GENOVA DIAGNOSTICS
63 Zillicoa Street
Asheville, NC 28801

METHYLATION PANEL

Patient:
DOB:
Sex:
MRN:

3534 Methylation Panel - Plasma & Whole Blood

Interpretation At-a-Glance

Methylation

- Homocysteine ▲
- SAH ▲
- SAM ▲
- Choline ▲
- Betaine ▲
- DMG ▲
- Sarcosine ▲

Genetic Polymorphism

DOWNREGULATING SNPS

MTHFR
- C677T ⊖⊖
- A1298C ⊕⊕

COMT
- V158M ⊖⊕

MTRR
- A66G ⊕⊕

MAT1A
- D18777A ⊖⊕

SHMT1
- C1240T ⊖⊕

UPREGULATING SNPS

MTR
- A2756G ⊖⊖

CBS
- C699T ⊖⊕

BHMT
- G742A ⊖⊖

GNMT
- C1289T ⊖⊕

GENOMICS AVAILABLE AS OPTIONAL ADD-ON

Transsulfuration

- Glutathione ▼
- Cystathionine ▲
- Cysteine ▲

Methylation Status

SAM/SAH Ratio

Low — High

Methylation Balance

Un-methylated Metabolites — Methyl Group Donors

Met/Sulf Balance

Transsulfuration — Methylation

3534 Methylation Panel - Plasma & Whole Blood

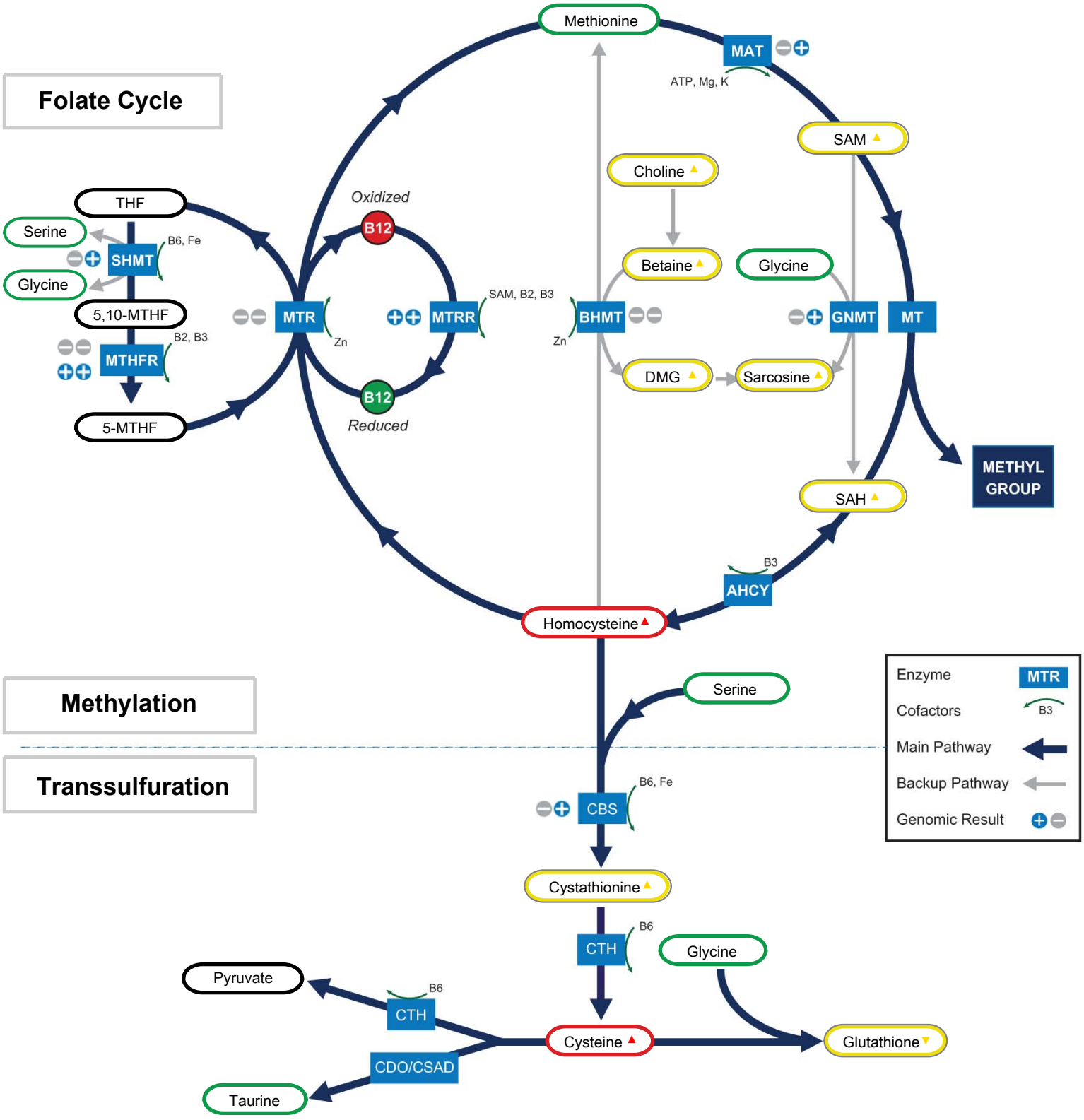
Methodology: LCMSMS & Colorimetric

	Results micromol/L	QUINTILE DISTRIBUTION (1st 2nd 3rd 4th 5th)	Reference Range
Methylation Capacity			
Ratios			
1. Methylation Index (SAM/SAH Ratio)	3.3		2.2-6.4
2. Methylation Balance Ratio	1.04		1.03-1.20
3. Met/Sulf Balance Ratio	0.63		0.55-0.64
4. Betaine/Choline Ratio	5.2		2.6-7.7
Methyl Group Donors			
5. S-adenosylmethionine (SAM)	137		65-150 nanomol/L
6. Methionine	30		23-38
7. Choline	12.0		5.2-13.0
8. Betaine	62		21-71
9. Serine	125		91-161
Methyl Group Metabolites			
10. S-adenosylhomocysteine (SAH)	41		16-41 nanomol/L
11. Homocysteine †	12.0 H		3.7-10.4
12. Dimethylglycine (DMG)	5.0		1.6-5.0
13. Sarcosine	6,485		3,670-6,743 nanomol/L
14. Glycine	317		181-440
Transsulfuration Metabolites			
15. Cystathionine	321		74-369 nanomol/L
16. Cyst(e)ine	439 H		271-392
17. Taurine	104		50-139
18. Glutathione †	836		>=669

†These results are not represented by quintile values.

Tests were developed and their performance characteristics determined by Genova Diagnostics. Unless otherwise noted with ◆, the assays have not been cleared by the U.S. Food and Drug Administration.

Methylation / Transsulfuration Pathway



Energy Production

Detoxification

3535 Add-on Methylation Genomics - Buccal sample

Methodology: DNA Sequencing

BHMT G742A

Your Genotype:

Allele 1	Allele 2
G	G
Wild Type -	Wild Type -

Potential Impact:

No Upregulation

Genotypes	Amino Acid
G G	Arg Arg
G A	Arg Gln
A A	Gln Gln

Amino Acid Position: 239

Arginine to Glutamine

cGA → cAA

DNA Position: 821

SNP

GAGGCTGCC c(G or A)A CTGAAAGCT

Amino Acid Codon

Rs Number: rs3733890

Location: Chromosome 5q14.1

*** Frequency:**

Population Category	GG	GA	AA
EUR	48%	41%	11%
EAS	52%	41%	7%
AFR	55%	41%	4%
AMR	32%	52%	16%
SAS	52%	43%	5%

Betaine-homocysteine S-methyltransferase

Betaine-homocysteine methyltransferase (BHMT) is the enzyme responsible for remethylation of homocysteine via an alternate pathway using betaine as a methyl donor.[5] BHMT acts as a backup pathway to maintain SAM levels and is expressed primarily in the liver and kidney.[6]

Health Implications

- The BHMT G742A polymorphism results in increased BHMT activity (also referred to as “upregulation”). Upregulation of BHMT may lead to lower levels of homocysteine as well as less dependency on folate and vitamin B-12 as methyl donors.
- Because this BHMT polymorphism results in increased activity, research suggests that this SNP is protective against many of the clinical conditions related to elevated homocysteine and folate deficiency.
- This G742A SNP has been associated with reduced all-cause mortality in breast cancer and decreased birth defect risk in some studies.[1-4]
- However, the overuse of choline as a substrate for methylation may have a negative metabolic consequence, because choline is needed for many other processes in the body.
  - For example, SNPs for this enzyme may result in decreased choline availability for the PEMT pathway, which is responsible for acetylcholine and phospholipid synthesis.[5]
- Abnormal choline metabolism may be associated with congenital abnormalities such as Down syndrome and neural tube defects.[7] These risks may be exacerbated by homozygous positive findings combined with low folate intake.

Clinical Considerations

- Check choline and betaine levels; consider supplementation if applicable. Ensure adequate dietary choline intake.
- Assess likelihood of zinc insufficiency; evaluate plasma zinc and zinc/copper ratio.
- Assess SAM/SAH ratio and Methyl Balance Ratio to rule out excessive SAM production.

References

1. Boyles AL, et al. *Environ Health Perspect.* 2006;114(10):1547-1552.
2. Shaw GM, et al. *BMC Med Gen.* 2009;10:49.
3. Mostowska A, et al. *J Med Gen*. 2010;47(12):809-815.
4. da Costa KA, et al. *FASEB J.* 2014;28(7):2970-2978.
5. Obeid R. *Nutrients*. 2013;5(9):3481.
6. Sunden SL, et al. *Arch Biochem Biophys.* 1997;345(1):171-174.
7. Jaiswal SK, et al. *Eur J Clin Nutr.* 2017;71(1):45-50.

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3535 Add-on Methylation Genomics - Buccal sample

Methodology: DNA Sequencing

CBS C699T

Your Genotype:

Allele 1	Allele 2
C	T
Wild Type -	Variant +

Potential Impact:

Upregulation

Genotypes	Amino Acid
C C	Tyr Tyr
C T	Tyr Tyr
T T	Tyr Tyr

Amino Acid Position: 233

Tyrosine to Tyrosine

TA C ⟶ TA T

DNA Position: 944

SNP

TGGCTCAC**TA(C** or **T)** GACACCACCG

Amino Acid Codon

Rs Number: rs234706

Location: Chromosome 21q22.3

*** Frequency:**

Population Category	CC	C T	TT
EUR	42%	48%	10%
EAS	95%	5%	<1%
AFR	59%	33%	8%
AMR	72%	25%	3%
SAS	44%	46%	10%

Cystathionine beta-synthase

Cystathionine beta-synthase (CBS) is the enzyme responsible for homocysteine's irreversible conversion to cystathionine. This is the first step in the transsulfuration pathway that ultimately leads to glutathione production.

Health Implications

- The CBS enzyme is strongly regulated by the availability of SAM. Adequate SAM levels leads to an upregulation of the CBS enzyme, allowing homocysteine to be irreversibly committed to the transsulfuration pathway.[1]
- Most literature suggests that CBS C699T polymorphisms result in upregulation of CBS activity favoring transsulfuration and lowering homocysteine.[2,3]
- One study demonstrated the opposite effect in a Chinese population where CBS polymorphisms resulted in increased plasma homocysteine.[4] Therefore, debate exists regarding the impact of C699T polymorphism on enzyme activity.
- Despite the lack of agreement on enzyme activity, multiple studies demonstrate clinical associations with the C699T polymorphism. These include:
  - Reduced risk of lymphoma [5]
  - Reduced risk of venous disease [6,7]
  - Protective effects against deep vein thrombosis [6]
  - Decreased risk of coronary artery disease [8]

Clinical Considerations

- Since this polymorphism is mostly considered to be protective, evaluate homocysteine levels in patients with "wild-type" (negative) CBS genotypes and address causes of elevated homocysteine.
- Some clinicians consider CBS polymorphisms to potentially "drain" methylation metabolites into the transsulfuration cycle. Evaluate overall methyl balance ratios and consider methylation support if warranted.
- Reduce levels of oxidative stress which further upregulate the CBS enzyme.
- Evaluate other transsulfuration metabolites (taurine, cystathionine, and glutathione) to determine if upregulation of CBS is likely. Assess met/sulf balance ratio.
- Ensure adequate supply of vitamin B-6 and iron, as these are cofactors for the CBS enzyme.

References

1. Stabler SP, et al. *Blood*. 1993;81(12):3404-3413.
2. DeStefano Vea. *Ann Hum Genet*. 1998;62(6):481-490.
3. Aras Ö, et al. *Clin Genet*. 2000;58(6):455-459.
4. Wu X, et al. *Hered Cancer Clin Pract*. 2014;12(1):2.
5. Li Q, et al. *Cancer Causes Control : CCC*. 2013;24(10):1875-1884.
6. Ayala C, et al. *Biomedica*. 2010;30(2):259-267.
7. Hendrix P, et al. *J Neurosurg*. 2017:1-7.
8. Kruger WD, et al. *Mol Genet Metab*. 2000;70(1):53-60.

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3535 Add-on Methylation Genomics - Buccal sample

Methodology: DNA Sequencing

GNMT C1289T

Your Genotype:

Allele 1	Allele 2
C	T
Wild Type -	Variant +

Potential Impact:

Upregulation

Genotypes	Amino Acid
C C	Non-Coding
C T	Non-Coding
T T	Non-Coding

Amino Acid Position: Untranslated Region

DNA Position: 4962

SNP

AGTGCTTATG (C or T) TTTAAGTGCG

Rs Number: rs10948059

Location: Chromosome 6p21.1

* Frequency:

Population Category	CC	C T	TT
EUR	29%	47%	24%
EAS	70%	28%	2%
AFR	23%	43%	34%
AMR	50%	44%	6%
SAS	36%	47%	17%

Glycine N-methyltransferase

Glycine n-methyltransferase (GNMT) is an enzyme that plays a critical role in the disposal of excess s-adenosylmethionine (SAM), which is the body's main methyl donor. GNMT removes methyl groups from SAM by conjugating them with glycine to form the byproduct sarcosine.

Health Implications

- GNMT acts as a SAM/SAH buffer by disposing excess SAM through conjugation with glycine. This process is downregulated in response to low 5-MTHF and SAM levels. Increased GNMT activity could potentially lead to increased sarcosine levels, which has been associated with prostate cancer risk in several studies.[1-3]
  - However, in one study of Taiwanese men (where GNMT polymorphism is less common), GNMT polymorphism showed a protective effect on prostate cancer risk, which highlights the differences in SNP frequencies in different populations.[4]
- The C1289T polymorphism results in upregulation of the GNMT enzyme which increases the rate of SAM disposal and sarcosine creation. This may limit SAM availability for methylation reactions and reduce its regulatory effects on the transsulfuration and/or folate pathways.
- GNMT is also involved in detoxification and antioxidant pathways. This may play a role in the increased cancer risk demonstrated in homozygous negative individuals and in animal models.
- GNMT SNPs have been shown to play a role in elevating plasma homocysteine, particularly with folate-restriction.[5]

Clinical Considerations

- Evaluate methylation balance, SAM/SAH, and sarcosine levels.
- Ensure adequate levels of glycine, as this is a substrate for the reaction catalyzed by GNMT and is also involved in glutathione synthesis.

References

1. Lucarelli G, et al. *Prostate*. 2012;72(15):1611-1621.
2. Jentzmik F, et al. *Eur Urol*. 2010;58(1):12-18.
3. Sreekumar A, et al. *Nature*. 2009;457(7231):910.
4. Chen M, et al. *PloS one*. 2014;9(5):e94683.
5. Beagle B, et al. *J Nutr*. 2005;135(12):2780-2785.

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3535 Add-on Methylation Genomics - Buccal sample

Methodology: DNA Sequencing

MAT1A D18777A

Your Genotype:

Allele 1	Allele 2
G	A
Wild Type -	Variant +

Potential Impact:

Downregulation

Genotypes	Amino Acid
G G	Non-Coding
G A	Non-Coding
A A	Non-Coding

Amino Acid Position: Untranslated Region

DNA Position: 23777

SNP

GCTTTTCTCT(G or A)TAATGTGTCA

Rs Number: rs3851059

Location: Chromosome 10q22.3

* Frequency:

Population Category	GG	GA	AA
EUR	50%	43%	7%
EAS	36%	48%	16%
AFR	62%	34%	4%
AMR	52%	40%	8%
SAS	42%	45%	13%

Methionine adenosyltransferase

Methionine adenosyltransferase (MAT) is the enzyme that catalyzes the conversion of methionine into the body's main methyl donor, s-adenosylmethionine (SAM). This enzyme requires magnesium as a cofactor and is downregulated by oxidative stress, such as alcohol and free radical damage.

Health Implications

- Methionine adenosyltransferase (MAT) activity is critical to methylation. There are a few MAT1A genetic polymorphisms studied that lead to MAT1A deficiency (also known as Mudd's Disease), but this condition is extremely rare.
- The D18777A SNP is fairly common in the human population and has associations with cardiovascular disease risk.[1]
- Although literature is scant on this mutation, some studies have demonstrated higher homocysteine levels with this polymorphism.[2] Another study also demonstrated that this correlation was modulated by overall dietary fat intake.[3]
- Another study demonstrated that the D18777A SNP was associated with higher rates of stroke independent of homocysteine levels, which was hypothesized to be due to methylation activity impairment.[1]

Clinical Considerations

- Evaluate methylation balance, SAM/SAH, and sarcosine levels.
- Reduce levels of oxidative stress, such as free radical exposure and alcohol intake as these can further impair the MAT1A enzyme.
- Ensure adequate levels of MAT1A cofactors such as magnesium and potassium. Consider testing RBC magnesium an potassium.
- Patients with this polymorphism may have higher homocysteine in response to dietary fat intake than those without.[3] Monitor advanced cardiovascular risk markers if clinically appropriate.

References

1. Lai CQ, et al. *Am J Clin Nutr*. 2010;91(5):1377-1386.
2. Beagle B, et al. *J Nutr*. 2005;135(12):2780-2785.
3. Huang T, et al. *Nutr Metab Cardiovasc Dis*. 2012;22(4):362-368.

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3535 Add-on Methylation Genomics - Buccal sample

Methodology: DNA Sequencing

MTR A2756G

Your Genotype:

Allele 1	Allele 2
A	A
Wild Type -	Wild Type -

Potential Impact:

No Upregulation

Genotypes	Amino Acid
A A	Asp Asp
A G	Asp Gly
G G	Gly Gly

Amino Acid Position: 919

Aspartate to Glycine

GAC → GGC

DNA Position: 3179

SNP

ATTAGACAG G(A or G)C CATTATGAG

Amino Acid Codon

Rs Number: rs1805087

Location: Chromosome 1q43

Methionine synthase

Methionine synthase (MS/MTR) is responsible for converting homocysteine back into methionine by using 5-MTHF as a methyl donor. This reaction requires zinc and active B-12 (methylcobalamin) as cofactors and is the main pathway responsible for homocysteine recycling in every cell.

Health Implications

- The A2756G polymorphism is the most common MTR SNP discussed in the literature.
- It is generally accepted that this SNP upregulates the MTR enzyme leading to lower homocysteine levels.[1]
- The impact of this SNP on global DNA methylation is debated in the literature, however clinical associations with the A2756G polymorphism include congenital birth defects such as spina bifida, cleft lip/palate, and cardiac defects.[2-4]
- One hypothesis is that as the MTR enzyme is at the junction between the folate pathway and the methylation pathway, upregulation of MTR may shunt folate groups to the methylation cycle at the expense of other folate needs, such as purine/nucleotide synthesis.
- Several epidemiological studies on MTR polymorphism have demonstrated risk associations with various cancers, evidence remains controversial.[5-7] Many of these risk associations appear to be population/ethnicity specific, which could be due to gene-gene interactions with MTRR and MTHFR.

Clinical Considerations

- Compare any MTR polymorphisms with MTHFR and MTRR genetic results.
- Evaluate homocysteine, SAM/SAH ratio, and monitor biomarkers for vitamin B-12 and folate.
- Ensure adequate dietary intake of folate and vitamin B-12.

* Frequency:

Population Category	AA	A G	GG
EUR	69%	30%	1%
EAS	72%	25%	3%
AFR	47%	42%	11%
AMR	65%	33%	2%
SAS	42%	47%	11%

References

1. Ho V, et al. *Genes Nutr*. 2013;8(6):571-580.
2. Wang W, et al. *Genet Test Mol Biomarkers*. 2016;20(6):297-303.
3. Klerk M, et al. *Thromb Res*. 2003;110(2-3):87-91.
4. Doolin MT, et al. *Am J Hum Genet*. 2002;71(5):1222-1226.
5. Bleich S, et al. *Epigenomics*. 2014;6(6):585-591.
6. Hosseini M. *Pol J of Pathol*. 2013;64(3):191-195.
7. Jiang-hua Q, et al. *Tumour Biol*. 2014;35(12):11895-11901.

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3535 Add-on Methylation Genomics - Buccal sample

Methodology: DNA Sequencing

MTRR A66G

Your Genotype:

Allele 1	Allele 2
G	G
Variant +	Variant +

Potential Impact:

Downregulation

Genotypes	Amino Acid
A A	Ile Ile
A G	Ile Met
G G	Met Met

Amino Acid Position: 22

Isoleucine to Methionine

AT A → AT G

DNA Position: 203

SNP

CAGAAGAA **AT(A** or **G)** TGTGAGCAAG

Amino Acid Codon

Rs Number: rs1801394

Location: Chromosome 5p15.31

Methionine synthase reductase

Methionine synthase reductase (MTRR) is an enzyme that works in cooperation with methionine synthase (MTR) by reducing oxidized forms of vitamin B-12 to be reused. This allows MTR to continue to convert homocysteine back into methionine.

Health Implications

- MTRR polymorphisms result in decreased enzyme activity and therefore a decreased capacity to recycle oxidized cobalamin (vitamin B-12). This decreased enzyme activity can affect methylation capacity by limiting the amount of active B-12 available for homocysteine conversion.[1]
- Both MTRR polymorphisms can result in homocysteine elevation, independent of folate, B-12, or B-6 levels.[2]
- The A66G polymorphism is the most commonly studied MTRR SNP. It has been associated with numerous clinical conditions, such as various cancers, birth defects, metabolic syndrome, mood disorder, and elevated homocysteine.[3–5]
- The A66G polymorphism has also been shown to correlate with global DNA hypomethylation, which is a direct marker for methylation impairment.

Clinical Considerations

- Compare any MTRR polymorphisms with MTHFR and MTR genetic results Evaluate homocysteine, SAM/SAH ratio, and monitor biomarkers for vitamin B-12 and folate.
- Ensure adequate dietary intake of folate and vitamin B-12, consider repletion with methylcobalamin in these individuals.
- Ensure adequate vitamin B-2 and B-3 status, as they are cofactors for the MTRR enzyme.
- Assess antioxidant capacity, as oxidative stress impacts levels of methylcobalamin.

* Frequency:

Population Category	AA	A G	GG
EUR	38%	34%	28%
EAS	54%	37%	9%
AFR	59%	36%	5%
AMR	26%	57%	17%
SAS	N/A	N/A	N/A

References

1. Olteanu H, et al. *Biochemistry.* 2002;41(45):13378-13385.
2. Gaughan DJ, et al. *Atherosclerosis.* 2001;157(2):451-456.
3. Jamerson BD, et al. *Int J Geriatr Psychiatry.* 2013;28(9):925-932.
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3535 Add-on Methylation Genomics - Buccal sample

Methodology: DNA Sequencing

SHMT1 C1240T

Your Genotype:

Allele 1	Allele 2
C	T
Wild Type -	Variant +

Potential Impact:

Downregulation

Genotypes	Amino Acid
C C	Leu Leu
C T	Leu Phe
T T	Phe Phe

Amino Acid Position: 474

Leucine to Phenylalanine

CTC → TTC

DNA Position: 1631

SNP

CTTCGCCTCT (C or T) TC TTCCCTCT

Amino Acid Codon

Rs Number: rs1979277

Location: Chromosome 17p11.2

* Frequency:

Population Category	CC	C T	TT
EUR	45%	43%	12%
EAS	87%	13%	<1%
AFR	33%	47%	20%
AMR	59%	41%	<1%
SAS	N/A	N/A	N/A

Serine hydroxymethyltransferase 1

Serine hydroxymethyltransferase 1 (SHMT) is responsible for maintaining a relative balance of folate groups between the methylation cycle and the folate cycle. It uses serine and glycine to exchange methyl groups between THF and 5,10-MTHF as needed.

Health Implications

- SHMT1 is a bidirectional enzyme that can create a short-cut for methylation of homocysteine back to methionine through rapid creation of 5-MTHF. However, SHMT generally gives metabolic priority to nucleotide synthesis over SAM synthesis.[1]
- The C1240T polymorphism alters the SHMT1 enzyme function to favor the folate cycle over the methylation cycle to an even greater extent. Ultimately, this imbalance can cause reduced circulating folate (5-MTHF) levels and increased homocysteine.[2]
- This SNP adversely affects DNA synthesis, methylation systems, and causes genome instability. It eventually leads to oncogene overexpression and tumor suppressor gene inactivation.[1,3]
- The C1240T SNP has been associated with several clinical conditions, including various cancers and exacerbation of cardiovascular disease risk associated with MTHFR.[4-6]

Clinical Considerations

- Evaluate MTHFR SNP which may exacerbate CVD risk and low folate status.
- Consider supplementation with 5-MTHF and other methyl donors if high homocysteine or low SAM/SAH ratio.
- Consider additional B-vitamin supplementation to support MTHFR enzyme, such as vitamins B-2, B-3, and B-12.

References

1. Choi S-W, Mason JB. *J Nutr.* 2000;130(2):129-132.
2. Lightfoot TJ, et al. *Cancer Epidemiol Biomarkers Prev.* 2005;14(12):2999-3003.
3. Zijno A, et al. *Carcinogenesis.* 2003;24(6):1097-1103.
4. Wang Y-W, et al. *Chin J Cancer.* 2015;34(12):573-582.
5. Carmona B, et al. *Am J Clin Nutr.* 2008;88(5):1413-1418.
6. Wernimont SM, et al. *J Nutr.* 2011;141(2):255-260.

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3535 Add-on Methylation Genomics - Buccal sample

Methodology: DNA Sequencing

MTHFR C677T

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.

Your Genotype:

Allele 1	Allele 2
C	C
Wild Type -	Wild Type -

Potential Impact:

No Downregulation

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

Amino Acid Position: 222

Alanine to Valine

G C C → G T C

DNA Position: 894

SNP

TCTGCGGGA G(C or T) C GATTTCATC

Amino Acid Codon

Rs Number: rs1801133

Location: Chromosome 1p36.22

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
  - Various cancers [4]

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).

* Frequency:

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

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*Population frequency data is from 1000 GENOMES project as sourced from NCBI dbSNP. The population categories are listed below:
EUR (European): Americans with Northern and Western European Ancestry, Toscani, Finnish, British, Spanish
EAS (East Asian): Han Chinese (Beijing), Japanese (Tokyo), Southern Han Chinese, Chinese Dai, Kinh (Vietnam)
AFR (African): Nigerian, Kenyan, Gambian, Mendi (Sierra Leone), African American, African Caribbean
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

3535 Add-on Methylation Genomics - Buccal sample

Methodology: DNA Sequencing

MTHFR A1298C

Your Genotype:

Allele 1	Allele 2
C	C
Variant +	Variant +

Potential Impact:

Downregulation

Genotypes	Amino Acid
A A	Glu Glu
A C	Glu Ala
C C	Ala Ala

Amino Acid Position: 429

Glutamate to Alanine

G A A ⟶ G C A

DNA Position: 1515

SNP

ACCAGTGAA G(A or C)A AGTGTCTTT

Amino Acid Codon

Rs Number: rs1801131

Location: Chromosome 1p36.22

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 conversion to methionine, instead of nucleotide synthesis.

Health Implications

- The A1298C homozygous SNP mutation downregulates enzyme activity but may not independently affect folate or homocysteine levels.[1] However, a combined heterozygosity for both 677T and 1298C mutations does result in significant plasma homocysteine elevation.[1,2]
- Heterozygosity for only 1298 (-/+) has not been shown to affect overall MTHFR enzyme activity, however, homozygosity for 1298 (+/+) results in 30-40% reduction in MTHFR enzyme activity.[3]
- MTHFR A1298C SNPs have been associated with many disease processes including:
  - Cardiovascular disease [4-6]
  - Male infertility [7,8]
  - Increased risk of birth defects [9]
  - Certain cancer types[10-12]

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.[13]
- Evaluate the status of vitamin B-2 and B-3 (MTHFR enzyme cofactors).

* Frequency:

Population Category	AA	A C	CC
EUR	43%	45%	12%
EAS	63%	33%	4%
AFR	78%	21%	1%
AMR	62%	34%	4%
SAS	39%	44%	17%

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3535 Add-on Methylation Genomics - Buccal sample

Methodology: DNA Sequencing

COMT V158M

Your Genotype:

Allele 1	Allele 2
G	A
Wild Type -	Variant +

Potential Impact:

Downregulation

Genotypes	Amino Acid
G G	Val Val
G A	Val Met
A A	Met Met

Amino Acid Position: 158

Valine to Methionine

GTG →ATG

DNA Position: 721

SNP

TTTCGCTGGC (G or A)TGAAGGACAA

Amino Acid Codon

Rs Number: rs4680

Location: Chromosome 38.p12

Catechol-O-methyltransferase

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

Health Implications

- COMT polymorphisms result in decreased enzyme activity. Individuals with COMT SNPs may have an increased risk of inefficient methylation of catecholamines, estrogens, and toxins.[1,2]
- The most common genotype of COMT in most populations is heterozygous (+/-). Individuals with a homozygous positive (+/+) genotype for COMT have a 3-4-fold reduction in COMT activity.
- COMT polymorphisms have been implicated in mood disturbances such as anxiety, panic disorder, eating disorder, aggressiveness, anger, alcoholism, and severity of bipolar disorder.[3-5]
- COMT polymorphism has been implicated in risk of breast cancer, particularly in women with prolonged estrogen exposure;[6,7] or in women with low folate and high homocysteine.[8] Also, COMT SNPs have been shown to correlate with higher estrogen levels with estrogen replacement therapy.[9]
- Fibromyalgia and migraine have been associated with COMT polymorphisms as well.[10,11]

Clinical Considerations

- Evaluate methylation pathway to locate any potential backup.
- Ensure adequate B6, B12, folate, magnesium, betaine, and methionine to ensure adequate SAM production.
- SAM-e supplementation may be considered, as it is the cofactor for COMT, however, this therapy is contraindicated in bipolar disorder.
- Minimize stress, since catecholamine levels may already be high.
- Make sure to appropriately monitor estrogen levels and estrogen metabolites, especially if your patient is on estrogen replacement therapy.
- Consider additional antioxidant support, especially if low levels of glutathione are reported.

* Frequency:

Population Category	GG	G A	AA
EUR	22%	53%	25%
EAS	43%	47%	10%
AFR	46%	45%	9%
AMR	54%	37%	8%
SAS	37%	41%	22%

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3535 Add-on Methylation Genomics - Buccal sample

Commentary

This test has been developed and its performance characteristics determined by Genova Diagnostics, Inc. It has not been cleared by the U.S. Food and Drug Administration.

Commentary is provided to the practitioner for educational purposes, and should not be interpreted as diagnostic or treatment recommendations. Diagnosis and treatment decisions are the responsibility of the practitioner.

The accuracy of genetic testing is not 100%. Results of genetic tests should be taken in the context of clinical representation and familial risk. The prevalence and significance of some allelic variations may be population specific.

Any positive findings in your patient's test indicate genetic predisposition that could affect physiologic function and risk of disease. We do not measure every possible genetic variation. Your patient may have additional risk that is not measured by this test. Negative findings do not imply that your patient is risk-free.

DNA sequencing is used to detect polymorphisms in the patient's DNA sample. The sensitivity and specificity of this assay is <100%.