CBS Pathway

Genes, Key Issues and Regulatory Factors
Keynotes of This Presentation

- What are the key roles of the CBS pathway?
- How the gut works with CBS pathway?
- What links CBS pathway and energy cycle?
- What are genes related to CBS pathway?
- What factors regulate CBS pathway?
- Case Studies Related to CBS Pathway
Key Roles of the CBS Pathway
What is CBS pathway?

Cystathionine beta synthase is a homotetramer catalysing the conversion of homocysteine to cystathionine, the first step in the transsulfuration pathway.

Functions of CBS pathway

• Converting homocysteine to cystathionine

• Activated by adenosyl-methionine and uses pyridoxal phosphate as a cofactor (vitamin B6 related)

• A major contributor to cellular hydrogen sulfide production

• Help to regulate homocysteine (as a deficiency causes an increase in homocysteine.)

It is a B6 dependent pathway

CBS is a pyridoxine (vitamin B6)-dependent enzyme

- CBS – has a central core containing a P5P cofactor, C-terminal regulatory domain with 2 binding sites for SAM and N terminal with a B-type heme.

- Homocysteine (Hcy) is at the branch point between transsulfuration and methionine remethylation in the methionine metabolic cycle.

- CBS deficiency limits transsulfuration and results in:
  - Increased homocysteine: less converted to cystathionine
  - Increased methionine: more re-methylated

- CBS deficiency leads to homocystinuria

Elevated Homocysteine due to decreased CBS activity

- Homocystinuria caused by CBS deficiency (classic homocystinuria) is characterised by developmental delay/intellectual disability, myopia, skeletal abnormalities and thromboembolism.
- Marfan syndrome (joint flexibility) and scoliosis is seen.
- There may also be seizures, psychiatric problems, dystonia, hypopigmentation, malar flush and pancreatitis.
- The 2 most Common CBS pathogenic variants related to a down regulation of CBS causing elevated homocysteine:
  - P.Ile278Thr - rs.5742905 - B6 responsive
  - P.Gly307Ser – rs.12196492- B6 non-responsive

Effects of Elevated homocysteine

- Endothelial cell expression of leukocyte adhesion molecules
- Secretion of pro inflammatory cytokines and chemokines
- Release of matrix-degrading enzymes
- Activation of pro coagulants
  - This leads to:
    - Vascular inflammation
    - Accelerates the progression of atherosclerosis
    - Increases blood brain barrier permeability
    - Risk factor for neurodegenerative disease especially Alzheimer's.

Elevated Homocysteine due to decreased CBS activity

- Other reasons for elevated homocysteine:
  - Severe lack of dietary Vitamin B12
  - AHCY deficiency
  - Folate deficiency
  - Lack of B6
  - Lack of betaine

CBS deficiency and homocysteine

Dietary amino acids → Methionine

Folate / B12-dependent → Betaine*

Cystathionine β-synthase (CBS) enzyme (pyridoxine-dependent)

Homocysteine → Cystathionine → Cysteine

*Alternative pathway with betaine treatment

https://www.ncbi.nlm.nih.gov/books/NBK1524/
CBS controls the flux of sulphur from methionine to cysteine. This in turn allows the production of:

- Glutathione
- Taurine
- Hydrogen Sulphide which control cellular redox status and signalling.

Human CBS is activated by s-adenosylmethionine (AdoMet) which binds to the regulatory domain and triggers a conformational change that allows the protein to progress to an activated state.

The transulfuration pathway is present in the liver, kidney, pancreas, small intestine and immune cells (macrophages, T cells, dendritic cells). Not in spleen, testes, heart, and skeletal muscle.

Inhibition of the transulfuration pathway reduces cysteine and can reduce GSH by around 50%.
CBS and sulphur metabolism

Sulphur containing amino acids methionine and cysteine are precursors for the synthesis of important sulphur metabolites, such as hydrogen sulphide (H$_2$S) and glutathione (GSH)

- Methionine provides cells with methyl groups that are used in various methylation reactions
- Hcy is re-methylated to regenerate methionine by
  - Methionine synthase (MS), or
  - Betaine homocysteine methyltransferase (BHMT) (liver and kidney)
- Hcy is catalysed by CBS to form cystathionine; and cystathionine is used by γ-cystathionase/ cystathione y-lyase (CSE) to synthesize cysteine, then H$_2$S and GSH
- The exits of sulphur (methionine and cysteine) to form sulphur metabolites (H$_2$S and GSH) are irreversible

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4684266/
Sulphur metabolism in the intestine

Intestine is a significant place of sulphur metabolism

• Intestine metabolises about 20% of the dietary sulphur, and 25% of transmethylation and transsulfuration in the body

• It is a site of net homocysteine release and may contribute to homocysteinaemia

• Sulphur metabolism regulates gut growth and intestinal functions

• These functions include the digestion, absorption and metabolism of nutrients; the immune surveillance of the intestinal epithelial layer; and the regulation of the mucosal response to foreign antigens

• Sulphur deficiency suppresses intestinal mucosal growth and reduces intestinal epithelial cell proliferation, and contributes to the development of gastrointestinal diseases

Research on $\text{H}_2\text{S}$, cysteine & diabetes is new

- L-cysteine has an insulin-like action promoting glucose entry into adipose cells by means of its free sulfhydryl group
- It has a protective effect on pancreatic $\beta$-cells and to lower the oxidative stress and insulin resistance
- It can assist metformin in treating insulin resistance to reduce fatty acids, oxidative stress and inflammation
- Its downstream product taurine is emerging in treating diabetes due to its antioxidant, anti apoptosis, membrane stabilization, osmoregulation, and neurotransmission properties
- As supportive evidence, methionine levels are higher in poorly controlled diabetic patients, while GSH and GCL levels are higher in effectively treated diabetic animals

CBS and sulphur metabolism

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4684266/

www.mthfrsupport.com.au
CBS and glutathione synthesis

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3549305/
Hydrogen Sulfide (H2S)

- H2S is produced from the sulfur containing metabolites cysteine and homocysteine by the enzymes of the transsulfuration pathway, CBS and CSE and from 3-mercaptopyruvate by MST (mercaptopyruvate sulfurtransferase) and thioredoxin.

- Elevated homocysteine is expected to shift its utilization to the transsulfuration pathway and result in elevated H2S synthesis.

- H2S is involved in metabolic regulation & release of insulin

[https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3549305/](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3549305/)
https://www.slideshare.net/soorajben10/hydrogen-sulphide-as-a-gasotransmitter-loading
Hydrogen sulphide benefits

• **H₂S has multiple functions:**
  • Regulation of neuronal activity, vascular relaxation & oxygen sensing, protection from vascular inflammation
  • Protection of the heart, kidneys and brain from ischemic damages
  • Metabolic regulation and release of insulin

• **H₂S regulation through its metabolism can improve metabolic health**
  • Obesity is associated with elevated plasma cysteine
  • Diabetes is associated with reduced cysteine levels

Elevated Hydrogen Sulfide (H2S)

- Elevated H2S has been related to:
  - Conjunctival irritation
  - Upper airway irritation
  - Pulmonary oedema and respiratory paralysis, cardiovascular depression at higher levels
  - On the molecular level it is related to inhibition of mitochondrial Complex IV/cytotoxic hypoxia
  - Calcium mobilisation, iron mobilization, mitochondrial uncoupling, DNA damage, release of excitatory amino acids and intracellular acidification.
  - A hibernation-like state

H₂S and bacteria in the gut

• High levels of sulphide are produced by both enteric bacteria and human enteric cells
• Its detrimental or beneficial effects remain in debate
• On one hand, it protects the colon from the epithelial damage, oxidative stress and inflammation
• On the other exogenous H₂S is a potential player in the aetiology of intestinal disorders, inflammatory bowel diseases and colorectal cancer


www.mthfrsupport.com.au
**H₂S functions in the gut**

- Colonocytes utilize H₂S as a metabolic 'fuel'
- It suppresses contractile activity in jejunum by affecting directly smooth muscles
- It contributes to maintenance of mucosal integrity by increasing resistance to epithelial injury and accelerating repair, which may be signalling mediated by NF-kB
- It promotes resolution of inflammation, and restoration of normal tissue functions
- It protects the colon from oxidative stress

Sulphur and oxalates

Oxalate comes from its direct synthesis by the body, and higher intake of glyphosate in foods

• Glyphosate causes sulfur depletion in plant food, and systemic sulfur depletion in the body

• The sulfate ion transporter, Sat-1 plays an important role not only in sulfate transport but also in oxalate transport.

• Oxalate/glyoxalate compete with Sulfate for the Sat-1 transporter. High oxalate/glyphosate levels reduce sulfate.

• Sulphate is critical for bile acid formation and for detoxification of xenobiotics.

• Low Sulphate will affect those who have SULT SNP’s.

SUOX deficiency

SUOX mutations are found in patients with isolated sulfite oxidase deficiency.

- Homozygous SUOX mutations Y343X and Q364X, substituted residues such as I201L, R211Q, G305S, R309H, K322R, Q339R, and W393R; and a R319C as C>T transition. Other mutations are also found as c.713G > A, c.884G > A, and c.884G > A. But not all patients have symptoms.

- The cofactor for this gene is molybdenum.

- Other mutations affecting iron-sulphur cluster assembly and fatal infantile, NFU1, BOLA3, IBA57 and ABCB10

- Diets can affect sulphur metabolism through CBS gene deficiency, or insufficient sulphur in the diet; we need to ensure that we are not eliminating sulphur based foods like onion, garlic, broccoli, cabbage etc.

CBS is interacting with NO in Hcy & ammonia metabolism

- Elevated NO inhibits CBS and causes an increase in homocysteine that affects the kidney.
- CBS pathway and nitric oxide (NO) synthase cycle interact to assist metabolism and protect the body.
- NO binds to CBS quickly and tightly, and may have regulatory impact on CBS.
- CBS and CGL are secreted into the blood by the liver to metabolize Hcy and produce H₂S; when Hcy is high, it will disturb NO cycle to compromise vascular integrity.
- High ammonia levels in the brain, such as in chronic liver failure, can be toxic and lethal; this will trigger higher NO activities to detoxify ammonia and to increase protein nitration.

CBS and Nitric oxide

Nitric Oxide (NO) Dysregulation
- ↑iNOS, ↑3-NT
- Disrupted Barrier Integrity
- ↓claudin-5
- ↑permeability

Stress Conditions =
- ↓Cell Viability
- ↑DNA Damage

Liver

Restored NO Regulation
- ↓iNOS, ↓3-NT
- Disrupted Barrier Integrity
- ↑claudin-5
- ↓permeability

Vascular Lumen

Hcy

H₂S

CGL

Endothelium

Stress Conditions =
- ↑Cell Viability
- ↓DNA Damage

The citric acid cycle is a series of reactions also known as the tricarboxylic acid (TCA) cycle or the Krebs cycle. The TCA cycle is the final common pathway for the oxidation of fuel molecules—amino acids, fatty acids, and carbohydrates. Most fuel molecules enter the cycle as acetyl coenzyme A.

Functions of the TCA cycle

• Energy metabolism of the heart
• Energy metabolism of the skeletal muscles
• Energy provider for all processes in other cells

CBS pathway & the energy cycle

Novel information from a CBS silencing study

• CBS is responsible for mitochondrial respiration and ATP synthesis
• CBS affects the NAD/NADH ratio as a critical role in ATP synthesis
• TCA, the energy cycle, is involved in ATP synthesis to provide NADH
• CBS supports mitochondrial oxidative phosphorylation to generate the energy molecule ATP from ADP
• H₂S supports ATP synthesis under hypoxic stress.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3827285/
Serine, Taurine & CBS Pathway
Serine, Taurine & CBS Pathway

Both serine and taurine are important metabolites of CBS pathway.

What serine and taurine do in CBS pathway

• CBS catalyses the formation of cystathionine from homocysteine and serine
• CBS is responsible for cysteine and taurine biosynthesis
• CBS deficiency causes higher homocysteine and lower cysteine, serine and taurine

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC118034/
Only one transsulfuration pathway exists in mammals, i.e., from homocysteine to cysteine

- CBS directly involves in the removal of homocysteine from methionine cycle
- CBS converts homocysteine and serine to cystathionine
- Cystathionine is then used to synthesize cysteine


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC118034/
Taurine & CBS Pathway

• CBS is responsible for cysteine and taurine biosynthesis, as metabolites of homocysteine degradation

• Taurine is synthesized endogenously from cysteine or via conversion from methionine, but also provided by diet

• Taurine is the most abundant amino acid involved in many biological functions, including stabilization of cellular membrane, Ca2+ transport regulation, osmoregulation, antioxidant effects, anti-inflammation, and detoxification

• CBS deficiency leads to lower taurine production and higher cholesterol level

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4429143/
Taurine deficiency

- High blood cholesterol (as it supports lipid breakdown)
- Epilepsy, anxiety, seizures (it acts with glycine as an inhibitory neurotransmitter)
- Tinnitus – increases inhibitory tone and decreases noise in the auditory pathway
- Big drinkers – it reduces alcohol toxicity
- Inflammation
- Fat metabolism – it is involved in bile synthesis
- Regulator of Na⁺-K⁺ ATPase pump and calcium channels

https://www.ncbi.nlm.nih.gov/pubmed/?term=PMC3501277
Hydrogen peroxide ($H_2O_2$) & CBS Pathway

- Aerobic metabolism generates $H_2O_2$ and peroxynitrite, both with oxidation toxicity
- Cells respond to $H_2O_2$ and peroxynitrite challenges in a similar manner as an anti-oxidant response
- $H_2O_2$ can be metabolized by GSH peroxidase (GPx) in the cytosol and mitochondria, and by catalase in the peroxisome
- GSH has anti-oxidant function in redox signaling, to protect sensitive protein thiols from irreversible oxidation, and may serve to prevent the loss of GSH under oxidative conditions

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3549305/
Glutathione (GSH) and CBS pathway

Antioxidant defense
• Scavenging free radicals and other reactive species
• Removing hydrogen and lipid peroxides
• Preventing oxidation of biomolecules

Metabolism
• Synthesis of leukotriene's and prostaglandins
• Conversion of formaldehyde to formate
• Production of D-lactate from methylglyoxal
• Formation of glutathione-NO adduct
• Storage and transport of cysteine

Regulation
• DNA and protein synthesis, and proteolysis
• Cytokine production and immune response
• Mitochondrial function and integrity

http://jn.nutrition.org.simsrad.net.ocs.mq.edu.au/content/134/3/489/T1.expansion.html
Low GSH and its consequences

- Low homocysteine – need to replenish sulphur amino acids
- NAC
- Methionine
- Taurine
- Alpha lipoic acid
- Selenium

Antioxidant function of GSH

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3549305/
SNPs Related to CBS Pathway
<table>
<thead>
<tr>
<th>CBS related SNPs</th>
<th>Co-Factors</th>
<th>Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTH/CSE</td>
<td>P5P</td>
<td>β-Cyanoalanine (BCA), propargylglycine (PAG), &amp; L-aminoethoxyvinylglycine (AVG)</td>
</tr>
<tr>
<td>CDO</td>
<td>Fe(2+)</td>
<td>Cysteine</td>
</tr>
<tr>
<td>CSAD</td>
<td>P5P</td>
<td>Oxygen, nitric oxide (NO)</td>
</tr>
<tr>
<td>SUOX</td>
<td>Heme b</td>
<td>Cytochrome C</td>
</tr>
<tr>
<td>PDH</td>
<td>Thiamine diphosphate</td>
<td>Pyruvate, calcium, fluphenazine</td>
</tr>
<tr>
<td>MUT</td>
<td>Adenosylcobalamin</td>
<td>Ethylmalonyl CoA, cyclopropylcarbonyl CoA carboxylate, methylenecyclopropylacetyl CoA, and nitric oxide (NO)</td>
</tr>
<tr>
<td>GCL</td>
<td>Glutathione (GSH)</td>
<td>L-buthionine-[S,R]-sulfoximine (BSO)</td>
</tr>
<tr>
<td>GSS</td>
<td>Mg(2+)</td>
<td>CMBMB, Chloroquine</td>
</tr>
<tr>
<td>GST</td>
<td>Glutathione</td>
<td>4-hydroxyequilenin (4-OHEN); 4-hydroxyequilin (4-OHEQ)</td>
</tr>
<tr>
<td>GPX</td>
<td>Glutathione</td>
<td>(S)- and (R)-misonidazole</td>
</tr>
<tr>
<td>GSR</td>
<td>FAD</td>
<td>Glutathione</td>
</tr>
<tr>
<td>G6PD</td>
<td>NAD and NADP</td>
<td>Dehydroepiandrosterone (DHEA)</td>
</tr>
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</table>
MMAB, CTH, MUT and adenosylcobalamin

- MMAB (methylmalonic aciduria cblB) involves in the formation of adenosylcobalamin (AdoCbl) derived from B12; MMAB may also deliver AdoCbl to methylmalonyl CoA mutase
- CTH (Cystathionase) converts cystathione derived from methionine into cysteine, which is needed for glutathione synthesis in the liver
- MUT (methylmalonyl-CoA mutase) catalyzes methylmalonyl-CoA to succinyl-CoA, using adenosylcobalamin (vitamin B12) as a cofactor; meaning it is a B12 dependent enzyme
- MUT involves in the degradation of several amino acids, odd-chain fatty acids, and cholesterol via propionyl-CoA to the TCA cycle
- MUT mutations lead to various types of methylmalonic acidemia, which can also come from insufficient B12

https://www.ncbi.nlm.nih.gov/pubmed/?term=PMC3370288
Three CBS polymorphisms are compared

- CBS c.699C>T: rs234706
  - Down-regulate CBS gene transcription;

- CBS c.1080C>T: rs1801181
  - Down-regulate CBS gene transcription; increases Hcy levels

- CBS 844ins68 allele: rs5742905
  - Down regulates CBS gene transcription; increases Hcy levels
### Overview of CBS SNPs

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>SNP Name</th>
<th>Risk Allele</th>
<th>Allele Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2851391</td>
<td>CBS A13637G</td>
<td>T</td>
<td>Associated with higher Hcy level adjusted for folic acid concentration</td>
</tr>
<tr>
<td>rs1801181</td>
<td>CBS A360A</td>
<td>A</td>
<td><strong>Down-regulate gene expression</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increased risk of NHL through gene-nutrient interaction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Related to homocystinuria due to CBS deficiency</td>
</tr>
<tr>
<td>rs706209</td>
<td>CBS C*351T</td>
<td>A</td>
<td><strong>Down-regulate gene expression</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increased risk of clear cell renal cell carcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Related to homocystinuria due to CBS deficiency</td>
</tr>
<tr>
<td>rs4920037</td>
<td>CBS C19150T</td>
<td>A</td>
<td>Associated inversely with arsenic excreted as %DMA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increased risk of lung cancer in high %MMA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increased risk of CL/P</td>
</tr>
<tr>
<td>rs234706</td>
<td>CBS C699T</td>
<td>A</td>
<td><strong>Down-regulate gene expression</strong></td>
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<td></td>
<td></td>
<td></td>
<td>Reduced risk of CL/P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increased risk of preeclampsia</td>
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<tr>
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<td></td>
<td>Reduced risk of NHL in homozygous</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Associated with dysregulation of CBS</td>
</tr>
<tr>
<td>rs12613</td>
<td>CBS G*299A</td>
<td>T</td>
<td><strong>Down-regulate gene expression</strong></td>
</tr>
<tr>
<td>rs706208</td>
<td>CBS T*330C</td>
<td>G</td>
<td><strong>Down-regulate gene expression</strong></td>
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# CTH related SNP functions

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>SNP Name</th>
<th>Risk Allele</th>
<th>Allele Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs663649</td>
<td>CTH G25229T</td>
<td>T</td>
<td>Involved in folate/one-carbon metabolism</td>
</tr>
<tr>
<td>rs1021737</td>
<td>CTH S4031I</td>
<td>T</td>
<td>Associated with obesity in Indian children</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Related to higher level of plasma cystathionine only after methionine loading</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Related to mild plasma tHcy elevations</td>
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<tr>
<td>rs482843</td>
<td>CTH 1491</td>
<td>A</td>
<td>Participates in the development of preeclampsia</td>
</tr>
<tr>
<td>rs12723350</td>
<td>CTH T16147C</td>
<td>C</td>
<td>Associated with higher risk for neural tube defects</td>
</tr>
</tbody>
</table>

## CTH related SNP functions

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<tr>
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<tbody>
<tr>
<td>rs1145920</td>
<td>CTH A11886G</td>
<td>A</td>
<td>Associated with folate/one-carbon metabolism</td>
</tr>
<tr>
<td>rs515064</td>
<td>CTH A32114G</td>
<td>G</td>
<td>Associated with folate/one-carbon metabolism</td>
</tr>
<tr>
<td>rs663649</td>
<td>CTH G25229T</td>
<td>T</td>
<td>Associated with folate/one-carbon metabolism</td>
</tr>
<tr>
<td>rs10889869</td>
<td>CTH G6010A</td>
<td>A</td>
<td>Increased risk of neural tube defects</td>
</tr>
<tr>
<td>rs1021737</td>
<td>CTH S4031I</td>
<td>T</td>
<td>Increased risk of childhood obesity in early screening</td>
</tr>
<tr>
<td>rs12723350</td>
<td>CTH T16147C</td>
<td>C</td>
<td>Increased risk of neural tube defects</td>
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<tr>
<td>rs681475</td>
<td>CTH T8763C</td>
<td>T</td>
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## SUOX related SNP functions

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<th>Allele Effect</th>
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<tbody>
<tr>
<td>rs705702</td>
<td>SUOX 6821</td>
<td>A</td>
<td>Associated with higher risk of polycystic ovary syndrome in both Chinese Han, and White European populations</td>
</tr>
</tbody>
</table>

SNPs not on variant report but relevant:
- 121908009
- 121908008
- 1219008007

## MUT related SNP functions

<table>
<thead>
<tr>
<th>SNP ID</th>
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<th>Risk Allele</th>
<th>Allele Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6458690</td>
<td>MUT T24234C</td>
<td>G</td>
<td>Associated with elevated homocysteine level; and in linkage disequilibrium with rs4267943 in women</td>
</tr>
<tr>
<td>rs4267943</td>
<td></td>
<td>G</td>
<td>Associated with homocysteine level; and in linkage disequilibrium with rs6458690 in women</td>
</tr>
<tr>
<td>rs9369898</td>
<td></td>
<td>G</td>
<td>Associated with higher risk of stroke as small-vessel disease</td>
</tr>
</tbody>
</table>
Glutathione (GSH) provides a major source of thiol homeostasis critical to the maintenance of cell survival

Glutathione transferase (GST) is responsible for extra GSH detoxification

GSTP, as one of the newly found GSTs, can affect S-glutathionylation reaction to modify cysteine

GSTP is reversely affected by a number of redox sensitive proteins including glutaredoxin, thioredoxin and sulfiredoxin

GSTP involves in tumorigenesis & anticancer drug resistance

They decide how cells respond to oxidative stress exemplifying the broad importance of GSH/GST homeostasis in health conditions, such as cancer, ageing and neurodegenerative diseases

## GSS related SNP functions

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>rs6088659</td>
<td>GSS A5997G</td>
<td>T</td>
<td>Associated with higher risk of chronic obstructive pulmonary disease (COPD)</td>
</tr>
<tr>
<td>rs2236270</td>
<td>GSS C25447A</td>
<td>T</td>
<td>Associated with lower risk of SLE belonging to a 4-SNP protective haplotype with rs6087651, rs17092180 and rs2273684</td>
</tr>
<tr>
<td>rs28936396</td>
<td>GSS C373T</td>
<td>A</td>
<td><strong>Associated with higher risk of glutathione synthetase deficiency</strong></td>
</tr>
<tr>
<td>rs6060124</td>
<td>GSS G11705T</td>
<td>C</td>
<td>Related to lung function growth in children Associated with higher bladder cancer recurrence</td>
</tr>
</tbody>
</table>
## GSS related SNP functions

<table>
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<tr>
<th>SNP ID</th>
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<th>Risk Allele</th>
<th>Allele Effect</th>
</tr>
</thead>
</table>
| rs5751901| GGT1 T17549C              | T           | **Up regulating gene expression**  
Increased risk of pancreatic cancer, especially when combined with rs2017869.  
Causing an increased GGT level |
| rs4820599| GGT1/FAM211B A15496G      | G           | Increased risk of pancreatic cancer  
Increased risk of chronic pancreatitis  
Increased risk of diabetic retinopathy |
| rs2017869| GGT1                      | CC          | Increased GGT Level  
Statistically significant association with pancreatic cancer with 2017869 and 8135987 (CC allele). |

### GPX3 related SNP functions

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>SNP Name</th>
<th>Risk Allele</th>
<th>Allele Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs8177412</td>
<td>GPX3 129T&gt;C</td>
<td>C</td>
<td>Increased risk of differentiated thyroid cancer In older people the heterozygote TC was significantly associated with the thyroid cancer. &gt;45 years of age</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increased risk of arteriopathy stroke in children</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Results in a GPX 3 deficiency</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Also written as G allele in some research.</td>
</tr>
</tbody>
</table>

### GST related SNP functions

<table>
<thead>
<tr>
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<th>Risk Allele</th>
<th>Allele Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12068997</td>
<td>GSTM1 5419C&gt;T</td>
<td>T</td>
<td>Association with higher bladder cancer risk</td>
</tr>
<tr>
<td>rs4147567</td>
<td>GSTM1 7107A&gt;G</td>
<td>G</td>
<td>Association with higher bladder cancer risk</td>
</tr>
<tr>
<td>rs2239892</td>
<td>GSTM1 8869A&gt;G</td>
<td>G</td>
<td>Association with higher bladder cancer risk</td>
</tr>
<tr>
<td>rs7483</td>
<td>GSTM3 V224I</td>
<td>T</td>
<td>Associated with higher progression risk of prostate cancer, but lower risk of progression to CRPC and death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Associated with higher risk of Alzheimer interacting with rs1111875 in the HHEX / IDE/KIF11 gene</td>
</tr>
<tr>
<td>rs1138272</td>
<td>GSTP1 A114V</td>
<td>T</td>
<td>Associated with higher levels of PSA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Associated with higher risk of gastric cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Related to higher occurrence of docetaxel-induced peripheral neuropathy in cancer treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Related to higher risk of asthma associated with air pollution in children</td>
</tr>
<tr>
<td>rs1695</td>
<td>GSTP1 I105V</td>
<td>A</td>
<td>Associated with higher levels of PSA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Associated with higher risk of gastric cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Related to higher risk of asthma associated with air pollution in children</td>
</tr>
<tr>
<td>rs6591256</td>
<td>GSTP1 2950</td>
<td>A</td>
<td>Associated with increased diastolic blood pressure</td>
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</tbody>
</table>
## GPX related SNP functions

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</tr>
</thead>
<tbody>
<tr>
<td>rs8177412</td>
<td>GPX3</td>
<td>T</td>
<td>Related to higher arteriopathy stroke risk in children Associated with higher risk of differentiated thyroid cancer</td>
</tr>
<tr>
<td>rs8177417</td>
<td></td>
<td>G</td>
<td>Associated with higher risk of essential hypertension in Chinese Han</td>
</tr>
<tr>
<td>rs3828599</td>
<td></td>
<td>A</td>
<td>Associated with higher risk of essential hypertension in Chinese Han</td>
</tr>
<tr>
<td>rs10504050</td>
<td></td>
<td>A</td>
<td>Associated with obesity especially with morbid obesity</td>
</tr>
<tr>
<td>rs1050450</td>
<td>GPX1</td>
<td>C</td>
<td>Related to decreased DNA damage with increasing serum Se level in oxidative stress</td>
</tr>
</tbody>
</table>
## GSR related SNP functions

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>SNP Name</th>
<th>Risk Allele</th>
<th>Allele Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2551715</td>
<td>GSR A43851G</td>
<td>C</td>
<td>Associated with higher risk of SLE in Africans</td>
</tr>
<tr>
<td>rs3594</td>
<td>GSR G*1377T</td>
<td>A</td>
<td>Associated with higher risk of anaemia in clinical malaria infection in children</td>
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</tbody>
</table>

## GSS SNPs

<table>
<thead>
<tr>
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<th>Allele Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2273684</td>
<td>GSS A18836C</td>
<td>T</td>
<td>Decreased risk of SLE in Blacks in an early screening Reduced risk of death in SCLC in an early screening</td>
</tr>
<tr>
<td>rs6088659</td>
<td>GSS A5997G</td>
<td>T</td>
<td>Not found</td>
</tr>
<tr>
<td>rs28936396</td>
<td>GSS C373T</td>
<td>A</td>
<td>Increased risk of Glutathione synthetase deficiency</td>
</tr>
<tr>
<td>rs6060124</td>
<td>GSS G11705T</td>
<td>A</td>
<td>Associated with lung growth in children</td>
</tr>
</tbody>
</table>

http://www.ncbi.nlm.nih.gov/pmc/?term=rs2273684,
http://www.ncbi.nlm.nih.gov/pmc/?term=rs28936396,
http://www.ncbi.nlm.nih.gov/pmc/?term=rs6060124
Factors Down/Up Regulating CBS Pathway
Factors regulating CBS pathway

**CBS pathway up-regulators**
- SAM, zinc, proteasome inhibitors, s-glutathionylation and taurine-deficient diet

**CBS pathway down-regulators**
- Peroxynitrite, nitrogen dioxide, carbonate radical, methionine-deficient diet, rotenone, high-salt induced hypertension and pharmacological CBS inhibitors

**The GCL-GSH-NO axis**
CBS pathway: up-regulators

• **SAM** (S-adenosyl-L-methionine) markedly enhances CBS-mediated H2S production in vitro, especially when a combination of cysteine and homocysteine present

• **Zinc** enhances CBS activity in the liver and kidneys, to lower plasma homocysteine levels effectively

• **Proteasome inhibitors**, Bortezomib, and ONX0912 restore mutant misfolded CBS enzyme activities, and lower plasma homocysteine levels

• **S-glutathionylation** enhances CBS activity under oxidative stress, and increases cysteine and therefore glutathione synthesis

• **Taurine-deficient diet** up-regulates CBS mono-allele in gene knock-out mice

CBS pathway: down-regulators

- **Peroxynitrite**, the product of nitric oxide and superoxide radicals, inactivates CBS leading to nitration of Trp208, Trp43 and Tyr223 and loss of thiolate coordination
- **Nitrogen dioxide and carbonate radical** inactivate CBS
- **Methionine-deficient diet** induces post-transcriptional downregulation of CBS and transsulfuration shutdown
- **Rotenone**, a environmental toxin, down-regulates CBS function and H2S production in primary microgla probably via triggering ROS formation
- **High-salt induced hypertension** inhibits CBS/H2S pathway in renal tissues, might be an pathogenesis of salt-sensitive hypertension

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2783175
Nitric oxide down-regulates GCL and GSH

The GCL-GSH-NO axis plays important roles in CBS pathway

- NO protects endothelial cells against hydrogen peroxide induced toxicity.
- NO mediated protection towards hydrogen peroxide depends on the activity of glutathione peroxidase and glutamate cysteine ligase (GCL), the rate-limiting enzyme of GSH de novo biosynthesis.
- Under pro inflammatory conditions, both cellular NO synthesis and intracellular ‘free’ zinc are required to maintain the cellular GSH levels. (Because NO within cells induces a zinc release from proteins containing zinc-sulfur complexes).
- GSH is essential for iNOS which produces nitric oxide radical. Depletion of GSH below a critical level blocks hepatocyte NO formation.

Things to think about.
CBS issues

- Low zinc/high copper/low B6 – pyrroles/low GSH/oxalates/glyphosate?
- Low homocysteine
- Sleep issues – low serine getting to sleep
- Oxidative stress – low cysteine therefore low glutathione. Are they taking SAM? SAM activates CBS.
- Are there gut issues that might be increasing ammonia/histamine/producing endotoxins and so low NO?
- H2S important mediator of inflammation/vasodilator.
- High homocysteine – CBS mutations? Low B12/folate/Betaine/B6
- CBS mutations often present with an inability to tolerate onion, garlic, eggs, cabbage (i.e.: high sulphur foods) molybdenum will help this while you regulate the pathway. You don’t really need to restrict sulphur too much. Its important for detoxification
Questions to ask your patients

- Have you had a virus or gut issues lately? Could this have upset ammonia/histamine/H2S levels and therefore affected CBS?
- Do you have problems with sulphur sensitivity?
- Do you have urinary frequency or urgency? Oxalates?
- Do you have joint pain, arthritis? Oxalates?
- Any cardiovascular disease in the family? Elevated histamine
- Do you eat sulphur based foods? If no big alarm.
- What is your zinc level? If always low, could this be a GSH issue? Could HCL be low?
- Do you have elevated BP? Low NO/Low h2S? Low GSH.
- Do you have brain fog? Can’t think? Ammonia? NOS issues?
S/S Ammonia Toxicity In The Brain

- Chronic fatigue
- Headache
- Irritability
- Diarrhoea or nausea
- Lack of concentration
- Mental confusion
- Intellectual impairment
- Intolerance of foods – especially high protein ones
- Ataxia
- Stupor
- Low blood urea nitrogen (low BUN) levels
- Activation of NMDA receptors in the brain

S/S Taurine Deficiency

• High blood cholesterol (as it supports lipid breakdown)

• Epilepsy, anxiety, seizures (it acts with glycine as an inhibitory neurotransmitter)

• Tinnitus – increases inhibitory tone and decreases noise in the auditory pathway

• Big drinkers – it reduces alcohol toxicity

• Inflammation

• Fat metabolism – it is involved in bile synthesis

• Regulator of Na+K+ ATPase pump and calcium channels

• Don’t think CBS is high taurine as per Yasko – but is often in autistic children
Low Glutathione

- Low homocysteine – need to replenish sulphur amino acids
  - NAC
  - Methionine
  - Taurine
  - Alpha lipoic acid

**CAUTION:** needs to be done slowly and cautiously especially in those who have a CBS homozygous mutation

### Sulphur based foods

#### Trans-Sulfuration Pathway & Nutrition

Foods rich in sulfur

- Asparagus
- Avocado
- Blueberries
- Broccoli
- Brussel Sprouts
- Cabbage Garlic
- Cauliflower
- Cabbage
- Carrots
- Cheese
- Cherries
- Chives
- Coconut
- Eggs
- Garlic
- Grapes
- Grains
- Kale
- Leeks
- Legumes
- Meat Protein
- Mustard
- Nuts & Seeds
- Onions
- Pak Choi
- Parsley
- Radishes
- Red Peppers
- Rutabaga
- Shallot
- Swiss Chard
- Tomatoes
- Turnips
- Watercress
If pyrroles

Pyrroles – this is interesting and if you have someone who has low homocysteine, don’t give pyridoxine, it will stimulate the pathway too much. Give zinc/P5P.
Balance zinc/copper and then start with antioxidants NAC at low doses to support sulphur pathway.
Pyrroles are an issue with CBS and possibly oxalates. Chronic fatigue
Case Studies Related to CBS Pathway
Case History 1

23 year old female

Key issues:
1. Digestive issues – no appetite, gut feels sluggish, sensitive/intolerant to many foods. Always bloated/belching and burning feeling in my intestines. Nausea, stomach pain, wind. Previously had SIBO (multiple rounds of antibiotics and herbs) Been on the GAPS/FODMAP/PALEO diet for years.
   1. Kiwi fruit – tingling lips/red rash – high histamine/oxalates
   2. Fermented foods – burning pain in gut.
   3. Onion, garlic, brassica make her burp
   4. Tomatoes – burning pain
2. Stools varied. May be constipated and not go for 2-3 days. No energy/no vitality. Multiple dips in energy through the day. Most awake at 6pm.
3. Brain fog
4. Malaise/fatigue – could lay on the couch or in bed all day
5. Irregular periods
6. Anxiety – especially in social situations/loud noises
7. Also had dizziness/fainting/hair loss/dandruff/rash on face, elbows, knees
Case History

- Was on supplements with methyl's (12 and folate) and high dose zinc/P5P. But made her feel so unwell – nausea/fatigue/angry/depressed/anxious. But she was told by Naturopath who did her genetic test she needed the folate so she went back on but took niacin every day to mitigate the effects. She also started fermented foods – sauerkraut/ coconut kefir – made her feel like her intestines were being scrubbed out by a brush. Brain fog/nausea/very bad digestion/weight gain/hCG diet/ - fatigue, sore, twitchy muscles/heart palpitations. Lots of cruciferous on advise of Dr and Naturopath.
- HCL and digestive enzymes
- Fish oil
Case History

Current diet:

- Lunch – meat and veg. Steak with cruciferous vegetables, pumpkin and carrot
- Dinner – boiled eggs and more vegetables. Stewed fruit/baked apples/mandarin and homemade biscuits for dessert.
- 4 cups black tea with almond milk
- Doesn’t drink alcohol
- Never eats grains
- No foods make her feel good.
Case History

• Sleep issues – can’t fall asleep and sleep latency an issue. Never feel refreshed
• Hormones:
  • No regular cycle
  • Very angry at PMT
  • Fatigue and nausea
  • Menstrual Cramps
  • Endometriosis?? Never fully investigated.
• Anxiety 5/10 – gets shaking and sweating at times
• Pyrroles diagnosed at 18. Zinc and B6 didn’t make her feel better
Case History

- **Bloods:**
  - B12 – 1400 supplemented
  - RBC folate – 1754-1356 supplemented
  - Homocysteine – 4
  - Zinc – 10.3 (even though taking 75mg zinc for 4 years)
  - Copper 15
  - ESR 16
  - Albumin 47 – dehydration? Thyroid hypofunction? Adrenals?
  - Uric acid -.26 – low end – molybdenum deficiency perhaps?
  - Cholesterol – 3.9 low end – hormonal issues / fat metabolism
  - Iron – 12/ferritin 71/ saturation 19%
  - MCV – 89
  - RDW 11.7
  - ESR 18 – why? (WCC not elevated) CRP ok
Case History

• Genes
  o CBS C699T ++
  o MTHFR C677T +-
  o BDNF ++
  o MTHFD1 ++
  o MnSOD ++
Appointment 1

- Sulphur – gave molybdenum
- Low histamine/oxalate Diet
- Referred for Stool test/estrogen metabolism
- Removed methyl's, gave a B without B12/folate
- B12
- Niacin for next 24hours to reduce methyl's
- Magnesium
- Ammonia reducing formula with acetyl l carnitine/alpha keto gluturate
Appointment 2

3 weeks later

- Stomach pain has gone
- Knows when she breaks the diet because the stomach gets the raw feeling again. Had chocolate and really bad.
- Going to the toilet every day with the magnesium
- Energy improved from 1/10 to 7/10
- Brain fog good
- Mood better/Not as angry
- Sore breasts before period

- No results for CDSA or Oestrogen metabolism yet.
- Other Bloods that came back:
  - Vitamin D 74
  - Histamine .7
Appointment 3

- CDSA Results
  - Blastocystis
  - Yeast
  - Pancreatic enzymes low
  - Lysozyme elevated
  - SIGA low 7.6
  - Butyrate low

  Oestrogen Metabolism
  - 16OH very high
  - 2OH very high

- Gut protocol – herbs/caprylic
  - Vitamin A
  - Probiotics
  - Glutamine
  - Clay for die off
  - Aloe Vera

- Added:
  - Premular
  - ID3/DIM
Appointment 4

1 month later

- Not compliant with diet and so gut flared again.
- Energy good – back at uni and coping well
- Sleep good
- Brain fog completely gone

- Introduced 100mcg of methylfolate

Next Steps:
- Continue to heal gut and retest
- Increase methylfolate where possible.
  Smallest dose will do
Appointment 4

1 month later

• Next Steps:
  • Continue to heal gut and retest
  • Increase methylfolate where possible. Smallest dose will do
  • Get hormones balanced
  • Support CBS and GSH production
  • Increase homocysteine
Case History 2
Case 2

• 55 year old woman. Presents with:
  • Weight issues. Has never been able to lose weight even though she’s gone on multiple diets. Just puts it straight back on.
  • Vitiligo started 8 years ago. Her mum has it.
  • Tinnitus and hearing problems – had it for 10 years. Is getting worse
  • Pain in every joint
  • Red splotches on her legs and body
  • Recurrent UTI’s
  • Finds it hard to speak sometimes
Case 2

- 55 year old woman. Presents with:
  - Sleep issues. Cant fall asleep
  - Gut issues – very bad bloating with every meal, pain, cramping, stools float. Had blastocystis and dientamoeba. Stools change a lot.
  - Anxiety with new situations
  - Mood is good but Hx of depression.
  - Mum also has vitiligo, ear issues, pain in legs, arthritis, diabetes, back and thyroid issues. Had a hysterectomy due to heavy periods. Had depression.
  - Sister – bulimia, eczema, depression. All very self motivated.

http://www.mthfrsupport.com.au
Case 2

• Allergies to eggs (makes her body ache) and gluten. Cannot eat garlic, cabbage or high sulphur foods.

• Currently on a Pyrrole formula and juices with high greens, magnesium and probiotic. Been on pyrrole for 1 year and juices for 5 years.

• Not having periods any more but when she did she would get vomiting, migraines, very heavy periods and cysts.

• History of frequent colds, frequent UTI’s, migraines, headaches, eczema.

• Gets shortness of breath/tired/low energy/brain fog really bad

• One child with ADHD/mild Asperger's. Another with anaphylaxis
Case 2

- Homozygous C677T
- Pyrroles were 86 in 2015
- Homocysteine 7.8
- Zinc was very low.

Kidney function reduced – EGFR 52 *** (She has kidney pain a lot)
• Appointment 1

• So step 1 is to fix sulphur issue. Remember sulphur disturbed will get worse when methyl's are put into the picture. Sulphur is probably an issue due to gut dysfunction. Also pyrrole formula is speeding up the pathway and creating more sulphur.

• Diet – lots of greens, juicing and vital greens on tope for breakfast. Mushrooms, spinach and halloumi or amaranth with nuts, almond milk. Lemon juice on rising. Lunch – salmon with salad, sushi, chicken and salad. Dinner meat and veggies and sometimes ice-cream after dinner. What's the problem here?
Appointment 1

- Diet – low histamine/low oxalate
- Put in MTHFR cofactors – B12 – hydroxocobalamin/ B’s without B12/folate to support pathways
- Molybdenum – to suck up sulphur that’s currently an issue. But eat sulphur foods.
- Treat Blastocystis and dientamoeba.
- Oestrogen metabolism test and further bloods.
- Continue with magnesium.
- Gave her zinc 25mg/selenium 150mcg
Case 2

- Appointment 1
  - Continue with a probiotic – rhamnossis
  - Get rid of ammonia – acetyl l carnitine (will also help energy) and alpha ketoglutarate.
  - SAMe – 100mg
  - Stop all the methyl's in her food. Stop juicing
  - Take niacin for the next few days.
• 4 days later received an email.
  • With low histamine / oxalate diet she immediately lost 1 kg. Having aches in different places but not concerned about it.
  • SAMe – 100mg 4 days seems to be ok. No issues.
  • Continue with a probiotic – rhamnasis
  • Get rid of ammonia – acetyl l carnitine (will also help energy) and alpha ketoglutarate.
• **Appointment 2** – 2 weeks later
  • Stopped SAMe as her mood decreased.
  • Craving sugar and lollies
  • Getting constipated – stool very light in colour
  • Aches and pains have gone.
  • Reacted quite violently to the gut killing protocol – reduced the dose and gave clay to help eliminate toxins. Need to get her going to the toilet.
  • Put a small amount of vitamin C and increased the magnesium. Swapped SAMe to 100mcg of methyl folate per day.
Case 2

- **Appointment 3** – 2 weeks later
  - Diet good except for chips. Has a craving
  - Sleeping much better and her hearing seems to have improved a little.
  - Stools still fluctuating but not constipated.
  - Kidney pain has gone.
  - Speaking much better. Lisp has gone
  - No headaches
  - Now lost 2kg
  - Thinks the vitiligo on her hands has stopped spreading.
Case 2

- **Appointment 3** – 2 weeks later
  - Feeling pretty good emotionally
  - Continue to increase methylfolate and introduced Vitamin D
  - Oestrogen metabolism shows high levels of 2OH/16a/4OH
  - Introduced ID3/CDG
  - No other changes.
Case 2

- **Appointment 4** – 3 weeks later
  - Notice I am seeing her regularly until I know her reaction with the methylfolate.
  - Feeling really good.
  - Has lost 4.6 kg since she first started to see me.

- Up to ½ methyl folate ie: 300mcg per day. Seems to be good. Continue to increase.
  - Gut protocol continues to be good.
  - Eating sulphur based foods now.
Next steps:

- Take the folate as high as she will tolerate
- Support glutathione production but don’t increase B6 too high.
THANK YOU AND QUESTIONS

CONTACT US:

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