A Deeper Dive into the Data: L-methylfolate and Depression

STAR*D: Unresolved Symptoms Following Antidepressant Treatment

Percent of Patients

- ~67% Mild symptoms
- ~28% Moderate symptoms
- ~23% Severe symptoms
- ~12% Very severe symptoms

Depressive Symptoms (QIDS-SR score) After up to 12 Weeks of Antidepressant Treatment

Remission ~33%

MADRS = Montgomery-Asberg Depression Rating Scale.

Epigenetic Modification:
Genes Have a Right to Remain Silent

Epigenetics – Interaction of Genes and Environment in MDD

Gene Activation and Silencing
MDD is Associated with Altered Methylation of Inflammatory Genes

- IL-6 and CRP levels were elevated among those with lifetime depression and among those with depression only.
- IL-6 DNA methylation showed an inverse correlation with circulating IL-6 and CRP among those with lifetime depression.

Tying it All Together: ELA* Promotes Both Depression and Obesity

- In a 32-year longitudinal study of 1037 individuals followed from birth, ELA increased the risk of developing signs of metabolic disorder in adulthood, including overweight, high blood pressure, high total cholesterol, low high-density lipoprotein cholesterol, high glycated hemoglobin, and low maximum oxygen consumption levels adjusted for body weight.

A Predictive Marker in MDD?

- MTHFR
Folate Metabolism

DHF Reductase

Dihydrofolate

Tetrahydrofolate

10-formyl-THF

5,10 Methylenyl THF

L-methylfolate

MTHFD

BBB

MTHFR

L-methylfolate

Synthetic Folic Acid

Graphical representation of study by:
Krajinovic M. Pharmacogenomics. 2008; 9(7):829-832.

MTHFR TT Genotype Predicts Risk of Depression

26 studies including 4992 depression cases and 17,082 controls.

MTHFR Reduces Antidepressant Response

Genetic Predictors of Response to Treatment with Citalopram in Depression Secondary to Traumatic Brain Injury

With respect to the MTHFR C-(677)T SNP, individuals with one or two T SNPs had less of a response to treatment compared to C/C individual in the present study. Although the polymorphism in the MTHFR has not been previously linked to antidepressant treatment response, low folate levels have been shown to decrease response to fluoxetine. Folate, like MTHFR, is necessary for homocysteine metabolism and subsequent production of serotonin. These results suggest that another member of the 1-carbon metabolism pathway, the MTHFR, may also influence response to SSRIs.
MTHFR Polymorphism & Depression

- MDD is most likely a neurological, heterogeneous disorder.¹
- Genome-wide association studies have so far failed to identify specific genes involved in etiology of MDD.²
- MDD is most likely a product of complex interactions between multiple genes, epigenetic changes, and environmental adversity.¹,³
- MTHFR is associated with reduced antidepressant efficacy.³
- MTHFR polymorphism is associated with increasing the risk and severity of depression.⁴,⁵
- ELA interaction with MTHFR predicts increased risk of depression.⁶

Elevated BMI Associated with Poor Antidepressant Response

In a meta-analysis of 3 controlled clinical trials, patients with a BMI ≥30 had a lower antidepressant response rate compared to patients with a normal BMI.

Elevated BMI Predicts Poor Antidepressant Response

Patients with MDD had a significantly higher body mass index (BMI) compared with healthy controls. Patients with high BMI (≥25) showed a significantly slower clinical response and less improvement in neuroendocrinology and cognition than did patients with normal BMI (18.5 ≤ BMI < 25) during antidepressant treatment.

Depression Increases with BMI

### Elevated BMI Predicts Development of Depression

<table>
<thead>
<tr>
<th>BMI Category</th>
<th>Study Details</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥30</td>
<td>Herva et al., 2006</td>
<td>1.63 (1.16, 2.29)</td>
</tr>
<tr>
<td>≥30</td>
<td>Anderson et al., 2007</td>
<td>2.00 (1.00, 4.01)</td>
</tr>
<tr>
<td>≥30</td>
<td>Kasen et al., 2008</td>
<td>3.96 (1.23, 12.75)</td>
</tr>
<tr>
<td>≥30</td>
<td>Koponen et al., 2008</td>
<td>0.77 (0.38, 1.56)</td>
</tr>
<tr>
<td>≥30</td>
<td>Bjerkeset et al., 2008</td>
<td>1.66 (1.23, 2.24)</td>
</tr>
<tr>
<td>≥30</td>
<td>van Gool et al., 2007</td>
<td>1.01 (0.63, 1.63)</td>
</tr>
<tr>
<td>≥30</td>
<td>Roberts et al., 2003</td>
<td>2.01 (1.25, 3.24)</td>
</tr>
<tr>
<td>≥30</td>
<td>Sachs-Ericsson et al., 2007</td>
<td>1.76 (0.47, 6.57)</td>
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</tbody>
</table>

### Obesity Increases with Severity of Depression

<table>
<thead>
<tr>
<th>PHQ Scores</th>
<th>Prevalence of Depression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-5</td>
<td>0%</td>
</tr>
<tr>
<td>5 to 9</td>
<td>10%</td>
</tr>
<tr>
<td>10 to 14</td>
<td>30%</td>
</tr>
<tr>
<td>15 or more</td>
<td>70%</td>
</tr>
</tbody>
</table>

### Normal and Overweight Examples

- **Normal:** BMI 22.0 to 24.9
- **Overweight:** BMI ≥25

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**Elevated BMI Predicts Development of Depression**

A negative association between BMI and Depression.

**Favors A:** a negative association between BMI and Depression

**Favors B:** a positive association between BMI and Depression

**Luppino FS et al.**

*Arch Gen Psychiatry* 2010;67(3):220-229.

**Obesity Increases with Severity of Depression**

**Females, n=4,641**

**PHQ Scores**

- 5 to 9
- 10 to 14
- 15 or more

**Prevalence of Depression (%)**

- 0%
- 10%
- 30%
- 70%

**Normal (1-22)**

**Overweight (≥25)**

- BMI 22.0 to 24.9
- BMI ≥25

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**Prevalence of Obesity**

**PHQ Scores**

- Normal (<25)
- Overweight (≥25)

---

**PHQ Scores**

- -5
- 5 to 9
- 10 to 14
- 15 or more

---

**Prevalence of Depression (%)**

- 0%
- 10%
- 30%
- 70%

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**BMI Categories**

- Normal: BMI 22.0 to 24.9
- Overweight: BMI ≥25

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Relationship Between BMI, Metabolic Syndrome and Depression

Association between the metabolic syndrome (MetS) and depression in each body mass index (BMI) category. Graph displays the odds ratio (OR) for depression after adjustment for age, gender, prior cardiovascular disease, employment status, marital status, smoking status, dietary score, and physical activity. Obesity was defined as a BMI ≥ 30 and overweight status as a BMI between 25 and 30 kg/m².


Elevated BMI and Depression

- Increased body weight is associated with a decreased response rate to antidepressants.1,2
- Greater body weight increased the risk, severity and chronicity of major depression.3-5
- A majority of psychiatric medications can generate weight gain which may lead to obesity in some patients.6
- Obese individuals with depression have higher risk of developing metabolic syndrome.7


Adiposity, Inflammation, and Depression

- High caloric intake in the diet leads to increased accumulations of lipids in adipocytes.
- Increased diet content results in an increased release of MCP-1, a chemoattractant that increases the infiltration of macrophages into adipose tissue.
- Both adipocytes and macrophages release inflammatory mediators such as IL-6 and TNF-α into the peripheral circulation.

HDL = high-density lipoprotein; LDL = low-density lipoprotein; ROS = reactive oxygen species; mmLDL = minimally-modified low-density lipoproteins; TLR = toll-like receptor; MCP-1 = monocyte chemoattractant protein 1

Inflammatory Markers Predict Development of Depression

In a cohort of 644 initially non-depressed females, 48 developed de novo MDD over an approximate 10-year follow up. Survival plot (Kaplan-Meier) showing the probability of remaining free of de novo major depressive disorder for women stratified into tertiles of hsCRP. The concentration of hsCRP in each tertile is: low, <1.12 mg/L; mid, 1.12-2.97 mg/L; and high, >2.97 mg/L.


Inflammatory Markers Predict Symptom Severity in Depression

Comparison of 9 MDD Patients With 9 Matched Healthy Controls

A. CONCENTRATION
B. GUILT
C. SADNESS
D. SELF-ESTEEM
E. SUICIDAL THOUGHTS
F. TIREDNESS

*Correlations of IL-6 level with guilt, self-esteem, and suicidal thoughts remained significant after Bonferroni correction.

IL-6=Interleukin-6; MDD=Major depressive disorder; VAS=Visual analogue scale.


Inflammatory Markers Predict Poor Response to Antidepressants

24 healthy controls and 28 patients with depression (HAM-D >20) after 6 weeks of SSRI treatment and 16 euthymic patients (previously resistant to SSRIs) currently successfully treated with an SNRI or an addition of lithium to SSRI treatment.

HAM-D=Hamilton depression score; MDD=Major depressive disorder; SNRI=Serotonin–norepinephrine reuptake inhibitor; SSRI=Selective serotonin reuptake inhibitor; TNF=TNF.

Elevation of CSF Cytokines Alter 5-HT and Dopamine Metabolism

- Inflammatory cytokines and monoamine metabolites were compared in 63 suicide attempters and 47 healthy controls.
- MADRS scores correlated significantly with CSF IL-6 levels.
- IL-6 and TNF-α correlated with CSF 5-HIAA and HVA.
- Higher cytokine levels were associated with increased suicidality.

The Role of Folate in Depression

Monoamine Synthesis in MDD

De Novo Synthesis of BH₄
Conditions of Inflammation Decrease BH4, a Critical Cofactor in Monoamine Synthesis

Inflammation/Oxidative Stress Decreases BH4


Conditions of Inflammation Increase Demand for L-MTHF to Regulate Monoamine Synthesis

Inflammation/Oxidative Stress Increases Demand for L-methylfolate


Folate Metabolism

Krajinovic M. Pharmacogenomics. 2008; 9(7):829-832.
### Genetic Variants of Folate Metabolism

- **Folic Acid**
- **Dihydrofolate**
- **Tetrahydrofolate**
- **10-formyl-THF**
- **Dihydrofolate**
- **5,10-methenyl-THF**
- **5,10-methylene-THF**
- **Tetrahydrofolate**
- **L-methylfolate**
- **MTHFD**
- **MTHFR**
- **MTRR MTR**
- **SHMT**
- **DHFR DHFR**
- **Purine biosynthesis**
- **5-formyl-THF** (folinic acid)
- **Thymidine biosynthesis**
- **Homocysteine**
- **Serotonin Dopamine**
- **Norepinephrine**
- **Neurotransmitter biosynthesis**
- **DNA Methylation; RNA, protein & phospholipid biosynthesis**
- **L-methylfolate Crosses the Blood Brain Barrier**
- **Risk Factors for Low L-methylfolate**

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### L-methylfolate Crosses the Blood Brain Barrier

- Synthetic folic acid blocks L-methylfolate from crossing the blood brain barrier (BBB).
- High dose synthetic folic acid has led to an increase in depression, neurological complications, suppression of the immune system, and a decrease in monoamines.
- L-methylfolate, in the absence of synthetic folic acid, passes more readily into the CNS to regulate neurotransmitter synthesis in depressed individuals.


### Risk Factors for Low L-methylfolate

- **Drugs**
  - Anticonvulsants such as lamotrigine and valproate, methotrexate, polyethylene glycol, oral contraceptives, maflitoxin, nicotine, benzodiazepines, fluoxetine, and tebufenozin.
  - Antithyroid drugs, estrogen replacement therapy, oral contraceptives, and selective serotonin reuptake inhibitors.
  - Anticonvulsants, antiparkinsonian drugs, and antipsychotics.

- **Disease**
  - Diabetes, atrophic gastritis, Crohn's, colitis, renal failure and hypothyroidism.

- **Lifestyle**
  - Excessive alcohol, smoking, and poor nutrition.

- **Aging**
  - L-methylfolate in the brain decreases with age.

- **Genes**
  - MTHFR CT/TT Polymorphism.

- **Obesity**
  - Body Mass Index (BMI) ≥ 30.
Consequences of Folate Deficiency Impact Both Brain and Body

Folate deficiency can have various impacts on both the brain and the body. This can be caused by dietary, genetic, or drug-induced factors. Low CNS 5-MTHF can lead to decreased 5-MTHF, increased homocysteine, and impaired neurotransmitter metabolism.

CNS Disorders include depression, dementia, seizures, developmental delay, neuropathy, and myelopathy.

Decreased BH4 and decreased SAM/SAH ratio can lead to increased dUMP and impaired methylation of DNA, proteins, and phospholipids. DNA damage, endothelial dysfunction, excitotoxicity, and oxidative stress can also occur.


Up to 70% of MDD patients have a genetic mutation reducing conversion of folic acid to L-methylfolate.

The Interaction of L-methylfolate with Genes and Other Biomarkers in the Depressed Brain


Methods

Patient Selection
- Adults aged 18-65 years and meeting DSM-IV criteria for a current episode of MDD
- QIDS-SR ≥ 12 at screening and baseline visits
- SSRI given current episode for ≥ 8 weeks at adequate doses
- Must have been on a stable SSRI dose for the past 4 weeks
- Excluded if failed >2 adequate antidepressant trials during current episode

Study Design
- Multi-center, randomized, double-blind study
- Two 30-day treatment phases using a sequential parallel comparison design (SPCD)

Markers were evaluated as moderators of L-methylfolate response on HDRS-28:
- Plasma L-methylfolate levels > or < the median for the study population
- Plasma Hcy levels > or < the median for the study population
- BMI ≥ 30 kg/m² or < 30 kg/m²
- SAM/SAH ratio (methylation)
- CRP (inflammation)
- 4HNE (oxidative stress)
- Whole blood MTHFR C677T and MTR A2756G genotypes

Sequential Parallel Comparison Design

Trial 2: Adjunctive 15 mg L-methylfolate

Pooled (combined and averaged) efficacy results: 30-day results from groups 1+2 vs 30-day results from groups 3+4+5.

Group 1, Group 2, Group 3, Group 4, Group 5, Group 6

Adjunctive 15 mg L-methylfolate
Pooled Mean Score Reduction – 30 Days

Pooled Mean Change in Score

HDRS-17
P=0.05
QIDS-SR
P=0.04
CGI-S
P=0.01

HDRS = Hamilton Depression Rating Scale.
QIDS = Quick Inventory of Depressive Symptomatology.
CGI = Clinical Global Impression.
Adjunctive 15 mg L-methylfolate Response Rates – 30 Days

Adjunctive 15 mg L-methylfolate Tolerability

Baseline Mean Change Treatment Effect Stratified by BMI ≥30 Compared to General Study Population
Baseline Mean Change Treatment Effect Stratified by BMI ≥30 Compared to General Study Population

Examining the Statistical Concept of NNT

How many patients you would need to treat with Agent A instead of Agent B before you would encounter 1 additional outcome of interest, such as 1 response?

\[ f_A = \text{rate of outcome for Agent A} \]
\[ f_B = \text{rate of outcome for Agent B} \]

\[ \text{RD} = f_A - f_B \]
\[ \text{NNT} = \frac{1}{\text{RD}} \]

The smaller the NNT, the larger the differences between the 2 agents.

Larger numbers mean more patients need to be treated to see the effect.

Number Needed to Treat for Response Adjunctive Atypicals in MDD

Note: lower NNT numbers denote better response rates.

OFC = olanzapine-fluoxetine combination.
Number Needed to Treat for Response
Adjunctive Bupropion in MDD

Number Needed to Treat for Response
Adjunctive L-methylfolate in MDD

Number Needed to Treat for Response
Adjunctive L-methylfolate in MDD
Conclusion

- Depression is multi-factorial in nature, with genes, early life adversity, environment, obesity, and inflammation all playing a critical role.
- Treatment results are often sub-optimal, and there is critical need to improve treatment outcomes.
- Emerging knowledge about the interaction among genes, environment and adjunctive L-methylfolate 15mg as a nutritional solution holds promise for patients who sub-optimally respond to antidepressants.