METHYLATION CYCLE BLOCK: GLUTATHIONE DEPLETION

CLINICAL TREATMENT STUDY
IN PATIENTS WITH CHRONIC FATIGUE SYNDROME AND FIBROMYALGIA
A tribute to Dr. Rich van Konynenburg
SHEICON 2015
October 15, 2015
Presented by Neil Nathan MD
Objectives

Upon completing the session, learners will be able to:

- Understand the research behind methylation treatments for chronic fatigue and fibromyalgia.
- Understand the possible side effects of treatments and how to manage them.
- Understand the more global benefits of evaluating and treating methylation in chronic illness.
Got Methylation?
With Gratitude to the Ratna Ling Study Group
CONFLICT OF INTEREST?

I have NO financial ties to any laboratory, medication, or supplement product to be discussed in this presentation.

- The person inherits a genetic predisposition (polymorphisms in several of certain genes) toward developing CFS. (This genetic factor is more important for the sporadic cases than for the cluster cases of CFS.)

- The person then experiences some combination of a variety of possible stressors (physical, chemical, biological, psychological/emotional) that place demands on glutathione.

- Glutathione levels drop, producing oxidative stress, removing protection from B12, allowing toxins to accumulate, and shifting the immune response to Th2.

• Toxins react with B12, lowering the rate of formation of methylcobalamin.

• Lack of sufficient methylcobalamin inhibits methionine synthase, placing a partial block in the methylation cycle.

• Sulfur metabolites drain through the transsulfuration pathway excessively, pass through sulfoxidation, and are excreted.

• An interaction is established between the methylation cycle block and glutathione depletion, and the disorder becomes chronic.
We can demonstrate that the majority of our patients with fibromyalgia (FM) and chronic fatigue syndrome (CFS) have dysfunctional methylation chemistry, and that treating this disordered methylation chemistry clearly results in significant clinical improvement in our patients.
Methylation Cycle Block

• Can we document Glutathione and SAM deficiencies in chronic fatigue and fibromyalgia? Yes.

• Can we successfully treat these deficiencies with a biochemically appropriate protocol? Yes.

• Can we improve on those results by further study of, and treatment of, methylation imbalances? Yes.
METHYLATION
Methylation:
- Addition of a carbon atom to a molecule

- Methyl donor = S-adenosylmethionine (SAM)

- S-adenosylhomocysteine (SAH) inhibits methylation

- There are > 150 methylation reactions (e.g. DNA, histones, proteins, phospholipids, neurotransmitters)

- Methylation controls gene expression during development.

- Oxidative stress inhibits methylation
(basic) **Methylation cycle** (15-20)

- Methionine
- **S-adenosyl-Homocysteine** (SAH)
- Methionine
- **S-adenosyl-Methionine** (SAME)
- **MTs**
- **S-adenosyl-Homocysteine** (SAH)
- ACHY
- **MAT**
- MTR, MTRR
- Homocysteine
From: Autism: Effective Biomedical Treatments

**Region 1**
- Met → SAM
- ATP → P + Pi

**Region 5**
- THF → MS → Cbl → 5MeTHF
- Met → DMG
- BHMT → TMG

**Region 4**
- Hcy
- Serine → P5P
- Cystathionine → P5P
- Cysteine → GSH

**Region 2**
- SAM → methylation
- SAH

**Region 3**
- Adenosine → ADA → Inosine

**Abbreviations**
- SAM = S-adenosylmethionine
- MS = methionine synthase
- Cbl = cobalamin
- P5P = pyridoxal 5-phosphate
- MET = methionine
- SAH = S-adenosylhomocysteine
- Hcy = homocysteine
Methylation cycle with BHMT pathway added (15-20)

[Note that BHMT is found only in liver and kidney cells(22).]
(basic) Folate cycle (15-20)

TS → THF → SHMT → 5,10-methylene THF → MTHFR → THF → MTR MTRR → Me-B12 → 5-Methyl THF → 5,10-methylene THF → TS
Methionine Transsulfuration to Cysteine and Glutathione

1. Folate Cycle
2. Methionine Cycle
3. Transsulfuration Pathway

1. 5,10-CH₂THF
2. MTHFR
3. 5-CH₃THF

1. Methionine
2. SAM
3. SAH

1. Methylation Potential (SAM/SAH)
2. Cell Methylation
3. Adenosine

1. Homocysteine
2. Antioxidant Redox Potential (GSH/GSSG)
3. Cysteine

1. CBS
2. Antioxidant Redox Potential (GSH/GSSG)
“Obvious” Interventions

• *Consuming homocysteine* by pulling it to methionine with supplemental MeCbl.

• *Helping folate chemistry* and MeCbl supply by supplementing DMG or folinic acid.

• Assisting these are antioxidant, anti-inflammatory measures that lower cytokine levels and allow more Hcy to be processed into methionine. This improves methylation, energy transport to G proteins and cell membrane PLs.
“Obvious” Interventions

- Bolstering processes that reduce adenosine, by supplementing DPP4, expelling mercury, and avoiding milk or casein.
- Consuming homocysteine by pulling it to cysteine with megadoses of pyridoxine and pyridoxal phosphate.
- Consuming homocysteine by pulling it to methionine with supplemental TMG.
DPP4
DIPEPTIDYLPEPTIDASE IV

- SAME AS: CD26=ADENOSINE DEAMINASE BINDING PROTEIN
- DIGESTS BETA CASOMORPHINS, MORPHICEPTIN & GLIADINOMORPHIN
- LYMPHOCYTE RECEPTOR INVOLVED IN SIGNAL TRANSMISSION
- ASSISTS ADA IN PROCESSING ADENOSINE
- IMPAIRED BY: Hg, Pb, Cd, Zn+, milk allergy
THE SUBSET

• All patients have been diagnosed with fibromyalgia and chronic fatigue, and treated for 1-12 years with a protocol that includes evaluation and treatment of adrenal, thyroid, sex hormone, food allergy, intestinal dysbiosis, magnesium, heavy metal toxicity, infections (EBV, Lyme, Mycoplasma) mold exposure, and other nutritional imbalances.
Earlier Clinical Experience

• 51 patients with FM/CFS treated with methylation supplements for 2-3 months:
  • 38/51 reported improvement: 75%
  Of these:
  • 10/51 reported marked improvement: 20%
  • 18/51 reported side effects initially: 35%
Earlier Clinical Experience: Interesting Sidebars

- 3 of 3 patients with primary depression and fatigue “all my life” reported marked improvement.
- 5/5 patients with autism and ADHD reported significant improvement.
Methylation Study: Part 1

The Protocol:
• Signed consent
• Protocol Handout and Explanation
• Outcome Measures
• FACT at baseline, 3 and 6 months
• Methylation chemistry: B, 3 and 6 months
• Genomics: baseline
• Thyroid studies, T3, T4, TSH: B, 3 & 6 mo
Methylation Study: Part 2

AFTER SIX MONTHS: INDIVIDUALIZED TREATMENT PROGRAMS BASED ON---

Methylation chemistry results
Genomic testing
FACT (Visual contrast testing)
Heavy metal toxicity
Mold toxicity
Methylation Study: Part 3

• After 9 months of treatment, 9 patients still had elevated adenosine levels. All volunteered to continue the study using acyclovir, at a dose of 200 mg 5 times daily for 2-3 months.
The Supplement Protocol

Treatment Consisted of 5 Supplements:

• 1. Folapro: ¼ tablet (200mcg) daily
• 2. Intrinsi/B12/folate: ¼ tablet daily
• 3. General Vitamin Neurological Health Formula: start ¼ tablet daily
• 4. Phosphatidyl Serine Complex: 1 cap/d
• 5. Activated B-12 Guard: 1 sl lozenge/d
Methylation Protocol

- **METHYLATION PROTOCOL**
- Adapted from a treatment program
devolved by Amy Yasko, N.D., Ph.D.
- **BASIC MATERIALS**
- FolaPro: ¼ tablet (200mcg) daily
- Intrinsi B12/folate: ¼ tablet daily
- General Vitamin Neurological Health Formula: start with ¼ tablet and work up
dosage as tolerated to 2 tablets daily
- Phosphatidyl Serine Complex: 1 softgel capsule daily
- Activated B12 Guard: 1 sublingual lozenge daily
- The first two supplement tablets are difficult to break into quarters. We recommend
that you obtain (from any pharmacy) a good-quality pill splitter to assist with this
process. They can, alternatively, be crushed into powders, then separated on a flat
surface, and the powders can be mixed together. They can be taken orally with
water, with or without food.
- Occasionally these can make patients sleepy, so some take them at bedtime.
They can be taken any time of day, with or without food.
- GO SLOWLY. Occasionally, as the methylation cycle blockages are released,
toxins are released and processed by the body, and this can lead to an exacerbation
of symptoms. IF THIS HAPPENS, try smaller doses, every other day. SLOWLY work
up to the full dosages. If you have questions, please call our office to discuss them.
The Supplements

1. Folapro: 800mcg (per tablet) as L-5-methyl-tetrahydrofolate
   Dose: ¼ tablet daily
The Supplements

2. Intrinsi/B12/folate: 800mcg (per tablet) as folic acid, L-5-methyl tetrahydrofolate and 5-formyl tetrahydrofolate (now available as Actifolate)
Vitamin B12 500mcg (per tablet) as cyanocobalamin
Intrinsic factor 20mg

Dose: ¼ tablet daily
The Supplements

• 3. General Vitamin Neurological Health Formula ¼ tablet daily working up to 2 tablets daily as tolerated

• 4. Activated B-12 Guard: 2000mcg B12 sublingual once sublingual daily (hydroxocobalamin)
The Supplements

5. **Phosphatidyl-Serine Complex**: Once/d
   Phospholipids 500mg
   Phosphatidylserine 20-22%
   Phosphatidylcholine 14-18%
   Phosphatidylethanolamine 10-12%
   Phosphatidylinositol 7-10%
   Essential fatty acids 748mg
BASIC INFORMATION

- METHYLATION PROTOCOL FOR FIBROMYALGIA
- AND CHRONIC FATIGUE SYNDROME

BASIC INFORMATION: STARTING DATE:_______________

- NAME:  ______________________________________________
- AGE:    ___________      SEX:   MALE_______FEMALE________
- DATE OF BIRTH:  __________________
- DATE OF DIAGNOSIS OF FIBROMYALGIA ______________________
- DATE OF DIAGNOSIS OF CHRONIC FATIGUE __________________
- DATE OF ONSET OF SYMPTOMS:  ________________
  - SUDDEN ONSET?    YES ___      NO__________
  - CAUSE OF ONSET KNOWN?    ____IF SO, WHAT?_____________
  - FAMILY HISTORY OF THESE CONDITIONS?  YES_______NO______
    - IF SO, WHICH RELATIVES? ________________________

OTHER DIAGNOSES:
- _______IBS (IRRITABLE BOWEL SYNDROME)
- _______MIGRAINE HEADACHES
- _______MULTIPLE CHEMICAL SENSITIVITIES
- _______ENDOMETRIOSIS
- _______INTERSTITIAL CYSTITIS
- _______CHRONIC VULVITIS
- _______RESTLESS LEG SYNDROME
- _______LYME DISEASE
- _______MOLD EXPOSURE AND/OR TOXICITY
- _______MONONUCLEOSIS (EPSTEIN-BARR VIRUS)
- _______CHRONIC SINUS INFECTIONS
- _______MYCOPLASMA INFECTIONS

CURRENT MEDICATIONS:

CURRENT SUPPLEMENTS:
SYMPTOM CHECKLIST

• Check if this is true for you, now, or in the past 3 months

F.A.C.T. RESULTS

• Would you say that your fatigue has not been present for your whole life, that it is present even without ongoing physical exertion, that it is not alleviated much by resting, and that it has caused a substantial reduction in your participation in occupational, educational, social or personal activities?

• Do you have impairment of short-term memory or concentration that is severe enough to have caused a substantial reduction in your participation in occupational, educational, social or personal activities?

• Sore throat

• Tender neck or axillary (armpit) lymph nodes

• Muscle pain

• Pain in multiple joints without joint swelling or redness

• New or different headaches

• Non-refreshing sleep

• Fatigue that lasts for more than 24 hours following exercise

• Have you had chronic widespread pain for more than 3 months in all four quadrants of your body (above and below your waist and on both sides of your body)? Have you also had axial pain? (around your spine or chest).

• When you exercise, do you feel worse afterwards and exhausted the next day?

• Weakness

• Fatigue:

Please rate your fatigue on a scale of 10: 0=no energy at all, 10=a full tank of “gas”, on average. Circle on the line:

0 5 10

Pain:

Please rate your pain, (average, daily) on a scale of 10: 0=no pain, 10=worst pain you can imagine. Circle on the line:

0 5
SYMPTOM CHECKLIST 2

- Confusion, disorientation
- Difficulty in word finding
- Impairment of concentration, difficulty assimilating new information
- Reduced task completion
- Hypersensitivity to bright light
- Night blindness
- Tearing, redness of the eyes
- Blurred vision
- Chronic aching muscles
- Joint pain, morning joint stiffness
- Pain in weight bearing joints
- Nausea
- Loss of appetite
- Weight gain (how much over what period of time?)
- Abdominal pain
- Chronic sinus congestion
- Chronic cough that mimics asthma
- Shortness of breath
- Ice-pick like pain, or electrical pain that shoots into a muscle
- Nosebleeds
- Metallic taste or other unusual taste
- Vertigo, dizziness
- Ringing in the ears (tinnitus)
- Rage or inappropriate anger
- Panic attacks or anxiety
- Depression
- Tingling, “needles and pins” sensations
- Increased sensitivity to touch
- Difficulty with sleep:
  - Getting to sleep difficulties
  - Difficulty staying asleep
- Mood swings
- Excessive thirst, or frequent urination
- Impotence
- Irregular vaginal bleeding
- Low body temperature
- Hypoglycemia
- Low blood pressure
- Chronic yeast infections
- Onset of menopause (if appropriate)
OUTCOME MEASURES

Please circle the number that rates your response

Date: __________________________

A. HOW IS YOUR ENERGY?
   1  2  3  4  5  6  7  8  9  10
   1=no energy          10=excellent energy

B. HOW DO YOU SLEEP?
   1  2  3  4  5  6  7  8  9  10
   1=no sleep            10=8 hours of sleep without waking

C. HOW IS YOUR MENTAL CLARITY?
   1  2  3  4  5  6  7  8  9  10
   1=worst possible, “brain fog”  10=good clarity

D. HOW BAD IS YOUR PAIN?
   1  2  3  4  5  6  7  8  9  10
   1=worst possible pain      10=pain free

E. HOW IS YOUR OVERALL SENSE OF WELL-BEING?
   1  2  3  4  5  6  7  8  9  10
   1=worst possible           10= excellent
STUDY RESULTS: 3 MONTHS

• ALL 30 PATIENTS COMPLETED THE STUDY REQUIREMENTS AT 3 MONTHS
• 25/30 PATIENTS REPORTED IMPROVEMENT: 83%
• AMONG THE GROUP THAT REPORTED IMPROVEMENT, 8/30 PATIENTS REPORTED MARKED IMPROVEMENT: 27%
OUTCOME MEASURES

ENERGY:
Mean Initial Score: 3.9
Mean Score At 3 months: 5.7
Mean Score At 6 months: 6.0
Mean Score At 9 months: 6.6
Number improved: 26/30 = 86%
Self-rated Energy Level: 30 patients initially

Duration of treatment (number of patients)
Energy Level
Self-rated Energy Level: 30 patients initially
Initial 3 mo. (30 pts.)
6 mo. (29 pts.)
9 mo. (25 pts.)

Mean+2*Std Dev
Mean-2*Std Dev
Mean+Std Dev
Mean-Std Dev
Mean
Outliers
Extremes
OUTCOME MEASURES

SLEEP
Mean Initial score: 4.7
Mean Score At 3 months: 5.9
Mean Score At 6 months: 6.2
Mean Score At 9 months: 6.0
Number improved: 22/30 = 73%
Self-rated Amount of Sleep: 30 patients initially

Duration of treatment (number of patients)

Amount of sleep: 1 = no sleep, 10 = 8 hours of sleep without waking

Initial 3 mo. (30 pts.) 6 mo. (29 pts.) 9 mo. (25 pts.)

Mean+2*Std Dev
Mean-2*Std Dev
Mean+Std Dev
Mean
Outliers
Extremes
OUTCOME MEASURES

MENTAL CLARITY

Mean Initial score: 5.0
Mean Score At 3 months: 6.6
Mean Score At 6 months: 6.5
Mean Score At 9 months: 6.3
Number improved: 22/30 = 73%
Self-rated Mental Clarity: 30 patients initially

Duration of treatment (number of patients)
Mental Clarity
Self-rated Mental Clarity: 30 patients initially
Initial 3 mo. (30 pts.) 6 mo. (29 pts.) 9 mo. (25 pts.)

Mean+2*Std Dev
Mean-2*Std Dev
Mean+Std Dev
Mean-Std Dev
Mean
Outliers
Extremes
OUTCOME MEASURES

PAIN RELIEF

Mean Initial score: 5.0
Mean Score At 3 months: 5.6
Mean Score At 6 months: 5.6
Mean Score At 9 months: 6.6
Number improved: 24/30=80%
Self-rated Freedom from Pain: 30 patients initially

- Duration of treatment (number of patients)
- Freedom from pain
- Self-rated Freedom from Pain: 30 patients initially

- Initial
- 3 mo. (30 pts.)
- 6 mo. (29 pts.)
- 9 mo. (25 pts.)

- Mean+2*Std Dev
- Mean-2*Std Dev
- Mean+Std Dev
- Mean-Std Dev
- Mean
- Outliers
- Extremes

Duration of treatment (number of patients)
OUTCOME MEASURES

OVERALL

Mean Initial score: 4.3
Mean Score At 3 months: 6.6
Mean Score At 6 months: 6.1
Mean Score At 9 months: 6.8
Number improved: 23/30=77%
Self-rated Overall Wellbeing: 30 patients initially

Duration of treatment (number of patients)

Initial 3 mo. (30 pts.) 6 mo. (29 pts.) 9 mo. (25 pts.)

Mean+2*Std Dev
Mean-2*Std Dev
Mean+Std Dev
Mean-Std Dev
Mean
Outliers
Extremes
OUTCOME MEASURES

SYMPTOM CHECKLIST of 39 possible:
Initial Symptoms: Average 21
After 6 months: Average 14
After 9 months: Average 11
ALL patients had a decrease in symptoms checked.
Number of symptoms reported: 30 patients initially

- Initial
- 6 mo. (29 pts.)
- 9 mo. (25 pts.)

- Mean + 2 * Std Dev
- Mean - 2 * Std Dev
- Mean + Std Dev
- Mean - Std Dev
- Mean
- Outliers
- Extremes
Clinical Improvement:

Average clinical improvement: 48%

At 9 months, 15/25 reported >50% improvement. 3/5 patients who dropped out reported >50% improvement, for a total of 60%.
OUR DROPOUTS #1

• All 30 patients completed the study at 3 months
• 29/30 patients completed the study at 6 months
• 25/30 patients completed the study at 9 months

• The patient who dropped out at 6 months reported 100% relief of pain and 60% overall improvement but her family insisted that she be followed at another clinic.
OUR DROPOUTS #2

- Two other patients reported complete relief of pain, complete relief of symptoms and returned to work at full capacity, and decided not to continue the study.
- One patient underwent bilateral hip replacement surgery and could not return for follow up.
- One patient was disappointed with her results and elected to discontinue her treatment.
TIME TO IMPROVEMENT: 5.6 weeks avg
   Range: immediate (rare) to 8 weeks
SIDE EFFECTS:
   16/30=53% reported side effects, usually within 3-4 days, but ranging up to 2 weeks.
   Most were mild.
   None discontinued usage.
SIDE EFFECTS

3 patients needed to decrease frequency for several days (to every third or second day) until they could take the full daily dosage.

Commonest side effects:

GI (pain, cramps, constipation, diarrhea)

6/30 = 20%

Increase in pain 4/30 = 13%
SIDE EFFECTS (Continued)

Increase in fatigue: 3/30=10%

Others: Decrease in appetite, poor sleep, weak legs, flu-like symptoms, increase in anxiety and depression

(one patient for each)
STUDY RESULTS: 3 MONTHS GLUTATHIONE

SERUM GLUTATHIONE INITIALLY LOW
(3.7 or less) in 22/30 patients: 73% below normal average starting GSH: 3.2
AFTER 3 MONTHS: GSH INCREASED
Improved in 29/30 patients: 96.6%
Average GSH at 3 months 3.8 (19% increase)
30% of patients still below normal (3.8-5.5)
STUDY RESULTS: 6/9 MONTHS GLUTATHIONE

AFTER 6 MONTHS:
Average GSH rose to 4.3 (nl 3.8-5.5)
17% of patients (5/29) still below normal

AFTER 9 MONTHS
Average GSH rose to 4.7 (nl 3.8-5.5)
47% increase from baseline
ALL patients had come up to normal range
Glutathione: 30 patients initially

Duration of treatment (number of patients)

Glutathione (plasma) (micromoles per liter)

Initial 3 mo. (30 pts.) 6 mo. (29 pts.) 9 mo. (25 pts.) Ref. range

Mean+2*Std Dev
Mean-2*Std Dev
Mean+Std Dev
Mean-Std Dev
Mean
Outliers
Extremes
STUDY RESULTS: 3 MONTHS

IMPROVEMENT IN GSH/GSSH RATIO
Average improvement of 1.00
25/30 patients improved: 83.3%

RBC FOLIC ACID LEVELS IMPROVED
27/30 Patients improved: 90%
Average improvement: 30 nmol/l
GSH/GSSG: 30 patients initially

Duration of treatment (number of patients)

Initial 3 mo. (30 pts.) 6 mo. (29 pts.) 9 mo. (25 pts.)

Mean ref. ratio
1.5
3
4.5
6
7.5
9
10.5
12
13.5
15
16.5
18

Mean+2*Std Dev
Mean-2*Std Dev
Mean+Std Dev
Mean-Std Dev
Mean
Outliers
Extremes
STUDY RESULTS: 3 MONTHS SAM

SAM (RBC) LEVELS (nl 221-256)
Initial levels are LOW in 20/30 patients (67%) Average starting level: 217
After 3 months, improvement in 27/30 (90%)
Average improvement 12.6 (micromol/dl)
STUDY RESULTS: 6/9 MONTHS SAM

AFTER 6 MONTHS
Average SAM levels rose to 238

AFTER 9 MONTHS
Average SAM levels rose to 241
Only one patient (1/25) had not normalized
S-adenosylmethionine (SAMe): 30 patients initially

Duration of treatment (number of patients)
S-adenosylmethionine (RBC) (micromoles per deciliter)

<table>
<thead>
<tr>
<th>Duration</th>
<th>Number of Patients</th>
</tr>
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<tr>
<td>Initial</td>
<td>30 pts.</td>
</tr>
<tr>
<td>3 mo.</td>
<td>30 pts.</td>
</tr>
<tr>
<td>6 mo.</td>
<td>29 pts.</td>
</tr>
<tr>
<td>9 mo.</td>
<td>24 pts.</td>
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Ref. range
- 165
- 180
- 195
- 210
- 225
- 240
- 255
- 270

Mean + 2*Std Dev
Mean - 2*Std Dev
Mean + Std Dev
Mean - Std Dev
Mean
Outliers
Extremes

Graph showing S-adenosylmethionine levels over time with reference range and statistical measures.
Are the 9-month results of this study consistent with the predictions of the Glutathione Depletion—Methylation Cycle Block hypothesis?

Yes, as follows:

1. The reduced glutathione levels were significantly below normal before treatment was begun.

2. There was a partial methylation cycle block before treatment was begun.

3. The methylation cycle block was partially lifted by treating with bioactive forms of vitamin B12 and folate, together with basic nutritional support, directed specifically at raising the activity of the enzyme methionine synthase, which is the enzyme hypothesized to be partially blocked.
Are the 9-month results of this study consistent with the predictions of the Glutathione Depletion—Methylation Cycle Block hypothesis? (continued)

4. Treating to lift the methylation cycle block not only improved the methylation capacity, but also raised glutathione (as well as the ratio of reduced to oxidized glutathione), suggesting that these two phenomena are indeed linked in an interactive mechanism in CFS, as they also appear to be in autism.

5. The mean level of reduced glutathione rose by 47%, while the mean level of oxidized glutathione rose less than 5%, suggesting that the main issue involving glutathione in CFS is a deficit in production, rather than a deficit in recycling. (However, it must be noted that B2 and B3 supplements were included in the protocol, which may have increased the activity of glutathione reductase to some extent.)
AFTER 6 MONTHS

WE CONTINUED THE METHYLATION SUPPLEMENTATION PROGRAM AND ADDED INDIVIDUAL TREATMENTS BASED ON GENOMICS, F.A.C.T. TESTING, AND EVIDENCE OF HEAVY METAL TOXICITY AND MOLD TOXICITY
GENOMIC DATA

POSITIVE METHYLATION SNPs

AHCY-01  8/30 patients
BHMT-08  24/30 patients
CBS      19/30 patients
COMT     25/30 patients
MTR      11/30 patients

All patients had at least one, avg 3 SNPs
Homocysteine

Decreased
CBS Enzyme Activity

Increased
CBS Enzyme Activity
METHYLATION CYCLE

Methionine → SAM → Methyl acceptor

SHT → 5,10 Methylene THF → JUMP → THF

5 Methyl THF → Glycine

Purine synthesis

FOLATE CYCLE

B12 → THF

MTHFR

TRANSSULFURATION PATHWAY

Glutathione → Cysteine → Oxidation

Sulfate → Hypotaurine → Taurine
CBS
Cystathionine beta-synthase

• CBS C699T mutations may result in a ten-fold increase in enzyme activity, draining valuable intermediates by this upregulation, and causing a high level of breakdown products.

• This would convert homocysteine more efficiently to cysteine, lowering homocysteine.

• However, this could also generate more sulfur breakdown products, with enhanced ammonia production and a lack of glutathione.
CBS mutations

• The level of cysteine helps to determine if glutathione or taurine will be produced from transulfuration.
• High cysteine levels favor conversion of cysteine to sulfate and taurine, which would decrease glutathione.
• Adding additional glutathione could potentially increase sulfur to excess, which might directly activate cortisol/stress response & deplete NTs
Dependence of SAMe + SAH on the CBS C699T polymorphism (Amy Yasko’s “bathtub draining” phenomenon): 30 patients initially (number of patients in each group shown at the bottom).

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<th>(-/-)init</th>
<th>(+/-)init</th>
<th>(+/+)init</th>
<th>(-/-)3m</th>
<th>(+/-)3m</th>
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<td>7</td>
<td>14</td>
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### Summary

- **Mean**: Represents the average value of the dataset.
- **Mean ± 2*Std Dev**: Indicates the range within which 95% of the data falls.
- **Mean ± Std Dev**: Represents the range within which 68% of the data falls.
- **Mean ± Std Dev**: Represents the range within which 95% of the data falls.
- **Outliers**: Data points that fall outside the range of 1.5 times the interquartile range from the first and third quartiles.
- **Extremes**: Data points that are considered outliers based on the standard deviation criteria.
Rapid report

Stabilization of S-adenosyl-\textsubscript{L}-methionine promoted by trehalose

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Abstract

S-adenosyl-\textsubscript{L}-methionine (SAM), an important metabolic intermediate of mammals, is a well-known therapeutic agent. The molecule is chemically unstable, both in solution and in dry state, and forms different degradation products. Because the chemical instability represents a real problem during the preparation of therapeutic formulations, we investigated the capacity of some sugars to improve the SAM stability over time. In the present work, we demonstrated that the disaccharide trehalose exercises a protective effect towards the lyophilized SAM slackening its degradation (65\% of SAM was detected after 50 days at 37 \textdegree C). A parallel study, performed to stabilize the SAM into lyophilized yeast cells enriched in the sulfonium compound, assessed the positive effect of trehalose also in whole cells, but in lesser measure.
VISUAL CONTRAST TESTING

INITIALLY: 21/30 patients abnormal
AFTER 3 MONTHS: 7/21 improved
14/21 unimproved (1 worse)

DETAILED REVIEW OF THESE PATIENTS
Included mercury/heavy metal toxicity, mold exposure, Lyme testing and viral exposure: incomplete but specific patient histories are available for study.
Measuring Visual Contrast Sensitivity
Measuring Visual-Pattern Detection Function
VCS in Non-Cases & Cases Before & After Treatment

Visual Contrast Sensitivity

- Non-Cases (N=11)
- Cases Before CSM (N=53)
- Cases After CSM (N=53)

Mean Number Symptoms
Non-Cases = 2.00±0.30
Cases Before
Rx = 10.81±0.15
Cases After
Rx = 1.08±0.53

Acuity – No Change
F(1,52)=0.11
p=0.74

VCS – Significant Difference
Treatment
F(1,52)=289.70
p<0.001
Treatment X SF
F(4,49)=43.58
p<0.001
CASE STUDY #1

- D.F. was a 49 yo wf with a 6 year hx of FM and CFS. Her initial glutathione 3.0 (nl 3.8-5.5) and SAM 217 (nl 221-256). Although she felt a little better after the first 3 months of supplements, glutathione was 2.8, SAM 226 on f/u. (CBS +) After 6 months, GSH 3.5 and SAM 240. Still minimal clinical improvement. After beginning NH3 RNA, Nucleotide and Trehalose (24 hr urine aa showed elevated taurine and cysteine) at 6 months…
CASE STUDY #1

- After just one month on those supplements, she felt so much better that she was able to resume full time work, which she had not been able to do for 5 years, successfully. She was free of pain and her energy was back to normal. 9 month levels of GSH were 4.0 and SAM 240.
CASE STUDY #2

• B.E. was an 84 yo wf with a two year history of FM and CFS. Her initial GSH was 2.7, SAM was 201. She reported no clinical improvement at 3 months, but had two episodes of severe infection requiring two rounds of antibiotics during that time. Her GSH was 3.2 and SAM 220 at 3 months. At 6 months, still no improvement and GSH only 3.0, SAM 233. Since she had a +FACT she was evaluated
CASE STUDY #2

• for heavy metal toxicity with DMPS challenge test (elevated levels of mercury were found) and treated with cholestyramine for mold exposure, and DMPS IV monthly with oral DMSA. By the completion of the study, she reported marked improvement, and was able to join her friends for a week-long visit to Paris, noting she was pain free and her energy had returned to almost normal. At 9 months, GSH 4.0 and SAM 232.
CASE STUDY #3

• S.H. was a 55 yo wf with FM and CFS for 8 years. She was on disability for those conditions. We had discovered an elevated level of mercury on DMPS testing 4 years prior (level: 34) but she elected not to treat it for financial reasons. Initial GSH level was 3.4, SAM 207. These rose steadily over the study period: GSH at 3 months 3.9, and 6 months 4.8 and at 9 months 4.6; SAM rose to 219
CASE STUDY #3

• At 3 months, 230 at 6 months and 267 at 9 months. While noting a 20% improvement in energy, sleep and well-being, no additional improvements were noted. She continued to decline to treat the elevated mercury level.

• Parenthetically, 3 patients in the study were on disability, and all three reported only minimal improvement, throughout.
AFTER 9 MONTHS OF STUDY: One Additional Component

• 9 of our 25 remaining patients still had significantly elevated adenosine levels.
• All agreed to a 2-3 month trial of acyclovir
• Dosage of acyclovir: 200mg 5x a day
• Results: 8 of 9 patients reported an additional 20% improvement in overall well-being, which held even when acyclovir was discontinued.
WHAT HAPPENED NEXT?

SO ALL OF OUR PATIENTS LIVED HAPPILY EVER AFTER………………

PERHAPS NOT ALL.

BUT THE SUPPLEMENT COMPANIES CAME KNOCKING….AND WE DECLINED
THIS GENERATED A LOT OF EXCITEMENT ABOUT CH3

We published our work in the Townsend Letter, December 2011

Dr. Marty Pall wrote a letter in March, 2012 to the Townsend Letter suggesting that our work proved his OONO hypothesis
RICH RESPONDED

In the April, 2012 Townsend Letter Rich clarified those arguments, agreeing that peroxynitrite metabolism was clearly related to methylation (it is) and you would expect nitrosamine levels to rise with inadequate methylation.
RICH LEFT US……

Rich died, almost exactly 3 years ago of an unexpected heart attack a day after kayaking in the Pacific Northwest in September, 2012

WE MISS HIM EVERY DAY!
AND WE KIND OF RESTED ON OUR LAURELS……..

…..until the outliers started to trickle in (What!?) and reminded me about how little we really knew
So I jumped at the chance to be here and learn more from all of you!
Have Methylation....Will Travel
Wire Nathan, Santa Rosa, CA
THANK YOU!

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QUESTIONS?

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- We are happy to share our protocols and details of treatment upon request