Introducing Genova's Innovative Methylation Panel

Discussion on Clinical Utility and Case Review

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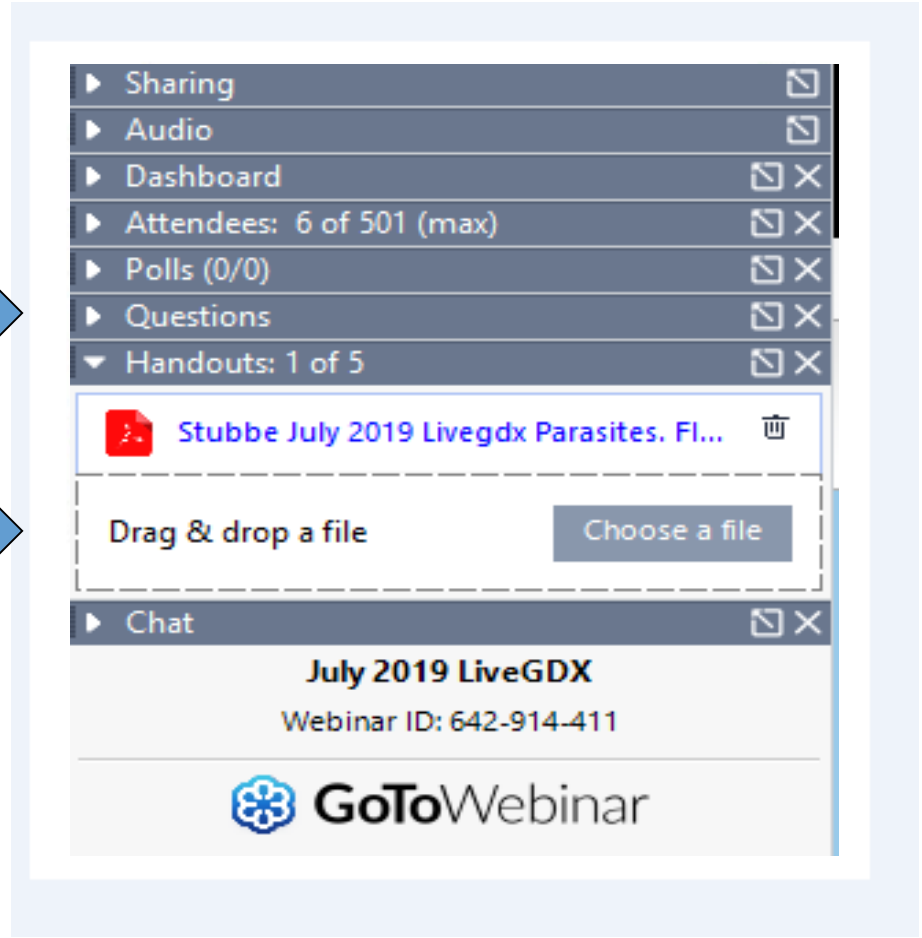
for Genova Diagnostics

Technical Issues & Clinical Questions

Please type any technical issue or clinical question into either the “Chat” or “Questions” boxes, making sure to send them to “Organizer” at any time during the webinar.

We will be compiling your clinical questions and answering as many as we can the final 15 minutes of the webinar.



DISCLAIMER: *Please note that any and all emails provided may be used for follow up correspondence and/or for further communication.*

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for more information and educational resources, including…

LEARN GDX – Brief video modules

LIVE GDX – Previous webinar recordings

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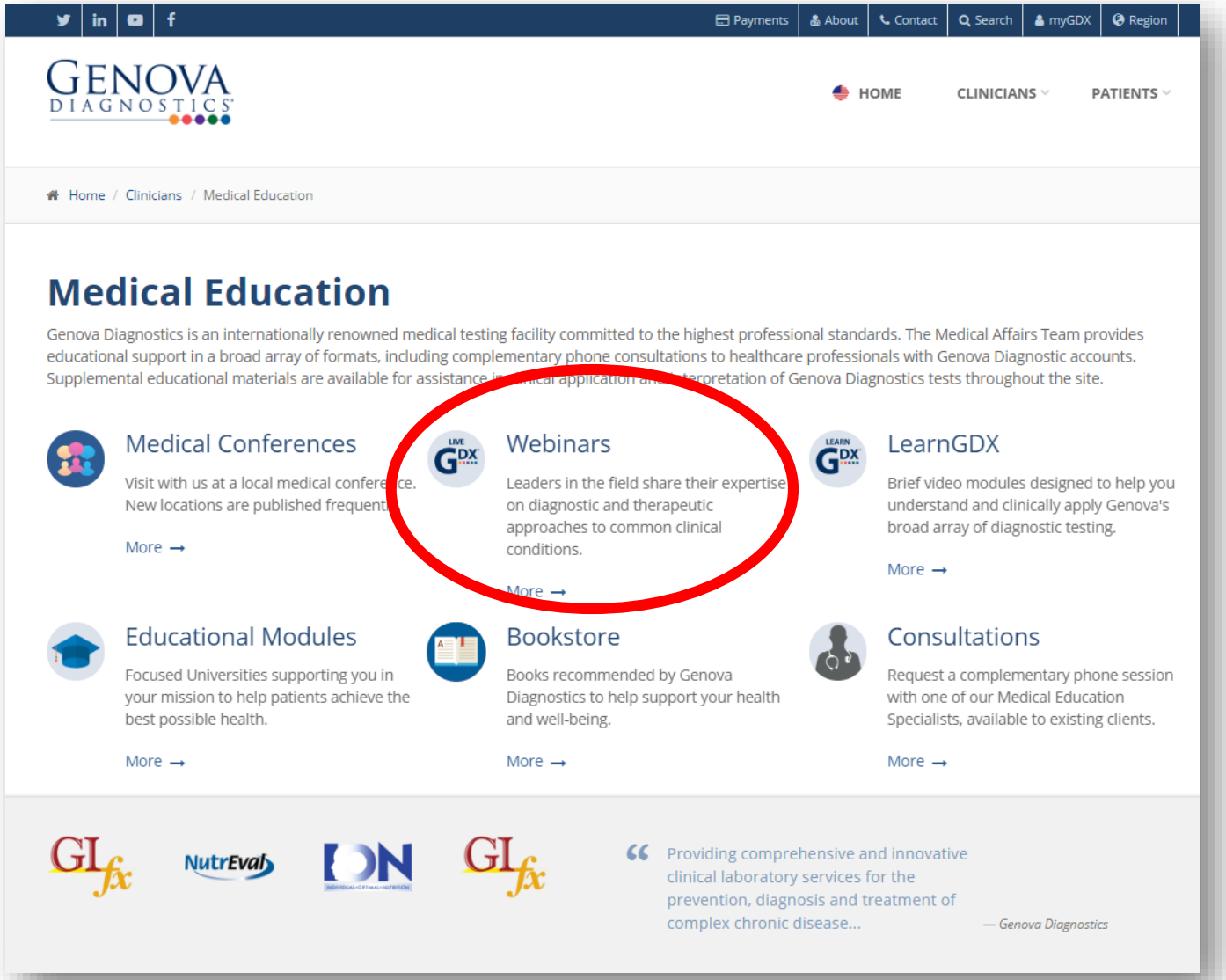
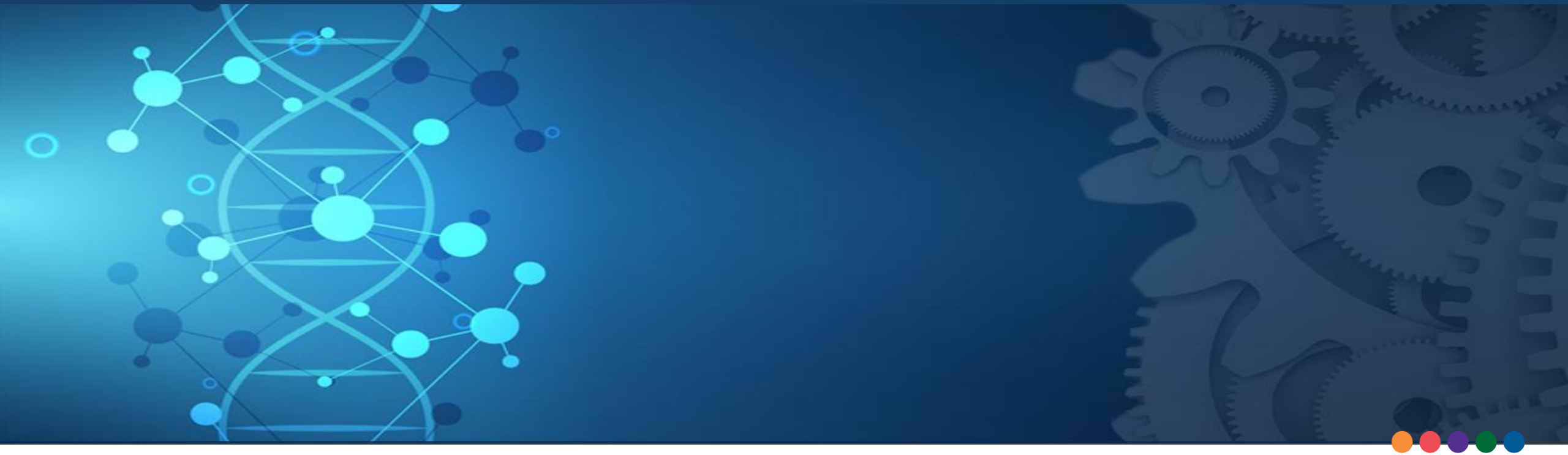




Introducing Genova's Innovative Methylation Panel

Discussion on Clinical Utility and Case Review

Michael Chapman, ND

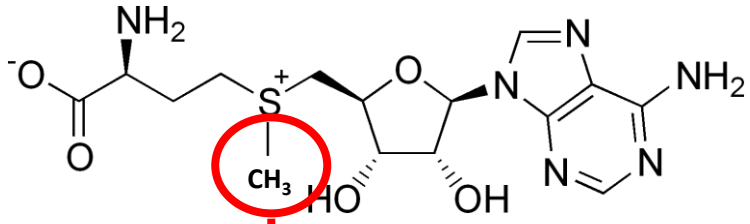
Learning Objectives for this Presentation

- Review the concept of 1-carbon metabolism and methylation reactions
- Discuss the interpretation and application of Genova's Methylation Panel along with case study review
- Apply nutritional and lifestyle therapies as potential therapeutic options for different methylation imbalances

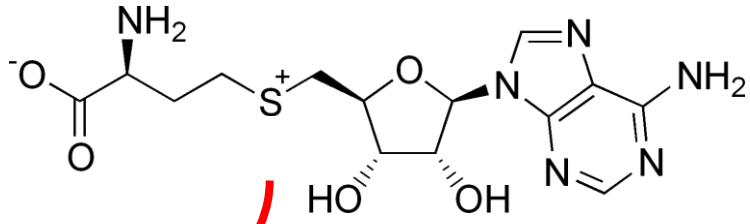
What is Methylation?

- Transfer of single carbon unit from molecule to molecule
  - This is why methylation is also referred to as “1-carbon metabolism”

S-Adenosylmethionine (SAM)

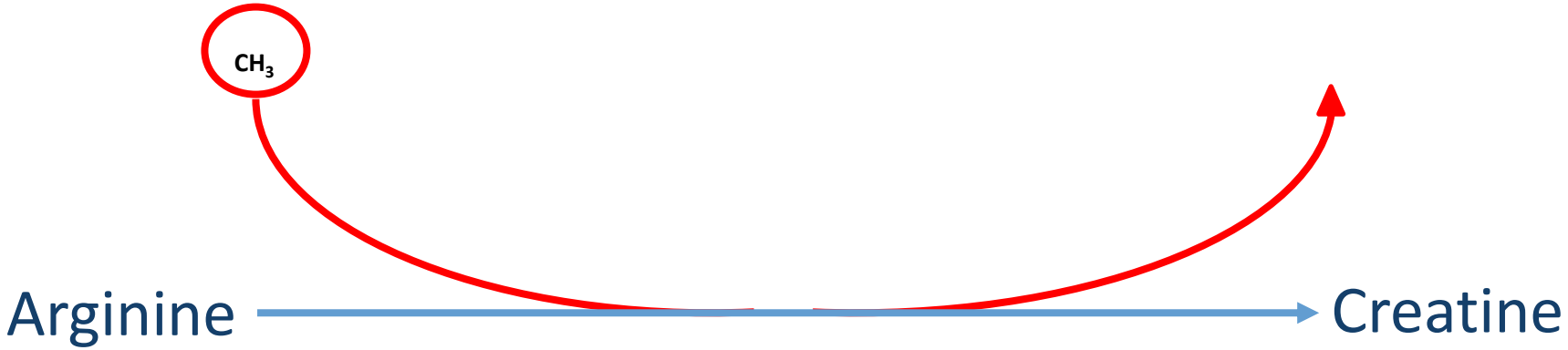


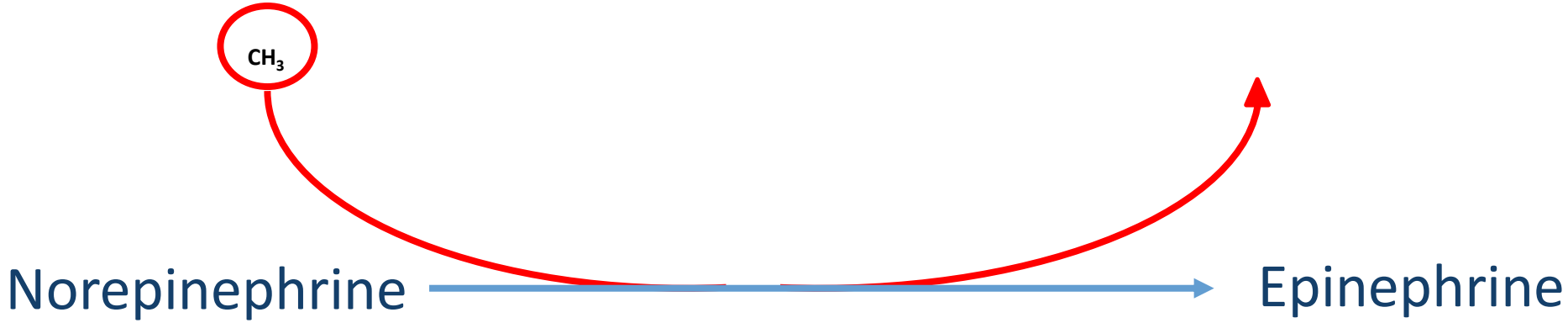
S-Adenosylhomocysteine (SAH)



4-hydroxyestradiol → 4-methoxyestradiol

What is Methylation?





What is Methylation?

- What makes this enzymatic reaction any more or less important than the rest?

- “If you want to know how important a single process is to a system, just look at how often that process is used within the system.”
  - Biochemical Proverbs by Michael Chapman, ND

- It’s not the methylation reaction that is special, but rather how many processes absolutely depend on methylation

What Systems Depend on Methylation?

- Creatine production: skeletal muscle contraction
- DNA and RNA synthesis
- Epigenetic gene regulation
- Hormone regulation and detoxification
- Energy production
- Cell membrane repair
- Lipid metabolism
- Neurotransmitter production
- Nitric oxide production: vascular endothelial function
- Immune function

Moore LD, et al. *Neuropsychopharmacology*. 2013;38(1):23-38.

Brosnan JT, et al. *Acta Biochimica Polonica (English Edition)*. 2004;51:405-14.

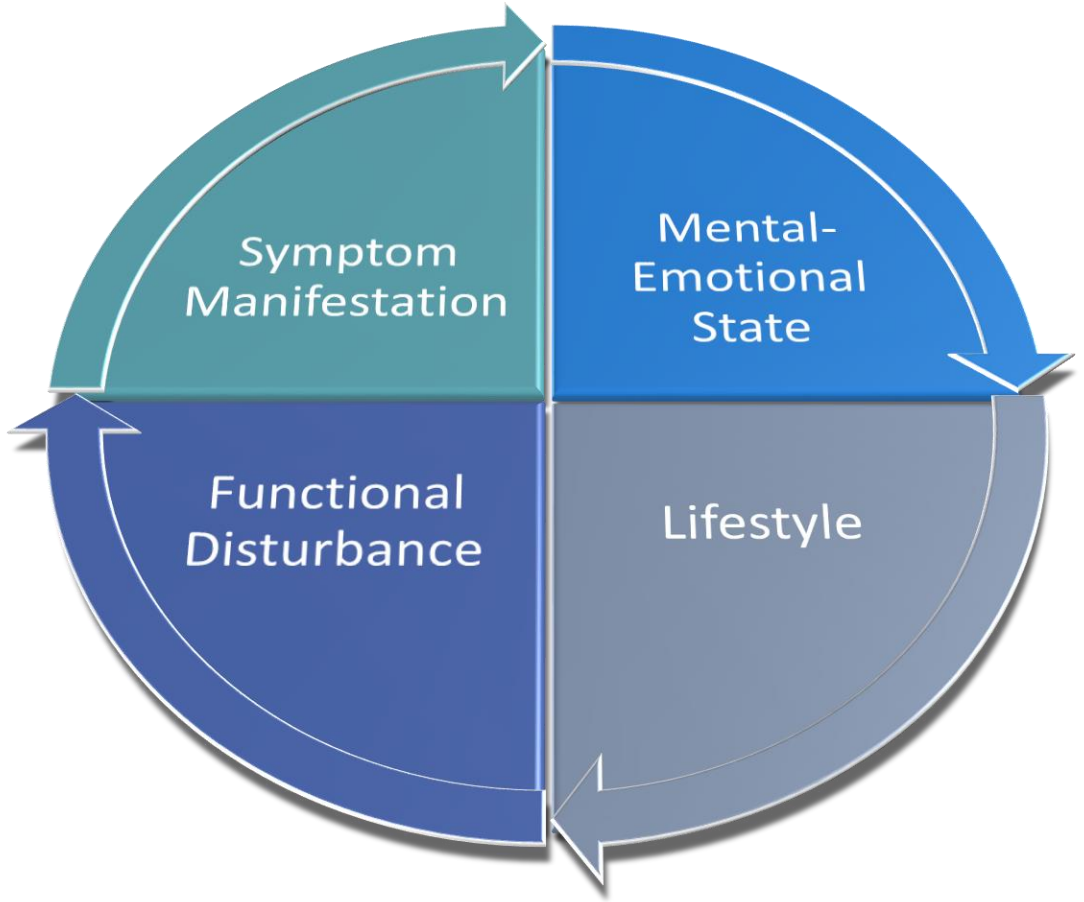
Smazal AL, Iowa State University; 2013.

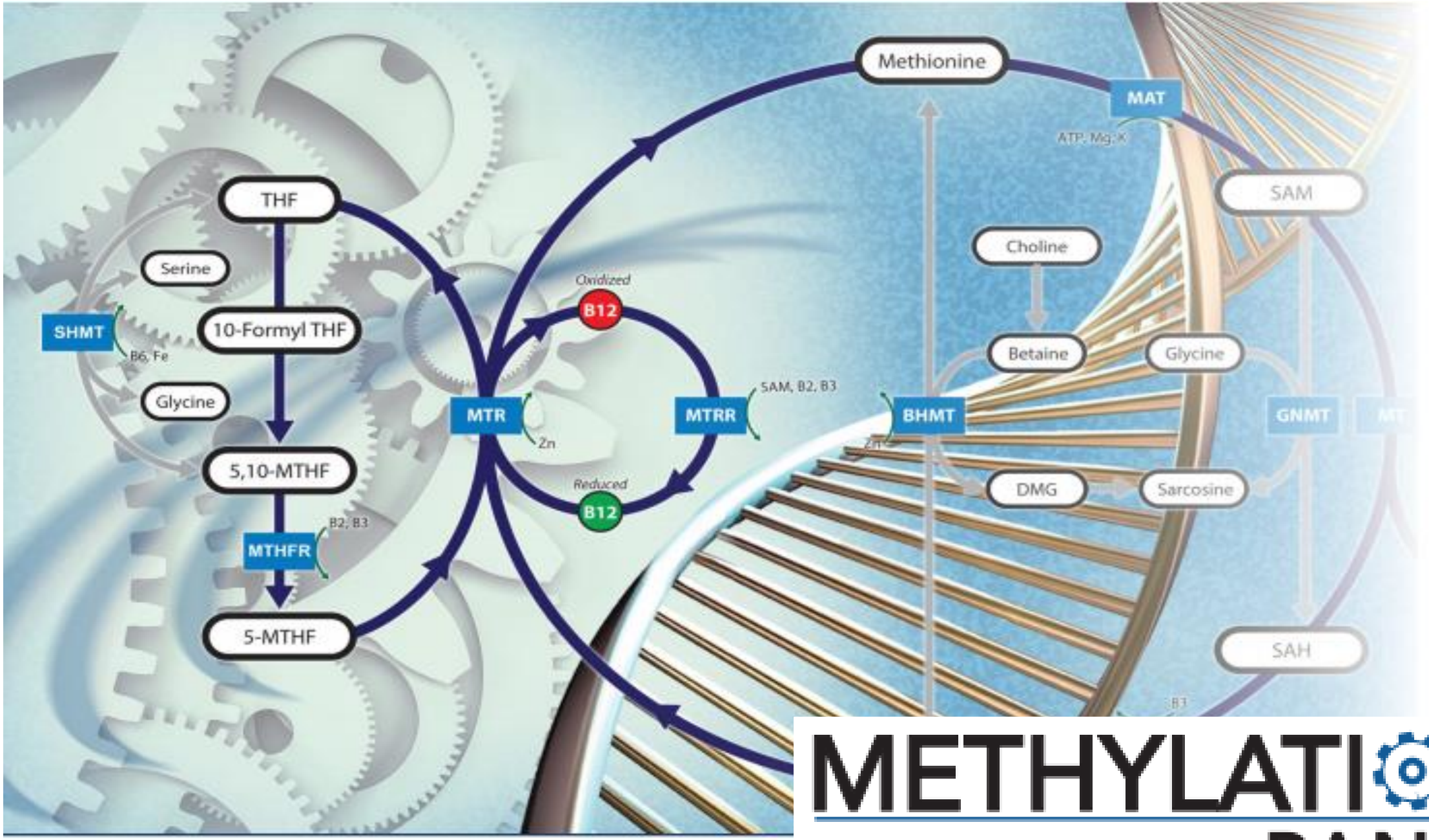
Lawson BR, et al. *Clin Immunol.* 2012;143(1):8-21.

Abu-Lebdeh HS, et al. *J Clin Endocrinol Metab.* 2006;91(9):3344-48.

Schalinske KL, et al. *Adv Nutr.* 2012;3(6):755-62.

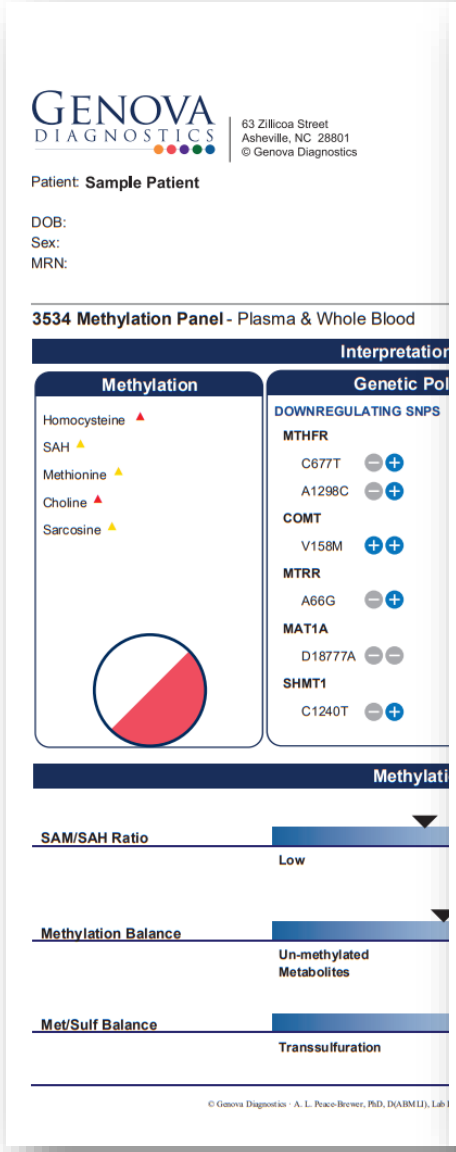
Translating Systems to Symptoms





METHYLATION PANEL

Report Review


GENOVA DIAGNOSTICS
63 Zillicoa Street
Asheville, NC 28801
© Genova Diagnostics

Patient: **Sample Patient**

DOB:
Sex:
MRN:

3534 Methylation Panel - Plasma & Whole Blood

Interpretation

Methylation

Homocysteine
SAH
Methionine
Choline
Sarcosine

Genetic Pol

DOWNREGULATING SNPS

MTHFR
C677T
A1298C

COMT
V158M

MTRR
A66G

MAT1A
D18777A

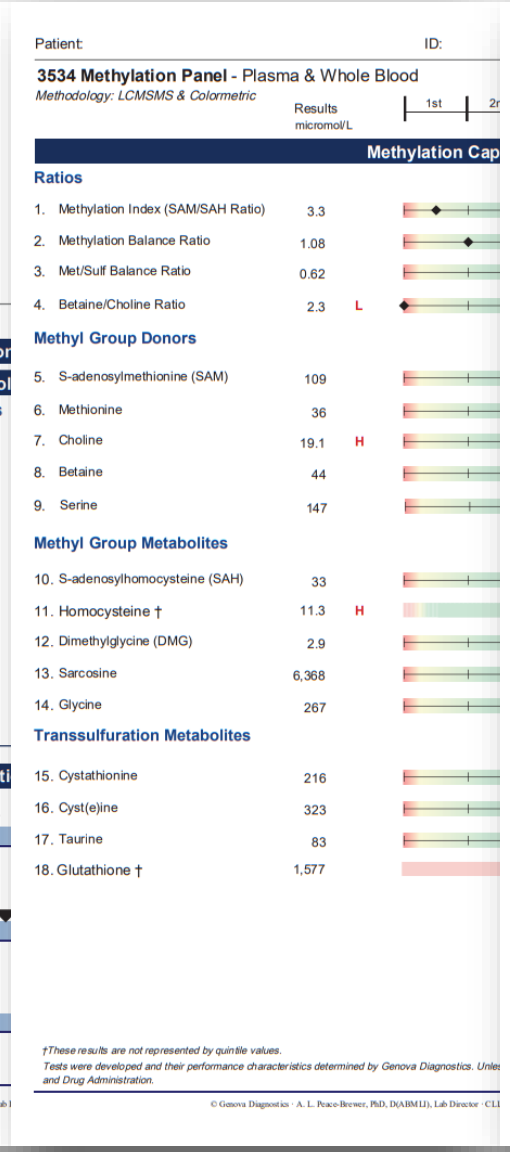
SHMT1
C1240T

Methylati

SAM/SAH Ratio
Low

Methylation Balance
Un-methylated Metabolites

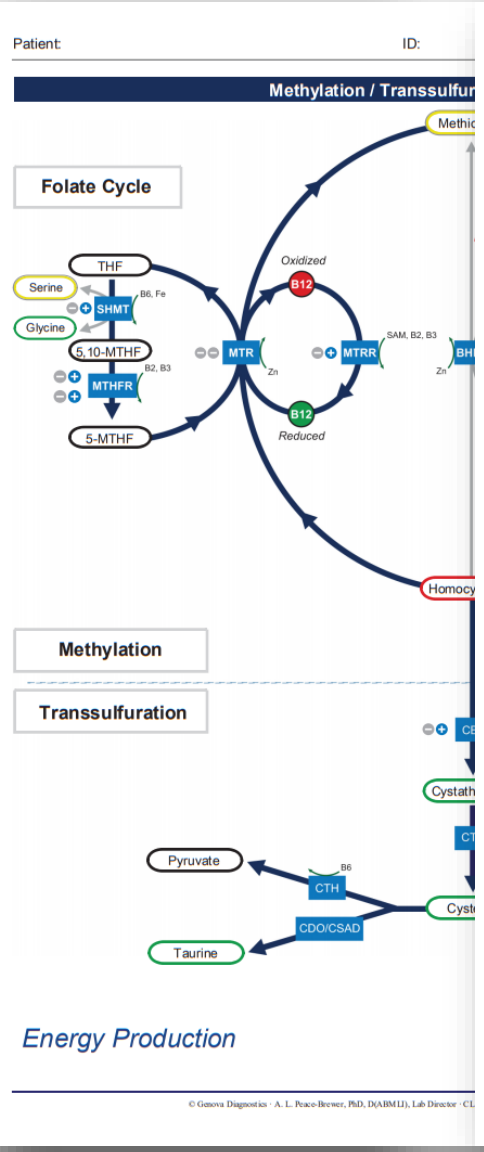
Met/Sulf Balance
Transsulfuration


Patient: ID:

3534 Methylation Panel - Plasma & Whole Blood
Methodology: LCMSMS & Colorimetric

		Results micromol/L	1st	2nd
Methylation Cap				
Ratios				
1.	Methylation Index (SAM/SAH Ratio)	3.3		
2.	Methylation Balance Ratio	1.08		
3.	Met/Sulf Balance Ratio	0.62		
4.	Betaine/Choline Ratio	2.3	L	
Methyl Group Donors				
5.	S-adenosylmethionine (SAM)	109		
6.	Methionine	36		
7.	Choline	19.1	H	
8.	Betaine	44		
9.	Serine	147		
Methyl Group Metabolites				
10.	S-adenosylhomocysteine (SAH)	33		
11.	Homocysteine †	11.3	H	
12.	Dimethylglycine (DMG)	2.9		
13.	Sarcosine	6,368		
14.	Glycine	267		
Transsulfuration Metabolites				
15.	Cystathionine	216		
16.	Cyst(e)ine	323		
17.	Taurine	83		
18.	Glutathione †	1,577		

†These results are not represented by quintile values.
Tests were developed and their performance characteristics determined by Genova Diagnostics. Unles
and Drug Administration.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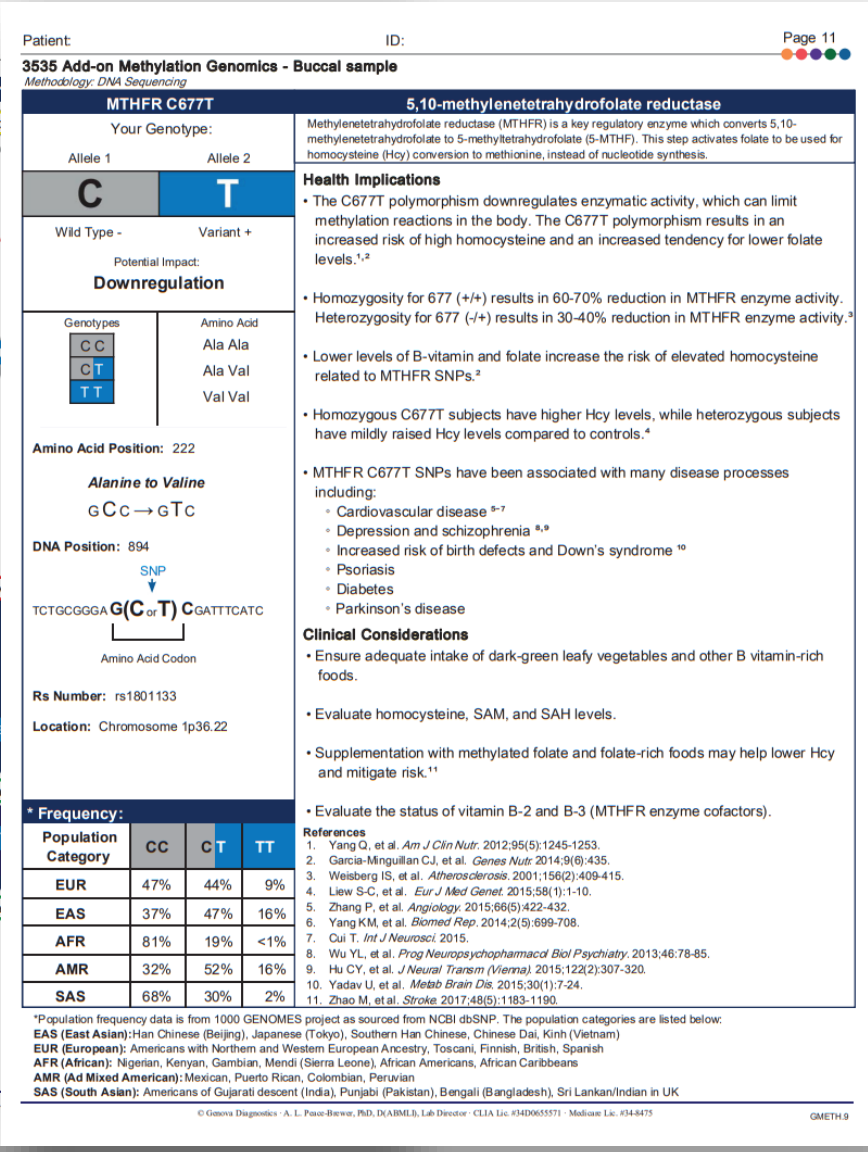
Methylation / Transsulfur

Folate Cycle

Methylation

Transsulfuration

Energy Production


Patient: ID: Page 11

3535 Add-on Methylation Genomics - Buccal sample
Methodology: DNA Sequencing

MTHFR C677T

Your Genotype:

Allele 1	Allele 2
C	T
Wild Type -	Variant +

Potential Impact: **Downregulation**

Genotypes	Amino Acid
C C	Ala Ala
C T	Ala Val
T T	Val Val

Amino Acid Position: 222

Alanine to Valine

GCC → GTC

DNA Position: 894

SNP
TCTGCGGGA G(C or T) CGATTTCATC
Amino Acid Codon

Rs Number: rs1801133

Location: Chromosome 1p36.22

*** Frequency:**

Population Category	CC	CT	TT
EUR	47%	44%	9%
EAS	37%	47%	16%
AFR	81%	19%	<1%
AMR	32%	52%	16%
SAS	68%	30%	2%

5,10-methylenetetrahydrofolate reductase

Methylenetetrahydrofolate reductase (MTHFR) is a key regulatory enzyme which converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-MTHF). This step activates folate to be used for homocysteine (Hcy) conversion to methionine, instead of nucleotide synthesis.

Health Implications

- The C677T polymorphism downregulates enzymatic activity, which can limit methylation reactions in the body. The C677T polymorphism results in an increased risk of high homocysteine and an increased tendency for lower folate levels.[1,2]
- Homozygosity for 677 (+/+) results in 60-70% reduction in MTHFR enzyme activity. Heterozygosity for 677 (-/+) results in 30-40% reduction in MTHFR enzyme activity.[3]
- Lower levels of B-vitamin and folate increase the risk of elevated homocysteine related to MTHFR SNPs.[2]
- Homozygous C677T subjects have higher Hcy levels, while heterozygous subjects have mildly raised Hcy levels compared to controls.[4]
- MTHFR C677T SNPs have been associated with many disease processes including:
  - Cardiovascular disease [5-7]
  - Depression and schizophrenia [8,9]
  - Increased risk of birth defects and Down's syndrome [10]
  - Psoriasis
  - Diabetes
  - Parkinson's disease

Clinical Considerations

- Ensure adequate intake of dark-green leafy vegetables and other B vitamin-rich foods.
- Evaluate homocysteine, SAM, and SAH levels.
- Supplementation with methylated folate and folate-rich foods may help lower Hcy and mitigate risk.[11]
- Evaluate the status of vitamin B-2 and B-3 (MTHFR enzyme cofactors).

References
1. Yang Q, et al. *Am J Clin Nutr.* 2012;95(5):1245-1253.
2. Garcia-Minguillan CJ, et al. *Genes Nutr.* 2014;9(6):435.
3. Weisberg IS, et al. *Atherosclerosis.* 2001;156(2):409-415.
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5. Zhang P, et al. *Angiology.* 2015;66(5):422-432.
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7. Cui T. *Int J Neurosci.* 2015.
8. Wu YL, et al. *Prog Neuropsychopharmacol Biol Psychiatry.* 2013;46:78-85.
9. Hu CY, et al. *J Neural Transm (Vienna).* 2015;122(2):307-320.
10. Yadav U, et al. *Metab Brain Dis.* 2015;30(1):7-24.
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EUR (European): Americans with Northern and Western European Ancestry, Toscani, Finnish, British, Spanish
AFR (African): Nigerian, Kenyan, Gambian, Mendi (Sierra Leone), African Americans, African Caribbeans
AMR (Ad Mixed American): Mexican, Puerto Rican, Colombian, Peruvian
SAS (South Asian): Americans of Gujarati descent (India), Punjabi (Pakistan), Bengali (Bangladesh), Sri Lankan/Indian in UK

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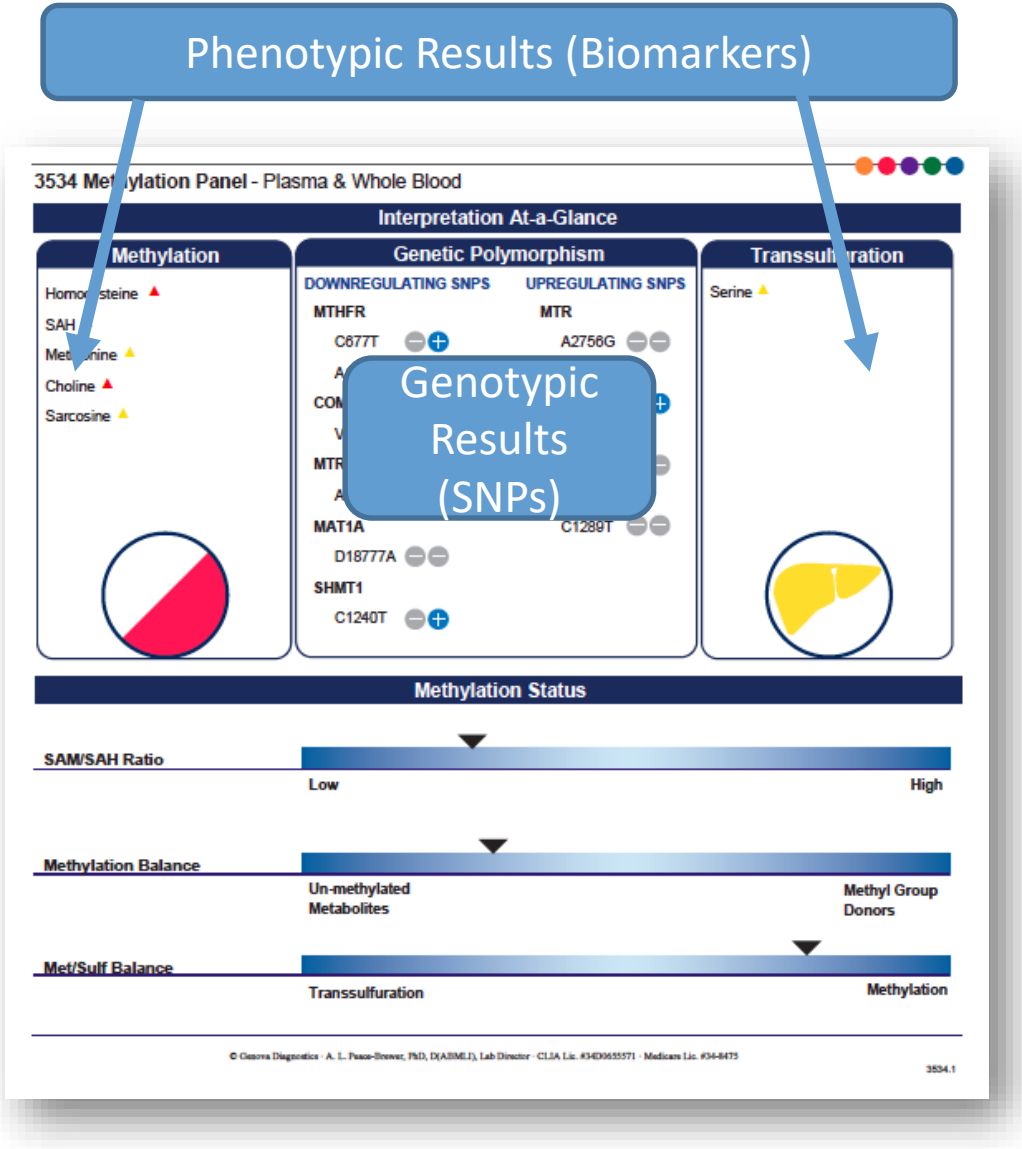
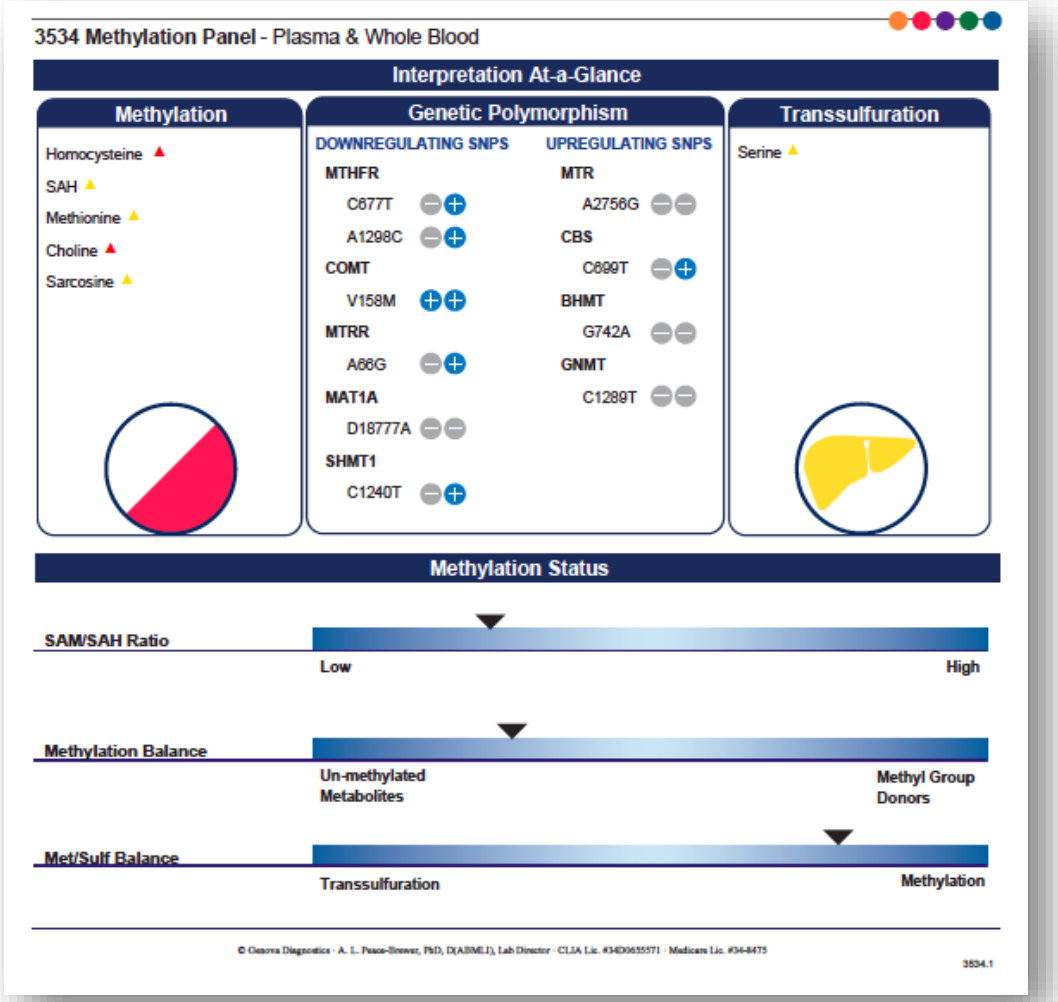
Combined Assessment: A Methylation Panel

- A comprehensive approach to systems biology assessment
- Genotypic and phenotypic evaluation of methylation
- Novel biomarkers and genomics involved in methylation pathway



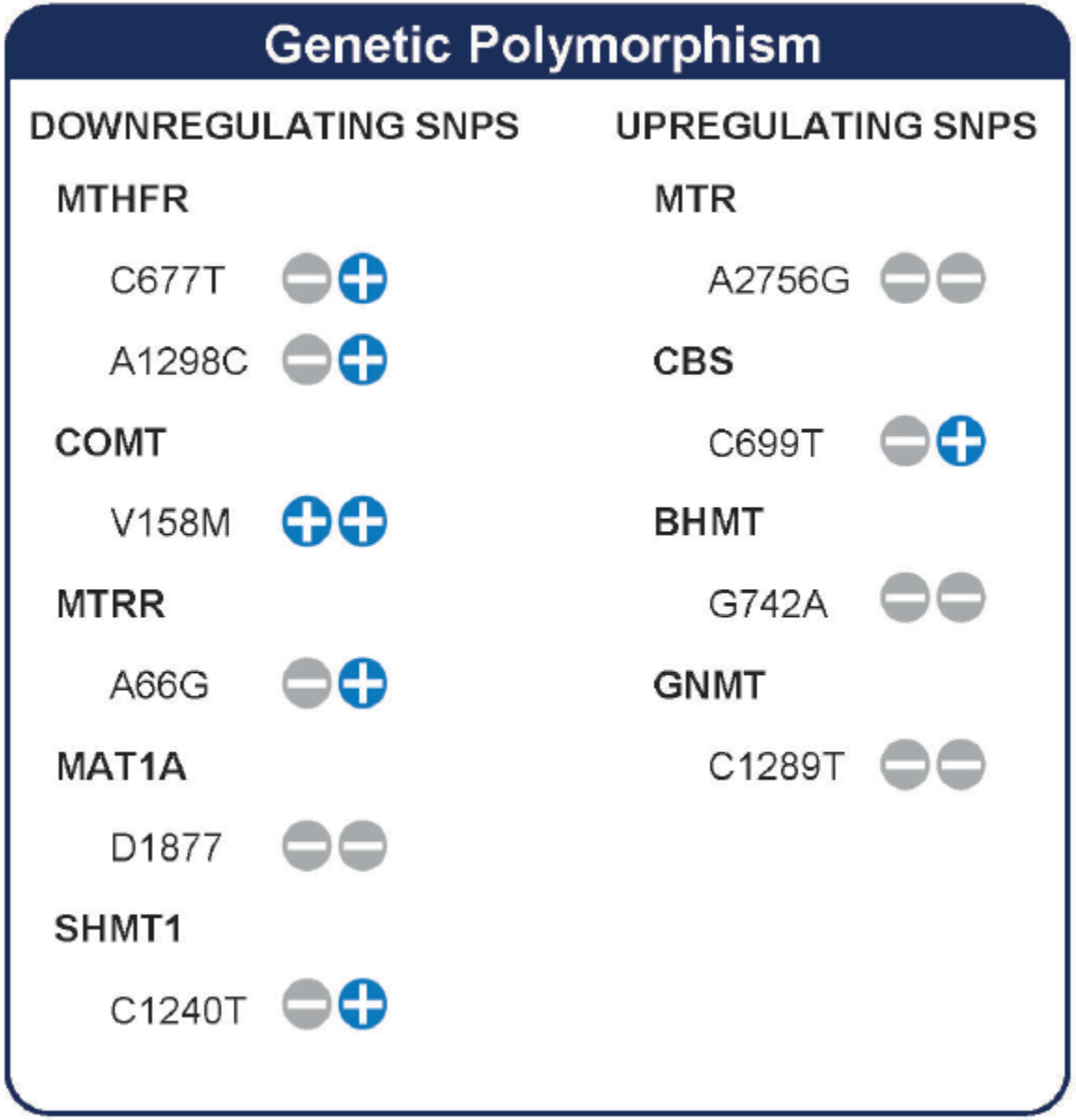
Interpretation-at-a-Glance

- 3 Pillars
  - Abnormal biomarkers are categorized as either problems in:
    - Methylation Cycle
      - Consider methylation support
    - Transsulfuration Pathway
      - Consider antioxidant support
  - Middle pillar are SNP results



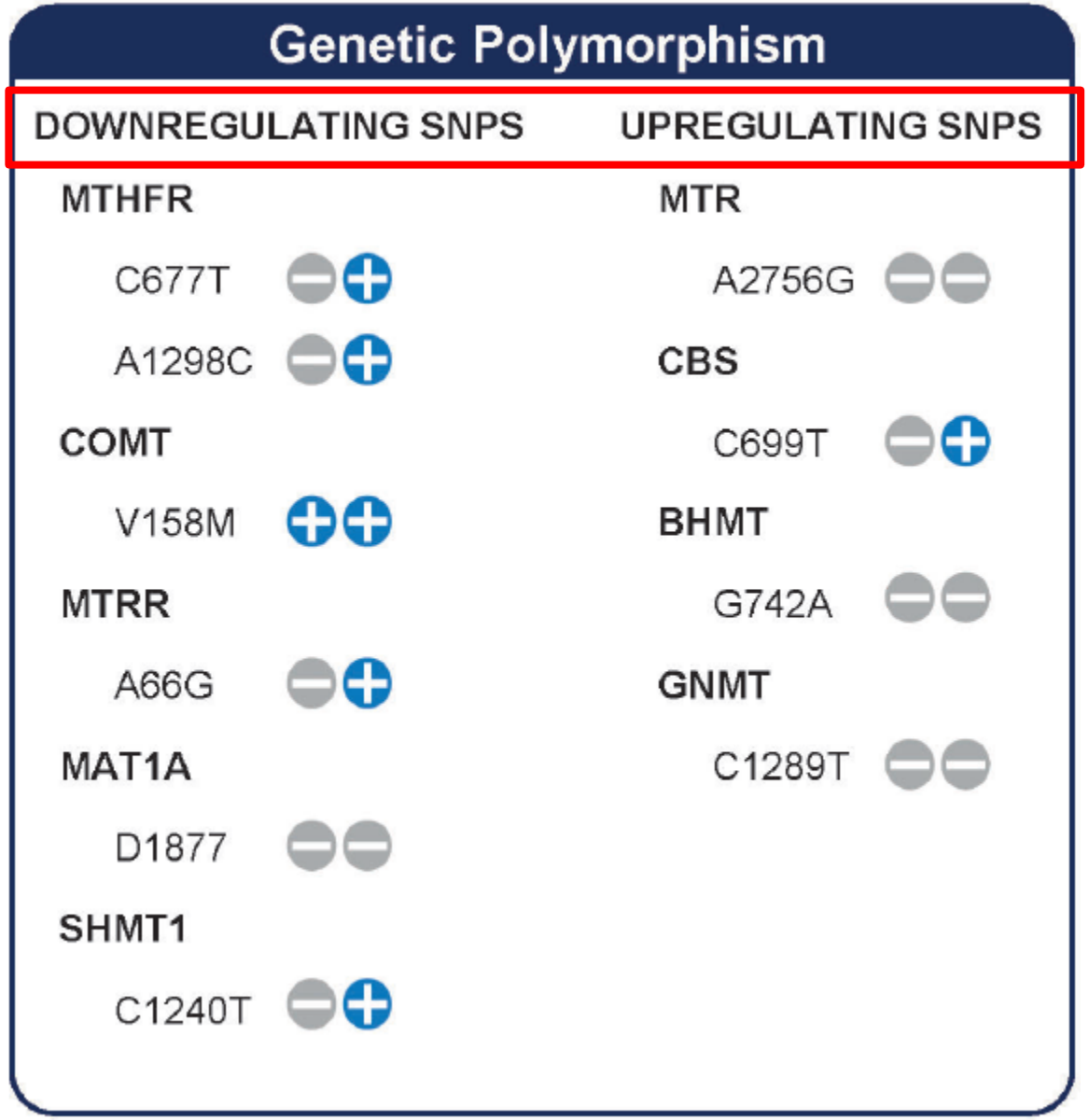
Methylation Panel Genomics

- Ten genomic SNPs relevant to methylation pathway function
  - MTHFR (2)
  - COMT
  - MTRR
  - MAT1A
  - SHMT1
  - MTR
  - CBS
  - BHMT
  - GNMT



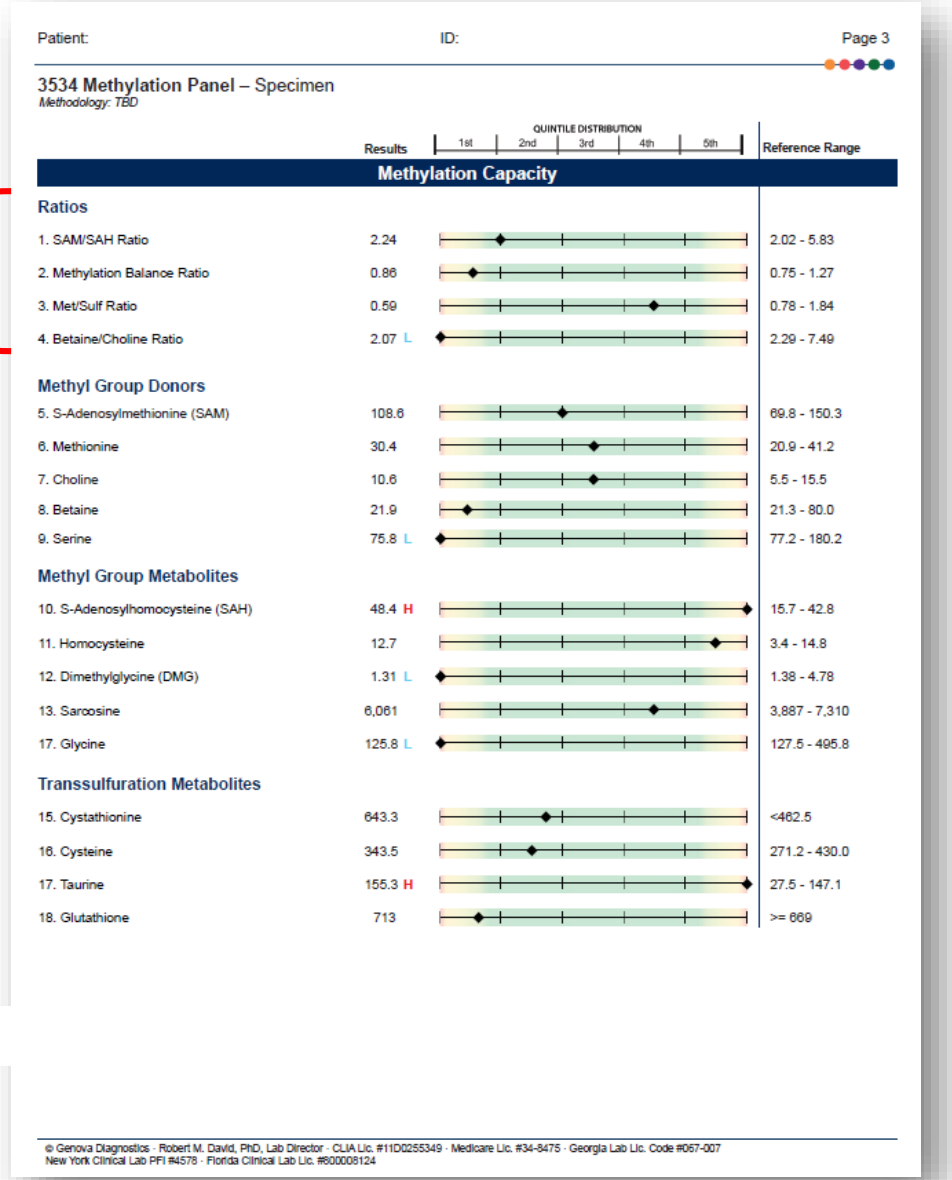
Methylation Panel Genomics

- SNPs are categorized into downregulating or upregulating SNPs
- This means that “positive” findings cause the enzyme to work slower (downregulating) or faster (upregulating)



Methylation Panel Biomarkers (cont)

- Functional Ratios
  - Discussed later
- Methyl Group Donors
  - SAM, Methionine, Choline, and Betaine
- Methyl Group Metabolites
  - SAH, Homocysteine, DMG, and Sarcosine
  - Generally, do not want elevated
- Transsulfuration Metabolites
  - Glutathione you want to be adequate

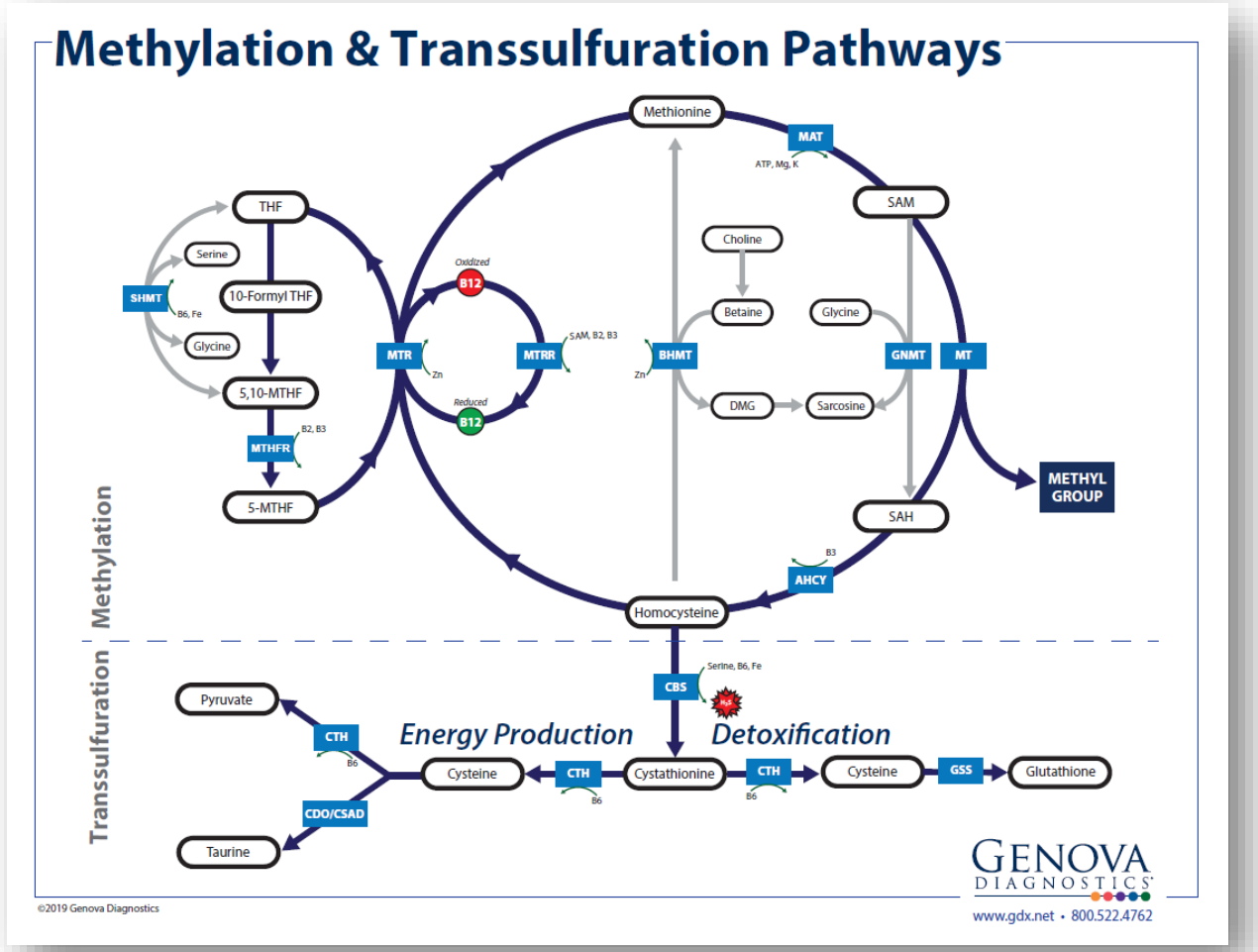


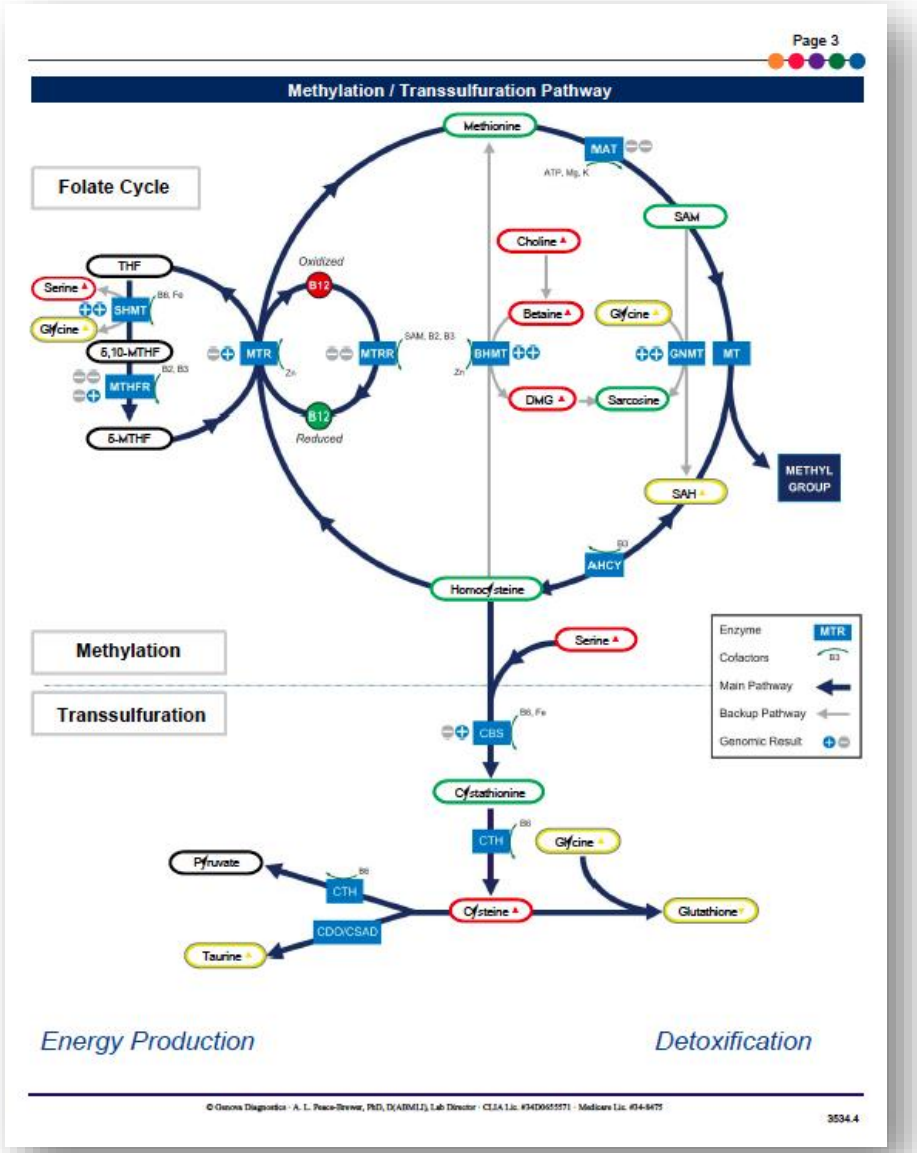
Patient: ID: Page 3

3534 Methylation Panel – Specimen
Methodology: TBD

	Results	QUINTILE DISTRIBUTION 1st 2nd 3rd 4th 5th	Reference Range
Methylation Capacity			
Ratios			
1. SAM/SAH Ratio	2.24		2.02 - 5.83
2. Methylation Balance Ratio	0.86		0.75 - 1.27
3. Met/Sulf Ratio	0.59		0.78 - 1.84
4. Betaine/Choline Ratio	2.07 L		2.29 - 7.49
Methyl Group Donors			
5. S-Adenosylmethionine (SAM)	108.6		69.8 - 150.3
6. Methionine	30.4		20.9 - 41.2
7. Choline	10.6		5.5 - 15.5
8. Betaine	21.9		21.3 - 80.0
9. Serine	75.8 L		77.2 - 180.2
Methyl Group Metabolites			
10. S-Adenosylhomocysteine (SAH)	48.4 H		15.7 - 42.8
11. Homocysteine	12.7		3.4 - 14.8
12. Dimethylglycine (DMG)	1.31 L		1.38 - 4.78
13. Sarcosine	6,061		3,887 - 7,310
17. Glycine	125.8 L		127.5 - 495.8
Transsulfuration Metabolites			
15. Cystathionine	643.3		<462.5
16. Cysteine	343.5		271.2 - 430.0
17. Taurine	155.3 H		27.5 - 147.1
18. Glutathione	713		>= 669

Pathway Interpretation – Page 3

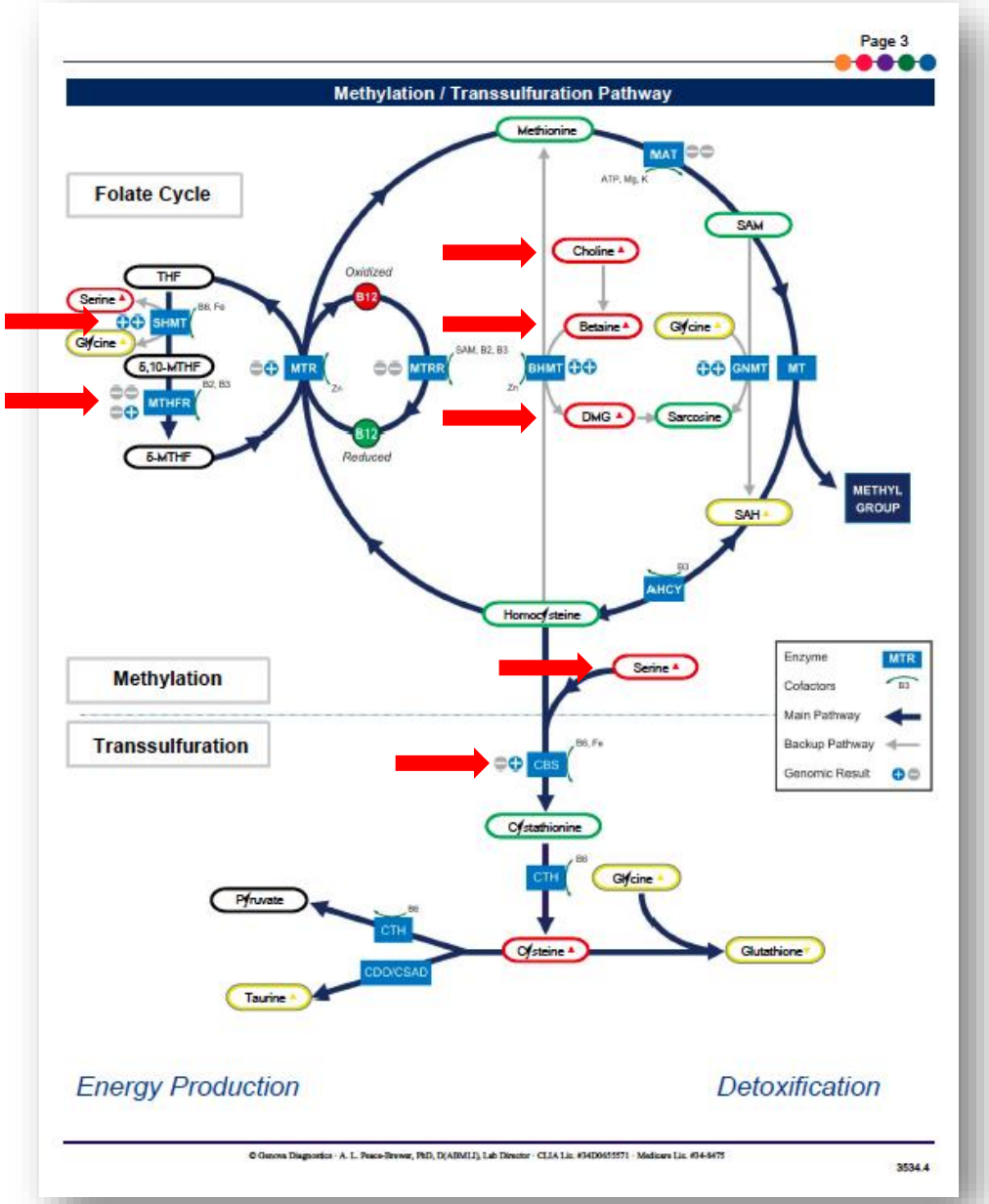




Methylation Pathway Format

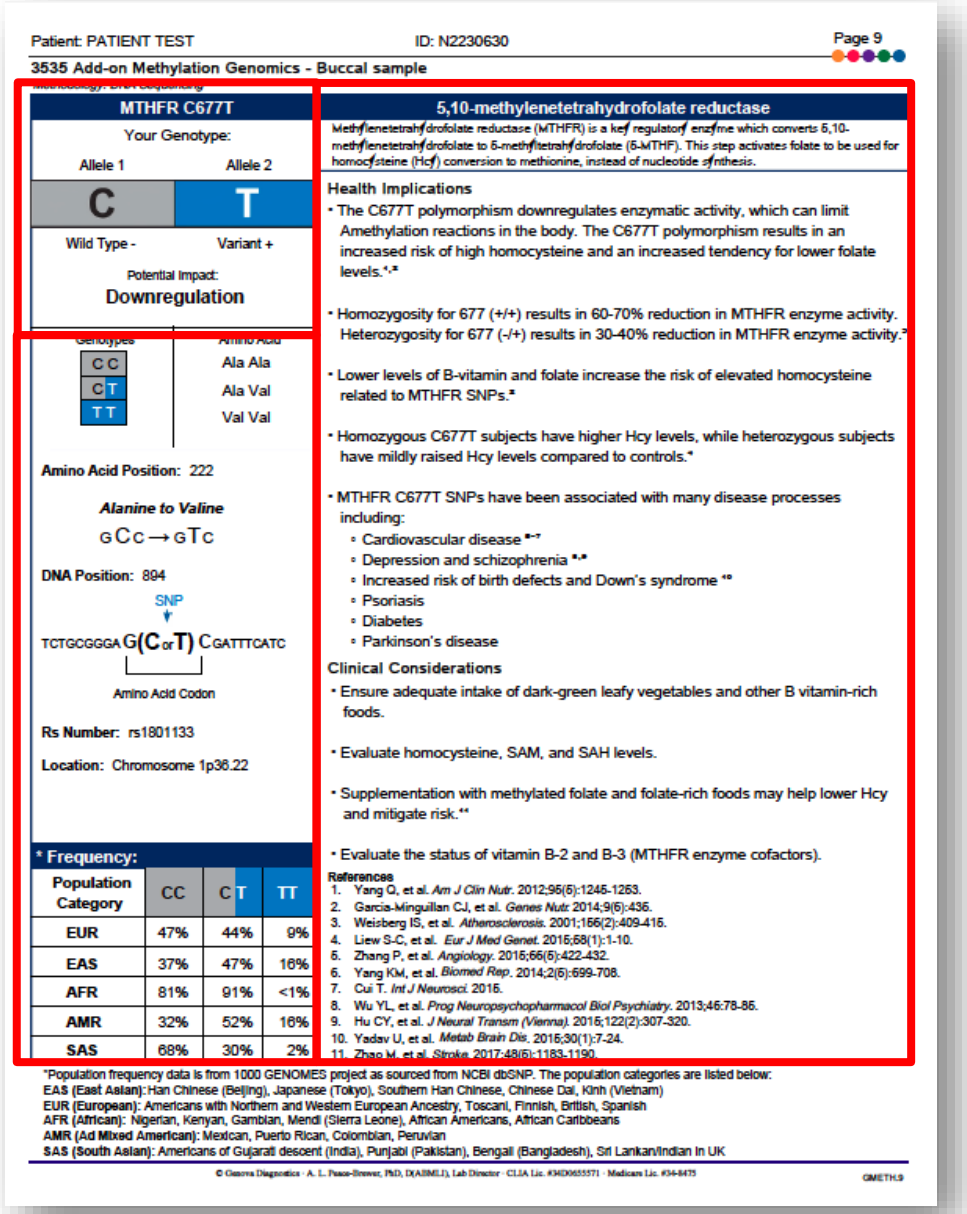
- Results placed in pathway format
  - Genomic Results
  - Abnormal Biomarkers

- Allows clinicians to visualize the delicate balance or abnormalities in pathway



Genomic Results – Pages 4+

- There will be a page for each SNP result
- Patient results at the top left
- General genomic information under patient result
- Commentary box on the right



Patient: PATIENT TEST ID: N2230630 Page 9

3535 Add-on Methylation Genomics - Buccal sample

MTHFR C677T

Your Genotype:

Allele 1	Allele 2
C	T
Wild Type -	Variant +

Potential Impact: **Downregulation**

Genotypes	Amino Acid
C C	Ala Ala
C T	Ala Val
T T	Val Val

Amino Acid Position: 222

Alanine to Valine

GCC → GTC

DNA Position: 894

SNP

TCTGCGGGA G(C or T) CGATTTCATC

Amino Acid Codon

Rs Number: rs1801133

Location: Chromosome 1p36.22

*** Frequency:**

Population Category	CC	CT	TT
EUR	47%	44%	9%
EAS	37%	47%	16%
AFR	81%	91%	<1%
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5,10-methylenetetrahydrofolate reductase

Methylenetetrahydrofolate reductase (MTHFR) is a key regulatory enzyme which converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-MTHF). This step activates folate to be used for homocysteine (Hcy) conversion to methionine, instead of nucleotide synthesis.

Health Implications

- The C677T polymorphism downregulates enzymatic activity, which can limit Amethylation reactions in the body. The C677T polymorphism results in an increased risk of high homocysteine and an increased tendency for lower folate levels.*,*
- Homozygosity for 677 (+/+) results in 60-70% reduction in MTHFR enzyme activity. Heterozygosity for 677 (-/+) results in 30-40% reduction in MTHFR enzyme activity.*
- Lower levels of B-vitamin and folate increase the risk of elevated homocysteine related to MTHFR SNPs.*
- Homozygous C677T subjects have higher Hcy levels, while heterozygous subjects have mildly raised Hcy levels compared to controls.*
- MTHFR C677T SNPs have been associated with many disease processes including:
  - Cardiovascular disease *-7
  - Depression and schizophrenia *,*
  - Increased risk of birth defects and Down's syndrome *9
  - Psoriasis
  - Diabetes
  - Parkinson's disease

Clinical Considerations

- Ensure adequate intake of dark-green leafy vegetables and other B vitamin-rich foods.
- Evaluate homocysteine, SAM, and SAH levels.
- Supplementation with methylated folate and folate-rich foods may help lower Hcy and mitigate risk.*4
- Evaluate the status of vitamin B-2 and B-3 (MTHFR enzyme cofactors).

References

1. Yang Q, et al. *Am J Clin Nutr.* 2012;95(5):1245-1263.
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AFR (African): Nigerian, Kenyan, Gambian, Mendi (Sierra Leone), African Americans, African Caribbeans
AMR (Ad Mixed American): Mexican, Puerto Rican, Colombian, Peruvian
SAS (South Asian): Americans of Gujarati descent (India), Punjabi (Pakistan), Bengali (Bangladesh), Sri Lankan/Indian in UK

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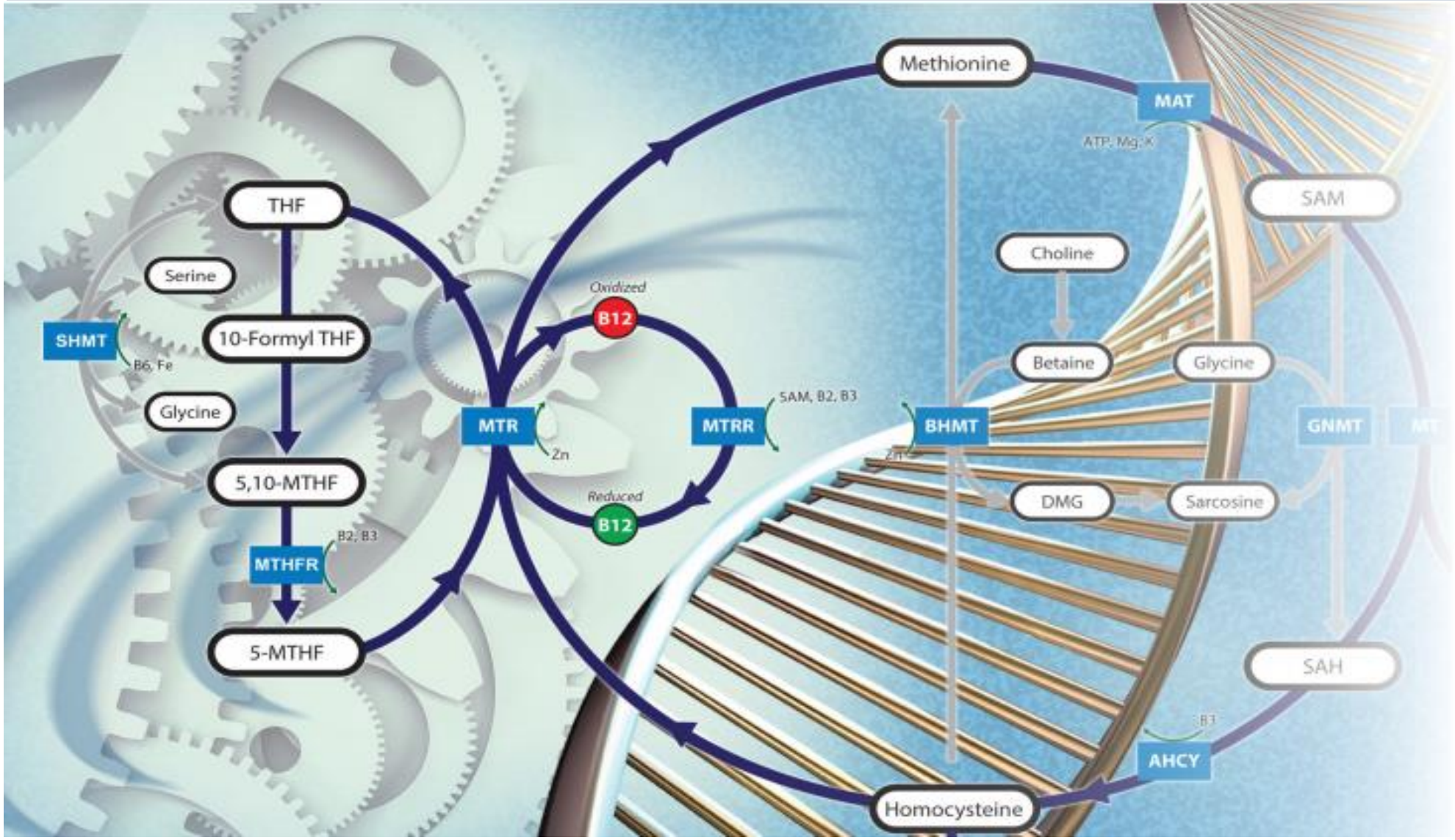
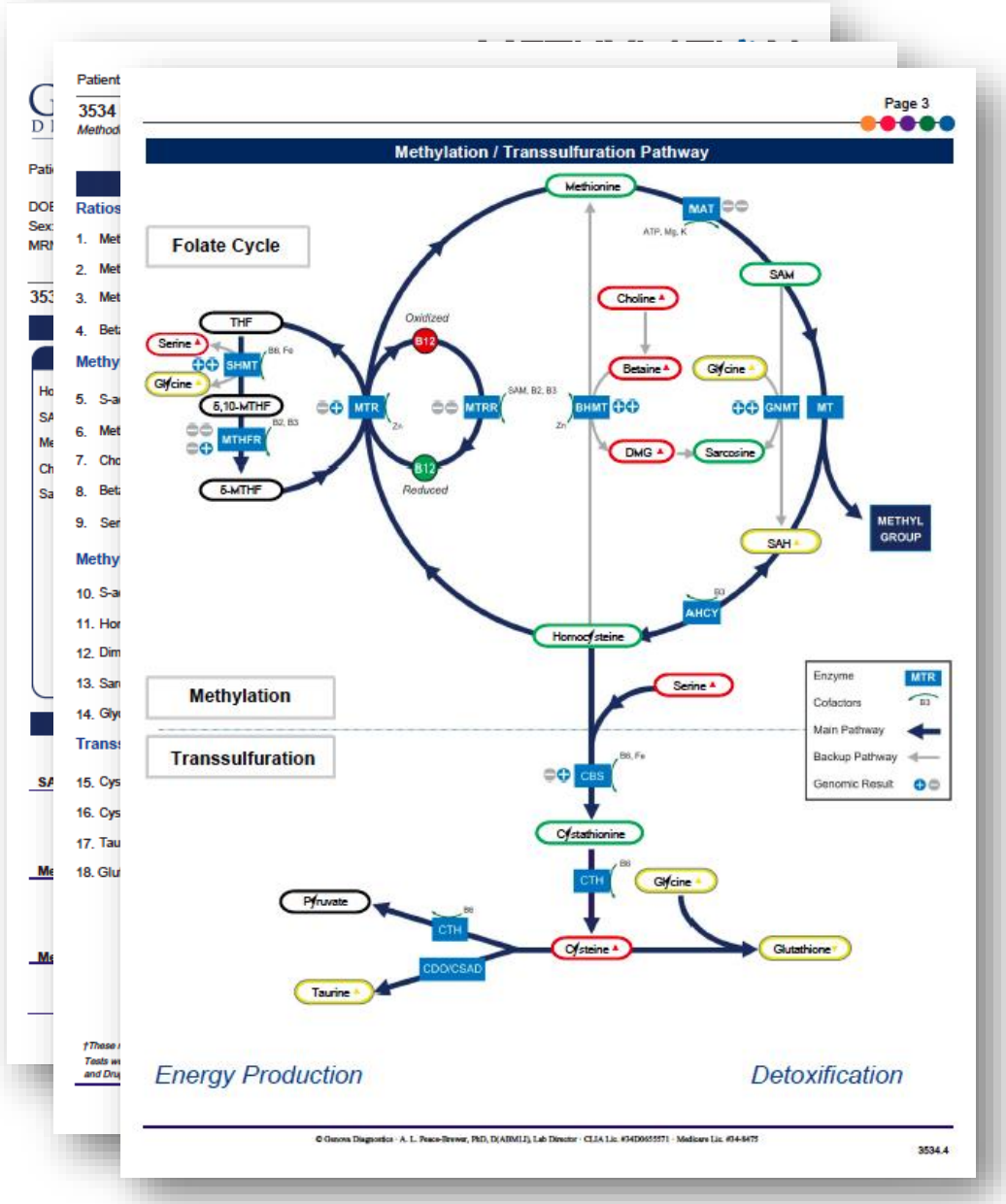


Methylation Panel Interpretation

Walkthrough

Interpretation: Where to Begin?

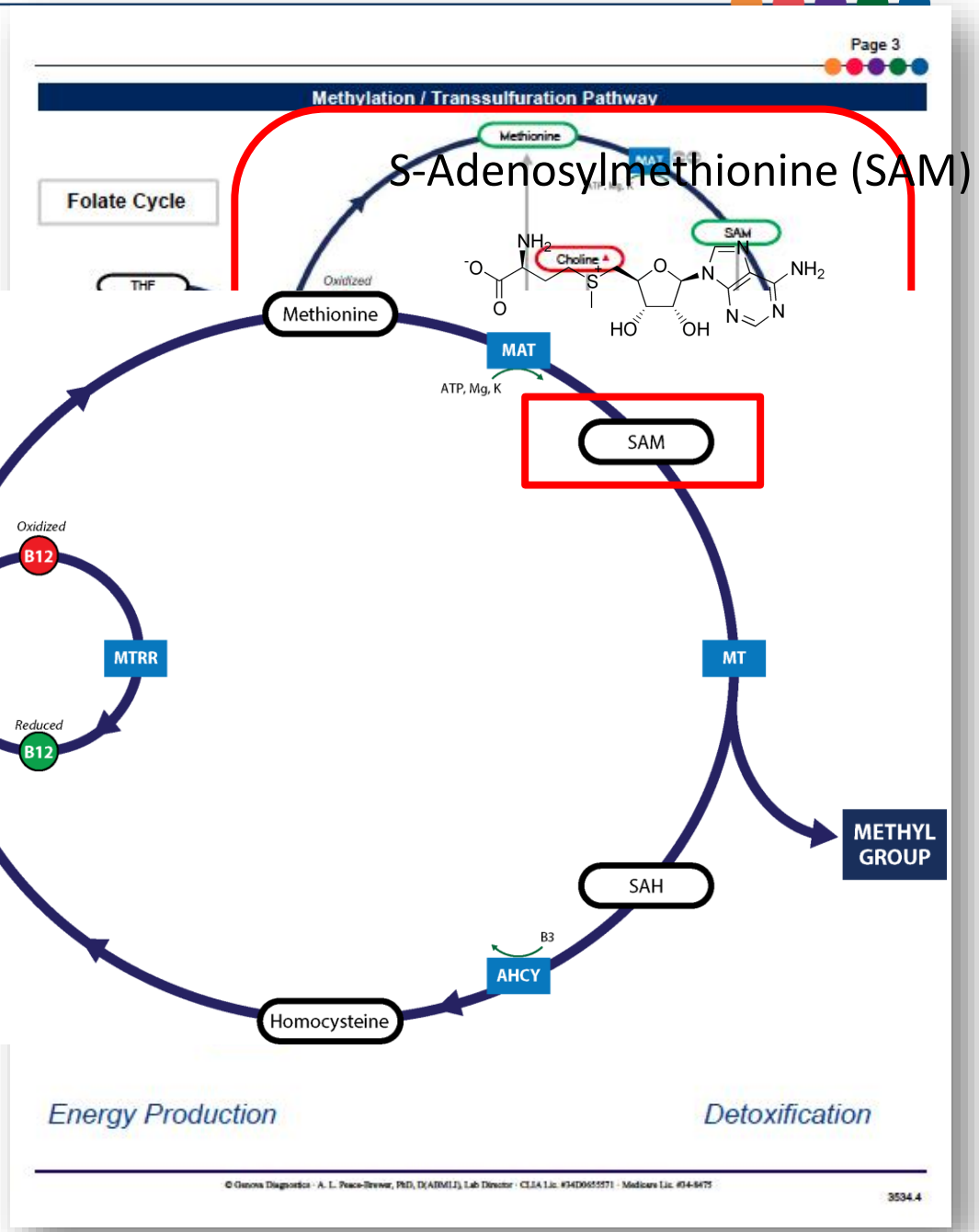
- Lots of places you could start
  - **Page 1: Interpretation-at-a-Glance**
    - A great overview once you are familiar with the test
  - **Page 2: Biomarker Results**
    - Helps to categorize the biomarkers and provides quantitative result findings
  - **Page 3: Pathway Analysis**
    - Great place to start when you are new to methylation testing




"If you truly love nature, you will find beauty everywhere." -Vincent Van Gogh
Photo by Gabe Swinney, 2018

1. Start with Methylation Cycle

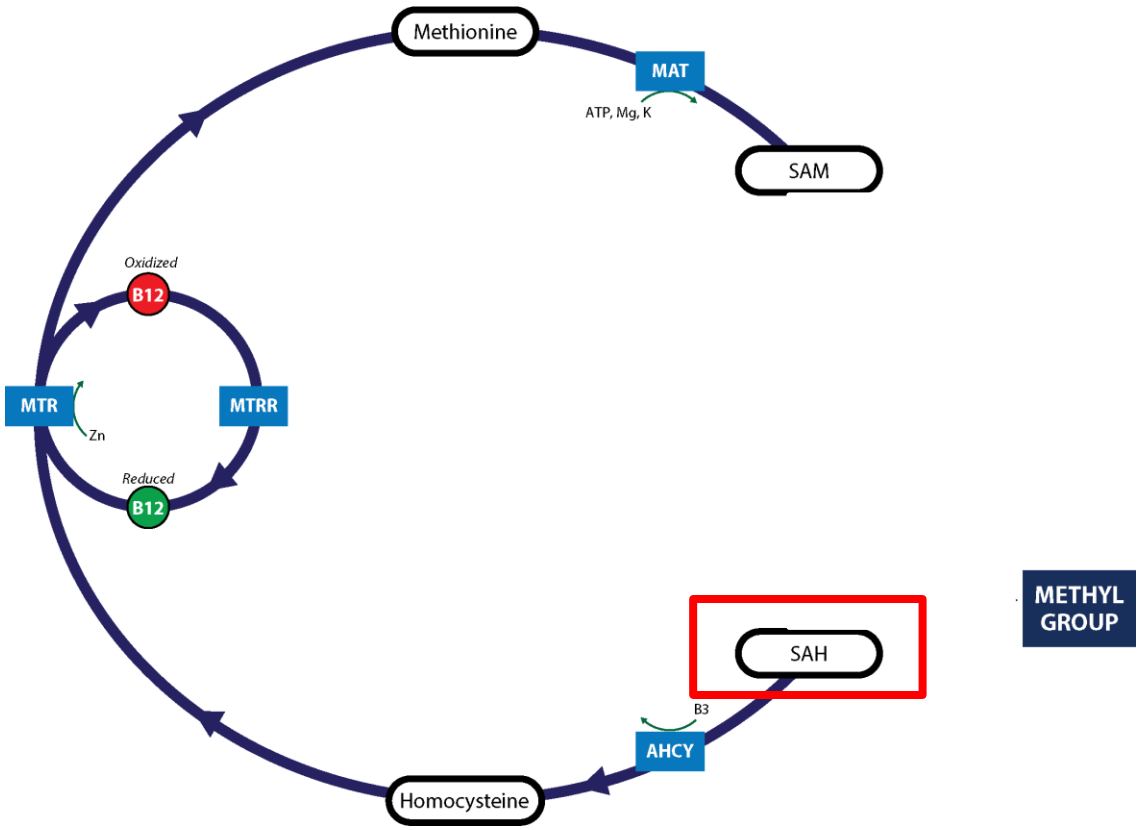
- Key takeaway:
  - The methylation cycle is all about making sure there is adequate SAM (S-adenosylmethionine)
  - SAM is overwhelmingly the body's main methyl donor
  - Think of SAM as the body's methylation currency!



S-Adenosylmethionine (SAM)

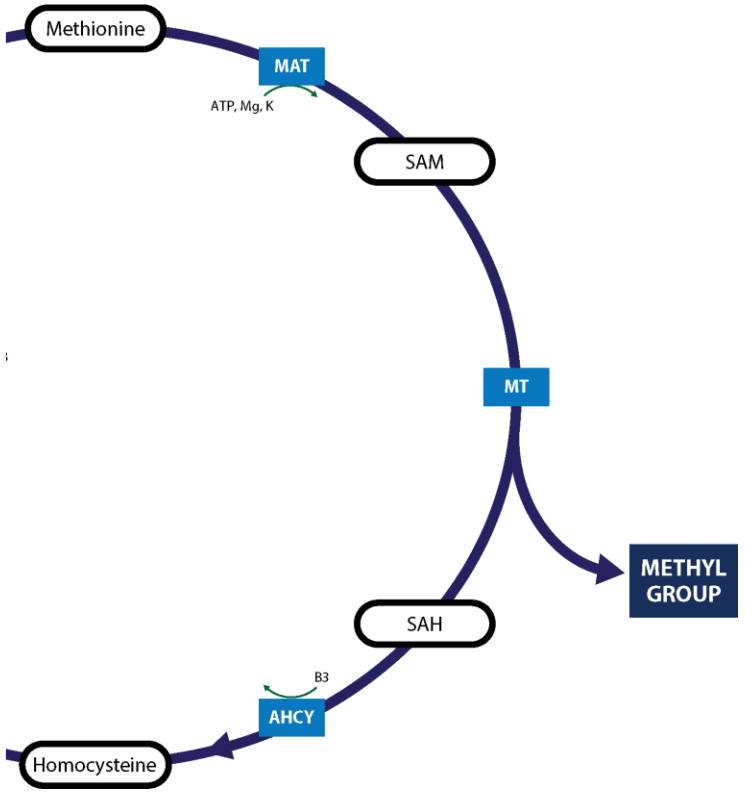
The Basic Methylation Cycle

- There and back again:
  - SAM can donate a methyl group wherever it is needed
  - Becomes SAH (S-adenosylhomocysteine)
  - SAH then breaks down into…
  - Homocysteine



Finishing the Cycle

- Homocysteine MUST be converted, or else, it will lead to build-up of SAH
- Main way to recycle SAM is by turning Hcy back into methionine
- This requires:
  - Activated Folate (5-MTHF)
  - Activated B-12
  - Zinc



Ho V, et al. *Genes Nutr.* 2013;8(6):571-580.

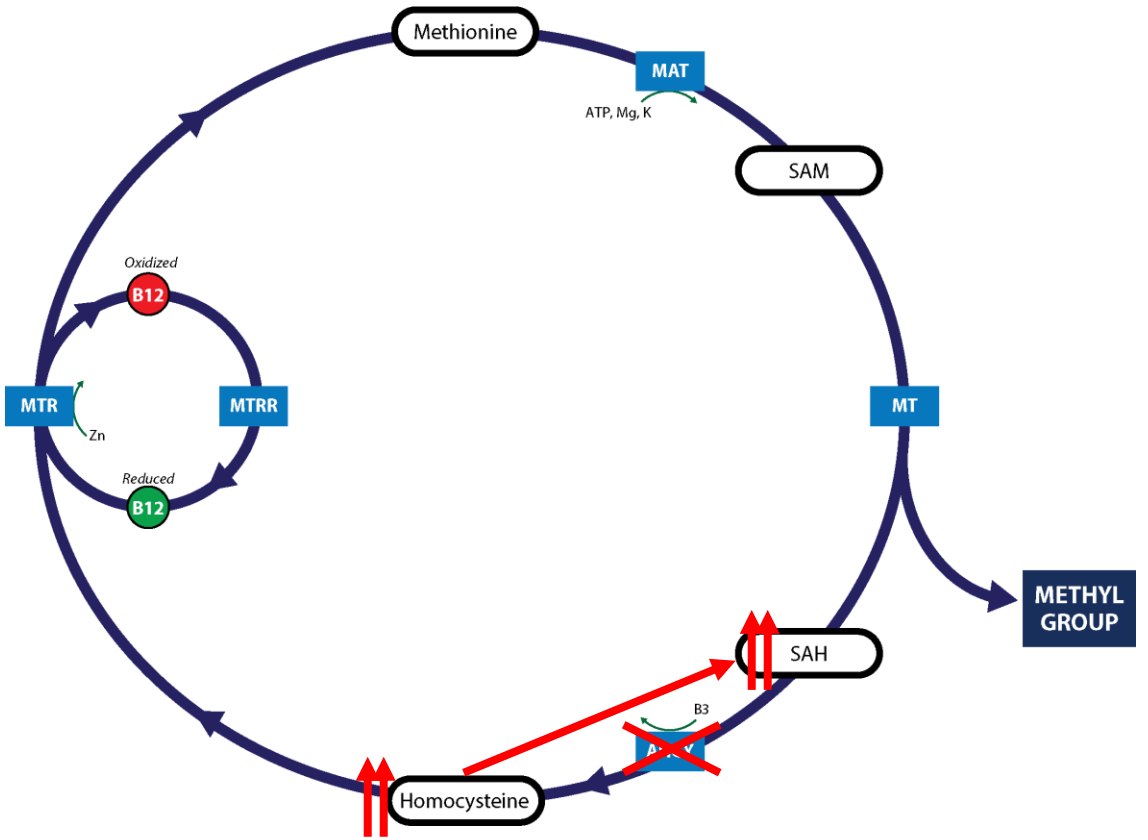
Homocysteine

- You may know homocysteine as a marker for cardiovascular risk
- Elevated levels are associated with:
  - Atherosclerosis and coronary artery disease
  - Osteopenia
  - Neurodegenerative conditions
  - Mood disorders
  - IBD and colon cancer risk
- Sound familiar?

Castro R, et al. *J Inherit Met Dis.* 2006;29(1):3-20.
Oussalah A, et al. *Aliment Pharmacol Ther.* 2011;34(10):1173-84.
Behera J, et al. *J Cell Phys.* 2017;232(10):2704-09.
Gariballa S. *Age Ageing.* 2011;40(6):702-05.

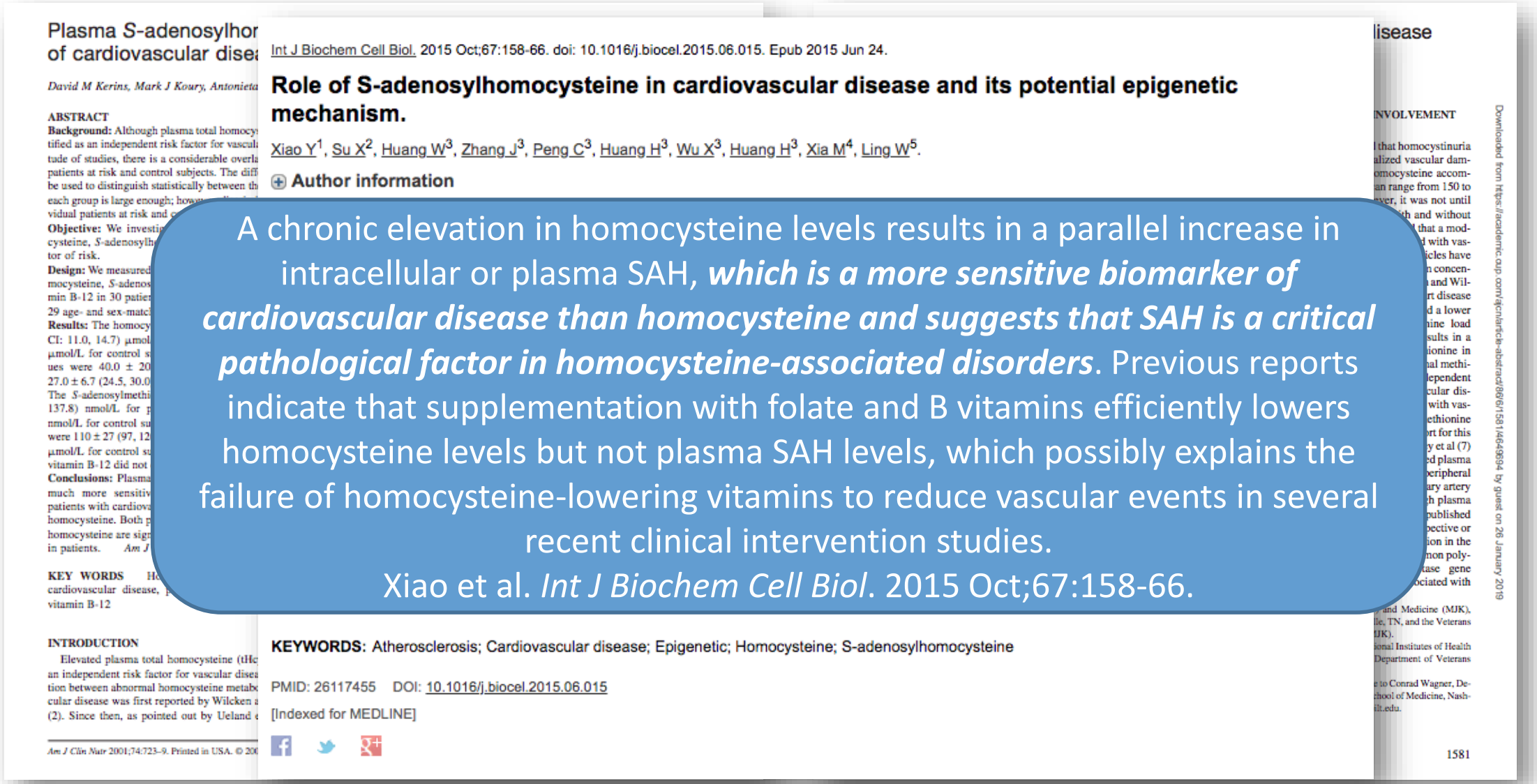
Homocysteine: a Biomarker for Methylation

- Homocysteine is often used as an indicator of methylation status
  - Clinicians aim for optimal: 2-10µmol/L
- Homocysteine must be recycled back into methionine
- Key takeaway!
  - Homocysteine → Higher SAH levels



Yi P, et al. *J Biol Chem.* 2000;275(38):29318-323.

Elevated SAH levels



Int J Biochem Cell Biol. 2015 Oct;67:158-66. doi: 10.1016/j.biocel.2015.06.015. Epub 2015 Jun 24.

Role of S-adenosylhomocysteine in cardiovascular disease and its potential epigenetic mechanism.

Xiao Y[1], Su X[2], Huang W[3], Zhang J[3], Peng C[3], Huang H[3], Wu X[3], Huang H[3], Xia M[4], Ling W[5].

Author information

A chronic elevation in homocysteine levels results in a parallel increase in intracellular or plasma SAH, ***which is a more sensitive biomarker of cardiovascular disease than homocysteine and suggests that SAH is a critical pathological factor in homocysteine-associated disorders***. Previous reports indicate that supplementation with folate and B vitamins efficiently lowers homocysteine levels but not plasma SAH levels, which possibly explains the failure of homocysteine-lowering vitamins to reduce vascular events in several recent clinical intervention studies.

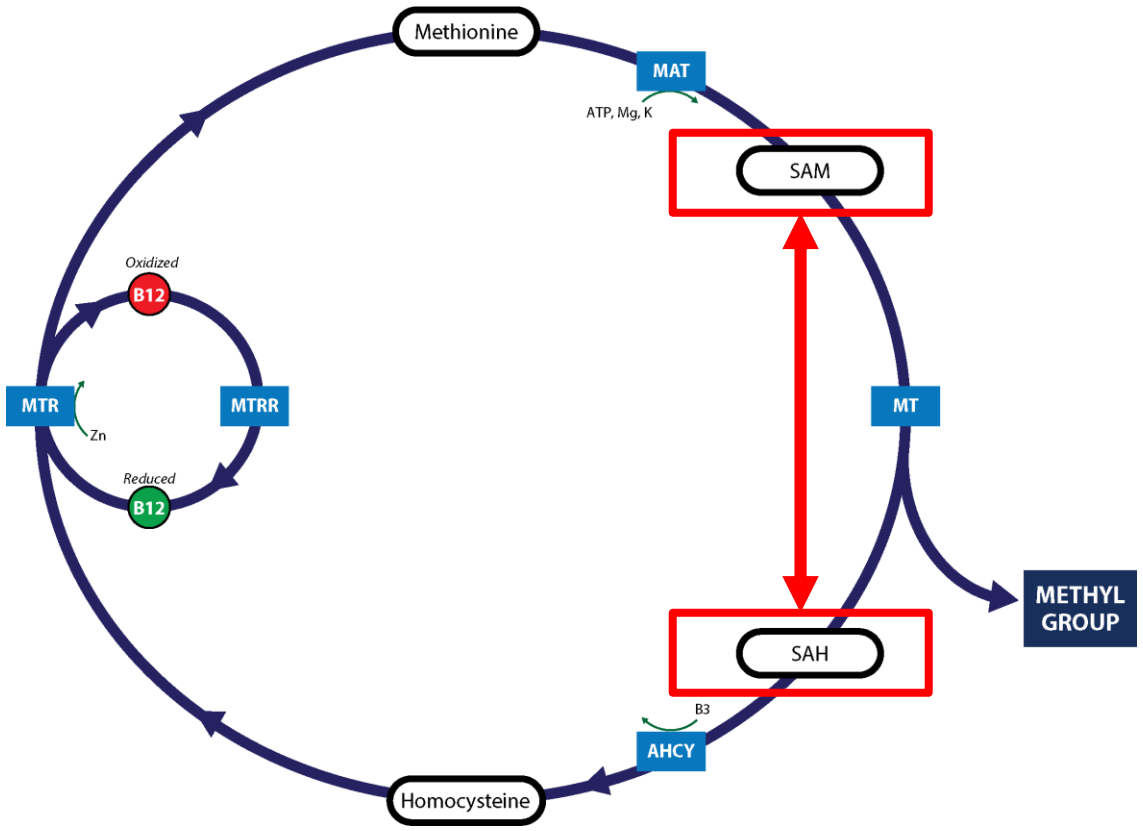
Xiao et al. *Int J Biochem Cell Biol*. 2015 Oct;67:158-66.

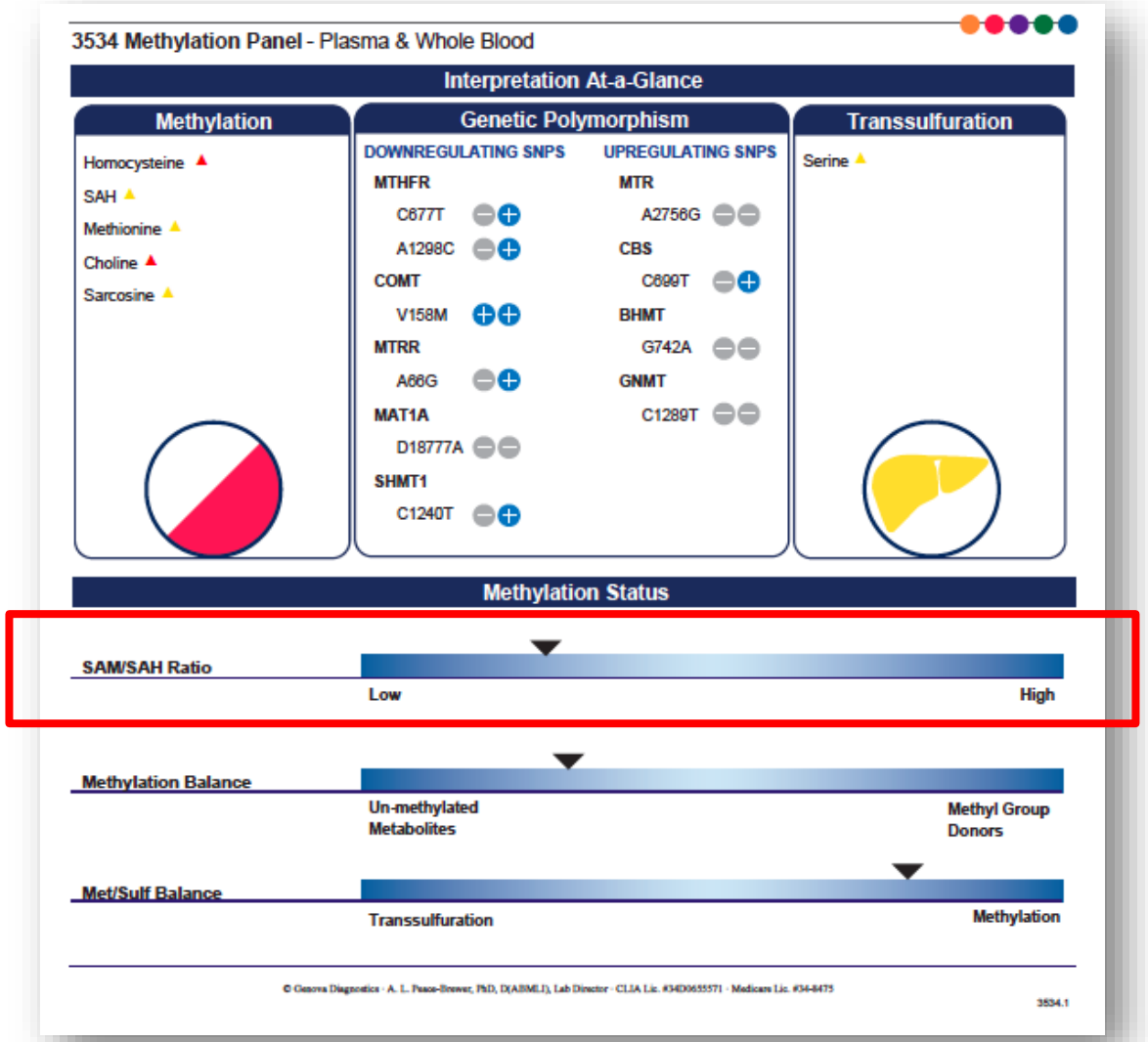
KEYWORDS: Atherosclerosis; Cardiovascular disease; Epigenetic; Homocysteine; S-adenosylhomocysteine

PMID: 26117455 DOI: 10.1016/j.biocel.2015.06.015

[Indexed for MEDLINE]

SAM/SAH Ratio





The SAM/SAH Ratio

- Believed to be a good indicator of cellular “methylation capacity”
  - Correlates well with intracellular SAM/SAH
- SAM tends to be under more homeostatic control
  - If you are running low on cash, you will withdraw money from other sources
- Fluctuations tend to be more due to relative SAH concentrations

Nutrients **2013**, *5*, 2457-2474; doi:10.3390/nu5072457

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Article

A Population Model of Folate-Mediated One-Carbon Metabolism

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* Author to whom correspondence should be addressed; E-Mail: hfn@duke.edu; Tel.: +1-919-684-2793; Fax: +1-919-660-7293.

Received: 12 April 2013; in revised form: 29 May 2013 / Accepted: 4 June 2013 / Published: 5 July 2013

Abstract: *Background*: Previous mathematical models for hepatic and tissue one-carbon metabolism have been combined and extended to include a blood plasma compartment. We use this model to study how the concentrations of metabolites that can be measured in the plasma are related to their respective intracellular concentrations. *Methods*: The model consists of a set of ordinary differential equations, one for each metabolite in each compartment, and kinetic equations for metabolism and for transport between compartments. The model was validated by comparison to a variety of experimental data such as the methionine load test and variation in folate intake. We further extended this model by introducing random and systematic variation in enzyme activity. *Outcomes and Conclusions*: A database of 10,000 virtual individuals was generated, each with a quantitatively different one-carbon metabolism. Our population has distributions of folate and homocysteine in the plasma and tissues that are similar to those found in the NHANES data. The model reproduces many other sets of clinical data. We show that tissue and plasma folate is highly correlated, but liver and plasma folate much less so. Oxidative stress increases the plasma *S*-adenosylmethionine/*S*-adenosylhomocysteine (SAM/SAH) ratio. We show that many relationships among variables are nonlinear and in many cases we provide explanations. Sampling of subpopulations produces dramatically different apparent associations among variables. The model can be used to simulate populations with polymorphisms in genes for folate metabolism and variations in dietary input.

Mato JM, et al. *Ann Hepatol.* 2015;12(2):183-89.

Duncan TM, et al. *Nutrients.* 2013;5(7):2457-74.

Why Haven't We Been Measuring SAM & SAH?

- Plasma concentrations are around 1/500th of Hcy
- Need specialized technology to accurately measure SAM and SAH
  - More labs are developing the capacity to measure these
- Stability concerns:
  - SAM & SAH are very unstable molecules
  - Have to be diligent about preserving and handling of samples

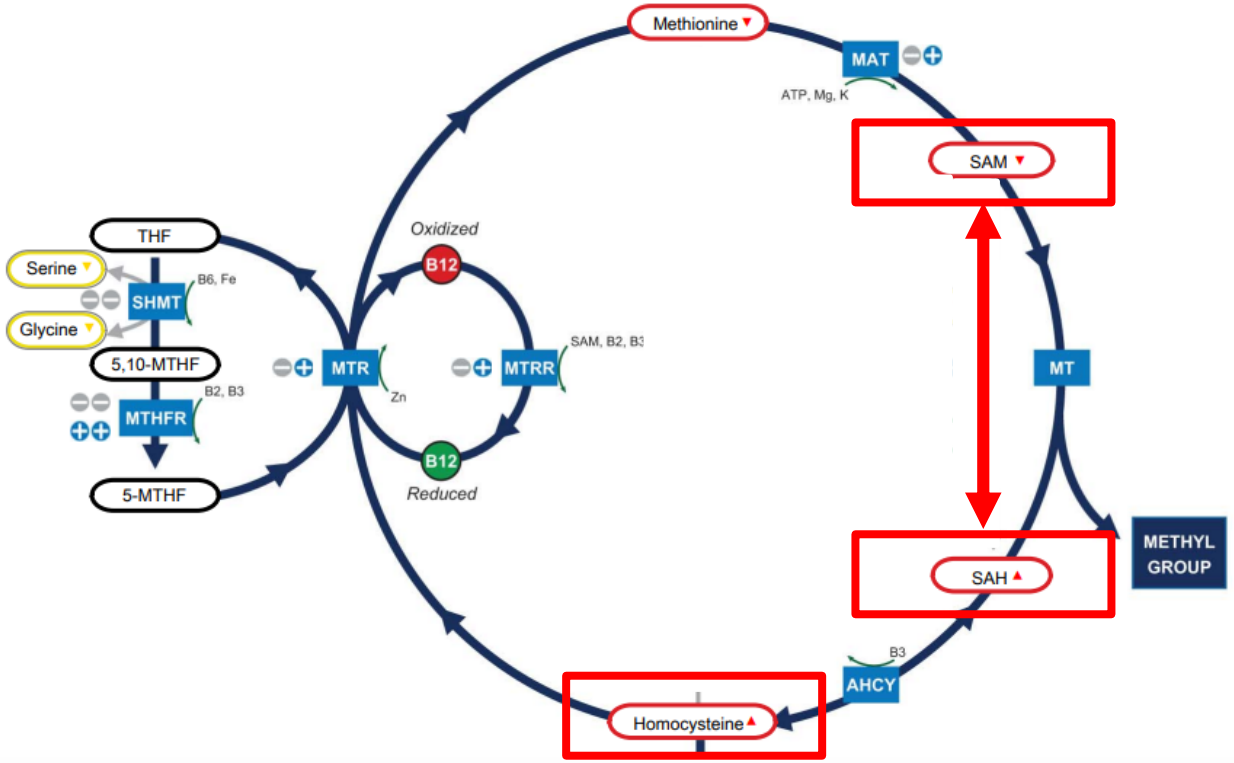
Let’s Do a Case!

75 yo Female: “Beth”

- Longstanding pernicious anemia
- Standard American Diet
  - Coffee for breakfast
  - Maybe eats some toast and bacon around lunchtime, or eats fast food
  - Processed microwave dinners
  - Regular nightly wine intake
- Depression/Anxiety
- Osteopenia
- Fatigue
- Osteoarthritis

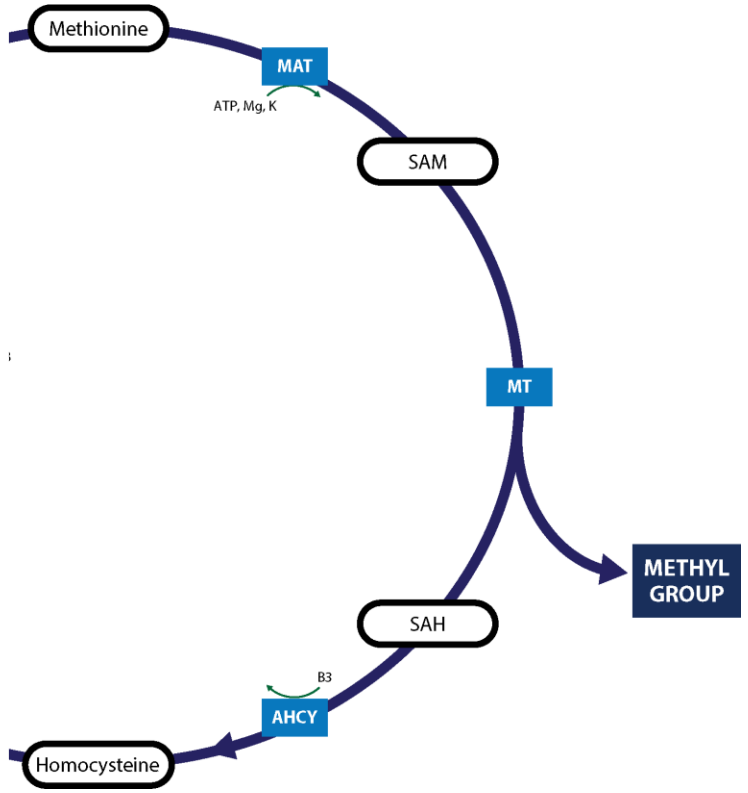
Report Results

- Where to start?
  - Low SAM: main methyl donor
  - High SAH: marker for CVD risk and poor methylation recycling
  - SAM/SAH Ratio
- Homocysteine
  - Elevated
- Even methionine is low



Treatment Considerations

- In this patient there is an obvious backup in SAH/homocysteine recycling
- Main way to recycle SAM is by turning Hcy back into methionine
- Consider:
  - Activated Folate (5-MTHF)
  - Activated B-12
  - Zinc



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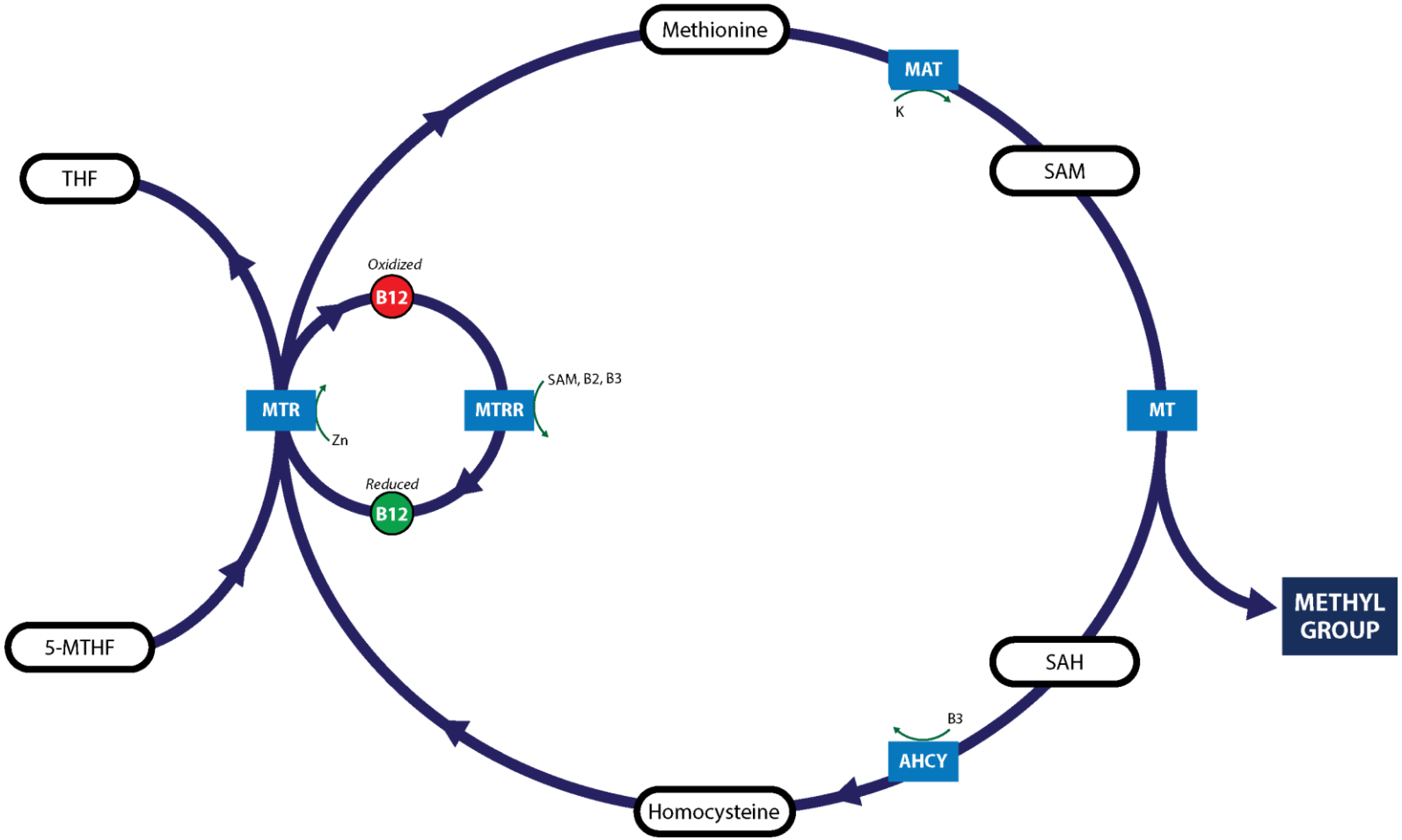
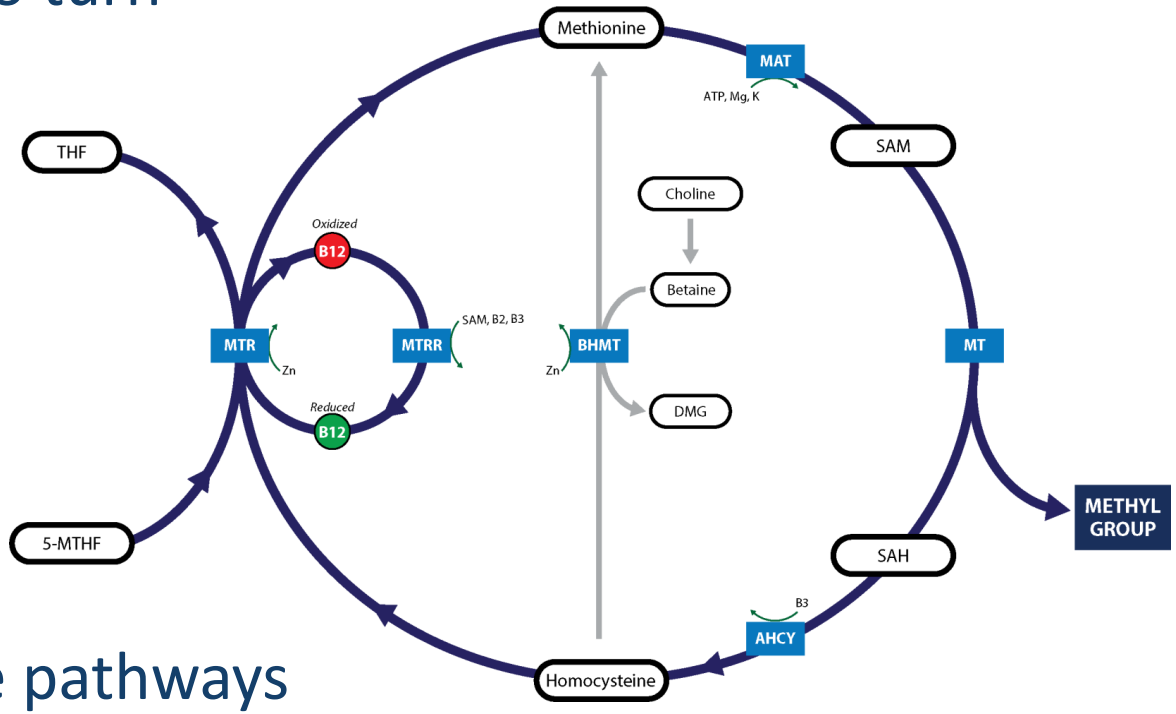
Adding Another Layer



Methylation Back-up Pathway to the Rescue!

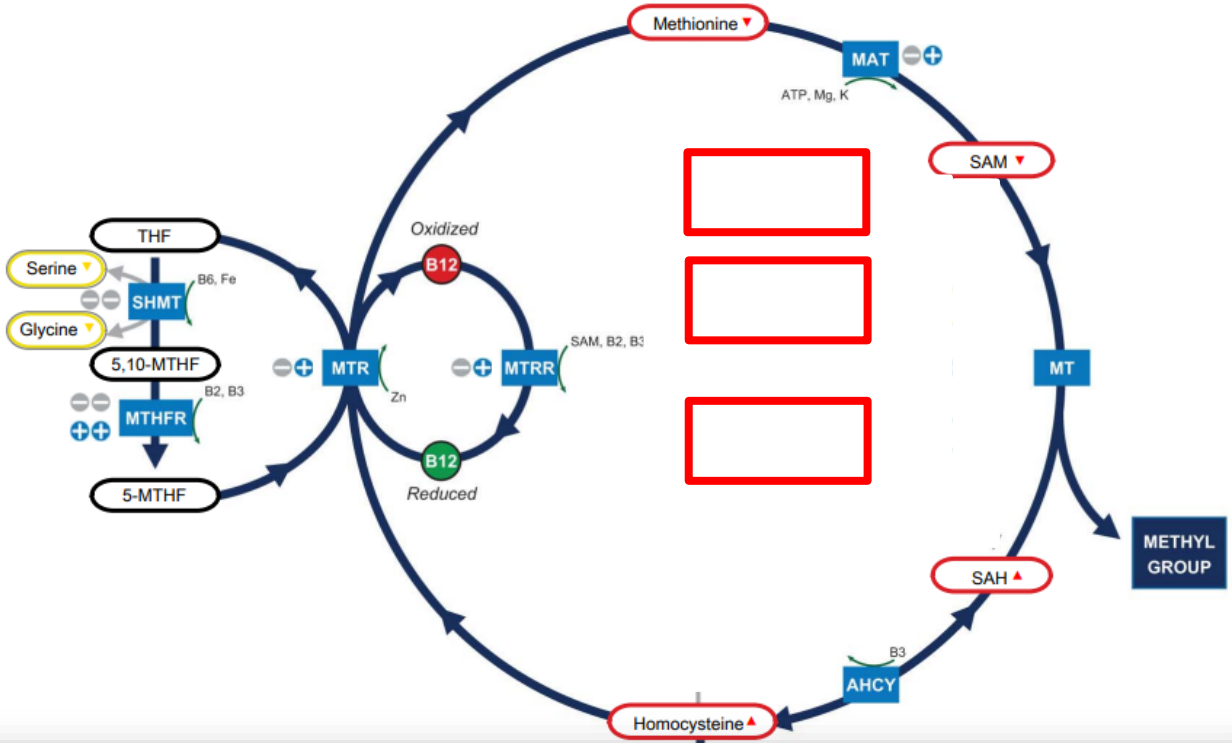
- Betaine (tri***methyl***glycine) can be used to turn homocysteine back into methionine
- This only takes place in the liver
  - Main pathway happens everywhere
- Betaine is derived from dietary choline
  - Meats, eggs, and beets
- Shunts choline AWAY from other choline pathways
  - Acetylcholine, cell membrane repair, etc.



Obeid R. *Nutrients.* 2013;5(9):3481-3495.

Report Results

- What about the backup pathway in this patient?
  - DMG is an independent indicator of BHMT (backup pathway) utilization
    - Here DMG is normal
  - Betaine is low
  - Betaine ultimately comes from choline
    - Here choline is normal, but…

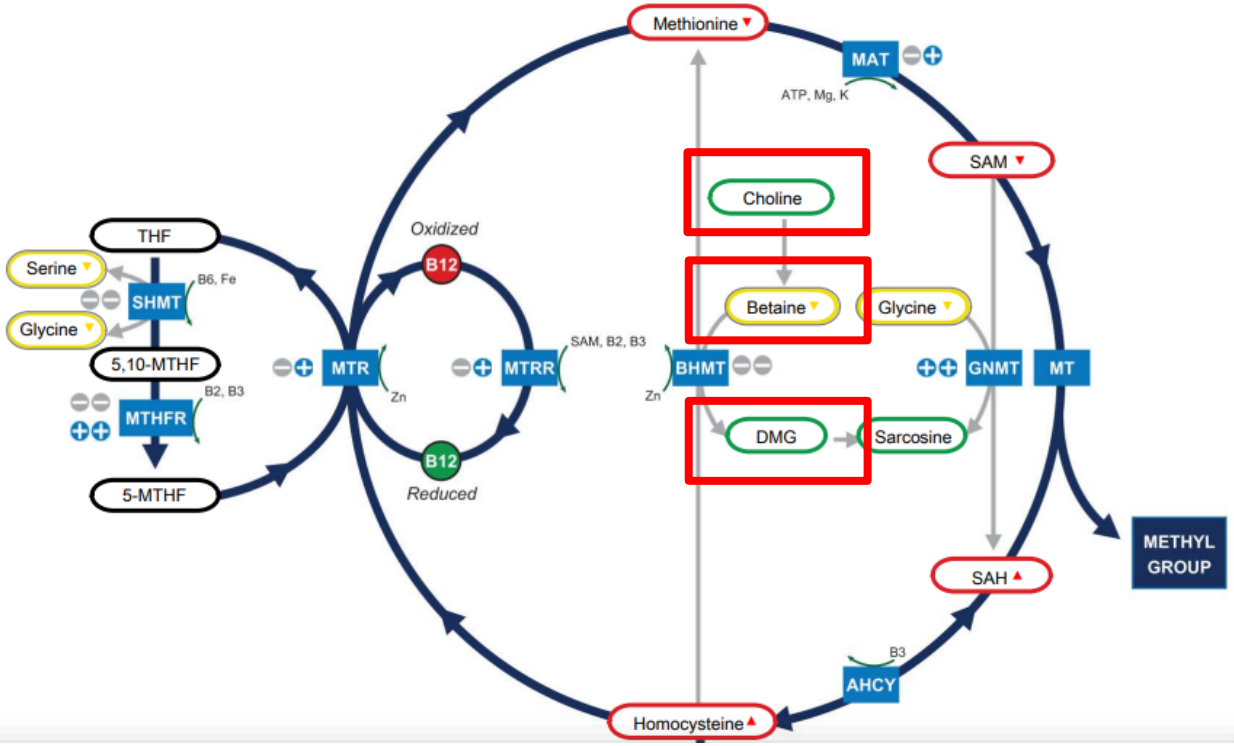


Ratios

1.	Methylation Index (SAM/SAH Ratio)	0.8	L	2.2-6.4
2.	Methylation Balance Ratio	0.93	L	1.03-1.20
3.	Met/Sulf Balance Ratio	0.60		0.55-0.64
4.	Betaine/Choline Ratio	3.9		2.6-7.7

Report Results

- What does all this tell me?
  - Despite the fact that there is obvious poor methylation recycling…
  - The backup pathway is not compensating like it should
- Consider betaine (TMG)



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The Dance of Betaine & Choline

- Novel biomarkers

- Choline and betaine are “quaternary” ammonium compounds
  - Choline: Eggs, beef, pork, liver, soybean, and wheat germ
  - Betaine: Wheat bran, wheat germ, spinach, and beets

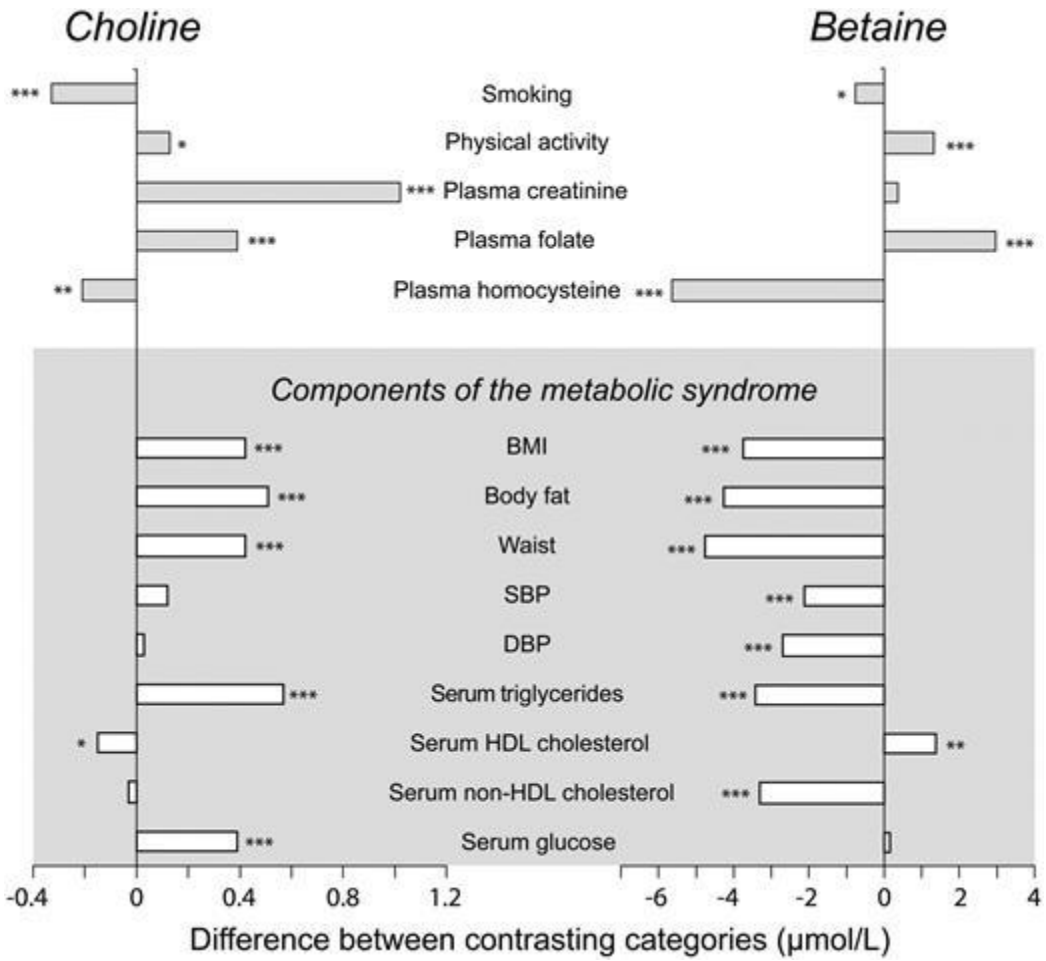
- Choline is used for:
  - Epigenetic gene regulation
  - Precursor to lipoproteins
  - Phospholipids
  - Acetylcholine

- Betaine is used for:
  - Methylation
  - Osmolyte, under cell stress (mainly in kidneys)

Betaine/Choline

- Choline is a major lipotrophe, responsible for creating VLDL
  - Elevated plasma choline is positively associated with:
    - Triglycerides
    - Glucose
    - BMI
    - Body fat
    - Waist circumference

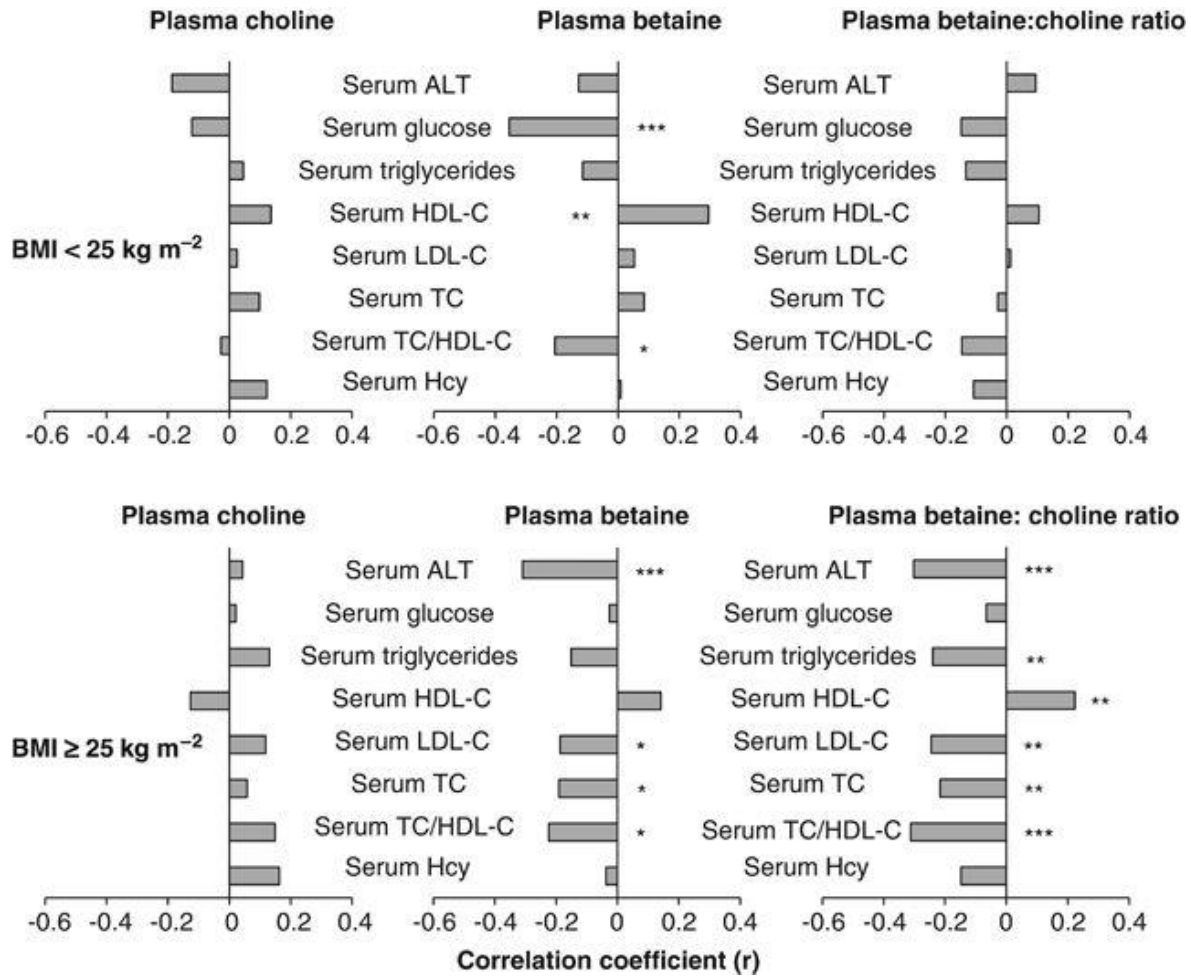
- Plasma betaine is *negatively* associated with the majority of these risk factors



https://academic.oup.com/jn/article/138/5/914/4670139

Research on the Betaine/Choline Ratio

- Plasma betaine:choline is statistically significant with almost every aspect of metabolic stress
  - Low betaine, high choline is a risk



https://www.nature.com/articles/nutd201223

Methylation Genomics (SNPs)

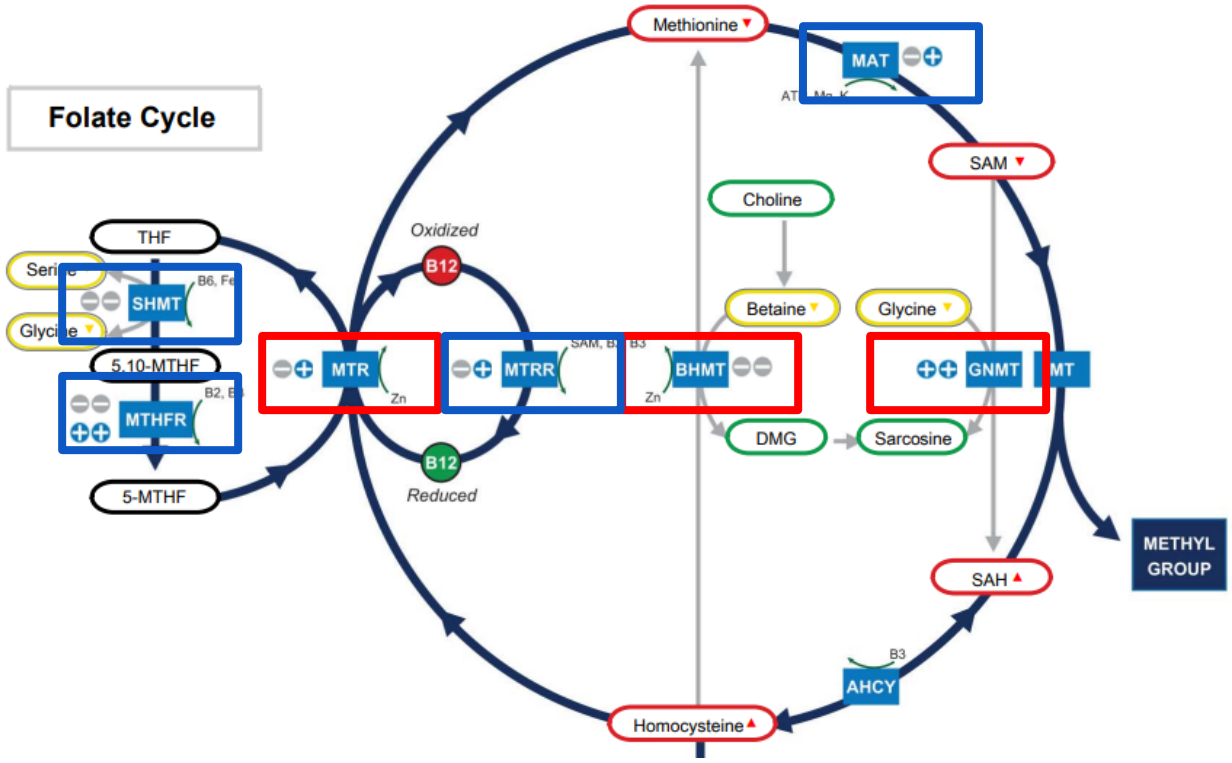
How to appropriately apply genomic information clinically

Single-Nucleotide Polymorphisms (SNPs)

- Common genetic variants
  - Most of us have millions of SNPs
- These SNPs may alter the activity of a particular enzyme
  - Upregulate
  - Downregulate
- Genes do NOT make your destiny!

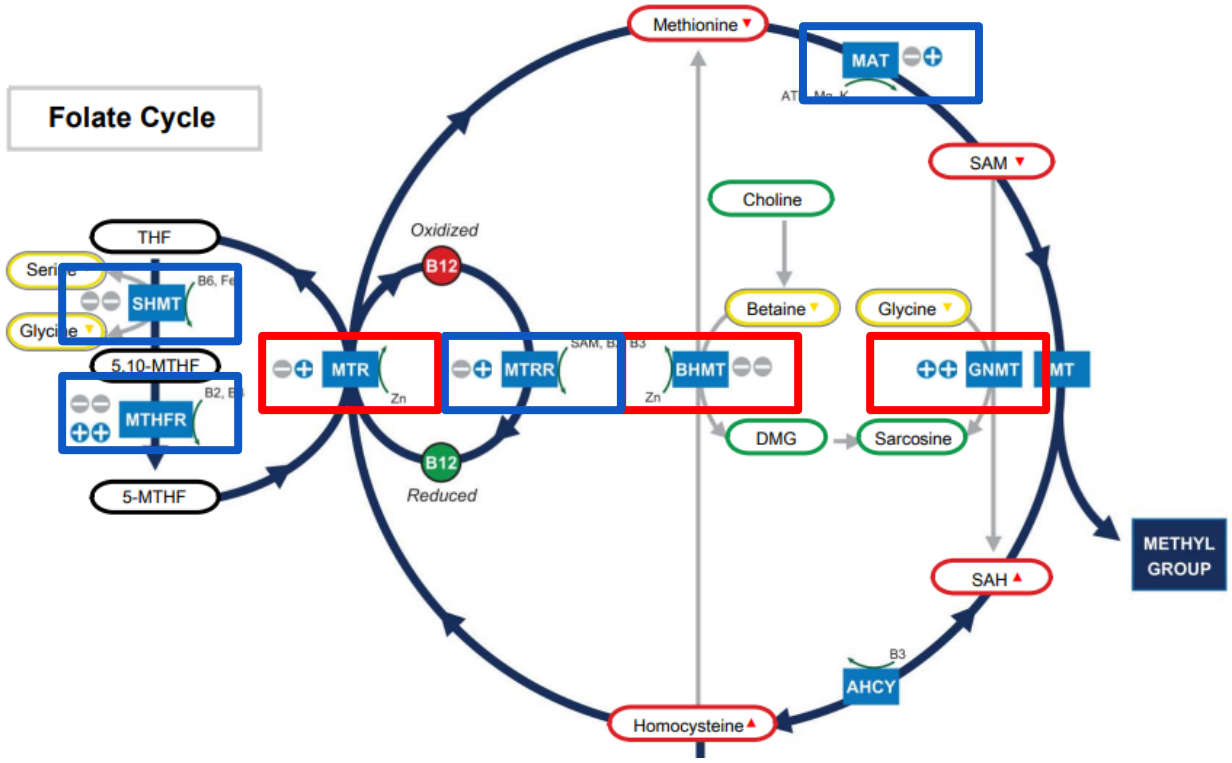
Methylation Pathway Genomics

- Remember which SNPs upregulate vs. downregulate
  - Three in the middle upregulate
  - All the rest downregulate
- Remember, SNPs are just a predisposition
  - MTR: actual upregulation of homocysteine conversion
  - BHMT: patient has tendency to under-utilize the backup pathway (makes sense with results)
  - GNMT: patient has tendency to dispose of SAM and make more SAH



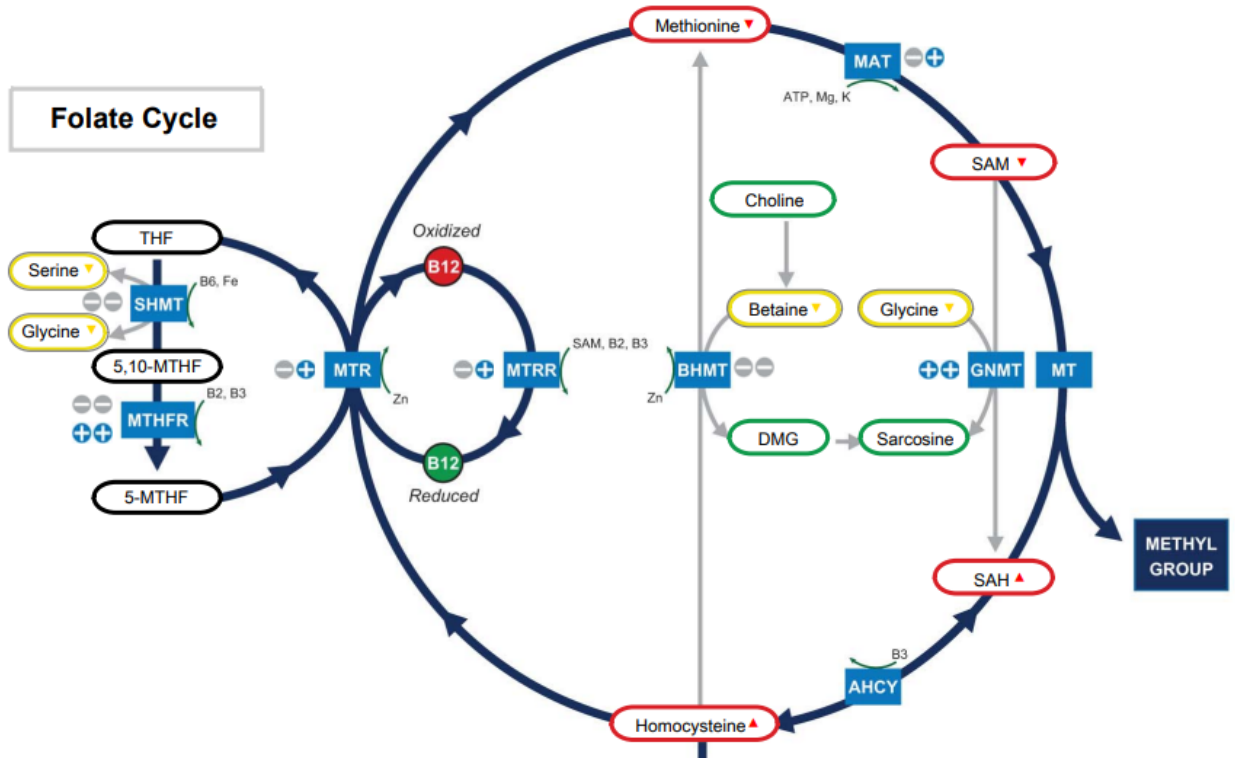
Methylation Pathway Genomics

- SNPs that downregulate:
  - MTHFR A1298C: reduced conversion to activated folate
    - Clinically consider reaching for your 5-MTHF
  - MTRR: predisposition toward slow repair of oxidized (inactive) B12
  - MAT1A: predisposition toward slow conversion of methionine to SAM
    - Consider short-term application of SAMe supplementation?

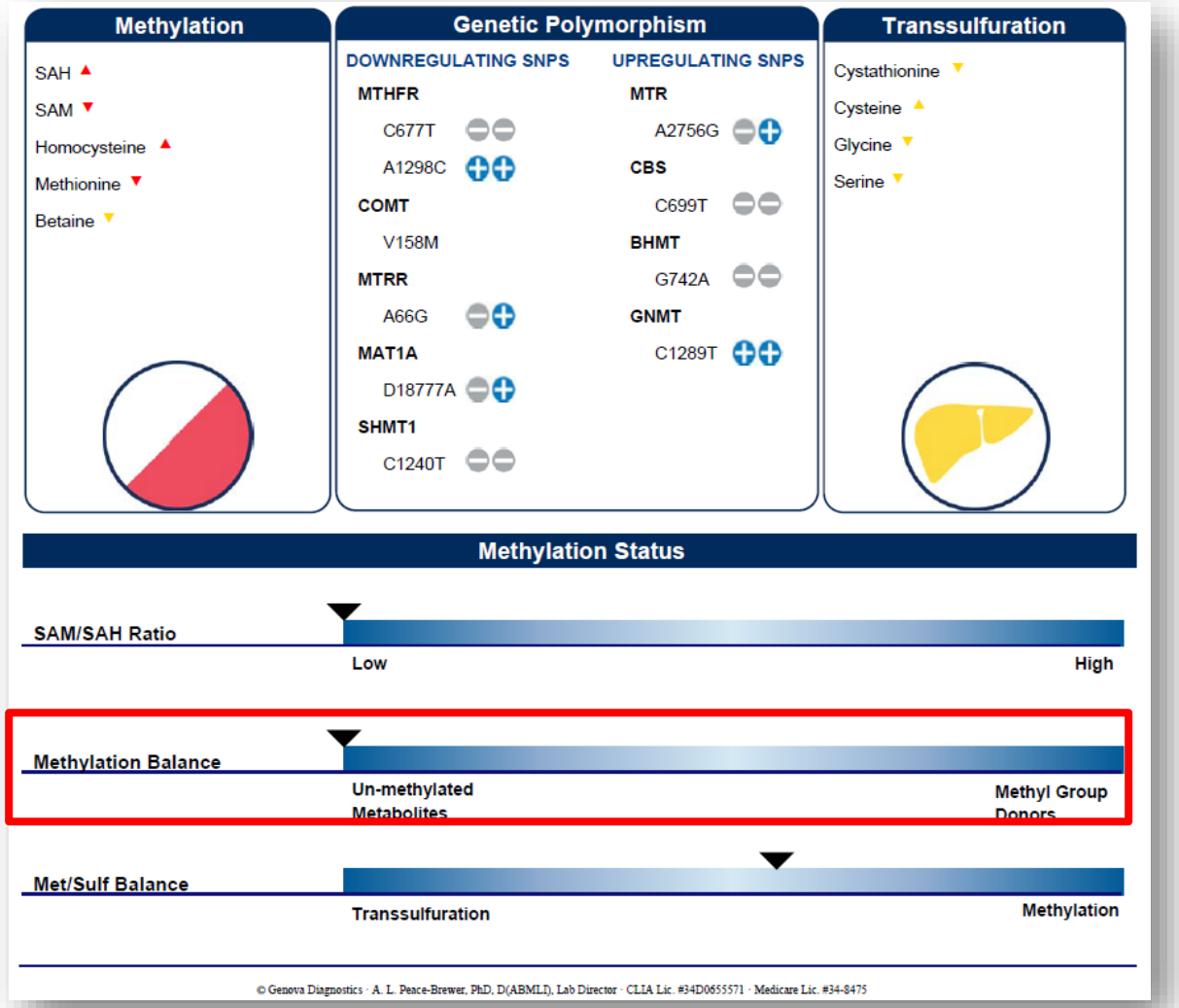


Case Analysis Thus Far

- Therapeutic Interventions:
  - Folate (consider 5-MTHF d/t MTHFR SNP)
  - B12 (consider methylcobalamin in this case)
  - Betaine to support backup pathway
  - May also consider SAMe short-term
  - Glycine?...to be continued



Methylation Balance Ratio

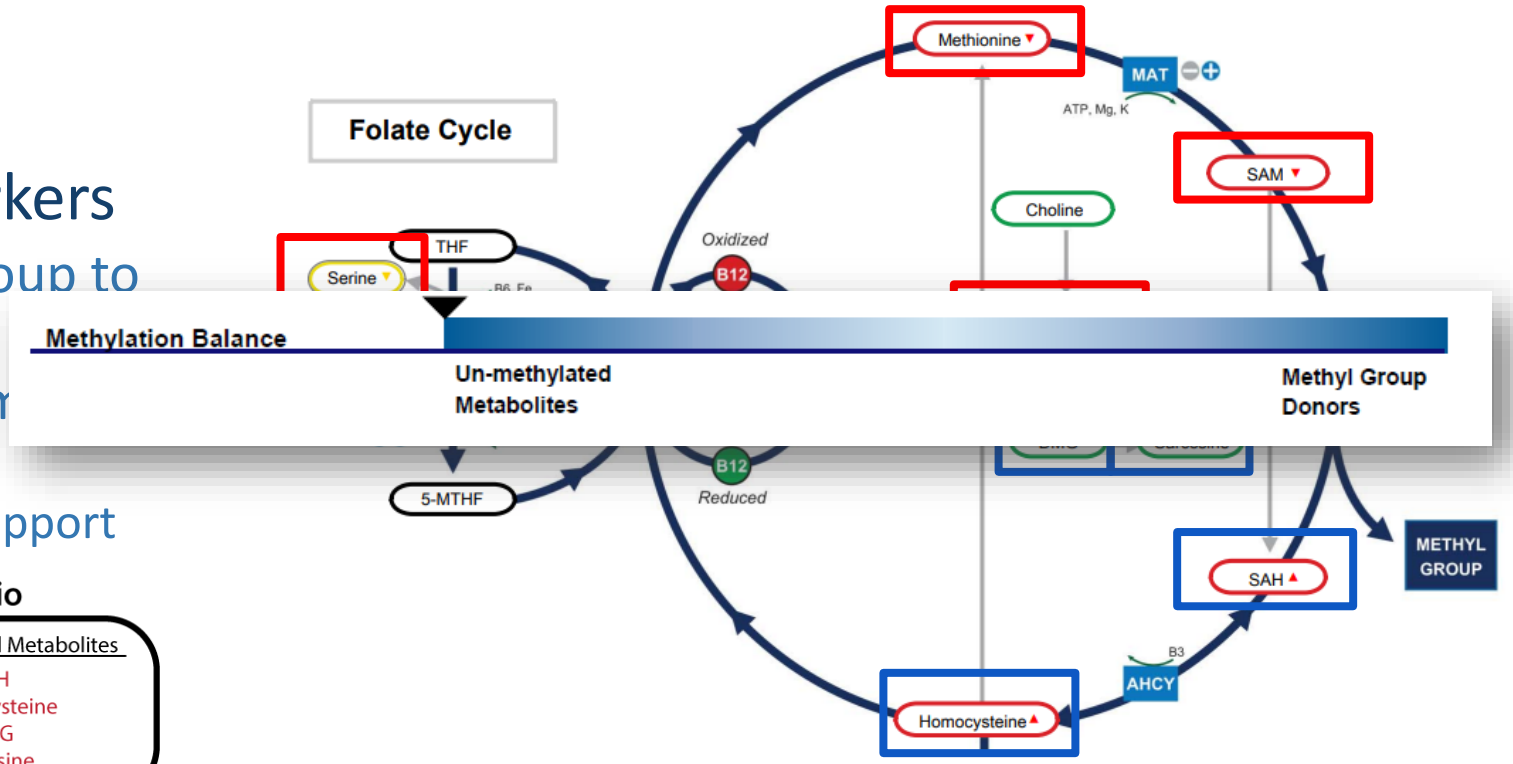


The Methylation Balance Ratio

- Novel functional assessment for overall methylation balance
- Compares 8 different biomarkers
  - 4 biomarkers with a methyl group to give
  - 4 biomarkers that have had a m group removed
    - An explanation will be in the support guide

Methylation Balance Ratio

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



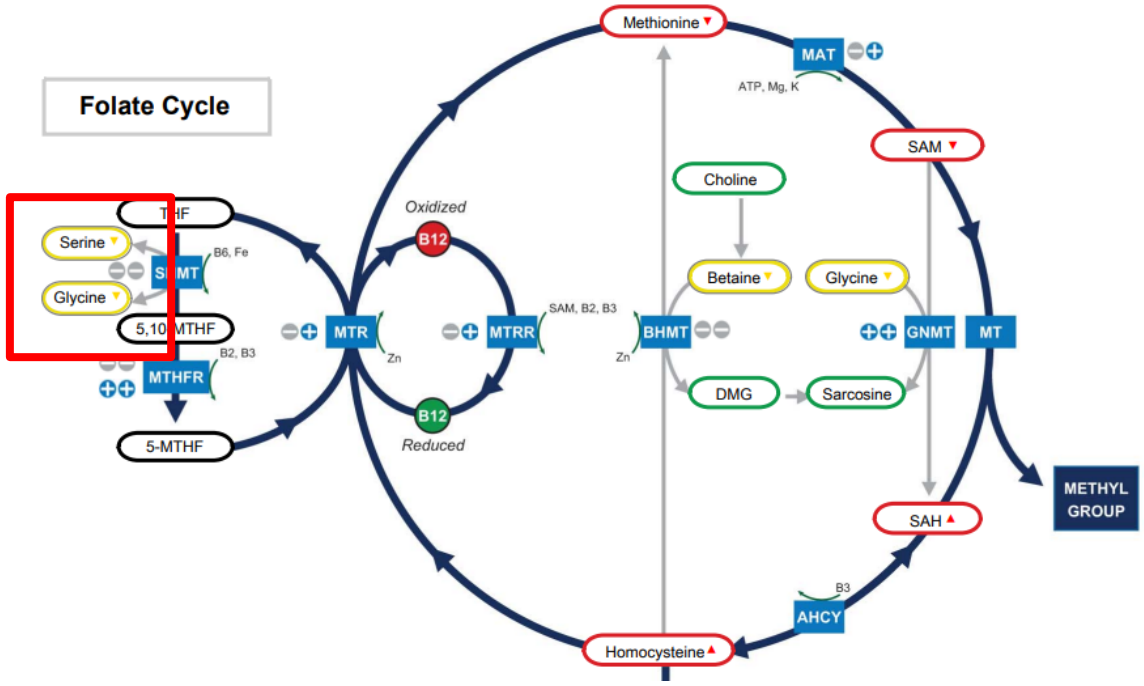
- This ratio is unique to GDX and is being researched

Mato JM, et al. *Ann Hepatol.* 2015;12(2):183-189.

Folate Analytes – Glycine/Serine

- These analytes are tricky because they are involved in so many different biochemical pathways

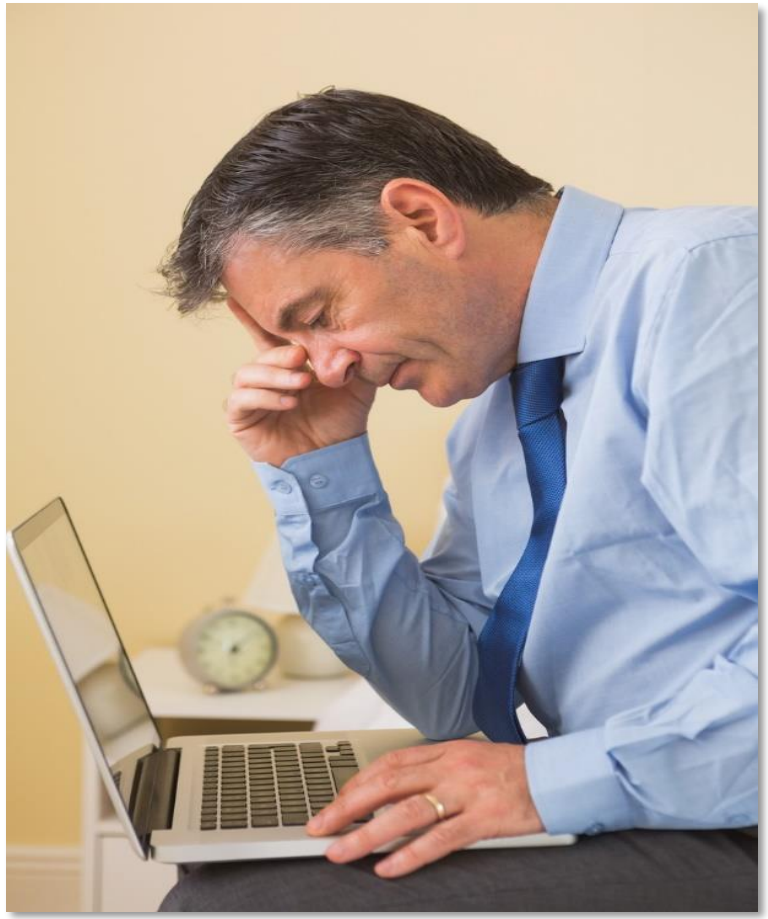
- Glycine:
  - Needed as glutathione precursor
  - Needed for SAM disposal
- Serine:
  - Primarily used in methylation balance calculation



Let’s Do Another Case!

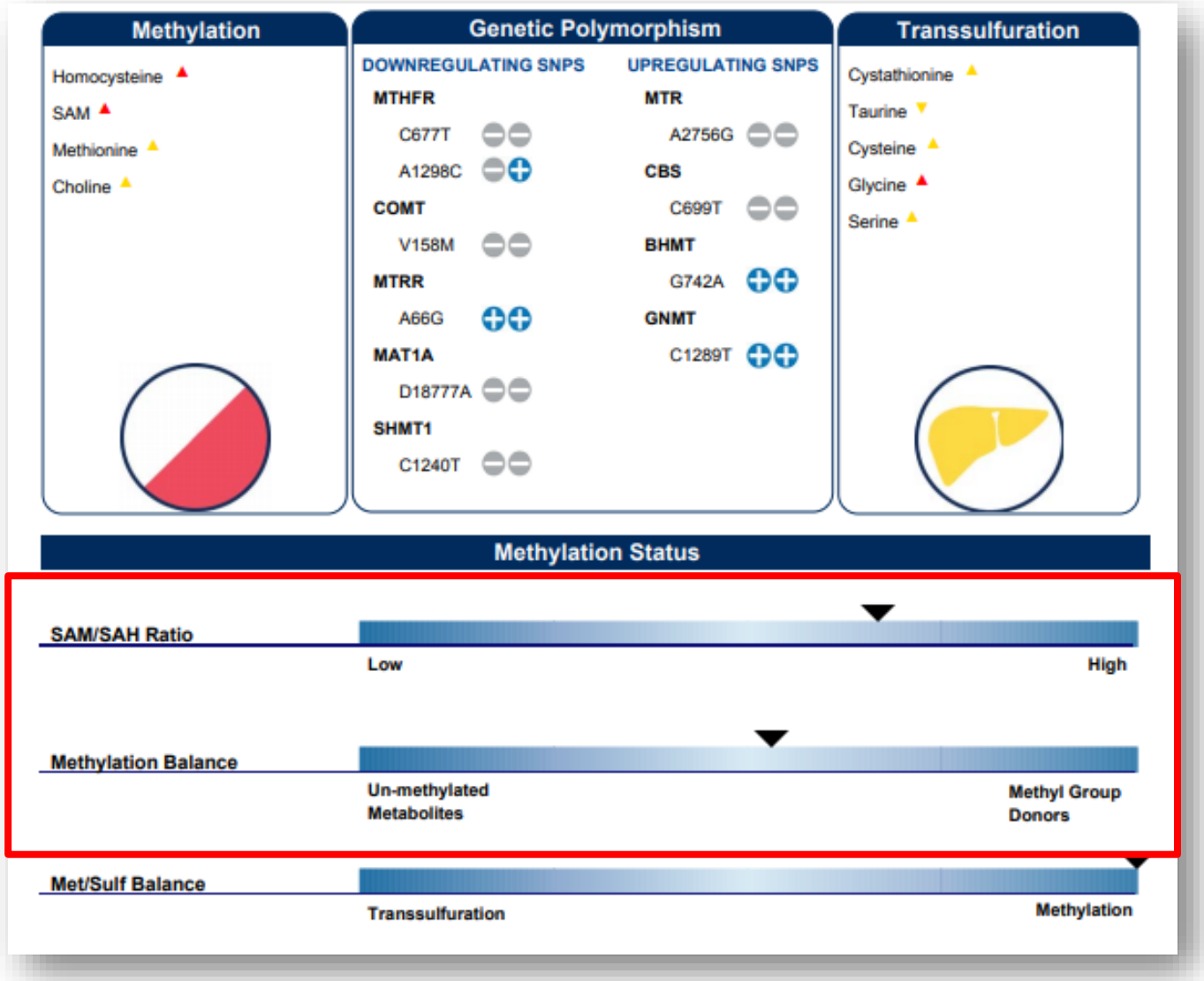
55 yo Male: “George”

- 10 year hx obesity
- Began Paleo Diet 3 mo ago for weight loss
  - Very high meat intake (not particular about quality of meat)
  - Very low complex carbohydrates/Low fiber
- Has been exercising 3-4x per week
  - Mostly anaerobic
- Family History
  - Cardiovascular Disease
  - Alcoholism



Let's Look at the Pathway

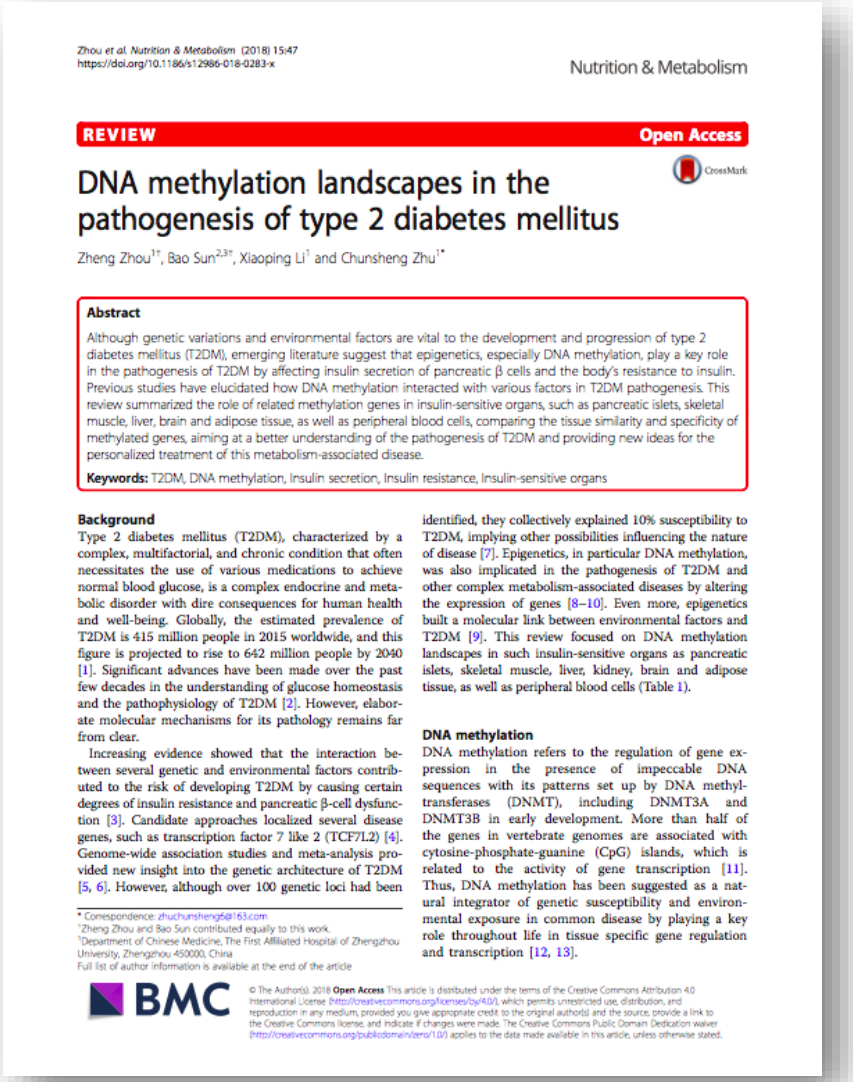
- High SAM/High Methionine
- High Homocysteine
- High Choline, Serine, Glycine
- A lot of these elevations correlate to both *high protein* and *overweight/obesity*



Over-Methylation and Plasma SAM/SAH

- Many articles report that higher levels of SAM are associated with…
  - Higher BMI
  - Higher protein intake
  - Higher caloric intake
  - Overweight/Obesity

- Is metabolic syndrome an over-methylation disorder?



Zhou et al. Nutrition & Metabolism (2018) 15:47
https://doi.org/10.1186/s12986-018-0283-x

Nutrition & Metabolism

REVIEW Open Access

DNA methylation landscapes in the pathogenesis of type 2 diabetes mellitus

Zheng Zhou[1†], Bao Sun[2,3†], Xiaoping Li[1] and Chunsheng Zhu[1*]

Abstract

Although genetic variations and environmental factors are vital to the development and progression of type 2 diabetes mellitus (T2DM), emerging literature suggest that epigenetics, especially DNA methylation, play a key role in the pathogenesis of T2DM by affecting insulin secretion of pancreatic β cells and the body's resistance to insulin. Previous studies have elucidated how DNA methylation interacted with various factors in T2DM pathogenesis. This review summarized the role of related methylation genes in insulin-sensitive organs, such as pancreatic islets, skeletal muscle, liver, brain and adipose tissue, as well as peripheral blood cells, comparing the tissue similarity and specificity of methylated genes, aiming at a better understanding of the pathogenesis of T2DM and providing new ideas for the personalized treatment of this metabolism-associated disease.

Keywords: T2DM, DNA methylation, Insulin secretion, Insulin resistance, Insulin-sensitive organs

Background

Type 2 diabetes mellitus (T2DM), characterized by a complex, multifactorial, and chronic condition that often necessitates the use of various medications to achieve normal blood glucose, is a complex endocrine and metabolic disorder with dire consequences for human health and well-being. Globally, the estimated prevalence of T2DM is 415 million people in 2015 worldwide, and this figure is projected to rise to 642 million people by 2040 [1]. Significant advances have been made over the past few decades in the understanding of glucose homeostasis and the pathophysiology of T2DM [2]. However, elaborate molecular mechanisms for its pathology remains far from clear.

Increasing evidence showed that the interaction between several genetic and environmental factors contributed to the risk of developing T2DM by causing certain degrees of insulin resistance and pancreatic β-cell dysfunction [3]. Candidate approaches localized several disease genes, such as transcription factor 7 like 2 (TCF7L2) [4]. Genome-wide association studies and meta-analysis provided new insight into the genetic architecture of T2DM [5, 6]. However, although over 100 genetic loci had been identified, they collectively explained 10% susceptibility to T2DM, implying other possibilities influencing the nature of disease [7]. Epigenetics, in particular DNA methylation, was also implicated in the pathogenesis of T2DM and other complex metabolism-associated diseases by altering the expression of genes [8–10]. Even more, epigenetics built a molecular link between environmental factors and T2DM [9]. This review focused on DNA methylation landscapes in such insulin-sensitive organs as pancreatic islets, skeletal muscle, liver, kidney, brain and adipose tissue, as well as peripheral blood cells (Table 1).

DNA methylation

DNA methylation refers to the regulation of gene expression in the presence of impeccable DNA sequences with its patterns set up by DNA methyltransferases (DNMT), including DNMT3A and DNMT3B in early development. More than half of the genes in vertebrate genomes are associated with cytosine-phosphate-guanine (CpG) islands, which is related to the activity of gene transcription [11]. Thus, DNA methylation has been suggested as a natural integrator of genetic susceptibility and environmental exposure in common disease by playing a key role throughout life in tissue specific gene regulation and transcription [12, 13].

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Full list of author information is available at the end of the article

Over-Methylation: Is it a Thing?

S/Sx associated with over-methylation:

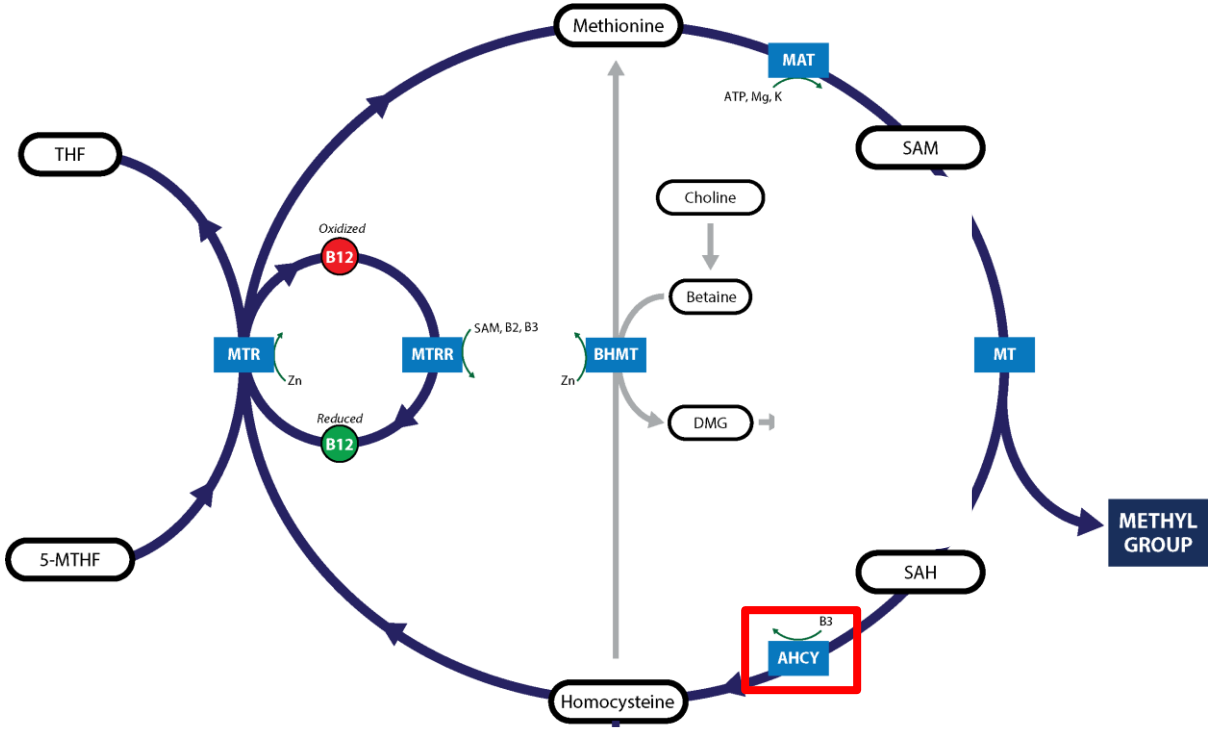
- Anxiety
- Depression
- Panic attacks
- Attention deficit hyperactivity disorder (ADHD)
- Behavior disorders
- Sleep disorders
- Restlessness
- Schizophrenia

- How does it happen?
  - Taking too much folate
  - Enzyme SNPs that upregulate remethylation of homocysteine
- Potential causes:
  - High SAM levels altering neurotransmitter metabolism?
- This has not been fully studied

Over-Methylation: Is it a Thing?

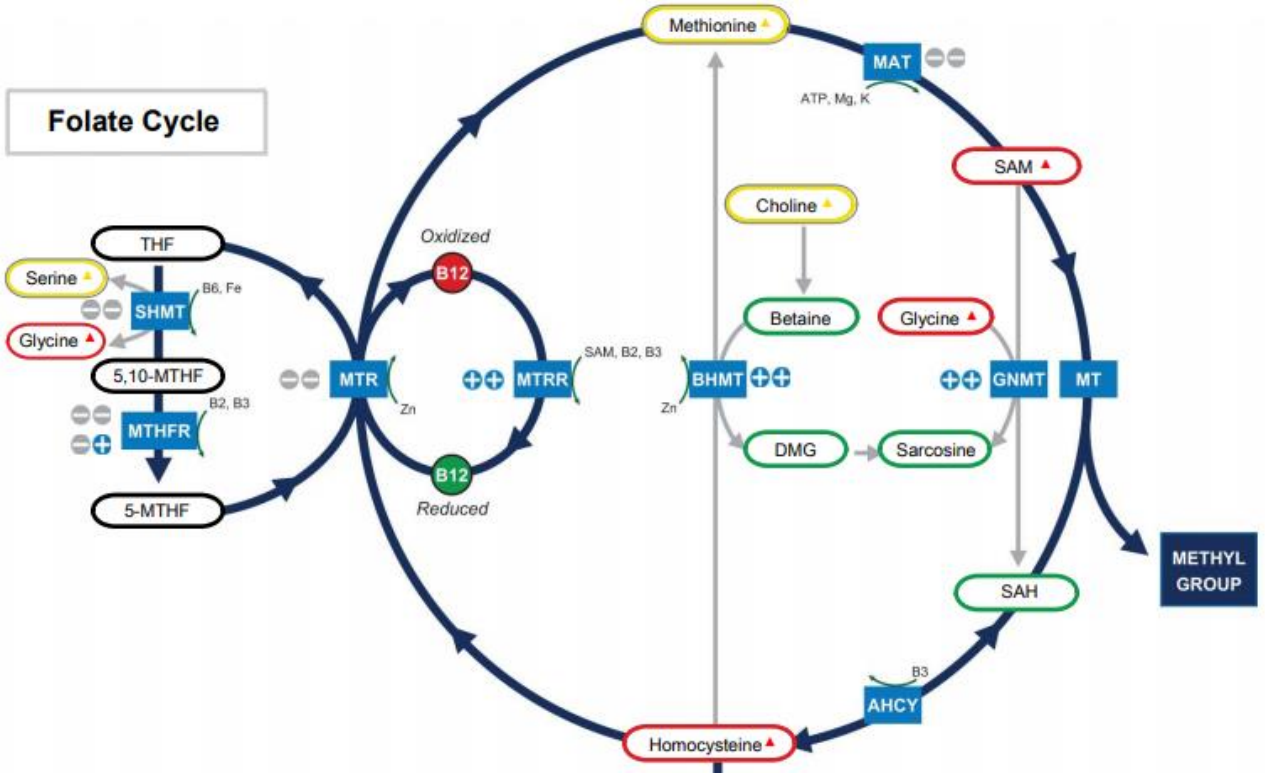
- What to look for on lab testing
  - Low Homocysteine (<2umol/L)
  - High SAM/SAH
  - High Sarcosine?

- Common clinical intervention:
  - Niacin
  - Glycine
  - Vitamin B-6



Therapeutic considerations

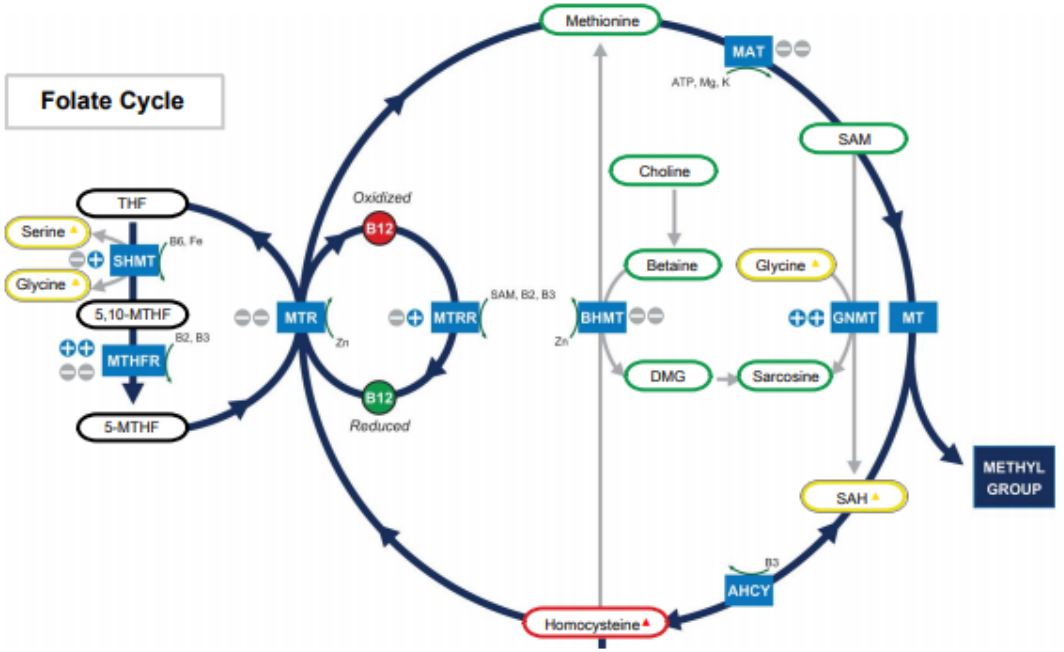
- Considering his diet and his results:
  - Ensure getting adequate dietary B-vitamins
    - Especially vitamin B12
  - Consider supplementation with Zn
    - SNP toward BHMT upregulation
- Reduce protein intake?
  - Betaine Supplementation?



4. Betaine/Choline Ratio	3.8		2.6-7.7

Let's Look at One More

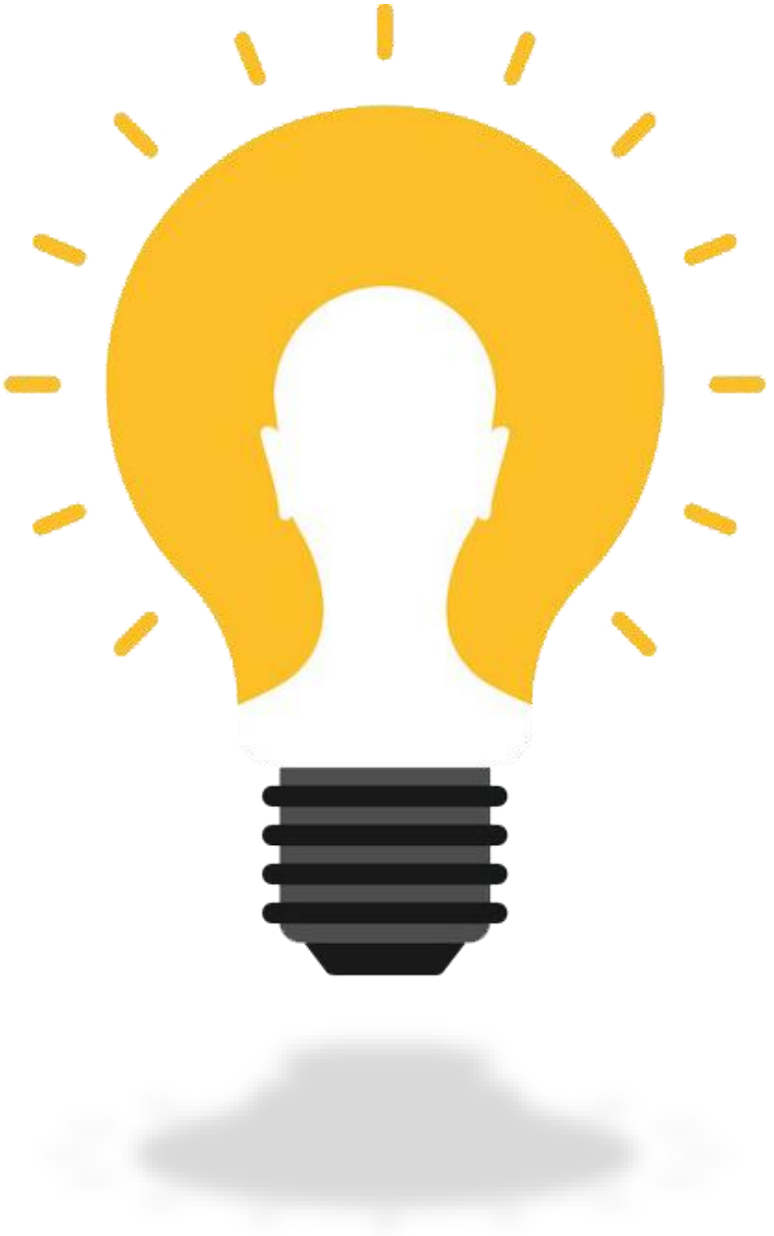
- High homocysteine
- Borderline high SAH
- SAM and methionine look good
  - SAM/SAH Ratio & Methylation Balance on the low side
- So what do we consider?
  - Transsulfuration support
  - Minerals: Mg, Zn
  - Vitar



Ratios			
1. Methylation Index (SAM/SAH Ratio)	3.2		2.2-6.4
2. Methylation Balance Ratio	1.09		1.03-1.20

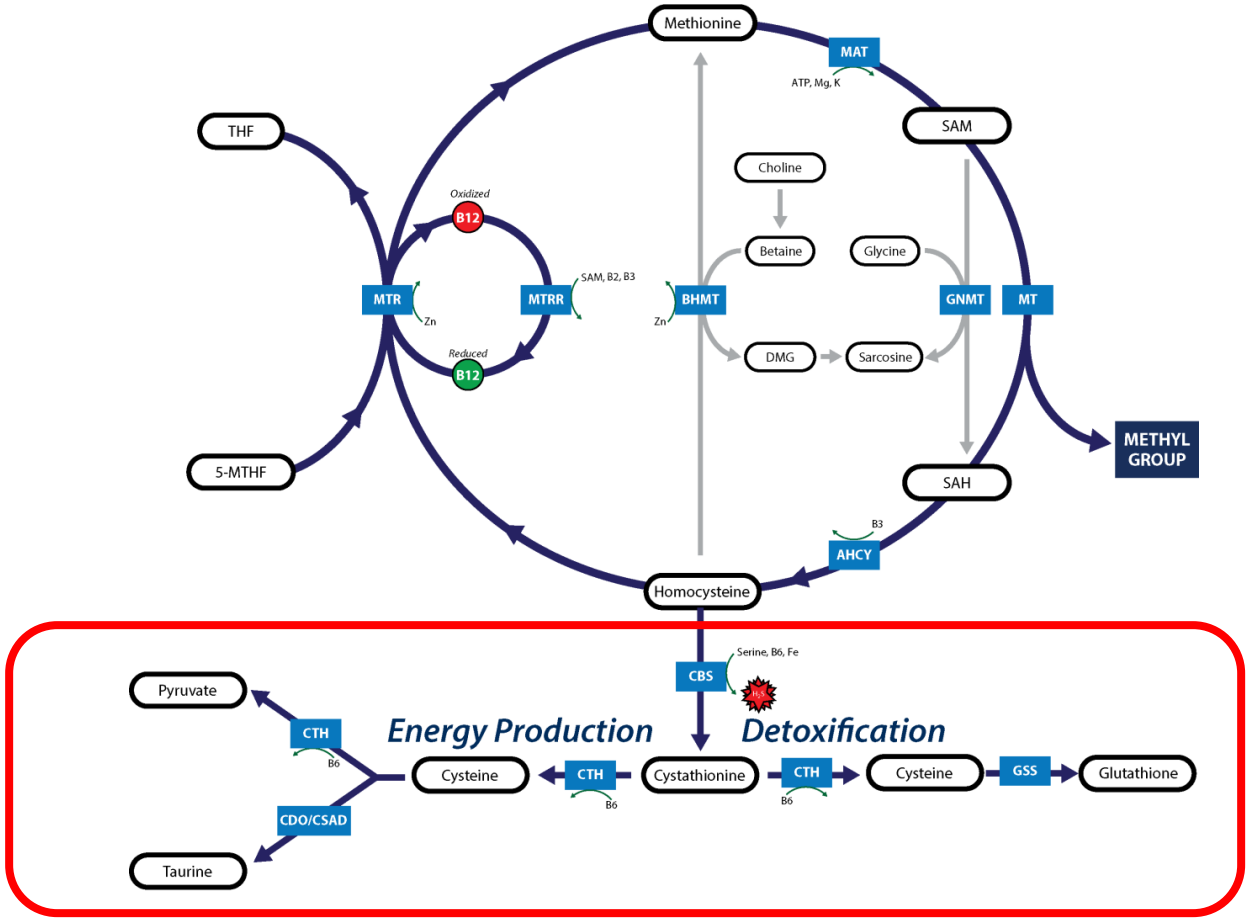
Transsulfuration

Now that you are all experts…



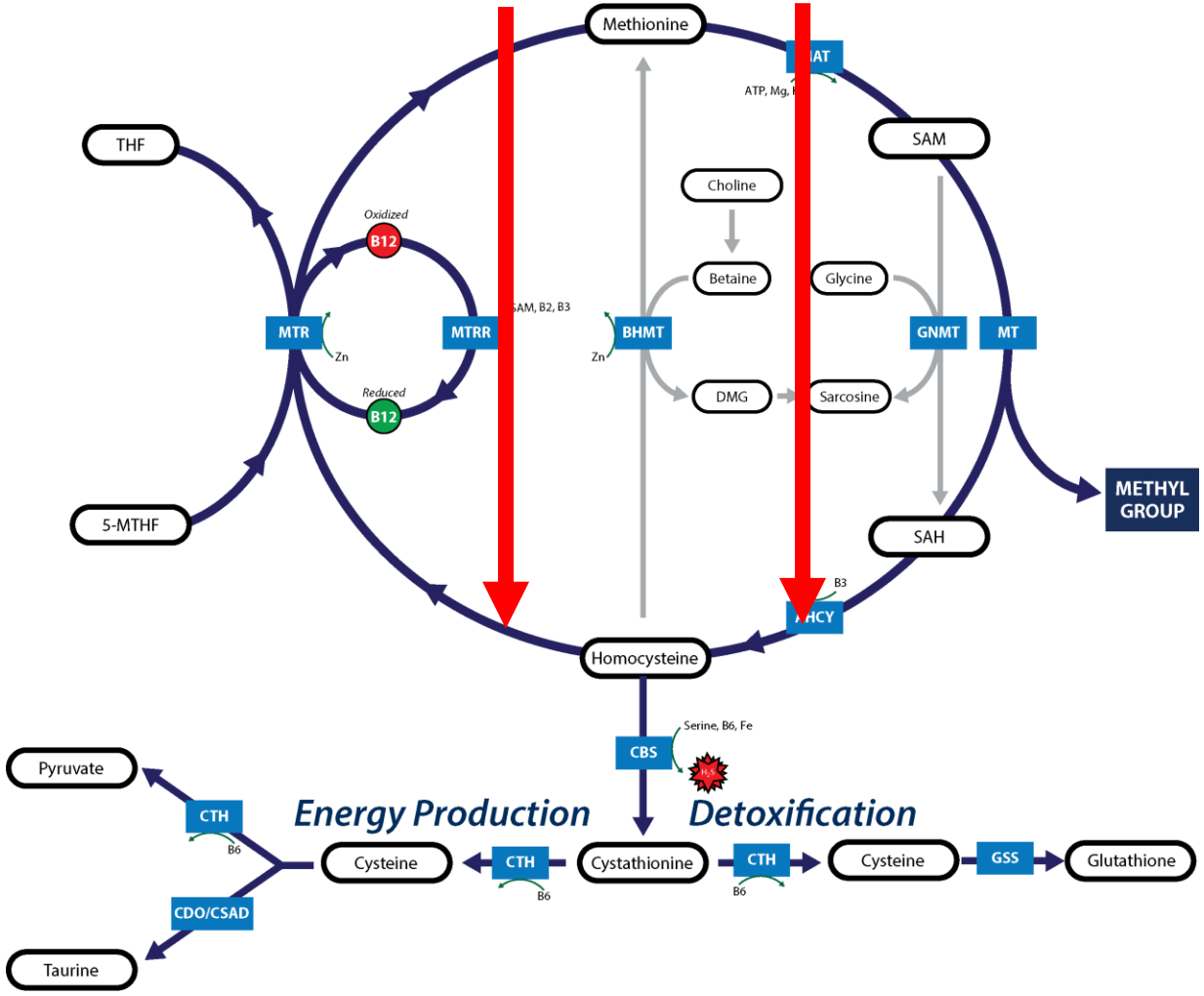
2. Moving to Transsulfuration

- What do we see under transsulfuration?
  - Glutathione
    - One of the body's *most* powerful *antioxidants*
  - Pyruvate
    - Cellular energy
  - Taurine



Transsulfuration

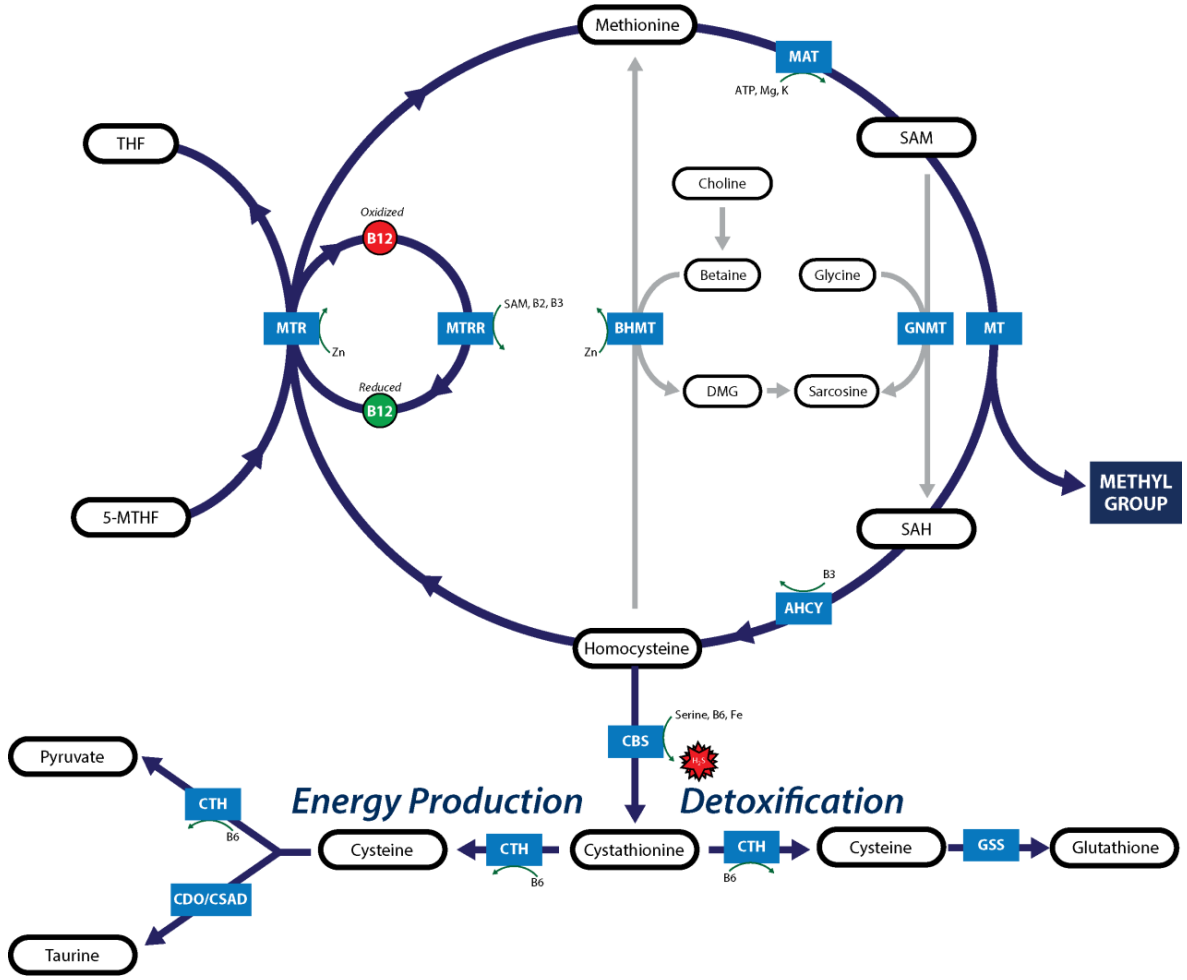
- **Key takeaway!**
- Transsulfuration is upregulated by two critical factors:
- Higher SAM levels
- Oxidative Stress



Stabler SP, et al. *Blood.* 1993;81(12):3404-3413.

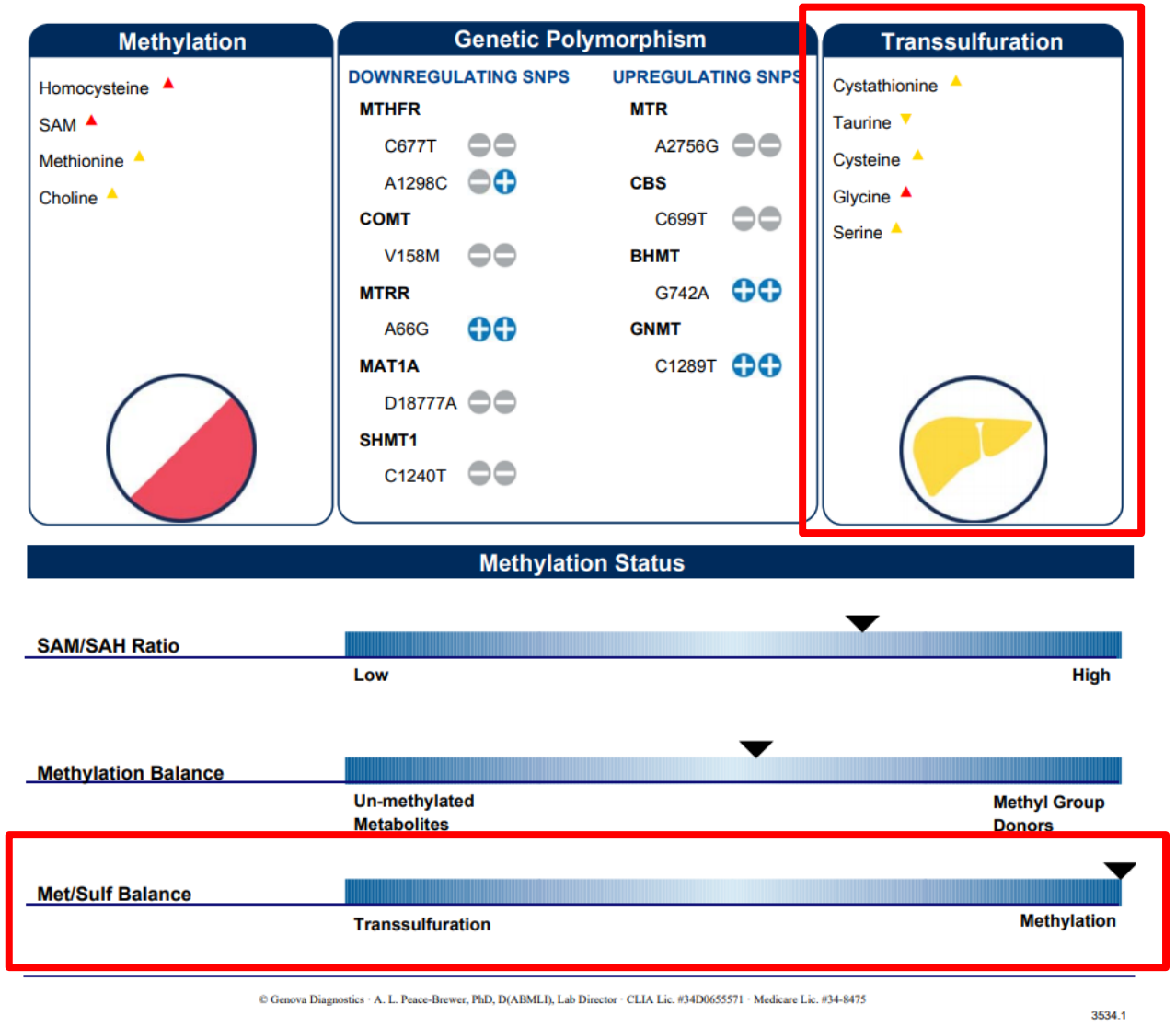
Transsulfuration

- Keep in mind:
- SAM availability determines the rate that homocysteine goes through transsulfuration
- Translation…
  - Glutathione production will not take priority over creating SAM for methylation reactions
- Low SAM → Low Glutathione



Stabler SP, et al. *Blood.* 1993;81(12):3404-3413.

Transsulfuration Interpretation



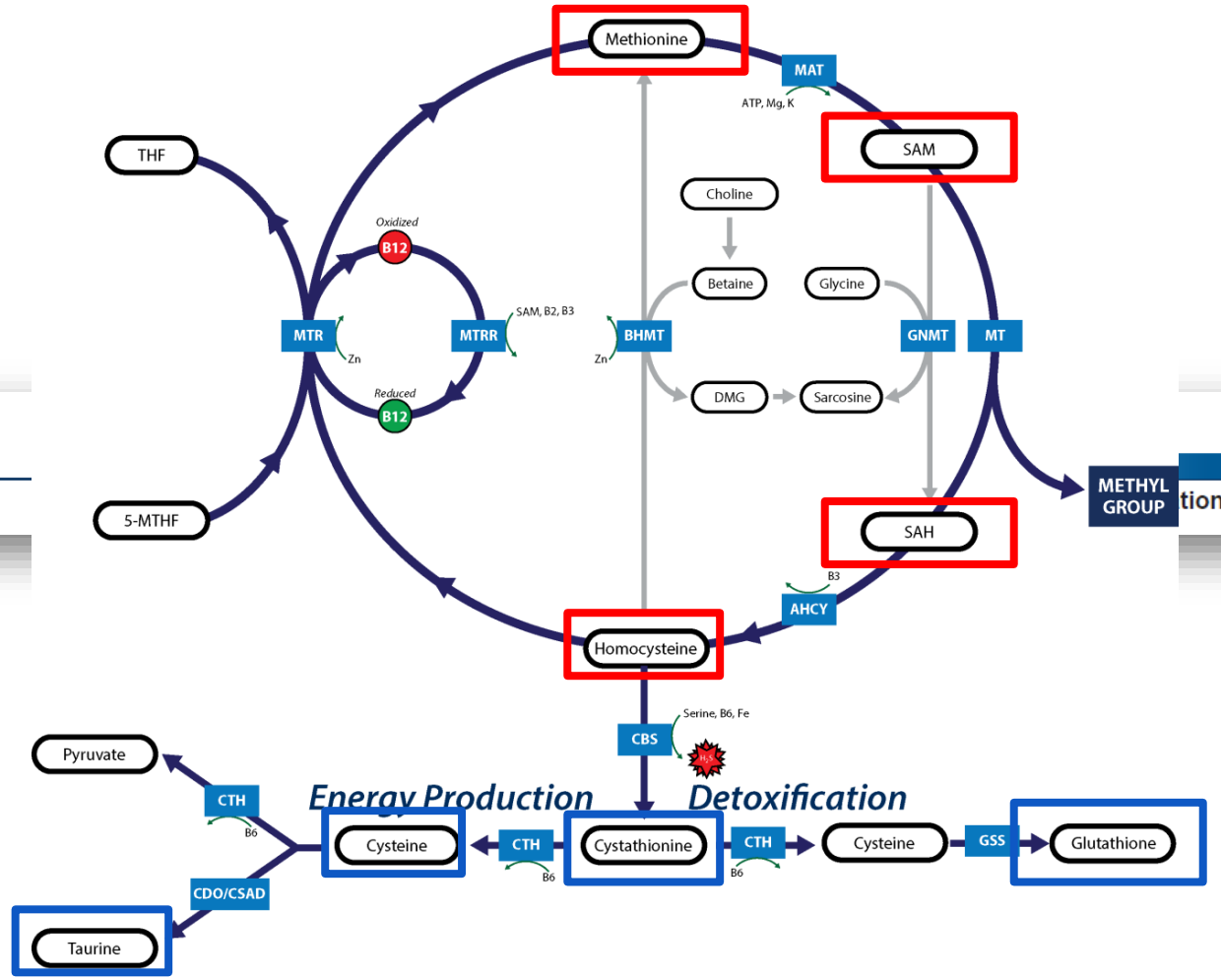
The Met/Sulf Balance Ratio

- Novel functional assessment for methylation v transsulfuration balance
- Compares 8 different biomarkers
  - 4 biomarkers from methylation pathway
  - 4 biomarkers from transsulfuration pathway
    - An explanation will be in the support guide

Met/Sulf Balance Ratio

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

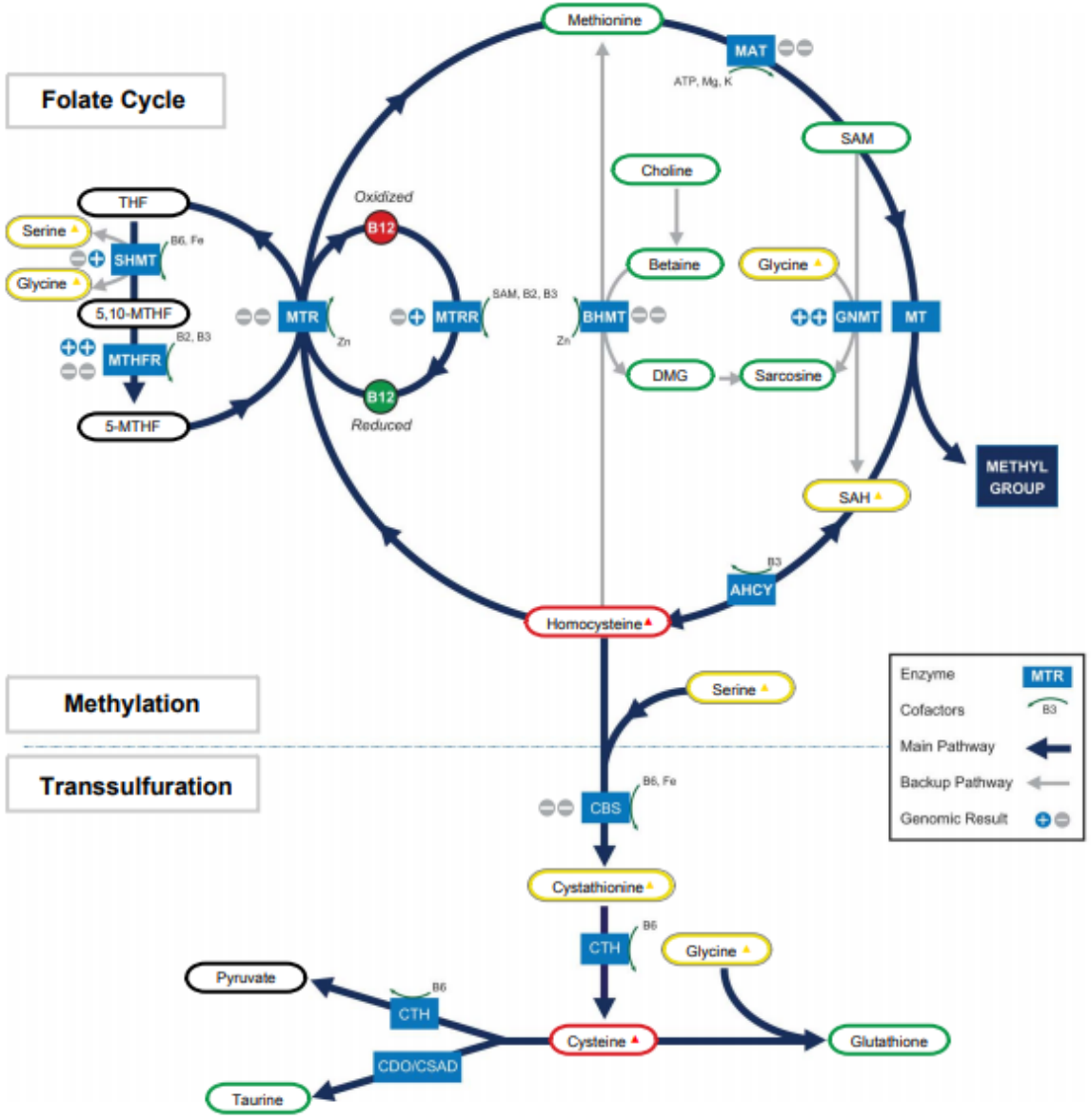
- This ratio is unique to GDX and is being researched



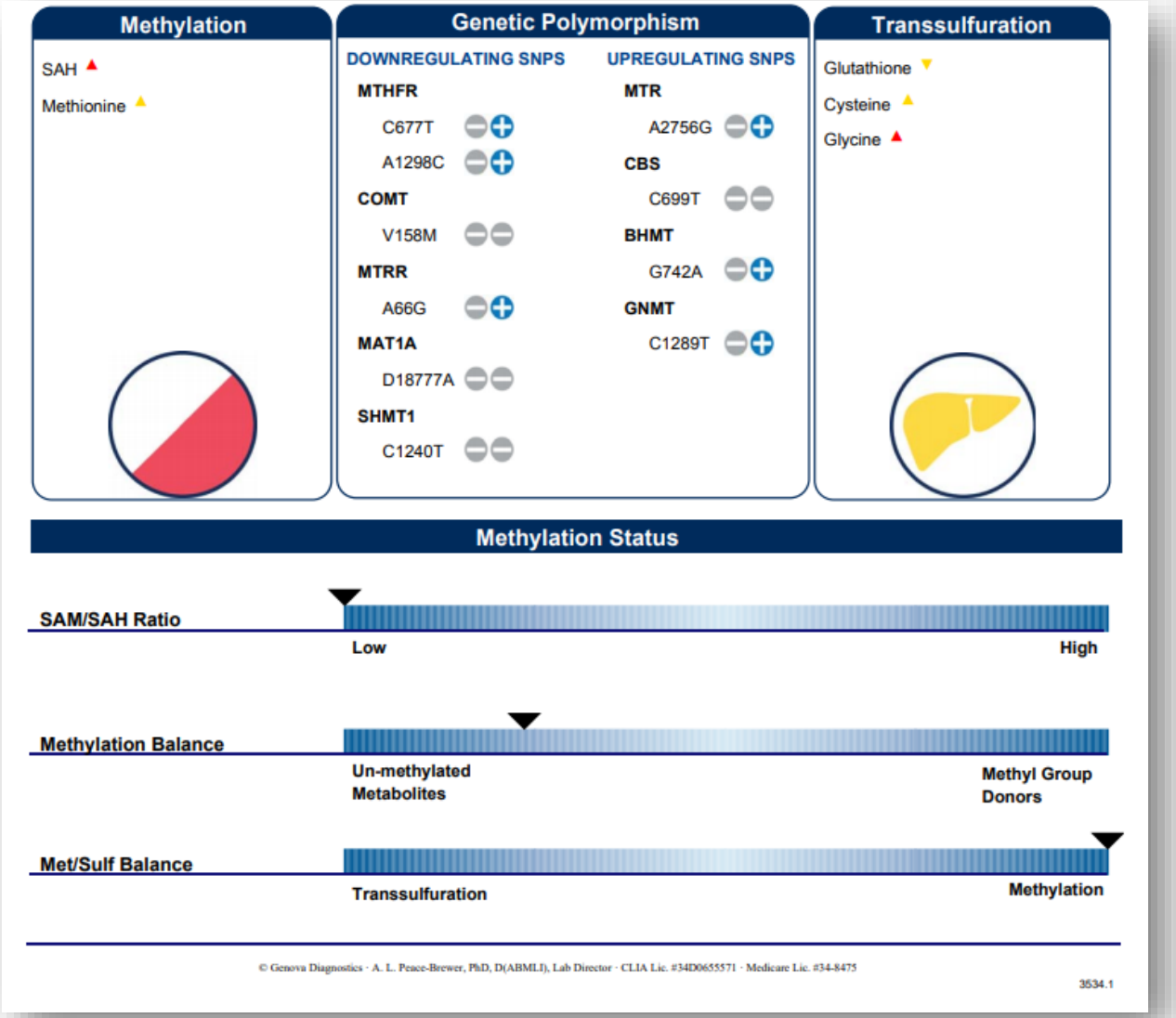
Mato JM, et al. *Ann Hepatol.* 2015;12(2):183-189.

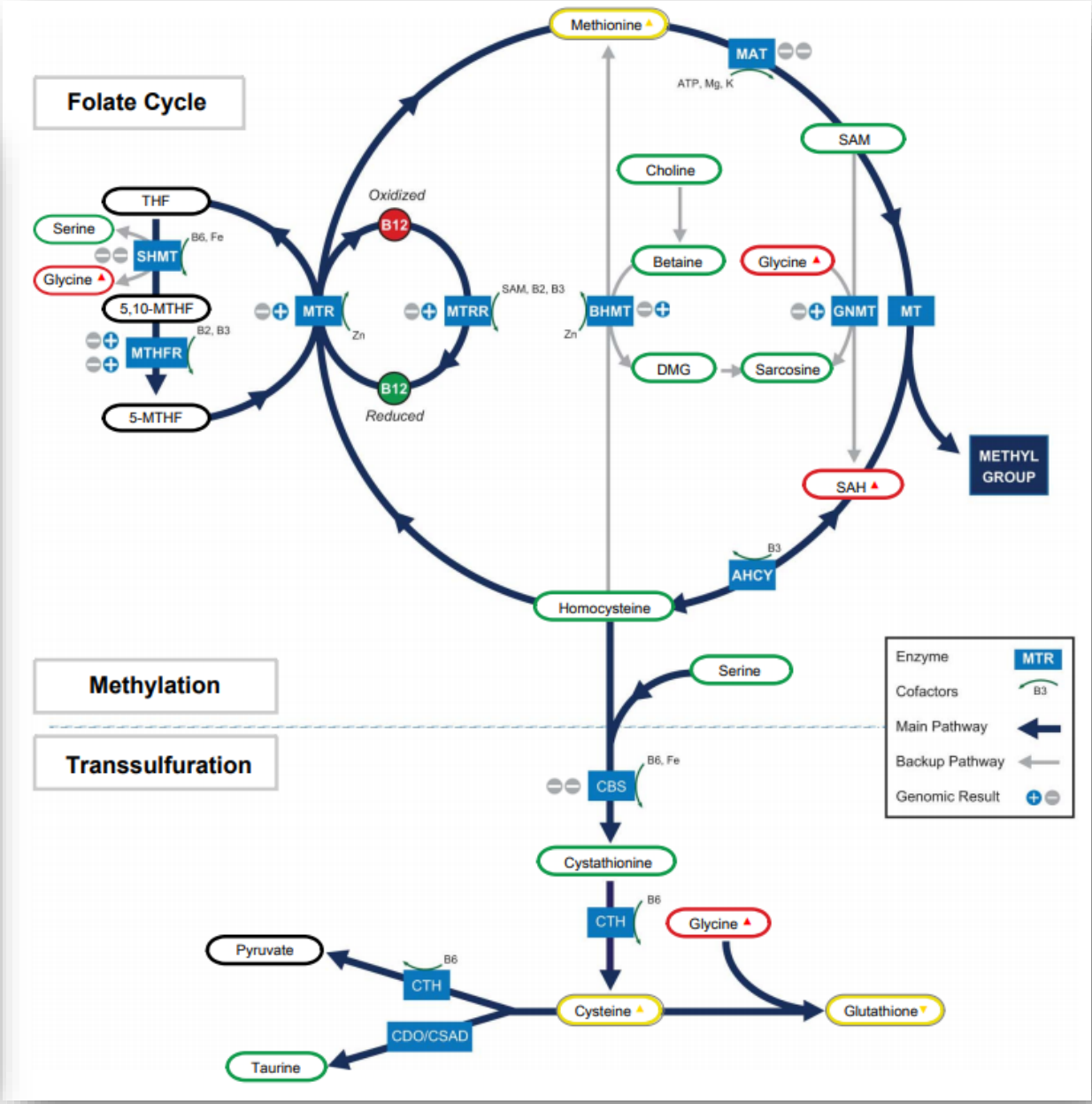
Putting it together

- SAM/SAH ratios look okay…
  - However, homocysteine is still high
- Met/Sulf balance is favoring *methylation* over *transsulfuration*
  - Remember, prefer to have all ratios toward the middle
    - Unless clinically you have a reason to push a particular pathway



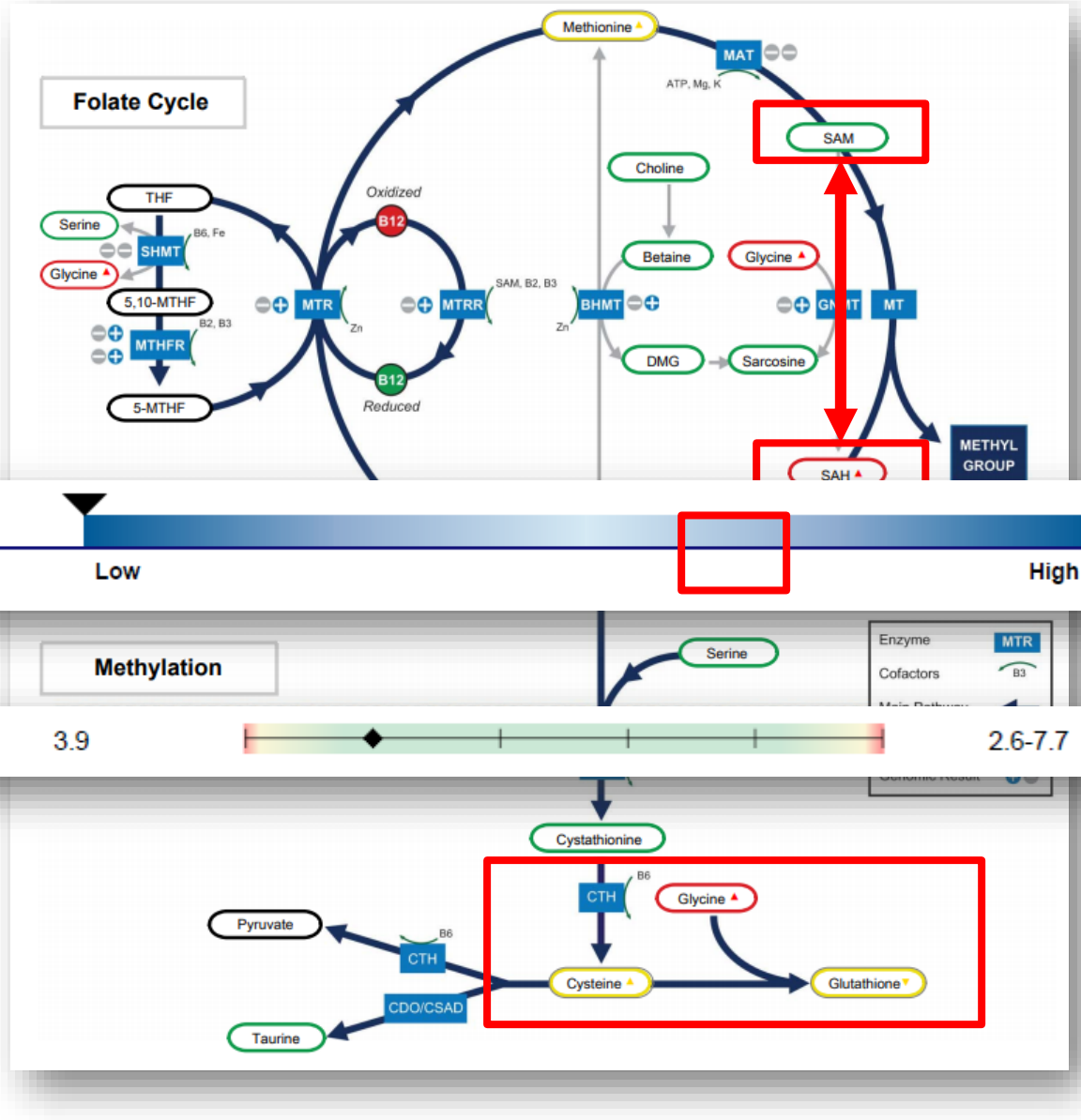
Blind Case – You Try!





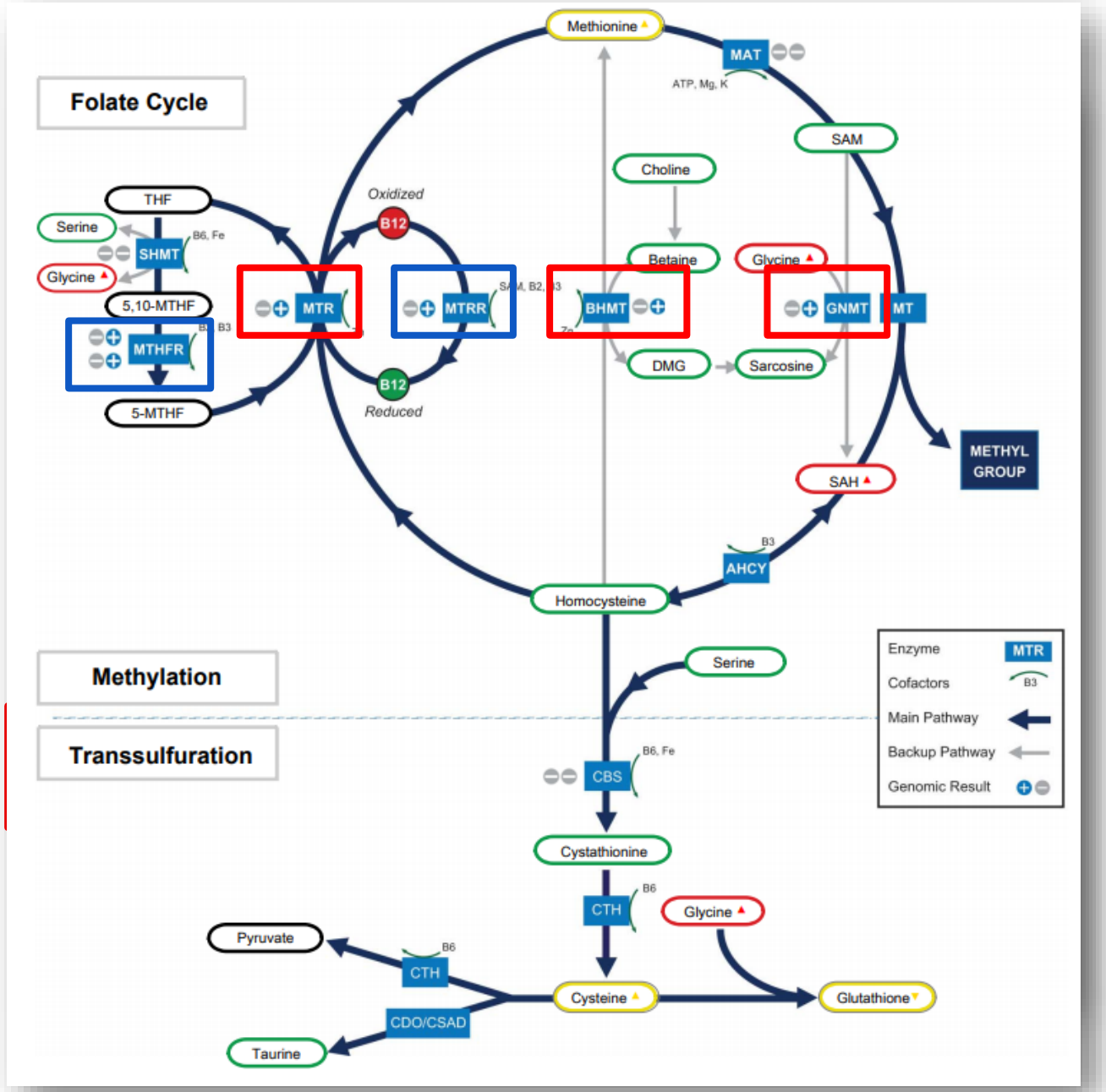
Where Do You Start?

- Always start with the analytes that are most clinical significant!
  1. SAM, SAH, SAM/SAH
     - Then try to determine where the backup is…
  2. Choline/Betaine Balance
     - Associated with many metabolic risks
  3. Glutathione Production/Transsulfuration
     - Then try to determine where the backup is…



Where Do You Start?

- Then widen the lens to look at overall pathway *balance*
  4. Methylation Balance Ratio
     - What is contributing most to the imbalance?
  5. Met/Sulf Balance Ratio
     - What is contributing most to the imbalance?
  6. Genomics
     - Explain why trouble spots may exist and prevention



Ratios Summary

- SAM/SAH Ratio
  - Most severe alteration of methylation balance
- Methyl Balance Ratio
  - May be able to detect methylation imbalance earlier
  - May indicate needs for vitamin B-12, folate, choline, or betaine
- Met/Sulf
  - Looks at balance between two interdependent pathways
  - May indicate needs for vitamin B-6, magnesium, and antioxidant support

Treating Methylation Dysfunction

Methylation and What To Do

- Methylation Imbalances
  - Methylation Support
    - Green leafy vegetables
    - Folate or 5-MTHF
    - Activated vitamin B-12
    - Betaine
    - Zinc
- Transsulfuration Imbalances
    - Glutathione
    - Antioxidants
    - Glycine
    - Vitamin B-6
    - Magnesium
- The Methylation Panel helps clinicians decided which area needs more support
  - We may even get more information to figure out what *forms* of B-vitamins are best for a patient

Folate

- Dietary intake of folate-rich foods
  - Leafy green vegetables, legumes, citrus fruits, beets, and whole grains
  - People with significant MTHFR SNPs may have difficulty converting dietary folates into 5-MTHF
- Folic acid
  - Lots of debate regarding this supplement
  - Has been vilified recently, perhaps unfairly
- Folinic acid
  - Used with 5-fluorouracil and methotrexate
- 5-MTHF
  - Irreversibly committed to the methylation cycle
  - Potential for over-methylation?

Gaby, A. *Nutritional Medicine*. Fritz Perlberg Publishing, 2017.

Vitamin B-12

- Deficiency common among elderly
  - Hypochlorhydria
  - Pernicious anemia
  - *H. pylori* infection
- Dietary sources: meat, poultry, fish, dairy, and eggs
- Methylcobalamin
  - Cyanocobalamin is synthetic and must be converted by the body into methylcobalamin
- Serum B-12 measurements may not tell the whole story
  - Methylmalonic acid

Gaby, A. *Nutritional Medicine*. Fritz Perlberg Publishing, 2017.

Vitamin B-6

- Particularly useful if showing poor glutathione production
- Depleted by environmental toxicants and certain medications
  - PCBs
  - Hydrazine
  - Oral contraceptives
- Dietary sources:
  - Potatoes, bananas, meat, and whole grains
- Pyridoxine vs P-5-P
  - Uncertain absorption of P-5-P
  - 10-200 mg/day

Gaby, A. *Nutritional Medicine*. Fritz Perlberg Publishing, 2017.

Zinc

- Very common mineral insufficiency
- Used in both main and backup methylation pathways
- Dietary sources: seafood, meats, whole grains, wheat germ, dairy, and legumes
- Competitive inhibition with copper
  - Also bound by calcium intake
  - May be depleted by ACE inhibitors, PPIs, and glucocorticoids

Gaby, A. *Nutritional Medicine*. Fritz Perlberg Publishing, 2017.

Choline/Betaine

- Assists with methylation pathway as well as adequate supply of choline for other pathways
- Useful in metabolic syndrome and NASH
- Dietary sources: organ meats, eggs, wheat germ, soybeans, and meat
  - Beets (Betaine)
- Soy lecithin

Gaby, A. *Nutritional Medicine*. Fritz Perlberg Publishing, 2017.

Glycine

- Has been used in multiple clinical conditions
  - Anxiety
  - Poor detoxification (glycination pathway)
  - Poor sleep quality
- Indirectly supports methylation pathways
- Tastes great!

Gaby, A. *Nutritional Medicine*. Fritz Perlberg Publishing, 2017.

SAMe

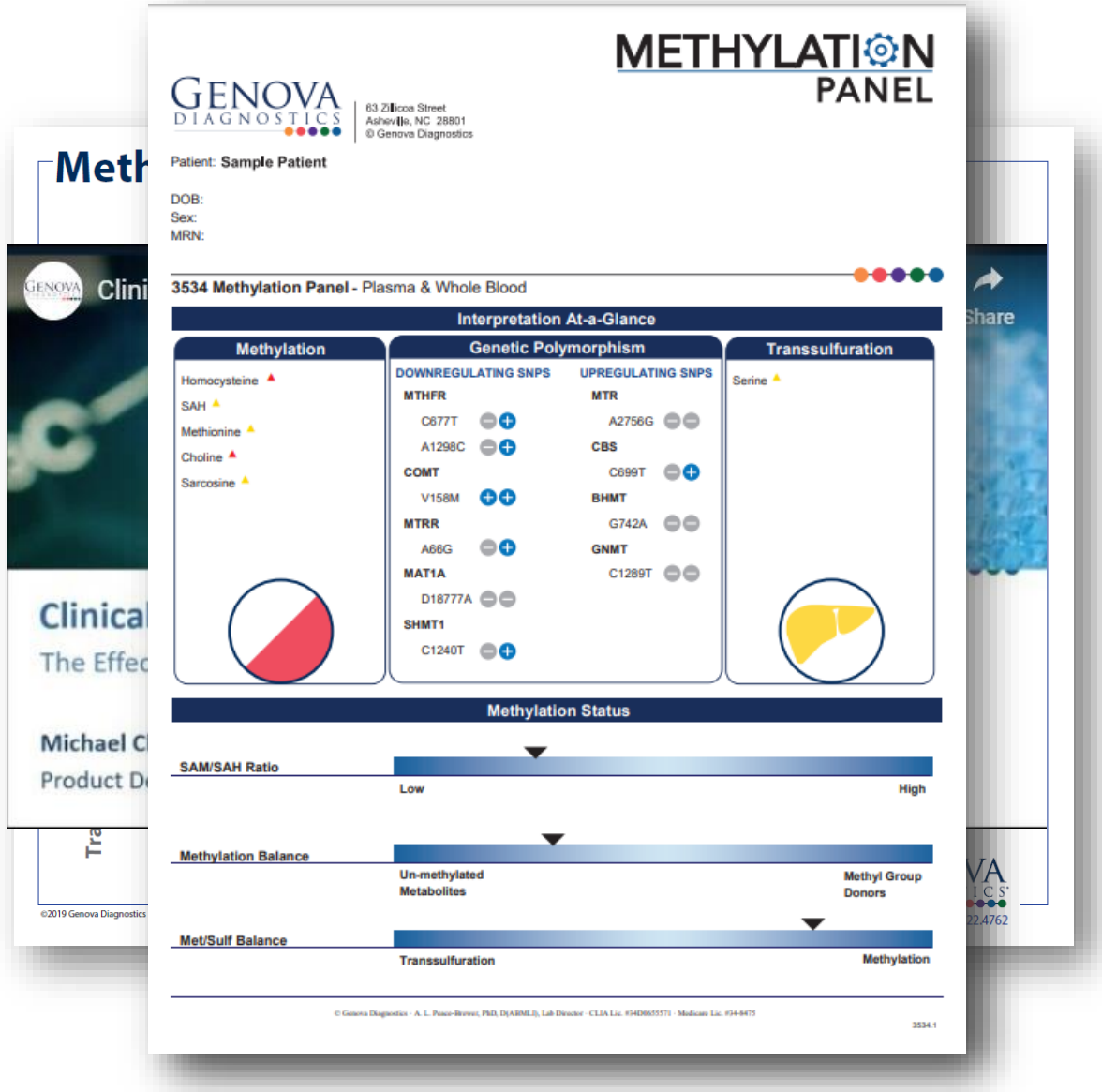
- Extensively researched in multiple conditions
- Directly supplies methyl donors
- Concerns:
  - **Bipolar and Schizophrenia**
  - Over-methylation
  - Expensive
  - Clinical trials use very high doses for mood and musculoskeletal conditions

Gaby, A. *Nutritional Medicine*. Fritz Perlberg Publishing, 2017.

Support Materials

- Website Materials:
  - Methylation Pathway Chart
  - Methylation Panel Support Guide
  - Intro to Methylation Video
  - Sample Report

- Case analysis and clinical consultation with medical education specialists
  - 800.522.4762 to schedule




“If you truly love nature, you will find beauty everywhere.” -Vincent Van Gogh
Photo by Gabe Swinney, 2018

Presenter:
Michael Chapman, ND

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Wednesday, September 25th 2019

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Kara Fitzgerald, ND,

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Introducing Genova's Innovative Methylation Panel

Discussion on Clinical Utility and Case Review

Michael Chapman, ND