MTHFR Polymorphisms
C677T/A1298C and Disease Risk

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Objectives

I. Define the two most studied MTHFR polymorphisms: C667T and A1298C

I. Diseases associated with MTHFR polymorphisms

I. Current treatments

I. Future biomarkers to enhance treatment
Methylenetetrahydrofolate Reductase (MTHFR)

- MTHFR is the gene responsible for production of the MTHFR enzyme, which is the rate-limiting enzyme in folate cycle
  - C677T and A1298C are the most well-known

- MTHFR enzyme is responsible for converting 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate
  - 5-methyltetrahydrofolate = bioactive form of folate
    - Needed for conversion of homocysteine (Hcy) to methionine
    - Necessary for production of neurotransmitters
    - Important in DNA methylation
MTHFR Polymorphisms

- Polymorphism = cytosine (C) is replaced by thymine (T) at position 677 or adenine (A) is replaced by cytosine (C) at position 1298.

- C667T Prevalence:
  - Homozygous affected (T/T): 11%
  - Homozygous unaffected (C/C): 49%
  - Heterozygous affected (C/T): 40%

- A1298C Prevalence:
  - Homozygous affected (C/C): 9%

- Compound heterozygotes involve both mutations: ~17% of the population

Population Prevalence (%) C677T Polymorphism

Caucasian

African American

Mexican American

http://webdev.nccd.cdc.gov/genomics/population/genvar/frequencies/MTHFR.htm
Population Prevalence (%) A1298C Polymorphism

http://webdev.nccd.cdc.gov/genomics/population/genvar/frequencies/MTHFR.htm
<table>
<thead>
<tr>
<th></th>
<th>Counts</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Count</td>
<td>392,993</td>
<td>100%</td>
</tr>
<tr>
<td>Wild Type C/C</td>
<td>190,349</td>
<td>48%</td>
</tr>
<tr>
<td>Heterozygote C/T</td>
<td>160,650</td>
<td>41%</td>
</tr>
<tr>
<td>Homozygote T/T</td>
<td>41,994</td>
<td>11%</td>
</tr>
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</table>
Prevalence of Elevated Homocysteine with MTHFR C677T – HDL, Inc.

<table>
<thead>
<tr>
<th></th>
<th>Count</th>
<th>% with Hcy &gt;= 11 &amp; &lt;13</th>
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</thead>
<tbody>
<tr>
<td><strong>Heterozygote</strong></td>
<td></td>
<td></td>
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<tr>
<td>C/T</td>
<td>42,654</td>
<td>27%</td>
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<tr>
<td><strong>Homozygote</strong></td>
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<td></td>
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<tr>
<td>T/T</td>
<td>11,127</td>
<td>26%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Count</th>
<th>% with Hcy &gt;= 11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heterozygote</strong></td>
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<td></td>
</tr>
<tr>
<td>C/T</td>
<td>34,781</td>
<td>22%</td>
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<tr>
<td><strong>Homozygote</strong></td>
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</tr>
<tr>
<td>T/T</td>
<td>10,708</td>
<td>25%</td>
</tr>
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</table>
Disease Associations with the MTHFR C677T Polymorphism

- Associated with the following disease states:
  - Cardiovascular disease
  - Renal failure
  - Congenital abnormalities and pregnancy outcome
  - Cancer
  - Psychiatric disorders, schizophrenia, dementia, depression
  - IBD
  - Autism
  - Down’s syndrome
  - Addictions: smoking, drugs, alcohol
  - Fibromyalgia
  - Chronic fatigue syndrome

Selhub J. J Nutr 2006;136:1726S-1730S.
Wierzbucki, AS. Diab Vasc Dis Res 2007;4:143-50
Disease Associations with the MTHFR A1298C Polymorphism

- Associated with the following disease states:
  - Cancer
  - Neural tube defects
  - Male infertility
  - Miscarriages
  - Diabetic nephropathy
  - Stroke
  - Schizophrenia, anxiety, depression, dementia

Selhub J. J Nutr 2006;136:1726S-1730S.
Wierzbucki, AS. Diab Vasc Dis Res 2007;4:143-50
Elevations in homocysteine can exacerbate an increase in fibrinogen levels and affect clotting, along with other clotting tests, such as prothrombin and Factor V Leiden:

- Factor V, Factor II & MTHFR mutation = 2x risk for DVT/PE

Homocysteine/Methionine Conversion

- Homocysteine is a non-protein AA
  - Synthesized from methionine by the removal of its terminal methyl group
  - Can be recycled into methionine OR converted into cysteine
- Cysteine can be further metabolized to glutathione by cystathione beta-synthase (CBS) and vitamin B6
- Glutathione (a tripeptide) = master antioxidant/detoxification
Homocysteine Pathway

- **Folate Cycle**
  - THF
  - Methylene-THF
  - MTHFR
  - Methyl-THF
  - B12
  - MS
  - B2

- **Methionine Cycle**
  - Methionine
  - DMG
  - BHMT
  - TMG
  - SAMe
  - SAH
  - R
  - Methylation
  - R-CH3

- **Homocysteine**
  - Cystathionine
  - CBS
  - B6
  - Cysteine
  - B6

- **1st**: methionine receives an adenosine group from ATP, a reaction catalyzed by S-adenosylmethionine-synthetase, to give S-adenosyl methionine (SAMe).

- SAMe then transfers the methyl group to an acceptor molecule (i.e., norepinephrine or to DNA methyltransferase for DNA methylation)

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CBS = cystathione beta synthase  
THF = Tetrahydrofolate  
Methylene-THF = 5,10-Methylenetetrahydrofolate  
Methyl-THF = 5-Methyltetrahydrofolate  
SAMe = S-adenosylmethionine  
SAH = S-adenosylhomocysteine

Ueland PM et al., Ed. Robinson K, Homocysteine and Vascular Disease, 2000, 59-84
Vitamins as Cofactors

► Vitamin B12: needed to for recycling of homocysteine to methionine
► Medications that can deplete B12 levels in the body:
  ► Inhalant, systemic, and topical corticosteroids
  ► Antibiotics
  ► Metformin
  ► Bile acid sequestrants
  ► Histamine H2 antagonists
  ► Proton pump inhibitors
► Other potential causes for depleted vitamin B12:
  ► Malabsorption disorders (Crohn’s/Ulcerative Colitis/IBS/Celiac Disease)
  ► Hyperthyroidism
  ► Lack of intrinsic factor (inherited, age, etc.)
  ► Pregnancy
  ► Vegan diet

http://www.umm.edu/altmed/articles/vitamin-b12-000717.htm
Vitamins as Cofactors

► Vitamin B6: needed to break down homocysteine into cystathionine and then cysteine

► Medications that can deplete B6 levels in the body:
  ► Antibiotics
  ► Corticosteroids
  ► Birth control medication

► Other potential causes for depleted B6 levels:
  ► Malabsorption disorders
  ► Alcoholism

http://www.umm.edu/altmed/articles/vitamin-b6-000721.htm
Vitamin B2: a required cofactor for the MTHFR enzyme to convert 5,10-MTHF to 5-MTHF

Medications that can deplete B2 levels in the body:
- Antibiotics
- Tricyclic anti-depressants

Other potential causes for depleted vitamin B2:
- Liver disease
- Alcoholism
- Long-standing infections
- Vegan diet
- Malabsorption disorders

http://www.umm.edu/altmed/articles/vitamin-b2-000719.htm
A1298C Disrupts Tetrahydrobiopterin Cycle

- BH4 = tetrahydrobiopterin
  - Chemical assists in breakdown of phenylalanine
  - Helps form neurotransmitters:
    - Serotonin
    - Melatonin
    - Dopamine
    - Norepinephrine
    - Epinephrine
  - Assists breakdown of ammonia
  - Cofactor to produce Nitric Oxide
A1298C Disrupts Tetrahydrobiopterin Cycle

DHPR = dihydropteridine reductase  NOS = Nitric oxide synthase  MTHFR = methylenetetrahydrofolate reductase
Potential Interventions:

- **Methylfolate:** many different forms
  - Methylfolate
  - L-MTHF
  - L-Methylfolate
  - L-Methylfolate Calcium
  - D-Methylfolate
  - D-5-Methylfolate
  - Levomefolic Acid
  - Metafolin
  - 5-MTHF, 5-Methylfolate
  - 5-Methyltetrahydrofolate
  - L-5-MTHF
  - L-5-Methyltetrahydrofolate
  - 6(S)-5-Methyltetrahydrofolate
  - 6(R)-5-MTHF
  - 6(R)-5-methyltetrahydrofolate
  - Quatrefolic

- **Vitamin B12 (methylcobalamin)**
- **Vitamin B6 (pyroxidal 5’phosphate)**
- **Betaine (TMG)**

http://mthfr.net/l-methylfolate-methylfolate-5-mthf/2012/04/05/
Potential Treatment Protocol

► Patients with multiple MTHFR mutations and significant symptoms of depression:
  ► May benefit from a prescription dosage of 5-MTHF (7.5mg or 15mg)

► Patients with few clinical symptoms and only a heterozygote mutation:
  ► Could reasonably start with a B-complex that contains approximately:
    ► 1 mg folate in the bioactive 5-MTHF form
    ► 500mcg B12 in the methylcobalamin form
    ► 10mg B6
    ► 100mg B2

► This dose can be titrated up to reach normal Hcy levels, if elevated. If Hcy remains elevated, consider betaine in dosages of 1-2grams.
Beneficial Forms of Methylfolate with Good Absorption

- L-Methylfolate Calcium
- Metafolin
- Levomefolic Acid
- L-5-MTHF
- L-5-Methyltetrahydrofolate
- 6(S)-L-MTHF
- 6(S)-L-Methyltetrahydrofolate
- Quatrefolic
  - Form of methylfolate that uses glucosamine instead of calcium to bind the L-methylfolate

http://mthfr.net/l-methylfolate-methylfolate-5-mthf/2012/04/05/
Question These Formulations of Methylfolate

► 5-MTHF
► 5-Methylfolate
► 5-Methyltetrahydrofolate
► D-5-MTHF
► D-5-Methyltetrahydrofolate
► 6(R)-L-MTHF
► 6(R)-L-Methyltetrahydrofolate

http://mthfr.net/l-methylfolate-methylfolate-5-mthf/2012/04/05/
L-Methylfolate Options:

- Prescription drugs containing Metafolin:
  - Metanx
  - Deplin
  - Cerefolin
  - CerefolinNAC
  - Neevo
  - NeevoDHA

- Supplements with *active* L- methylfolate
### Cost Analysis for OTC*

<table>
<thead>
<tr>
<th>Company</th>
<th>Product</th>
<th>Price/mcg</th>
<th>Price/90-days</th>
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<tbody>
<tr>
<td>Metabolic Maintenance</td>
<td>5-MTHF, 10mg</td>
<td>$0.01</td>
<td>$56.90</td>
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<tr>
<td>(<a href="http://www.metabolicmaintenance.com">www.metabolicmaintenance.com</a>)</td>
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<tr>
<td>Solgar Folate</td>
<td>L-methylfolate-Metafolin®, 400mcg</td>
<td>$0.01</td>
<td>$3.80</td>
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<tr>
<td>(<a href="http://www.solgar.com">www.solgar.com</a>)</td>
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<tr>
<td>Life Extension</td>
<td>Optimized Folate (L-Methylfolate), 1mg</td>
<td>$0.02</td>
<td>$21.00</td>
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<tr>
<td>(<a href="http://www.lifeextension.org">www.lifeextension.org</a>)</td>
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<tr>
<td>Thorne</td>
<td>5-MTHF, 5mg</td>
<td>$0.02</td>
<td>$27.30</td>
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<tr>
<td>(<a href="http://www.thorne.com">www.thorne.com</a>)</td>
<td>5-MTHF, 1mg</td>
<td>$0.03</td>
<td>$90.75</td>
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<tr>
<td>Seeking Health</td>
<td>Active B12 w/L-5-MTHF, 800mcg</td>
<td>$0.03</td>
<td>$29.90</td>
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<tr>
<td>(<a href="http://www.seekinghealth.com">www.seekinghealth.com</a>)</td>
<td>Homocystex (w/B6 + B12), 800mcg</td>
<td>$0.05</td>
<td>$35.90</td>
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*Based on Amazon.com prices on 1/24/2013
# Prescription L-Methylfolate

<table>
<thead>
<tr>
<th>Prescription</th>
<th>Ingredients</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deplin (<a href="http://www.deplin.com">www.deplin.com</a>)</td>
<td>L-methylfolate (Metafolin®), 7.5mg L-methylfolate (Metafolin®), 15mg</td>
<td>$144/90-day supply</td>
</tr>
<tr>
<td>MetaNX (<a href="http://www.metanx.com">www.metanx.com</a>)</td>
<td>L-methylfolate Calcium (as Metafolin®), 3mg Pyridoxal 5’-phosphate, 35mg Methylcobalamin, 2mg</td>
<td>$144/90-day supply</td>
</tr>
<tr>
<td>CerefolinNAC (<a href="http://www.cerefolinnac.com">www.cerefolinnac.com</a>)</td>
<td>L-methylfolate Calcium (as Metafolin®), 6mg Methylcobalamin, 2mg N-Acetylcysteine, 600mg</td>
<td>$144/90-day supply</td>
</tr>
<tr>
<td>Neevo DHA (<a href="http://www.neevodha.com">www.neevodha.com</a>)</td>
<td>--</td>
<td>$144/90-day supply</td>
</tr>
</tbody>
</table>
Proposed MTHFR Treatment Decisions

A1298C Mutation
- Yes
- C677T Mutation
  - Yes
  - Provide L-methyfolate (high dose + methylcobalamin 1000 mcg)
  - Check B6 levels + supplement prn
  - No
  - Symptoms of methylation or neurotransmitter deficiency (fatigue, depression, neurological symptoms, history of cancer)?
    - Yes
    - Provide L-methyfolate (1 mg + methylcobalamin 500 mcg)
    - Check B6 levels + supplement prn
    - No
    - Provide L-methyfolate (400 mcg + methylcobalamin 500 mcg)
    - Check B6 levels + supplement prn
  - No
  - Elevated Homocysteine
    - Yes
    - Encourage high dietary folate intakes
    - Discourage folic acid supplementation
    - Check B12, B6 and folate
    - No
    - Treat other CVD risk
Additional Biomarkers to Enhance Treatment

- Other SNPs associated with MTHFR gene (i.e. COMT gene)
- Additional genetic markers in methylation pathway
- Inactive vs. biologically active folate
- Vitamin B2, B6, and B12 (MMA) levels
- Glutathione levels
Thank you for listening

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Click here or on the link below for References