires necessary cofactors, such as vitamin B<sub>6</sub>, zinc, glycine, and magnesium. Therefore, transient elevations of this metabolite may indicate increased need for these cofactors.

## Cysteine

**Cysteine** is a nonessential sulfur-containing amino acid. It is obtained from the diet and is also endogenously made from cystathionine. Dietary cysteine sources include poultry, eggs, beef, and whole grains.<sup>106</sup>

Cysteine is an important glutathione component. Recent studies provide convincing data to support the view that cysteine is the limiting amino acid for glutathione synthesis in humans.<sup>107</sup> This conversion requires the enzyme glutathione synthetase (GSS). Cysteine can alternatively be converted to taurine (another amino acid) and the organic acid pyruvate, which are used in the mitochondrial citric acid cycle and/or excreted in the urine.<sup>108</sup>

Cysteine metabolism can assist in the production of either glutathione or taurine. Lower plasma cysteine levels favor its utilization in glutathione formation during oxidative stress, given the importance of glutathione. Conversely, high levels of cysteine in the absence of oxidative stress favor its metabolism towards pyruvate and taurine.<sup>109</sup>

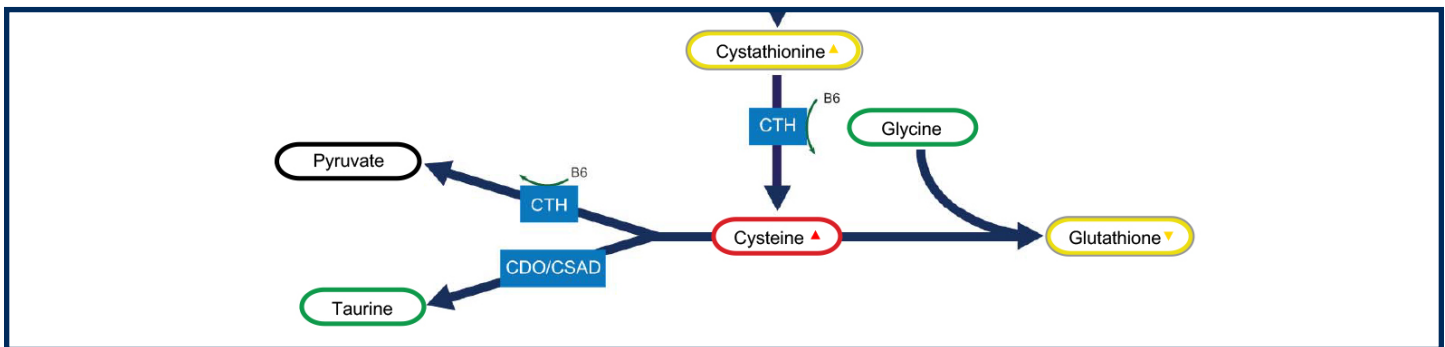
### CYSTATHIONINE

LOW	HIGH
<ul style="list-style-type: none"> <li>Vitamin B<sub>6</sub> cofactor need for the CBS enzyme<sup>80</sup></li> </ul>	<ul style="list-style-type: none"> <li>CBS SNP<sup>78,79</sup></li> <li>Elevated SAM which directly upregulates CBS enzyme<sup>17</sup></li> <li>DMG, Betaine supplementation<sup>17</sup></li> <li>High oxidative stress or inflammation<sup>104</sup></li> <li>Vitamin B<sub>6</sub> cofactor need for CTH enzyme<sup>105</sup></li> </ul>

### CYSTEINE

LOW	HIGH
<ul style="list-style-type: none"> <li>Vitamin B<sub>6</sub> cofactor need for the CBS and CTH enzymes<sup>80</sup></li> </ul>	<ul style="list-style-type: none"> <li>CBS SNP<sup>78,79</sup></li> <li>Elevated SAM which directly upregulates CBS enzyme<sup>17</sup></li> <li>High oxidative stress or inflammation<sup>104</sup></li> <li>Zinc cofactor need for GSS enzyme</li> </ul>

## Taurine



**Taurine** differs from other amino acids because a sulfur group replaces the carboxyl group of what would be the non-essential amino acid,  $\beta$ -alanine. It takes part in biochemical reactions and is not fully incorporated into proteins. In most tissues, it remains a free amino acid. Taurine's highest concentration is in muscle, platelets, and the central nervous system. Taurine is mainly obtained via dietary sources (dairy, shellfish, turkey, energy drinks), but can also come from sulfur amino acid metabolism (methionine and cysteine).<sup>110,111</sup> It has been proposed that taurine acts as an antioxidant, intracellular osmolyte, membrane stabilizer, and a neurotransmitter.<sup>112</sup>

In the CNS, taurine is second only to glutamate in abundance. Taurine is extensively involved in neurological activities, (calming neural excitability, cerebellar functional maintenance, and motor behavior modulation), through interaction with dopaminergic, adrenergic, serotonergic, and cholinergic receptors and through glutamate.<sup>112,113</sup>

In cardiovascular disease, taurine's benefits are multifactorial. Because taurine's main physiologic role is in bile acid conjugation in the liver, it has been demonstrated that taurine is capable of reducing plasma LDL, total lipid concentration, and visceral fat in diabetic, obese patients.<sup>113</sup> Taurine has been shown to be a protector of endothelial structure and function after exposure to inflammatory cells, their mediators, or other chemicals.<sup>113</sup> Taurine is thought to be involved in cell volume regulation and intracellular free calcium concentration modulation. Because of these effects, experimental evidence shows promise for taurine therapy in preventing cardiac damage during bypass surgery, heart transplantation and myocardial infarction. Moreover, severe taurine extravasation from cardiomyocytes during an ischemia-reperfusion insult may increase ventricular remodeling and heart failure risk.<sup>114</sup>

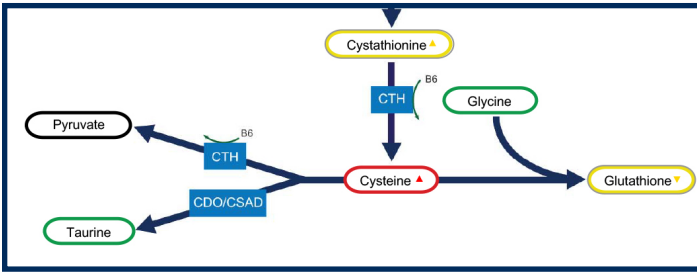
Recent work has revealed taurine's action in the retina as a photoreceptor cell promoter.<sup>4</sup> The human fetus has no ability to synthesize taurine. Taurine is found in breast milk, but it is also routinely added to infant formulas.<sup>4</sup>

Although taurine is very beneficial, it is often unnecessary to supplement. Dietary intake and sulfur amino metabolism are usually more than adequate to meet the body's needs. Newborns, patients with restricted diets, or patients with various diseases may be depleted in taurine and benefit from supplementation.

### TAURINE

LOW	HIGH
<ul style="list-style-type: none"> <li>• High oxidative stress<sup>109</sup></li> <li>• Elevated GSH requirement<sup>109</sup></li> <li>• Poor dietary intake, malabsorption/ maldigestion<sup>115</sup></li> </ul>	<ul style="list-style-type: none"> <li>• Dietary intake (i.e. energy drinks, dairy, shellfish, and turkey)<sup>111,115</sup></li> <li>• CBS SNP in absence of oxidative stress or inflammation, or adequate GSH levels<sup>116</sup></li> </ul>

## Glutathione (GSH)

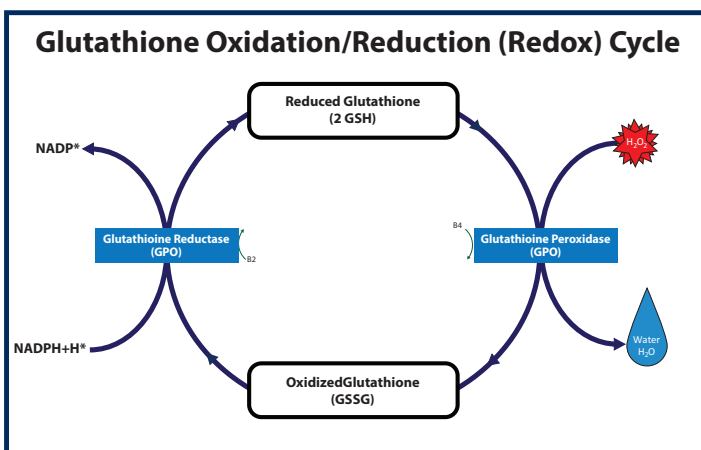


**Glutathione (GSH)** is a tripeptide comprised of three amino acids (cysteine, glycine, and glutamic acid). Glutathione is the body's most potent intracellular antioxidant. It exists intracellularly in either an oxidized or reduced state.

GSH acts as an antioxidant, free radical scavenger, and detoxifying agent. Excessive formation of reactive oxygen species (ROS), including hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), is toxic to the cell. Hence, the metabolism of these free radicals are critical, and they are tightly controlled.<sup>117</sup>

Availability of the amino acid cysteine is known to be rate-limiting for glutathione synthesis, and it is widely known that cysteine supplementation (in the form of N-acetylcysteine) can increase GSH levels. Recent literature has also suggested that adequate glycine levels are critical in maintaining glutathione levels, and glycine availability may modulate the production of glutathione.<sup>101</sup>

Glutathione's antioxidant function is accomplished largely by GSH peroxidase-catalyzed reactions. GSH neutralizes hydrogen peroxide and lipid peroxide, resulting in water and alcohol. By accepting a free radical electron, GSH is then oxidized. GSH continues to donate and accept electrons, forming a redox cycle to counter free radicals.<sup>118</sup>



Glutathione is also involved in phase II detoxification by conjugating hormones, toxins, and xenobiotics to make them water soluble for excretion.<sup>119</sup>

Nutritional deficiencies in GSH precursors can result in low GSH. Genetic polymorphisms (SNPs) can also affect the production of GSH. Without adequate GSH levels, oxidative stress and free radicals contribute to aging and disease. GSH deficiency and problems with GSH synthesis have been implicated in many diseases such as cancer, neuropsychiatric dysfunction, Parkinson's disease, HIV, liver disease, and cystic fibrosis.<sup>117</sup>

There is a transient increase in GSH plasma levels after intravenous supplementation and oral GSH ingestion, which may be useful under oxidative stress to counter free radical damage.<sup>120</sup> There are many foods which contain significant GSH sources including, but not limited to, asparagus, avocado, watermelon, ham, and pork.<sup>121</sup>

GSH inclusion in oral over-the-counter supplements may be of limited value, since the reduced state will not be maintained when exposed to normal atmospheric conditions and room temperature. Liposomal GSH has been shown to be an excellent alternative to raise GSH levels.<sup>122,123</sup> Additionally, increasing amino acid dietary intake and supplementation with sulfur-containing products (N-acetyl cysteine) and foods (cruciferous vegetables, such as asparagus, broccoli, cauliflower, Brussels sprouts) will support GSH synthesis.<sup>117</sup> The latter requires a healthy gastrointestinal ecosystem.

### GLUTATHIONE (GSH)

LOW	HIGH
<ul style="list-style-type: none"> <li>Decreased Hcy</li> <li>Decreased GSH precursors (cysteine, glycine, glutamine)<sup>104</sup></li> <li>Increased phase two conjugation in detox pathway<sup>104</sup></li> </ul>	<ul style="list-style-type: none"> <li>Dietary intake of amino acid precursors (cysteine, glycine, glutamine)</li> <li>Supplementation</li> <li>CBS SNP in the presence of oxidative stress or inflammation<sup>78,79</sup></li> <li>Inability to convert oxidized to reduced GSH (making excess due to inability to reduce it for use)<sup>118</sup></li> </ul>

## SAM/SAH Ratio

The **SAM/SAH ratio** is resistant to fluctuations in independent nutrients, given the interconnectedness of the pathways. SAM remains fairly stable due to de novo synthesis and the liver's tight control over SAM levels. Therefore, it is more likely that SAH variability will lead to alterations in the methylation index.<sup>57</sup>

Methylation reactions become compromised with a decreased SAM/SAH ratio and as SAH levels become elevated.<sup>63</sup> SAM/SAH ratio abnormalities should be addressed by ensuring adequate dietary methyl donor levels (i.e. methionine, SAM-e) and nutritional cofactors required for clearance of SAH and homocysteine (i.e. B<sub>6</sub>, B<sub>12</sub>, and folate).

It is important to note that while the Methylation Panel does not directly measure DNA methylation, the SAM/SAH ratio has been shown to correlate with DNA methylation. Many environmental and lifestyle factors have been shown to affect DNA methylation which can turn on/off gene expression. Exposure to tobacco smoke, arsenic, benzene, radiation, lead, cadmium, nickel and asbestos are all tied to DNA methylation defects.<sup>124,125</sup> Obesity, lack of physical activity, and shift work have also been shown to alter DNA and histone methylation, and thereby gene expression.<sup>125</sup> Chronic alcohol use is another pertinent lifestyle factor to evaluate as it can cause malabsorption of key B-vitamins necessary for methylation.<sup>125-127</sup>

- Elevated SAM/SAH Ratio (SAM elevation)
  - Potential Over-methylation: Review genetic markers, such as BHMT, that could lead to higher SAM levels<sup>128</sup>
    - » Review nutritional supplements that could be contributing to high SAM<sup>23</sup>
  - High Protein Intake<sup>37</sup>
  - High BMI: Elevated SAM has been correlated to higher BMI, however the degree of impact to SAH is unknown.
- Low SAM/SAH Ratio (SAH elevation)
  - Check SAH and Homocysteine Levels: If elevated, consider nutritional methylation support:
    - » Vitamins B<sub>12</sub>
    - » Vitamin B<sub>6</sub><sup>129,130</sup>
    - » Folate
    - » Zinc
    - » Other methylation support may be considered as well, especially with low levels of betaine and choline.

## Methylation Balance Ratio

The clinical utility of the **Methylation Balance Ratio** is that it represents a potential way to detect methylation imbalance prior to alterations in the SAM/SAH ratio. As previously mentioned, the SAM/SAH ratio is resistant to fluctuations due to multiple feedback mechanisms and backup pathways. Therefore, measuring the metabolites of these backup pathways may provide earlier insight into subtle fluctuations in methylation balance. This approach is still novel and is based solely on biochemical pathway analysis. However, early Genova data analysis of this biomarker has demonstrated its ability to distinguish a healthy cohort from an unqualified cohort. Genova will continue to conduct ongoing research on this novel biomarker's clinical application.

- More Methylation Group Donors
  - Potential Over-methylation: Review genetic markers, such as BHMT, that could lead to higher SAM levels
    - » Review nutritional supplements that could be contributing to high SAM, methionine, betaine or serine
  - High Protein Intake
- More Unmethylated Metabolites
  - Check SAH and Homocysteine Levels: If elevated, consider nutritional methylation support:
    - » Vitamin B<sub>12</sub>
    - » Vitamin B<sub>6</sub><sup>129,130</sup>
    - » Folate
    - » Zinc
    - » Other methylation support may be considered as well, especially with low levels of betaine and choline.



## Met/Sulf Balance Ratio

There is a relative balance that exists between the methylation and transsulfuration pathways. This is to ensure that adequate levels of glutathione are produced to counteract oxidative stress as well as ensure that an adequate amount of SAM is made for methylation reactions. In the presence of increased oxidative stress, more homocysteine is used for glutathione production because oxidative stress induces enzymes in the transsulfuration pathway. Also, transsulfuration is induced by higher levels of intracellular SAM. Therefore, poor methylation status (Methylation Balance Ratio and SAM/SAH Ratio) may be a risk for low glutathione production. As with the Methylation Balance ratio, the Met/Sulf ratio is based solely on biochemical pathway analysis. However, early Genova data analysis of this biomarker has demonstrated its ability to distinguish a healthy cohort from an unqualified cohort. Genova will continue to conduct ongoing research on this novel biomarker's clinical application.

- Balanced Shifted to Methylation
  - Look at Methylation Balance and SAM/SAH ratios; consider methylation support to correct low methylation status
  - Vitamin B<sub>6</sub>, especially if high homocysteine (consider adding vitamin B<sub>3</sub> if high SAH levels)
  - Evaluate whether high protein intake or high BMI could be contributing to elevated methylation metabolites
- Balance Shifted to Transsulfuration
  - May be a consequence of high levels of methyl products or potential “over-methylation”
    - » Look at SAM/SAH ratio and Methylation Balance Ratio
- May be due to adequate methylation status and increased oxidative stress
  - Reduce exposure to oxidative stress
  - Support with antioxidants

	LOW	HIGH
SAM/SAH	<ul style="list-style-type: none"> <li>• Poor methylation status</li> <li>• Consider 5-MTHF, vitamin B<sub>12</sub>, or SAM-e</li> <li>• Consider Mg, Zn support</li> <li>• Consider betaine supplementation</li> </ul>	<ul style="list-style-type: none"> <li>• Consequence of high BMI</li> <li>• Consequence of high protein intake</li> <li>• Potential over-methylation (evaluate supplementation)</li> </ul>
Methylation Balance Ratio	<ul style="list-style-type: none"> <li>• Poor methylation status likely</li> <li>• Consider 5-MTHF, B<sub>12</sub></li> <li>• Consider B<sub>6</sub>, Mg, iron</li> <li>• (With high Met/Sulf) Evaluate oxidative stress and consider antioxidant support</li> </ul>	<ul style="list-style-type: none"> <li>• Consequence of high BMI</li> <li>• Consequence of high protein intake</li> <li>• Potential over-methylation (evaluate supplementation)</li> <li>• MTR or BHMT SNPs</li> </ul>
Met/Sulf Ratio	<ul style="list-style-type: none"> <li>• Potential high oxidative stress</li> <li>• Ensure adequate methylation status</li> <li>• Decrease oxidative stress exposure</li> </ul>	<ul style="list-style-type: none"> <li>• Consider B<sub>6</sub>, Mg, iron</li> <li>• Consider antioxidant support</li> <li>• CBS SNP</li> <li>• May be consequence of high SAM/SAH</li> <li>• Evaluate oxidative stress and consider antioxidant support</li> <li>• Consider glutathione if needed</li> </ul>

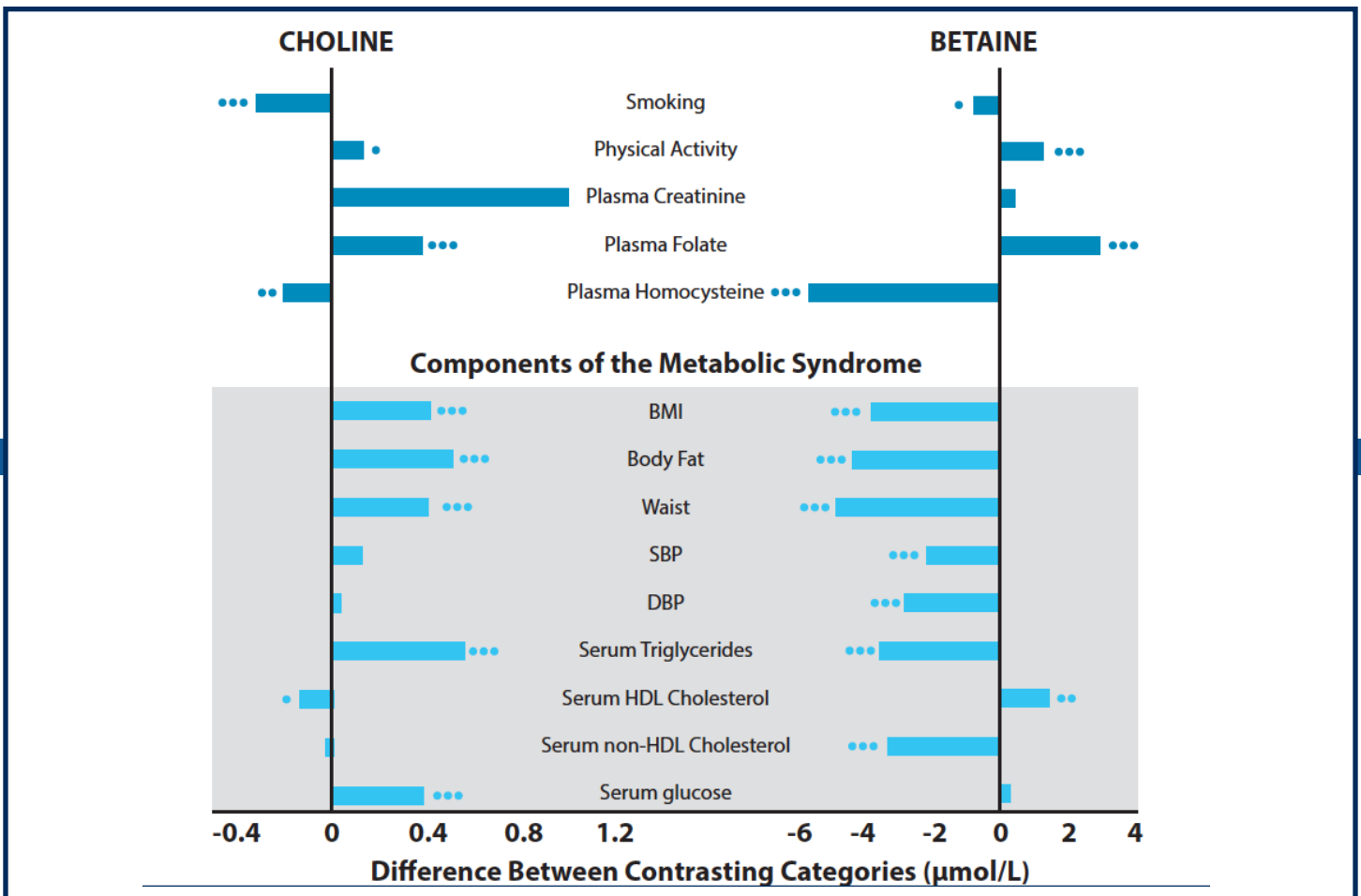
## Betaine/Choline Ratio

As outlined previously, betaine and choline can be obtained from the diet or synthesized de novo. Choline is a lipotrope, in that it helps to mobilize fat from the liver.<sup>131</sup> Phosphatidylcholine, a derivative, is required for the production of hepatic very-low-density lipoprotein and the mobilization of fat from the liver. Therefore, choline deficiency can result in fatty liver and liver abnormalities.<sup>132</sup>

In the methylation cycle, choline is oxidized to form betaine to donate methyl groups for many pathways. One of these pathways is the synthesis of phosphatidylcholine using the enzyme phosphatidylethanolamine N-methyltransferase (PEMT).<sup>132</sup> With this, the interplay between betaine and choline has shown clinical significance.

In spite of helping to mobilize fat from the liver, elevated plasma choline is positively associated with elevated triglycerides, glucose, BMI, body fat, and waist circumference. Plasma betaine is negatively associated with the majority of these risk factors.<sup>133</sup>

Literature has demonstrated that a low betaine/choline ratio is associated with many of the signature features of metabolic syndrome such as dyslipidemia, dysglycemia, and increased BMI. Therefore, it may serve as a useful independent biomarker of worsening metabolic function. Furthermore, supplementation with betaine may assist in correcting some of these metabolic concerns and should be considered with low plasma betaine levels.



# Folate Cycle

As mentioned previously, in the methylation cycle, homocysteine is remethylated to form methionine, and ultimately SAM. The main route of Hcy remethylation requires methylated folate (5-MTHF). The folate pathway is responsible for creating 5-MTHF from dietary folate. 5-MTHF is the predominant circulating folate in serum. Other non-circulating forms of folate precursors (i.e. 5,10-MTHF) are mainly found intracellularly, rather than in serum. These other forms of folate are vital in DNA synthesis and replication. Therefore, a delicate balance exists between the folate cycle and methylation pathway.<sup>134,135</sup>

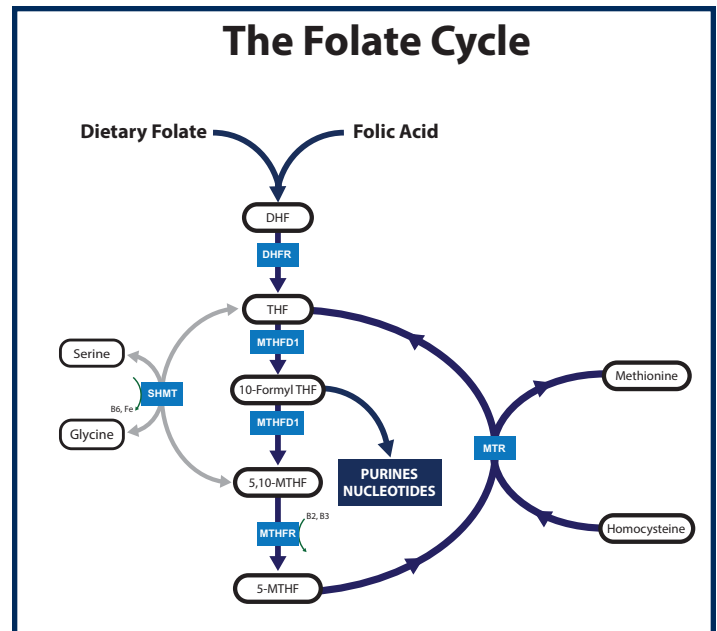
## Folate, Folic Acid, and Folinic Acid

**Folate (vitamin B<sub>9</sub>)** is a water soluble vitamin found in many foods, such as green leafy vegetables, asparagus, root vegetables, and beets.<sup>136</sup> Folate is also produced by certain commensal gut bacteria (*Bifidobacterium*).<sup>137</sup> Folic acid is the synthetic form often found in supplements and many fortified foods. Folate and folic acid must be converted to tetrahydrofolate to be used in biochemical reactions. Folinic acid is a reduced form of folate (also known as leucovorin calcium).<sup>138</sup>

In the diet, folates exist as polyglutamates. These need to be enzymatically converted into folate monoglutamates in the jejunal mucosa in order to be absorbed. In comparison, Folic acid used in supplements is two-fold better absorbed than dietary folates in the intestine.<sup>139</sup> Neither folate nor folic acid are biologically active until they are converted in the liver to dihydrofolate (DHF). DHF is then converted to tetrahydrofolate (THF) using the enzyme dihydrofolate reductase (DHFR) which can finally enter the folate cycle. Folinic acid can readily enter the folate cycle without being reduced by DHFR.<sup>140</sup>

DHFR has relatively low and variable activity in the liver which sets up the possibility that high intake of folic acid may result in elevated levels of unmetabolized folic acid in the blood stream. Recently, some clinicians have expressed concerns around the use of folic acid in dietary supplements and fortification. The concern is not around an excess of folates per se, but that high levels of folic acid could result in high levels of unmetabolized folic acid in the blood. Because the DHFR enzyme has low activity in humans, the competition for receptor binding may be potentially relevant.<sup>141</sup>

The debate whether unmetabolized folic acids levels contribute to disease is ongoing and literature is varied.<sup>142-146</sup> For example, a recent study aimed at finding an association between colorectal adenoma and UMFA's actually found that the increased risk was more likely related to higher levels of plasma methylated folate and not due to unmetabolized folic acid.<sup>142</sup> Another study



showed that increased UMFA's may reduce natural killer cell cytotoxicity, however the study was limited and was not associated with health outcomes.<sup>143</sup> It is clear, that with the program of folic acid fortification and increased folic acid supplementation in the United States, UMFA detection has become nearly ubiquitous and the health impact of UMFA needs to be further investigated.<sup>145</sup>

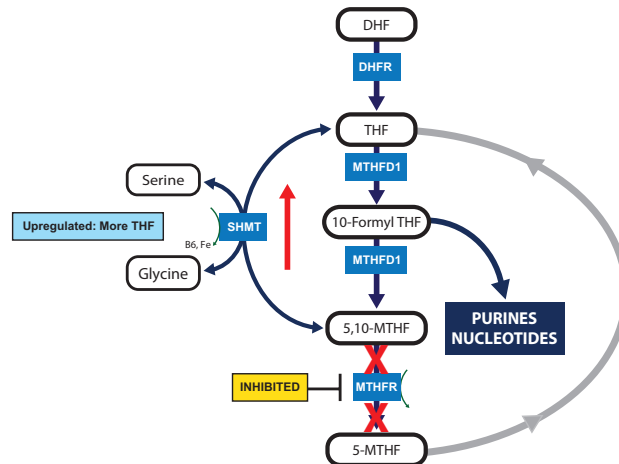
The eventual conversion of THF to 5,10 methylenetetrahydrofolate (5,10, MTHF) is a crucial step in the folate cycle. A methyl group from serine is transferred to THF using the SHMT enzyme to form 5,10 MTHF and glycine.<sup>46</sup> This reaction is reversible and favored for nucleotide synthesis based on methylation demand. Methylation demand takes precedence over purine synthesis.<sup>135</sup>

5,10-MTHF is a branch point in the cycle. The direction it takes depends on methylation demand. Methyltetrahydrofolate reductase (MTHFR) is an enzyme which irreversibly converts 5,10 MTHF to 5-MTHF, the main form of circulating folate in the plasma.<sup>80</sup> This enzymatic conversion essentially commits folate to the methylation cycle for SAM synthesis, at the expense

of purine and pyrimidine synthesis. With this, SAM can act as a negative feedback to MTHFR by slowing 5-MTHF creation and allowing folate to be used for DNA replication and repair. Cell culture studies have shown that under low folate conditions, the methionine cycle is metabolically favored.<sup>147</sup> SAM production ultimately takes priority.

Using SAM (along with vitamins B<sub>2</sub> and B<sub>3</sub>) the enzyme methionine synthase reductase (MTRR) is able to reduce vitamin B<sub>12</sub> and make it available as a cofactor for the MTR enzyme.

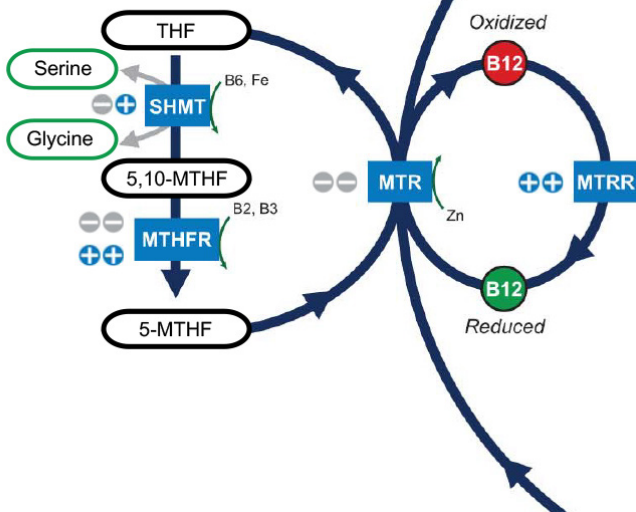
## Higher SAM Levels Favors Nucleotide Synthesis



To remethylate Hcy to methionine, 5-MTHF donates a methyl group, with vitamin B<sub>12</sub> and zinc as cofactors, using the enzyme methionine synthase (MTR). When 5-MTHF loses its methyl group, it then becomes tetrahydrofolate (THF). In order for this reaction to take place, vitamin B<sub>12</sub> must be in its reduced, not oxidized form.

If there is direct or functional vitamin B<sub>12</sub> deficiency, MTR cannot convert Hcy to methionine, nor can it return 5-MTHF to THF for use in the folate cycle. Multiple factors can lead to functional B<sub>12</sub> deficiency, such as genetic polymorphism and alcohol intake, therefore clinicians should be aware that supplementation with methylated folate may be ineffective for patients with poor B<sub>12</sub> status.

## Folate Cycle



# DNA Methylation

There is extensive research regarding methylation defects and clinical outcomes. Many outcome studies involve DNA hypo- and hyper-methylation.<sup>56,148-150</sup> Genova's Methylation Panel does not directly evaluate DNA methylation, however, abnormalities in the methylation cycle and the SAM:SAH ratio are comparable, and tightly linked to DNA methylation regulation and epigenetics.<sup>22</sup>

DNA methylation is a mechanism used by cells to turn genes on or off (epigenetics). DNA methylation refers to the addition of a methyl group to the DNA strand itself, often to the fifth carbon atom on a cytosine ring. DNA methylation is catalyzed by enzymes called DNA methyltransferases (DNMTs), and takes place along a gene's promotor region referred to as "CpG Islands."

When the CpG island is methylated, it is associated with repressing transcription (turning off the gene), whereas an un-methylated CpG island is associated with transcriptional activity (turning on the gene).<sup>3,151</sup>



# Single Nucleotide Polymorphisms (SNPs)

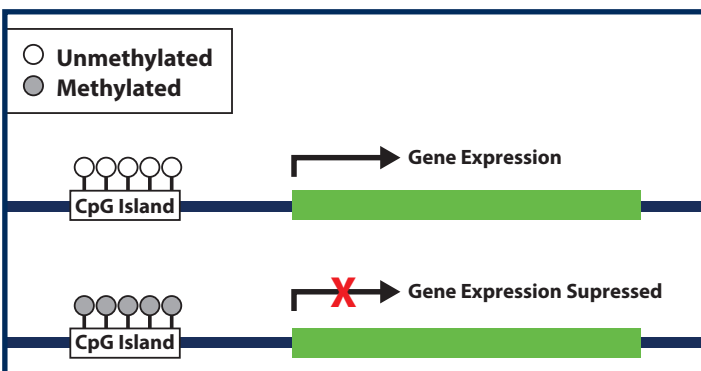
The Methylation Panel evaluates SNPs in several enzymes involved in the methylation cycle, folate pathway, and transsulfuration. Any SNP's clinical implication depends on the patient population studied, and often shows significant ethnic variance.

Genotypic SNPs do not always translate to phenotypic expression. SNPs represent genetic predispositions which are not always clinically manifested.

On the Methylation Panel, if an enzymatic SNP is present, abnormal biomarker results near that enzyme may reflect an enzymatic SNP abnormality. However, due to the interconnected nature of the methylation, folate, and transsulfuration cycles, abnormalities can be due to many factors unrelated to the SNP.

As with many SNPs, there is a degree of ethnic variation in prevalence. For each SNP, data is provided regarding SNP prevalence based on population studies using the latest information from NCBI dbSNP. The population categories are abbreviated as:

Population frequency data is from 1000 GENOMES project as sourced from NCBI dbSNP. The population categories are listed below:
<b>EUR (European):</b> Americans with Northern and Western European Ancestry, Toscani, Finnish, British, Spanish
<b>EAS (East Asian):</b> Han Chinese (Beijing), Japanese (Tokyo), Southern Han Chinese, Chinese Dai, Kinh (Vietnam)
<b>AFR (African):</b> Nigerian, Kenyan, Gambian, Mendi (Sierra Leone), African Americans, African Caribbeans
<b>AMR (Ad Mixed American):</b> Mexican, Puerto Rican, Colombian, Peruvian
<b>SAS (South Asian):</b> Americans of Gujarati descent (India), Punjabi (Pakistan), Bengali (Bangladesh), Sri Lankan/Indian in UK

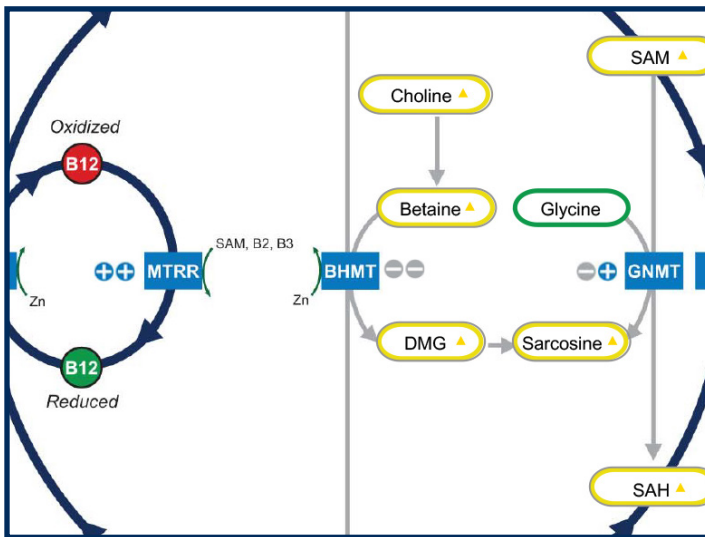




## Betaine-homocysteine S-methyltransferase (BHMT)

rs: 3733890 G742A

### Upregulation with Hetero/Homozygous SNP



The G742A SNP increases BHMT enzyme activity. Due to the SNPs effects on the enzyme kinetics, there is an increased affinity for betaine to be used in Hcy remethylation. Upregulation of BHMT may lead to lower levels of homocysteine as well as less dependency on folate and vitamin B<sub>12</sub> as methyl donors.

Because this BHMT polymorphism results in increased activity, research suggests that this SNP is protective against many of the clinical conditions related to elevated homocysteine and folate deficiency.

This polymorphism has been associated with reduced all-cause mortality in breast cancer and decreased birth defect risk.<sup>86,87,152,153</sup>

**The BHMT enzyme** is critically important to the methylation cycle because it directly converts Hcy back into methionine, using betaine as a methyl donor. When there is low folate availability for Hcy transmethylation, the zinc-dependent BHMT pathway actively provides the necessary methyl donors.<sup>85</sup> In this way, BHMT acts as a backup pathway in folate deficiency to ensure SAM production and methylation capacity.<sup>17</sup> The end-product of BHMT methylation is DMG, which is mostly converted to sarcosine. Therefore, elevated DMG and sarcosine levels are sometimes used as indicators of BHMT activation and betaine utilization.

Elevated SAM acts to inhibit this enzyme in a negative feedback mechanism to spare betaine and choline for other metabolic pathways.<sup>17</sup>

### G742A

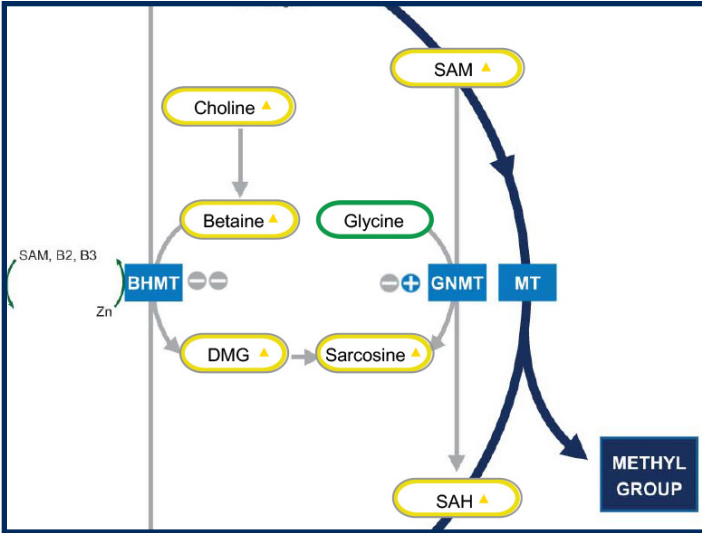
Population	Wild Wild (-/-)	Wild Variant (-/+)	Variant Variant (+/+)
EUR	48%	41%	11%
EAS	52%	41%	7%
AFR	55%	41%	4%
AMR	32%	52%	16%
SAS	52%	43%	5%

## Catechol-O-methyltransferase (COMT)

rs: 4680

V158M

Downregulation with Hetero/Homozygous SNP



COMT polymorphisms have been implicated in mood disorders such as anxiety, panic disorder, and eating disorders.<sup>156,157</sup> Other mood disturbances have also been documented in the literature such as aggressiveness, anger, alcoholism, and severity of bipolar disorder.<sup>158-160</sup> Fibromyalgia and migraines have also been associated with COMT SNPs.<sup>161,162</sup>

COMT is critical for phase 2 detoxification of estrogens. Therefore, decreased activity via genomic polymorphism has been implicated in breast cancer risk, particularly in women with prolonged estrogen exposure,<sup>163,164</sup> or in women with low folate and high homocysteine.<sup>165</sup> COMT SNPs have been shown to correlate with higher estrogen levels in patients on estrogen replacement therapy.<sup>166</sup>

**Catechol-O-methyltransferase (COMT)** is a key enzyme involved in the deactivation of catechol compounds, including catecholamines, catechol estrogens, catechol drugs such as L-DOPA, and various chemicals and toxins such as aryl hydrocarbons.

COMT SNPs result in decreased enzyme activity. Individuals with COMT SNPs may have an increased risk of inefficient methylation of catecholamines, estrogens, and toxins.<sup>154,155</sup>

The most common genotype of COMT in most populations is heterozygous (+/-). Individuals with a homozygous positive (+/+) genotype have a 3-4 fold reduction in COMT activity, leading to a significant predisposition for poor methylation.

### V185M

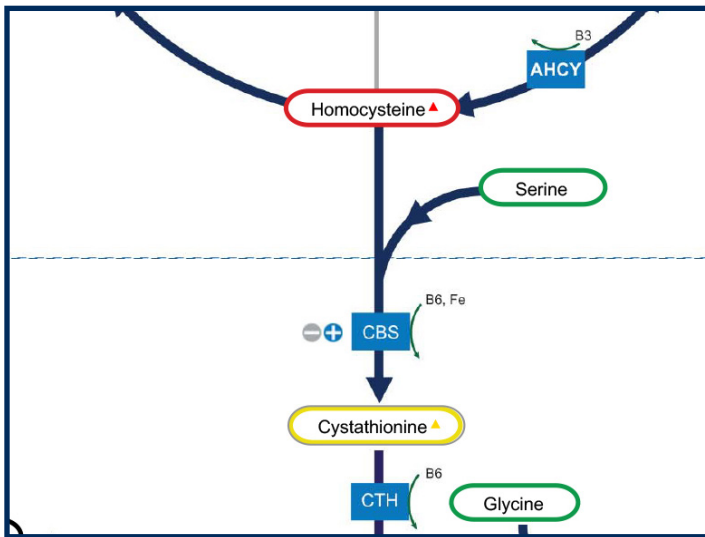
Population	Wild Wild (-/-)	Wild Variant (-/+)	Variant Variant (+/+)
EUR	22%	53%	25%
EAS	43%	47%	10%
AFR	46%	45%	9%
AMR	54%	37%	8%
SAS	37%	41%	22%

## Cystathionine beta-synthase (CBS)

rs234706

C699T

### Upregulation with Hetero/Homozygous SNP



**Cystathionine beta-synthase (CBS)** is the enzyme responsible for Hcy's irreversible conversion to cystathionine. In the general population, when excess methionine and SAM are present, CBS will be upregulated leading to an increase in transsulfuration. This causes an increase in homocysteine conversion to cystathionine. On the other hand, when methionine and SAM are scarce, CBS will be less active and methionine synthase will be upregulated. With this, more homocysteine will be remethylated to replenish methionine and S-adenosylmethionine levels.<sup>62</sup>

A SNP in this enzyme further upregulates CBS favoring transsulfuration. Genetically, there are nearly 200 known SNPs for CBS. The C699T SNP is relatively common.

Most literature reflects that the CBS C699T SNP upregulates Hcy metabolism, thereby lowering Hcy levels after a methionine load.<sup>78,79</sup> Homozygous C699T individuals are also more responsive to folate supplementation used to lower Hcy levels.<sup>167</sup> One study did show that the SNP slowed down the CBS enzyme in Chinese populations, causing Hcy elevation and increased breast cancer risk.<sup>168</sup> The discrepancy in these findings highlight the fact that different populations have variable prevalence and clinical relevance of individual SNPs.

Despite the lack of agreement on enzyme activity, multiple studies demonstrate clinical associations with the C699T polymorphism. These include:

- Reduced risk of lymphoma<sup>169</sup>
- Reduced risk of venous disease<sup>170,171</sup>
- Protective effects against deep vein thrombosis<sup>170</sup>
- Decreased risk of coronary artery disease<sup>167</sup>

Ammonia is a major transsulfuration pathway by-product. Theoretically, accelerating this pathway (CBS SNP) can increase ammonia, though urea cycle clearance makes this unlikely. Though often quoted by several methylation experts, there is no literature to support hyperammonemia as a result of upregulation by this CBS SNP.

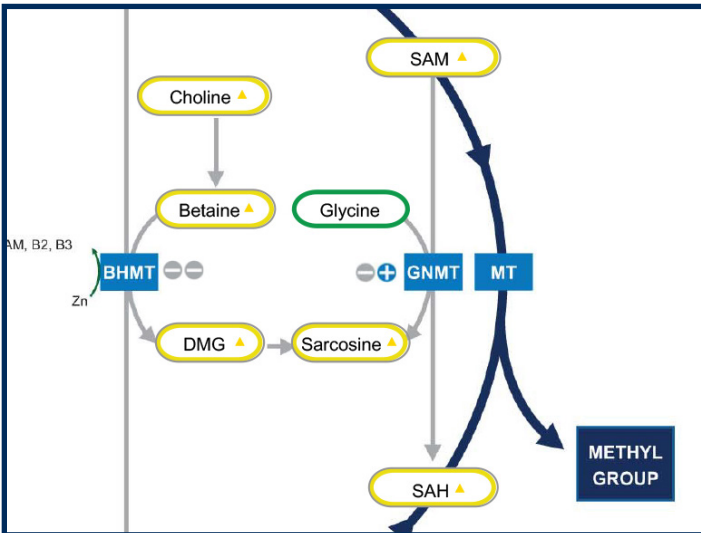
### C699T

Population	Wild Wild (-/-)	Wild Variant (-/+)	Variant Variant (+/+)
EUR	42%	48%	10%
EAS	95%	5%	<1%
AFR	59%	33%	8%
AMR	72%	25%	3%
SAS	44%	46%	10%

## Glycine N-methyltransferase (GNMT)

rs: 10948059 C1289T

### Upregulation with Hetero/Homozygous SNP



**Glycine n-methyltransferase (GNMT)** is an enzyme which plays a very important role in removing excess SAM that is synthesized during periods of elevated methionine and SAM levels. It catalyzes the methyl group transfer from SAM to glycine, ultimately forming sarcosine. This SAM removal process is down-regulated in response to low 5-MTHF and SAM levels.

GNMT is a key regulator in SAM/SAH metabolism, and it regulates genes related to liver detoxification and antioxidation pathways.<sup>172</sup> This may play a role in the increased cancer risk demonstrated in homozygous negative individuals and in animal models.

The GNMT polymorphism does not change Hcy levels at baseline. However, after folate restriction, Hcy levels increase. This is most evident in patients with the MTHFR C667T genotype. Folate supplementation can mitigate the effects of this SNP.<sup>100</sup>

In European populations, GNMT was found to be a tumor susceptibility gene for prostate cancer.<sup>172</sup> It was recently reported that sarcosine, which is regulated by GNMT, increased markedly in metastatic prostate cancer, though that literature is mixed.<sup>94-96</sup>

### C1289T

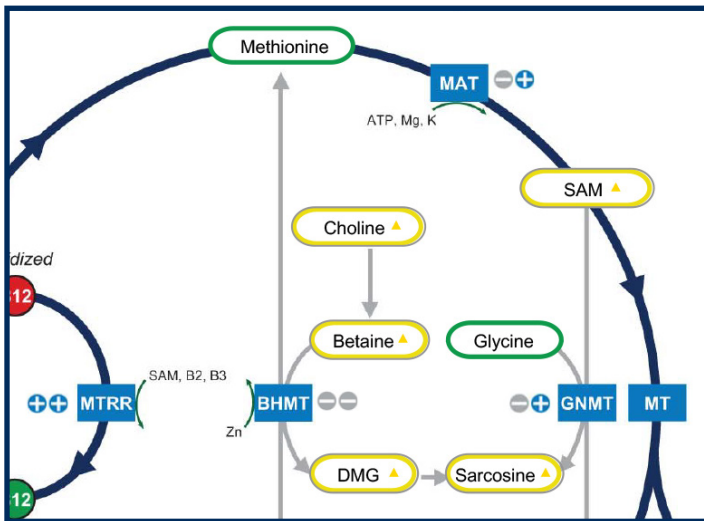
Population	Wild Wild (-/-)	Wild Variant (-/+)	Variant Variant (+/+)
EUR	29%	47%	24%
EAS	70%	28%	2%
AFR	23%	43%	34%
AMR	50%	44%	6%
SAS	36%	47%	17%

## Methionine adenosyltransferase 1A (MAT1A)

rs3851059

D18777A

### Downregulation with Hetero/Homozygous SNP



**Methionine adenosyltransferase (MAT)** is the enzyme that catalyzes methionine's conversion to SAM. The MAT enzyme is highly conserved and regulated. The D18777A MAT1A SNP downregulates the enzyme's activity, decreasing SAM production.

Although the D18777A MAT 1A SNP is fairly prevalent, there is very little literature regarding MAT SNPs and clinical outcomes. Some studies do show an impact in elevating Hcy levels, resulting in cardiovascular disease.<sup>173</sup> Additionally, this MAT SNP has been implicated in the inability to lower Hcy levels with vitamin B<sub>6</sub> supplementation.<sup>173</sup>

Omega-3 fatty acids are cardio-protective and have been shown to decrease Hcy, though literature has been inconsistent. Fish oil supplementation modifies plasma fatty acid profiles and decreases total VLDL triglyceride concentrations. However, there is concern as to whether omega-3 fatty acids increase LDL susceptibility to oxidation in some patients.<sup>174</sup> The relationship between omega-3 fatty acids and plasma Hcy is not yet fully understood. One study looked at enzyme activity and gene expression to explain the disparity. It showed that higher dietary fat intake is associated with significantly higher Hcy levels in patients with the D18777A SNP.<sup>175</sup> This supports a hypothesis that the MAT1A genotype may modulate polyunsaturated fatty acids' regulatory effect on Hcy metabolism.

Another study looked at the association between MAT1A variants as they relate to cardiovascular disease and stroke. The D18777A MAT SNP showed significantly higher stroke rates. The findings suggest that methylation activity impairment, independent of Hcy levels, have an effect on cardiovascular risk.<sup>173</sup>

### D18777A

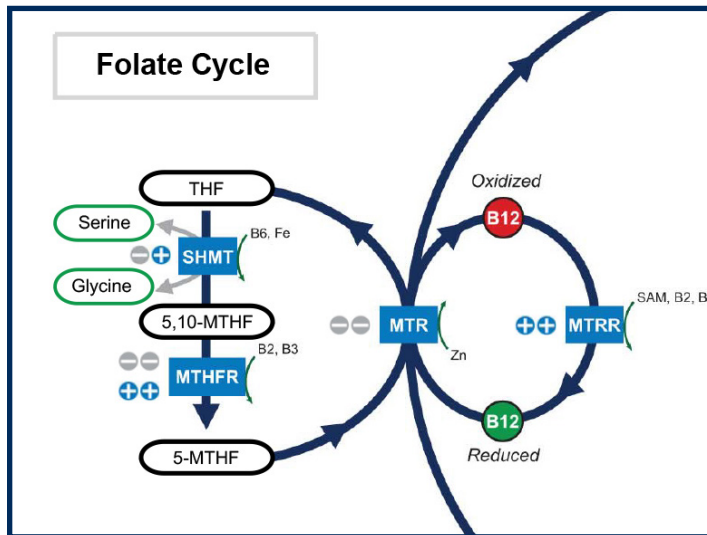
Population	Wild Wild (-/-)	Wild Variant (-/+)	Variant Variant (+/+)
EUR	50%	43%	7%
EAS	36%	48%	16%
AFR	62%	34%	4%
AMR	52%	40%	8%
SAS	42%	45%	13%



## Methionine synthase (MTR)

rs: 180587 A2756G

### Potential Upregulation with Hetero/Homozygous SNP



**Methionine synthase (MTR)** is the enzyme responsible for Hcy recycling back to methionine. This enzymatic conversion requires using 5-MTHF (folate) as a methyl donor, and activated vitamin B<sub>12</sub> (methylcobalamin) as a catalyst.<sup>11</sup> Therefore, adequate Hcy remethylation is dependent on both methylated folate and methylcobalamin.

An additional enzyme, methionine synthase reductase (MTRR), is responsible for maintaining activated (reduced) B<sub>12</sub> levels. Consequently, a unique enzymatic relationship between MTR, MTHFR, and MTRR is essential in Hcy remethylation.

The A2756G polymorphism is the most common MTR SNP discussed in literature.

Most literature suggests that the MTR A2756G polymorphism may increase enzyme activity, which would enhance Hcy's conversion to methionine, leading to lower Hcy levels.<sup>11</sup> There are a few studies which suggest that this SNP results in lower activity causing Hcy elevation and DNA hypomethylation.<sup>176</sup> Variance in these studies may be due to the interconnectedness of MTHFR, MTR, MTRR, and the complexity of epigenetic lifestyle factors. However, in general, it is accepted that this SNP upregulates MTR and lowers Hcy levels.<sup>11</sup>

Studies looking at this MTR SNP's clinical associations across various populations reveal congenital birth defects such as spina bifida, cleft lip/palette, and cardiac defects.<sup>177-179</sup> There are a large number of epidemiologic studies on MTR polymorphisms' role in cancer risk, though much of it is still controversial.<sup>176,180,181</sup>

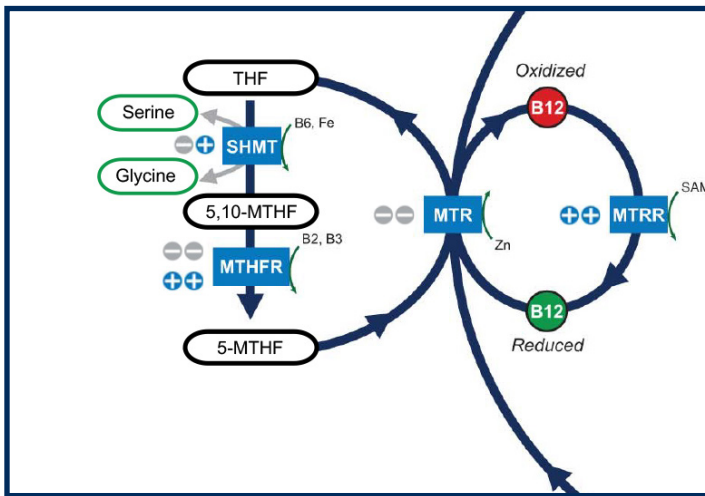
### A2756G

Population	Wild Wild (-/-)	Wild Variant (-/+)	Variant Variant (+/+)
EUR	69%	30%	1%
EAS	72%	25%	3%
AFR	47%	42%	11%
AMR	65%	33%	2%
SAS	42%	47%	11%

## Methionine synthase reductase (MTRR)

rs: 1801394 A66G

### Downregulation with Hetero/Homozygous SNP



As mentioned previously, **methionine synthase reductase (MTRR)** is a critical enzyme in Hcy's remethylation back to methionine. MTRR functions to maintain MTR activity by reducing oxidized cobalamin to be used as a cofactor.<sup>182</sup>

SNPs in MTRR result in decreased enzyme activity, and therefore a decreased capacity to recycle oxidized cobalamin (vitamin B<sub>12</sub>). This decreased enzyme activity can affect methylation capacity by limiting the amount of active B<sub>12</sub> available for homocysteine conversion.<sup>182</sup>

The most commonly studied polymorphism is the A66G mutation. The A66G SNP has been shown to result in elevations of Hcy, independent of serum folate, vitamin B<sub>12</sub>, and vitamin B<sub>6</sub> levels.<sup>81</sup>

The A66G polymorphism is the most common studied MTRR SNP. It has been associated with numerous clinical conditions, such as various cancers, birth defects, metabolic syndrome, mood disorder, and elevated homocysteine.<sup>183-185</sup> The A66G polymorphism has also been shown to correlate with global DNA hypomethylation, which is a direct marker for methylation impairment.

Population studies show SNP prevalence may depend on ethnicity. This should be taken into account when addressing clinical associations, since clinical significance varies between populations. For example, a meta-analysis conducted on an Iranian population demonstrated no association between MTRR SNPs and colorectal cancer, which was in contradiction to associations found in a Czech Republic population, which did demonstrate risk.<sup>186,187</sup>

In spite of the population variances, SNPs in MTRR have been implicated in many diseases as mentioned previously.

### A66G

Population	Wild Wild (-/-)	Wild Variant (-/+)	Variant Variant (+/+)
EUR	38%	34%	28%
EAS	54%	37%	9%
AFR	59%	36%	5%
AMR	26%	57%	17%
SAS	-	-	-

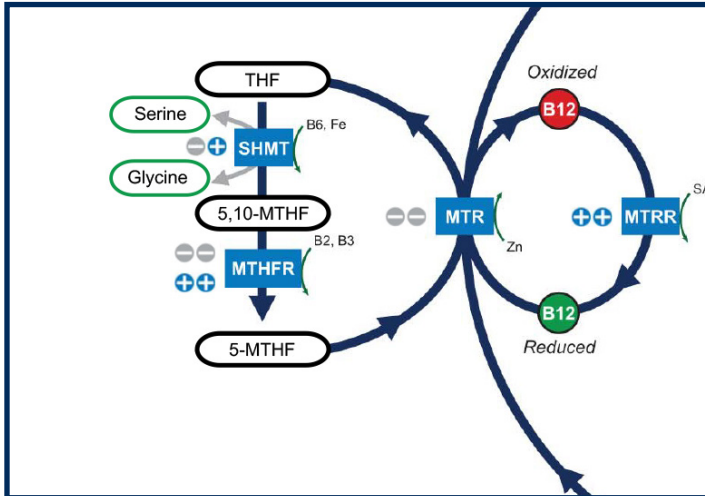
## Methylenetetrahydrofolate reductase (MTHFR)

rs: 1801133 C677T

Downregulation with Hetero/Homozygous SNP

rs: 1801131 A1298C

Downregulation only with Homozygous SNP



**Methylenetetrahydrofolate reductase (MTHFR)** is a key regulatory enzyme in folate and homocysteine metabolism. It converts 5,10-methylenetetrahydrofolate to 5, methyltetrahydrofolate (5-MTHF), which donates a methyl group for homocysteine conversion back to methionine. Therefore, the enzyme commits 5,10-MTHF to homocysteine metabolism and methionine formation, instead of nucleotide synthesis.<sup>188</sup>

MTHFR polymorphisms result in reduced enzyme activity, thus a decreased ability to remethylate Hcy back to methionine.

Because methylated folate (5-MTHF) is also an important coenzyme in DNA synthesis, a SNP in the MTHFR enzyme may be clinically significant in cancer development.<sup>188</sup> MTHFR SNPs do not guarantee methylation defects, only a genetic predisposition. Gene expression is highly dependent on epigenetic influences such as diet, smoking, and alcohol consumption.

Two common genetic variations have received the most attention in scientific literature: MTHFR C677T and A1289C

## C677T

The C677T polymorphism downregulates enzymatic activity, resulting in a predisposition to lower serum folate (5-MTHF) and higher homocysteine.<sup>189</sup>

Genetic Polymorphic Variation	MTHFR 677 C/C --	MTHFR 677 C/T - +	MTHFR 677 T/T ++
Physiologic Action	Baseline "normal" MTHFR activity <sup>190</sup>	Moderately decreased MTHFR activity (30-40%) <sup>190</sup>	Substantially decreased MTHFR activity (60-70%) <sup>190</sup>

MTHFR's inability to catalyze the conversion of 5,10-MTHF to 5-MTHF leads to a rise in Hcy levels. Furthermore, homozygous C677T subjects have higher Hcy levels, while heterozygous subjects have Hcy levels only mildly raised compared to controls.<sup>191</sup>

MTHFR C677T SNPs have been associated with many disease processes including:

- Cardiovascular disease<sup>192-200</sup>
- Depression<sup>201</sup> and schizophrenia<sup>202</sup>
- Increased risk of birth defects<sup>203-206</sup> and Down's syndrome
- Psoriasis
- Diabetes
- Parkinson's disease
- Various cancers<sup>191</sup>

This SNP's prevalence is highly variable, depending on the ethnicity and location.

## C677T

Population	Wild Wild (-/-)	Wild Variant (-/+)	Variant Variant (+/+)
EUR	47%	44%	9%
EAS	37%	47%	16%
AFR	81%	19%	<1%
AMR	32%	52%	16%
SAS	68%	30%	2%

## A1298C

The A1298C SNP mutation does not significantly affect folate or homocysteine levels.<sup>207</sup> However, a combined heterozygosity for both 677T and 1298C mutations does result in significant plasma homocysteine elevation.<sup>207,208</sup>

Genetic Polymorphic Variation	MTHFR 1298 A/A --	MTHFR 1298 A/C - +	MTHFR 1298 C/C ++
Physiologic Action	Baseline "normal" MTHFR activity <sup>190</sup>	Baseline "normal" MTHFR activity unless 677CT genotype also present (40-50% reduced activity) <sup>190</sup>	Moderately decreased MTHFR activity (30-40%) <sup>190</sup>

MTHFR A1298C SNPs have been associated with many disease processes including:

- Cardiovascular disease<sup>209-211</sup>
- Male infertility<sup>212,213</sup>
- Increased risk of birth defects<sup>206</sup>
- Certain cancer types<sup>214-216</sup>

MTHFR SNPs (both C677T and A1298C) have been associated with many disease processes including cardiovascular disease, Down's syndrome, infertility, neural tube defects, psoriasis, diabetes, Parkinson's disease, and many cancer types.<sup>191</sup> The main factor in disease association appears to be Hcy elevation.<sup>191</sup> Supplementation with methylated folate and folate-rich foods may help lower Hcy and mitigate risk.<sup>217</sup>

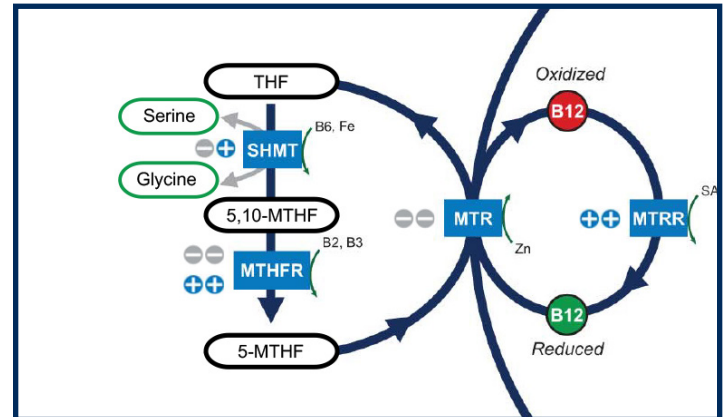
## A1298C

Population	Wild Wild (-/-)	Wild Variant (-/+)	Variant Variant (+/+)
EUR	43%	45%	12%
EAS	63%	33%	4%
AFR	78%	21%	1%
AMR	62%	34%	4%
SAS	39%	44%	17%

## Serine hydroxymethyltransferase 1 (SHMT1)

rs: 1979277 C1420T

### Downregulation with Hetero/Homozygous SNP



**Serine hydroxymethyltransferase (SHMT)** is an enzyme that is critical to the folate cycle. It regulates the availability of 5,10-MTHF to act as a substrate for MTHFR. The enzyme uses serine as a methyl donor; serine then becomes glycine. This enzyme is bidirectional. It constantly balances the needs for methylation substrates versus tetrahydrofolate (THF), which is vital for nucleotide synthesis.<sup>218</sup>

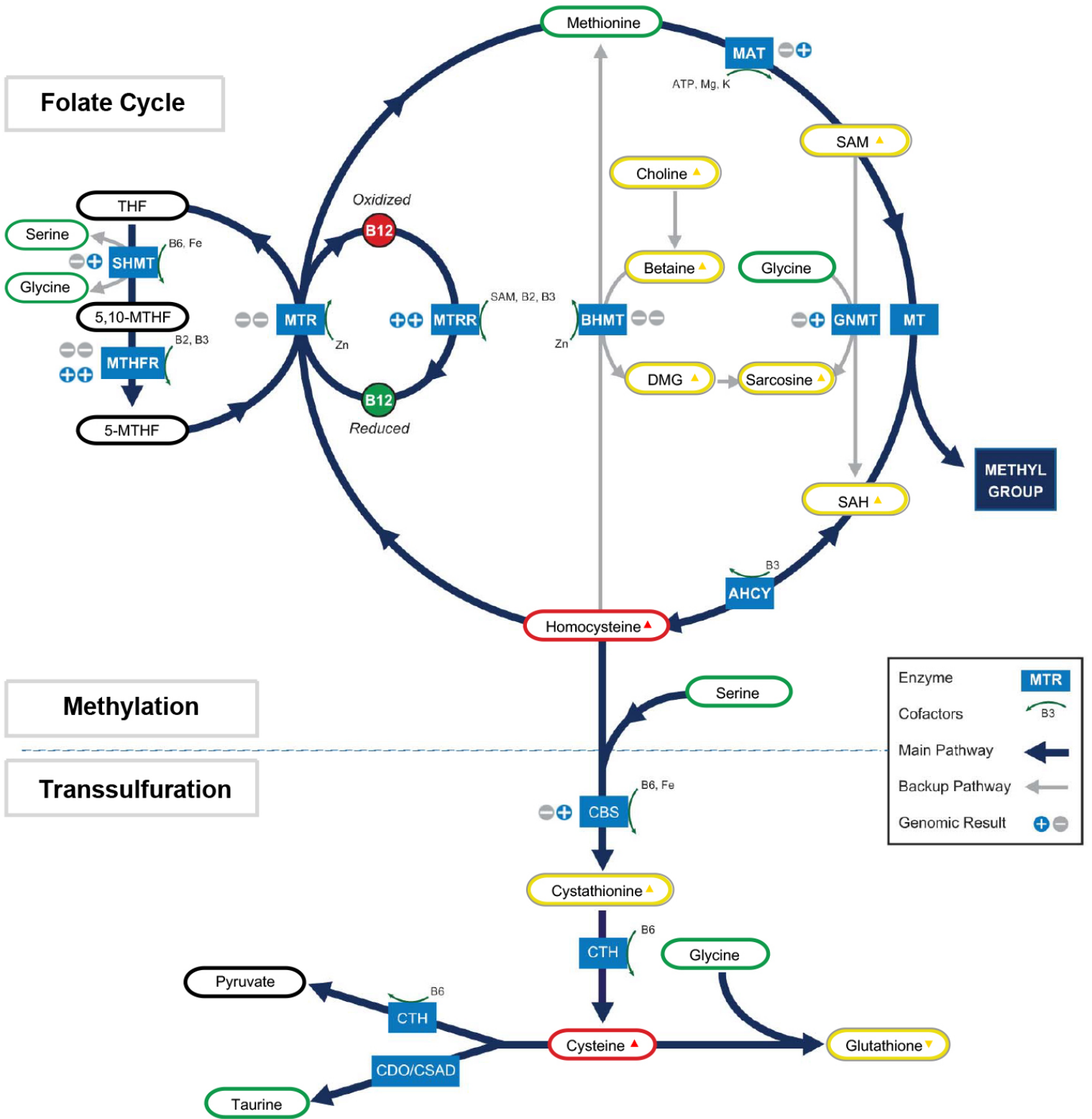
SHMT is a vitamin B<sub>6</sub>-dependent enzyme, and gives metabolic priority to nucleotide synthesis over SAM synthesis.<sup>219</sup> This is why it is implicated not only in disturbances in the methylation cycle, but also in cancer.<sup>219</sup>

The C1420T polymorphism in SHMT1 downregulates the enzyme and creates an imbalance within the folate cycle. This adversely affects DNA synthesis, methylation systems, and causes genome instability. It eventually leads to oncogene overexpression and tumor suppressor gene inactivation.<sup>219,220</sup> Additionally, the polymorphism can cause reduced circulating folate (5-MTHF) levels and increased homocysteine.<sup>218</sup>

## C1420T

Population	Wild Wild (-/-)	Wild Variant (-/+)	Variant Variant (+/+)
EUR	45%	43%	12%
EAS	87%	13%	<1%
AFR	33%	47%	20%
AMR	59%	41%	<1%
SAS	-	-	-

# Methylation / Transsulfuration Pathway



Energy Production

Detoxification



# References

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