Homocysteine and its Catabolism

UNDERSTANDING THE METHYLATION PATHWAY

Objectives

- Undearstand the basics of methylation
- Learn the three disposal routes of homocysteine catabolism
- Understand the clinical importance of optimizing methylation

What is Methylation?

- "The attachment of methyl groups to DNA at cytosine bases; correlated with reduced transcription of the gene and thought to be the principal mechanism in X-chromosome inactivation and imprinting"
  - *http://ghr.nlm.nih.gov/glossary=methylation*

But Really, What IS Methylation?

- A methyl group contains 1 carbon and 3 hydrogens
- Methylation is therefore the process of adding or subtracting methyl groups from different compounds.

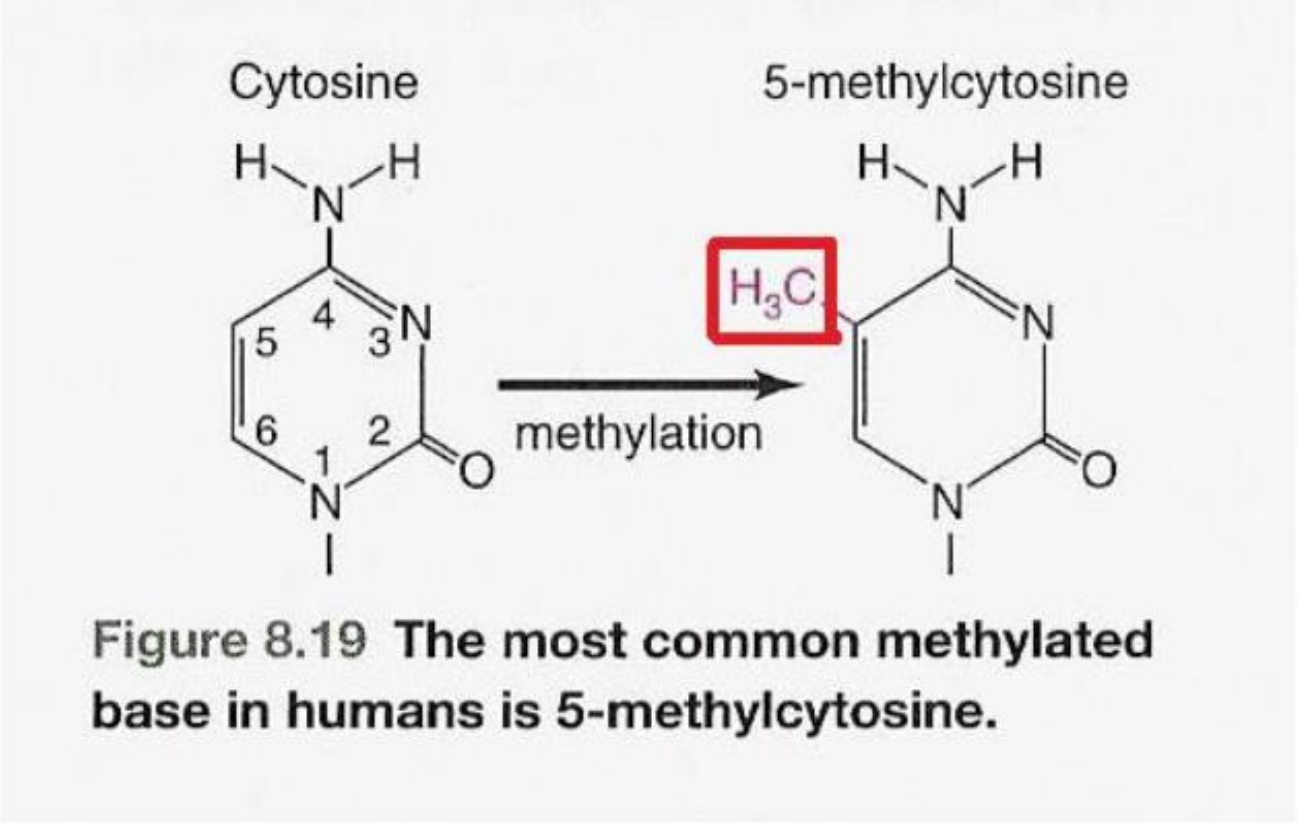


Figure 8.19 The most common methylated base in humans is 5-methylcytosine.

Devlin, Thomas. M. *Textbook of Biochemistry with Clinical Correlations.* Edition 7. Copyright 2011.

What is the Function of Methylation?

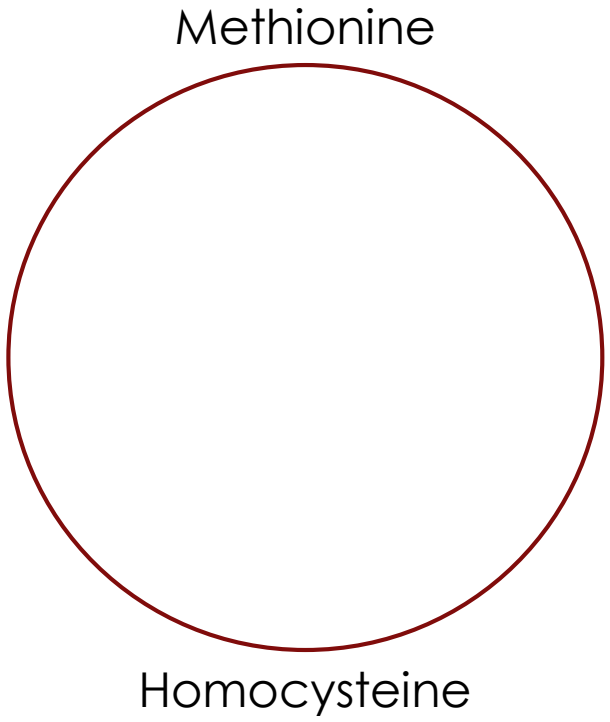
- Gene regulation
- Biotransformation
- Neurotransmitter synthesis
- Hormone catabolism (estrogen)
- Build immune cells such as T cells and NK cells
- DNA and histone synthesis
- Energy production
- Creation of myelin sheath on nerve cells
- Build and maintain cell membranes (phosphatidylholine)

What Negatively Impacts Methylation?

- Insufficient cofactors or substrates
- Medications that block absorption (antacids, methotrexate, metformin, etc.)
- High dose niacin depletes methyl groups
- Environmental toxins, heavy metals, and chemical exposure
- Too much substrate causes feedback inhibition
- Genetic mutations
- Mental state

Homocysteine

- Homocysteine is at the center of methylation.
- It is produced from the amino acid methionine.



KEY POINT:
Methionine IS methylated homocysteine.

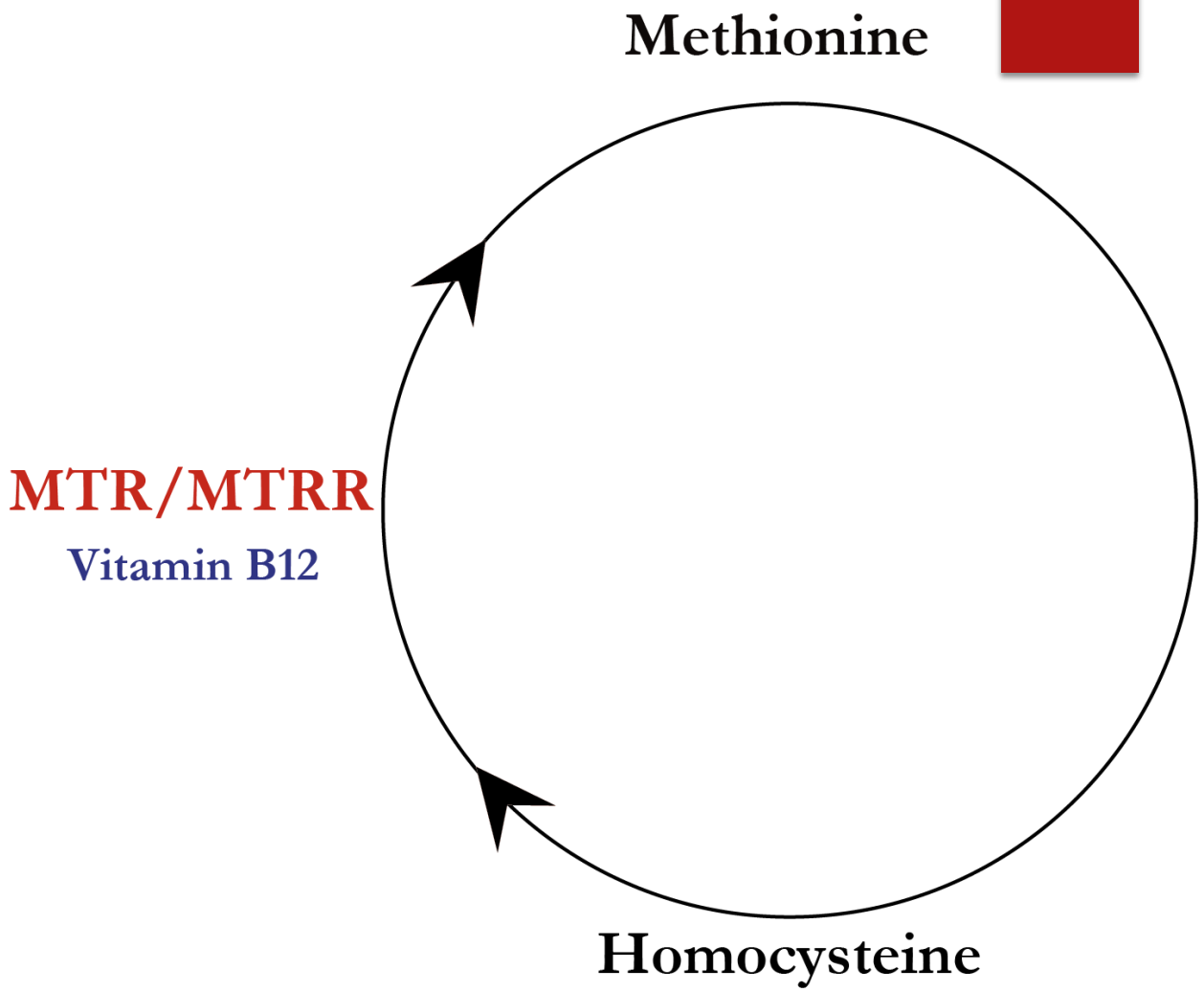
Methionine

Three Disposal Routes of Homocysteine

- MTR/MTRR: (50%)Vitamin B12 and folate
- BHMT: TMG and Zinc
- CBS: (50%)Vitamin B6 (Only eliminating route)

MTR/MTRR

- Methionine Synthase
- Methionine Synthase Reductase
- Accounts for half of homocysteine catabolism.
- Recycles homocysteine back to methionine.



Methionine Synthase (MTR)

- MTR is vitamin B12 dependent
- Catalyzes the transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine to produce methionine and tetrahydrofolate
- Maintains adequate intracellular methionine required for production of S-adenosyl methionine (SAM)
- Responsible for maintaining sufficient intracellular folate pools and regulating homocysteine levels

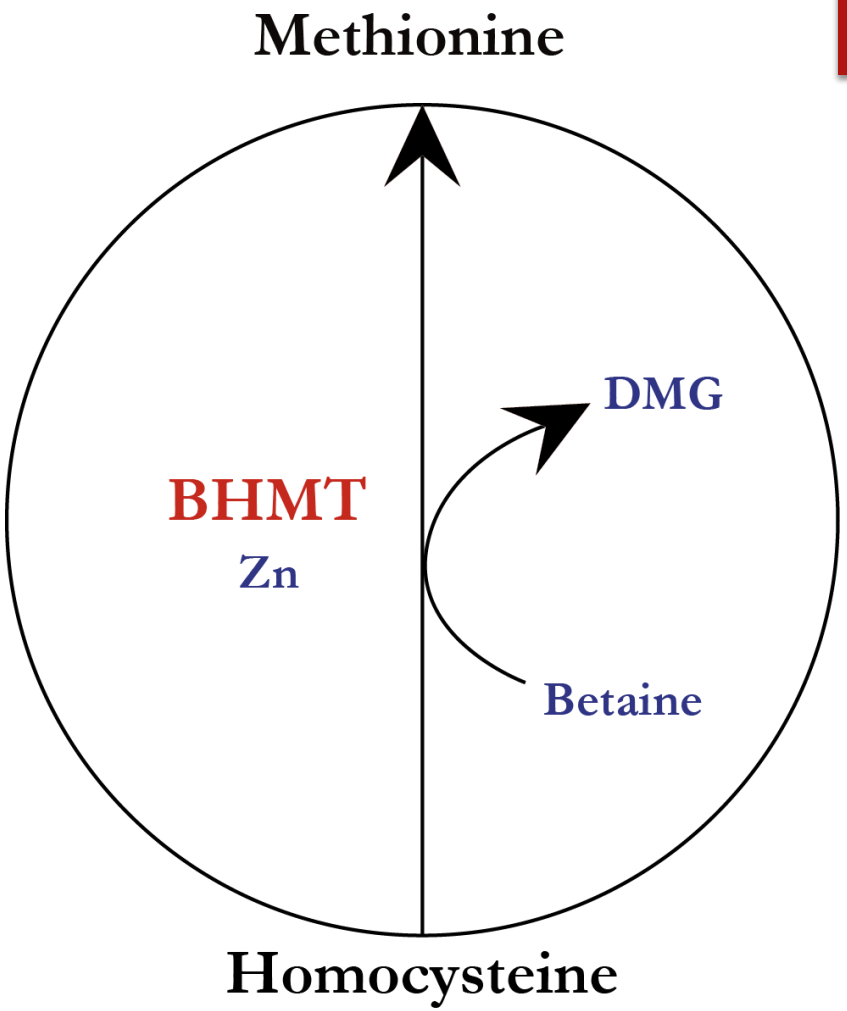
Methionine Synthase Reductase (MTRR)

- Responsible for maintaining sufficient levels of methyl B12 required for homocysteine remethylation
- MTRR A66G polymorphism increases homocysteine levels and may also contribute to dyslipidemia in men(Zhi et al., 2016). This is particularly true in combination with the MTHFR C677T polymorphism.

Zhi, X., Yankg, B., Fan, S., Wang, Y., Wei, J., Zheng, Q. and Sun, G. (2016) Gender-specific interactions of MTHFR C677T and MTRR A66G polymorphism with overweight/obesity on serum lipid levels in a Chinese Han populations. *Lipids in Health and Disease*. 15, 185.

BHMT

- Betaine-Homocysteine S-Methyltransferase
- The short cut through the methylation cycle.
- Supplies a methyl group to convert homocysteine back to methionine and betaine to dimethylglycine (DMG).



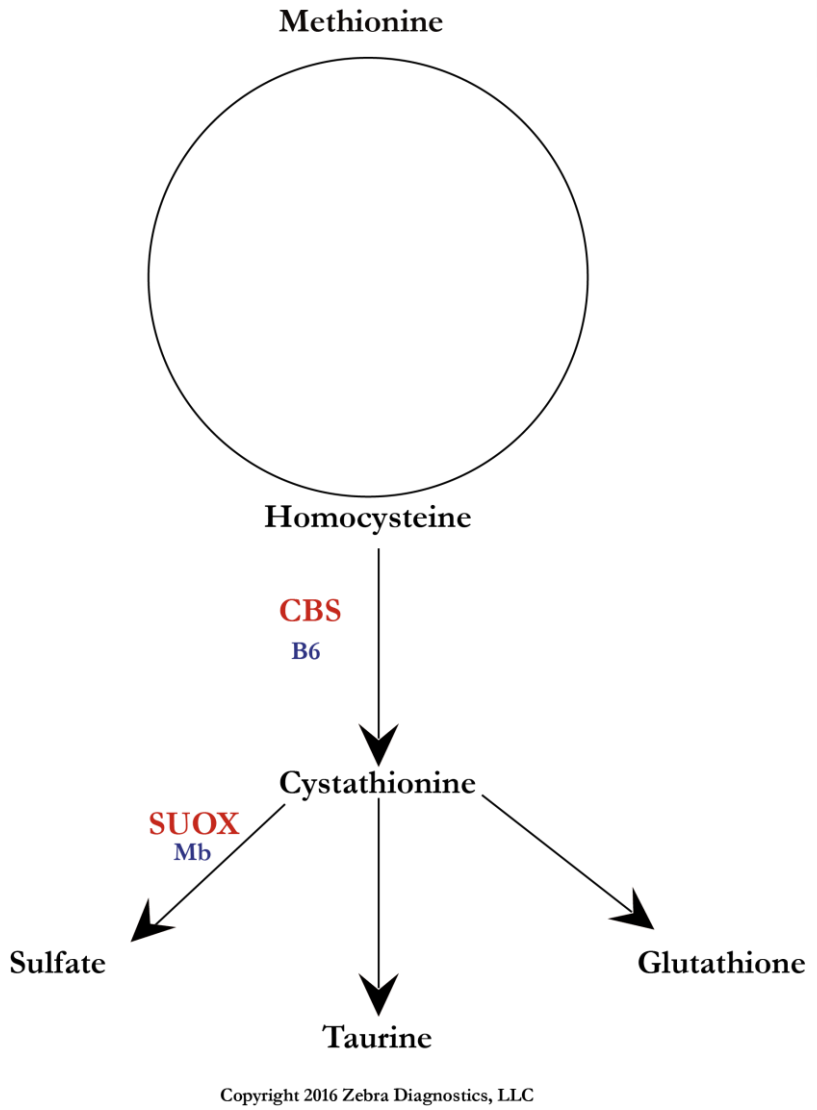
BHMT

- SNPs typically have no clinical affect on homocysteine levels
- Requires zinc as its cofactor and betaine
- Betaine may be found in foods such as wheat, spinach, shell fish and beets
- Betaine may also be created from choline endogenously
- In addition to catabolizing homocysteine, BHMT and MTR also work to increase availability of methionine so that it may be used to produce SAM the universal methyl donor (Ganu et al., 2015).

Ganu, R.S., Ishida, Y., Koutmos, M., Kolokotronis, S., Roca, A.L., Garrow, T.A. and Schook, L.B. (2015) Evolutionary Analyses and Natural Selection of Betaine-Homocysteine S-Methyltransferase (BHMT) and BHMT2 Genes. *PLOS One*. DOI: 10.1371/journal.pone.0134084.

CBS

- Cystathionine Beta Synthase
- Accounts for half of homocysteine catabolism.
- The only true disposal route.
- Converts homocysteine to cystathionine.



CBS

- Cofactor: **Vitamin B6 (pyridoxal-5-phosphate) and Magnesium**
- **Blocked by lead**
- Product: Hydrogen sulfide (H2S) and cystathionine
- Upregulation variants: CBS C699T, C1080T, and C1985T
- Down regulation variants: CBS A360A and CBS C9150T

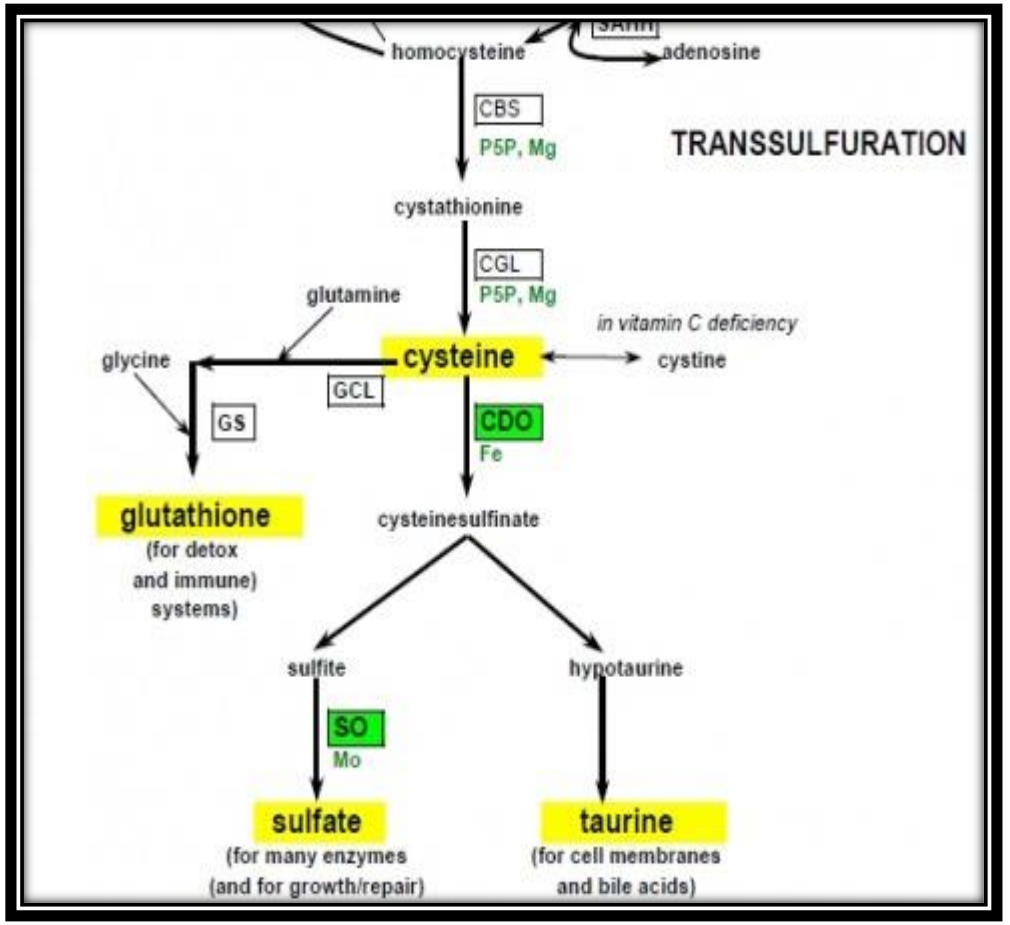
De Stefano V, Dekou V, Nicaud V, Chasse JF, London J, Stansbie D, Humphries SE, Gudnason V. (1998(Linkage disequilibrium at the cystathionine beta synthase (CBS) locus and the association between genetic variation at the CBS locus and plasma levels of homocysteine. The Ears II Group. European Atherosclerosis Research Study. *Ann Hum Genet*. 62(Pt 6):481-90.

CBS Upregulation

- Decreased levels of vitamin B6 and magnesium
- Decreased levels of homocysteine, GSH

(compromised ability to recycle homocysteine back the universal methyl donor SAM)

- Increased levels of ammonia drain methyl donors and increases levels of nitric oxide to counteract increased ammonia.
- Hydrogen Sulfide increases cortisol and glutamate (excitotoxicity)



CBS Upregulation Clinical/Lab Findings

- Elevated urinary sulfates: typically greater than 800. If consistently greater than 1000 then high CBS activity is likely; Sulfur flatulence, sulfur food intolerance, Sulfa drug reactions and halitosis. (Brain Fog!)
- Elevated ammonia and/or sulfate levels along with elevated Orotate, Citrate and Isocitrate on an Organic Acids Test (OAT) indicate elevated ammonia. (Ammonia depletes BH4, leading to insufficient dopamine and serotonin production; increased risk for cancer).

CBS Upregulation Clinical/Lab Findings

- Typically has an elevated xanthurenate on an OAT test
- Also will have high Taurine - Taurine is synthesized via Cysteine – found on amino acids tests.
- To combat sulfur intolerance: Try a reduced sulfur diet (cruciferous vegetables, eggs) and reduced protein diet for a few days along with molybdenum, the cofactor for SUOX.

CBS Downregulation

- Hypertension/Cardiovascular disorders
- **Potentially elevates homocysteine levels and moderately elevates xanthurenate—check MTHFR status.**
- **Consider N-acetyl cysteine (NAC) to help increase GSH levels, magnesium and P5P.**
- Decreased risk of breast cancer with CBS A360A

Stevens VL, McCullough ML, Pavluck AL, Talbot JT, Feigelson HS, Thun MJ, Calle EE.(2007) Association of polymorphisms in one-carbon metabolism genes and postmenopausal breast cancer incidence. *Cancer Epidemiol Biomarkers Prev.* 16(6):1140-7.

CBS Case Study

- TD is a six-year-old boy with autism spectrum disorder and intellectual disability. He has poor language development, though described by his mother as "a happy boy." He has been on the GAPS diet for two years with no noticeable changes. His mother notes that he often has foul, stinky gas and often appears bloated.

CBS Case Study Continued

- GAPS diet
  - **High in cruciferous vegetables, onions, garlic, animal proteins and other high sulfur foods.**
- He has foul, stinky gas and often appears bloated
- What SNP is associated with these clinical symptoms?
  - **CBS C699T**
- He has been diagnosed with Autism – thinking back the clinical associations of CBS upregulation, what occurs when there is excess glutamate?
  - **Excitotoxicity**

CBS Case Study Continued

- **Key Laboratory Markers**
  - Xanthurenate: 0.57 (<0.46)
    - Vitamin B6 levels are insufficient
  - Cystathionine: 40 (6-33)
    - Points to CBS upregulation
  - Methylmalonic acid: elevated
    - Cellular B12 deficiency
  - Which SNPs are likely?
    - CBS C699T and possibly MTR/MTRR

CBS Case Study Continued

- Should you run a serum homocysteine to confirm that it is low?
  - Yes
  - Homocysteine: 2 (4-15 µmol/L)
- Homocysteine will be low, cystathionine will be elevated and xanthurenate will be elevated due to the enzyme upregulation of CBS C699T
- Methylmalonic acid being high with low homocysteine confirms that it is a catabolism issue and likely a SNP in methionine synthase.

CBS Case Study Two

- You have a 23andme report for a 20 year old female who complains about chemical sensitivities. You notice that she has a homozygous SNP to CBS A360A. Additional testing reveals the following:
  - Xanthurenate: elevated
  - Glutathione: low
  - RBC Magnesium: low
  - Glutamine: Low
  - Glycine: Low
  - Cystine: High
  - Cysteine: Low
- Is this an upregulation or downregulation CBS case? How would you intervene?

Homocysteine and Stress Case Study

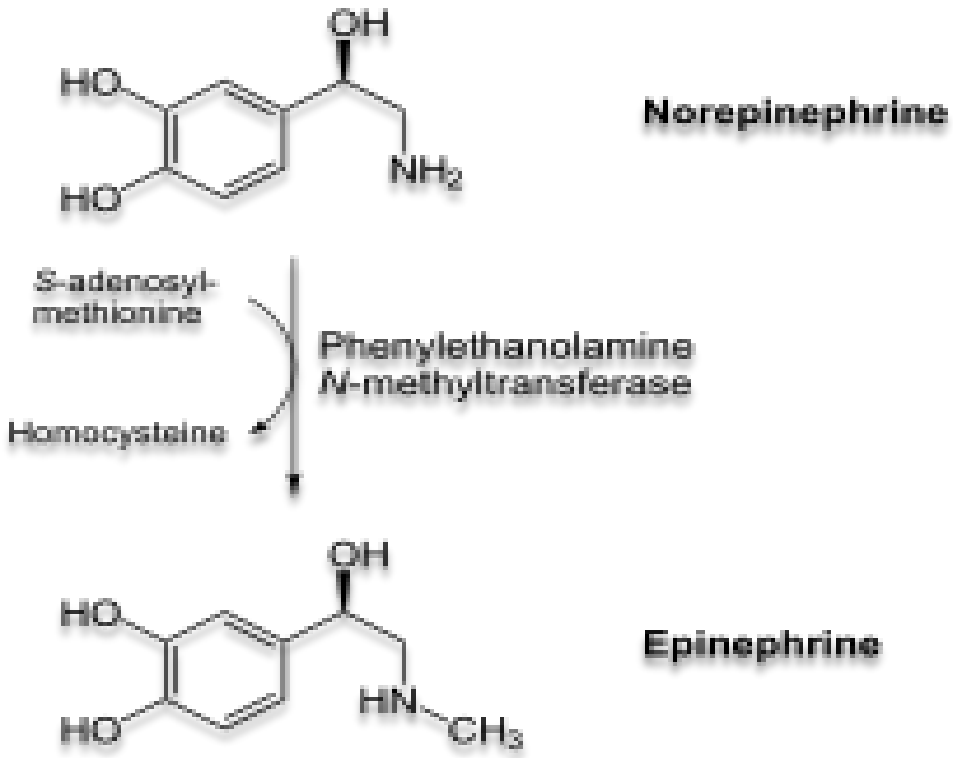
- John is a busy executive working 70 hours per week. His symptoms include insomnia, stress and has occasional diarrhea and anxiety.
  - You ran an initial four-point adrenal stress index (ASI) which indicated that cortisol is elevated throughout the day.
  - A serum homocysteine was also run and was lab high at 16 mmol/L (range is 4-16 mmol/L).

Homocysteine and Stress Case Study

- An organic acids test was also run which includes methylmalonic acid (MMA), formiminoglutamate (FIGLU) and xanthurenate which assess vitamin B12, folate and vitamin B6 status respectively. When elevated these markers indicate a cellular insufficiency. While xanthurenate levels were normal, both MMA and FIGLU were elevated.
- How would you proceed? Are there any SNPs that you would expect to find? Do you think that there is a relationship between stress and homocysteine?

Stress and Methylation

Talk to me about what you see here…What is the chemical difference between Epinephrine and Norepinephrine? What enzymatic process is being catalyzed by PNMT? What do you think it means for those under chronic stress? What do you think about their methylation status? How about their Hcy Levels?



Review of SNP Effects on Homocysteine

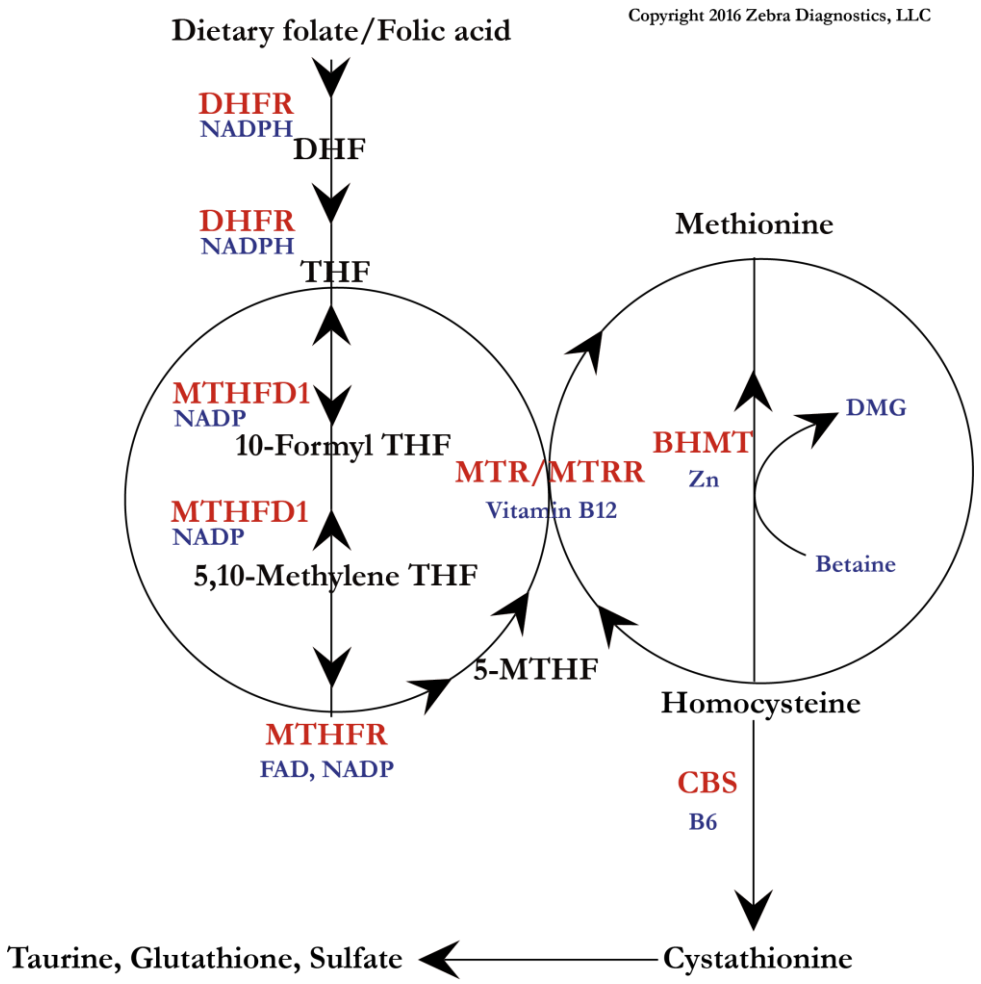
- MTR/MTRR: Likely Increased
- BHMT: Likely no effect
- CBS C699T: Likely decreased
- CBS A369A: Likely increased
- MTHFR C677T: Likely increased
- MTHFR A1298C: Likely no effect

Review of SNP Clinical Ramifications

- MTR/MTRR: Decreased folate and B12 status; macrocytic anemia
- BHMT: Likely no effect; possibly increased need for zinc and trimethylglycine
- CBS C699T: Sulfur intolerance, decreased B6 status, increased cystathionine, and decreased glutathionine.
- CBS A369A: Decreased B6, cystathionine and glutathione
- MTHFR C677T: Decreased folate, increased risk for CVD and miscarriage
- MTHFR A1298C: Decreased folate, depression

Relevant Lab Markers Related to Homocysteine

- Methylmalonic Acid (MMA): B12 status
- Formiminoglutatamate (FIGLU): folate status
- Xanthurenate: B6 status
- Complete Blood Count (CBC)
  - Increased MCV: above 100 is diagnostic, above 92 is a functional increase
    - B12 or folate deficiency
  - Decreased MCV: below 80 is diagnostic, below 84 is a functional decrease
    - Iron of B6 deficiency



One Carbon Metabolism and Methylation

Remember: Always remember to look at the intersection of pathways.

Questions?

Thank you for attending our webinar.