# Lifestyle Medicine and the Methylome

## Holistic Strategies for Optimising the Epigenome and Methylome

Presented by Kara Fitzgerald, ND

GENOVA DIAGNOSTICS®

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**Physician and Clinic Director at The Sandy Hook Clinic**

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# Technical Issues & Clinical Questions

Please type any technical issue or clinical question into either the “Chat” or “Questions” boxes, making sure to send them to “Organizer” at any time during the webinar.

We will be compiling your clinical questions and answering as many as we can the final 15 minutes of the webinar.


DISCLAIMER: *Please note that any and all emails provided may be used for follow up correspondence and/or for further communication.*

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**WWW.GDX.NET**

*for more information and educational resources, including…*

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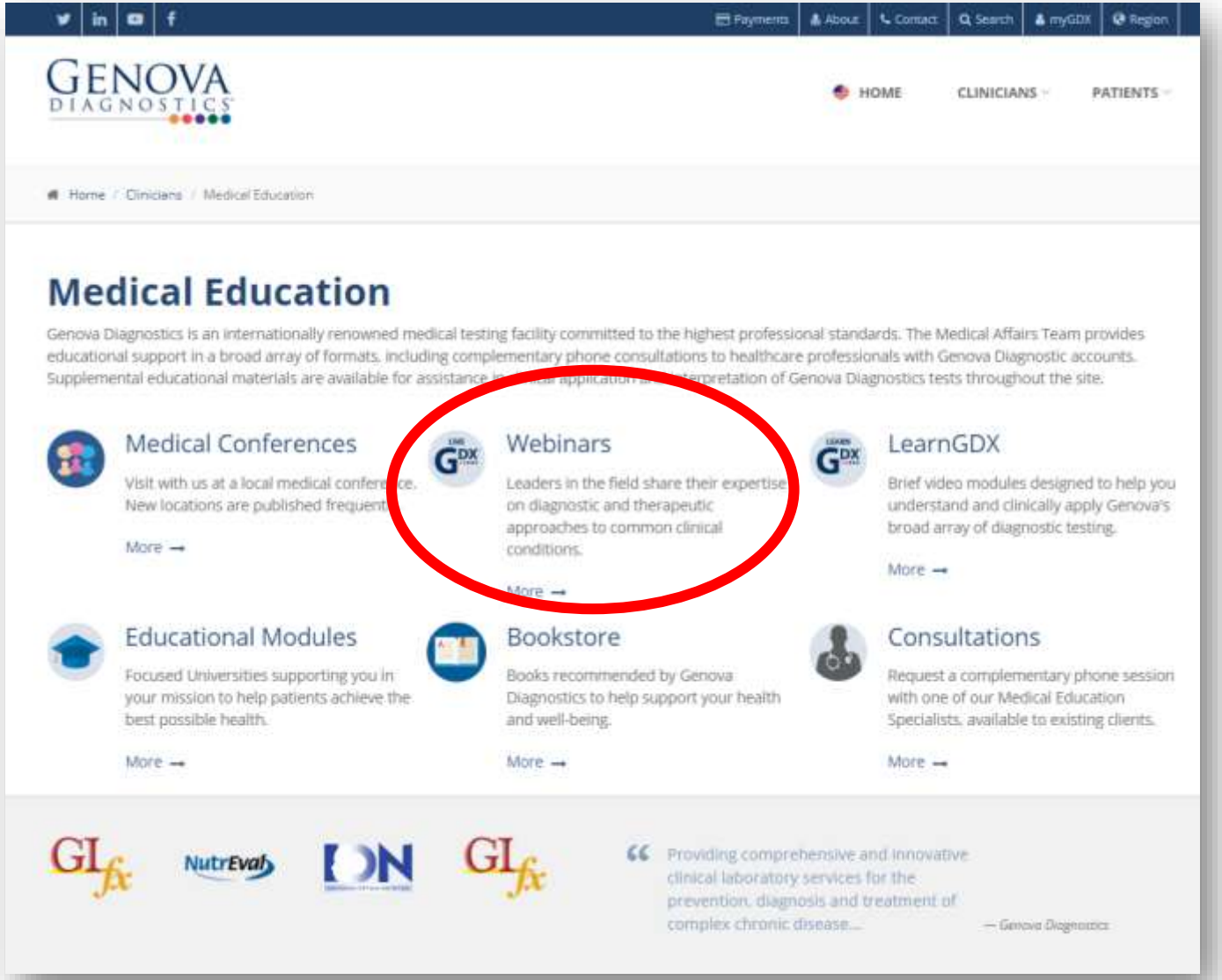
MY **GDX** – Order materials and get results

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# Lifestyle Medicine and the Methylome

## Holistic Strategies for Optimising the Epigenome and Methylome

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# Objectives for This Presentation

- Review DNA methylation and its importance to aging and health span
- Discuss the wide range of factors that influence methylation status
- Provide practical assessment and intervention tools to support balanced methylation, including supplements, diet, and lifestyle
- Learn what's next in assessing the methylome in clinical practice


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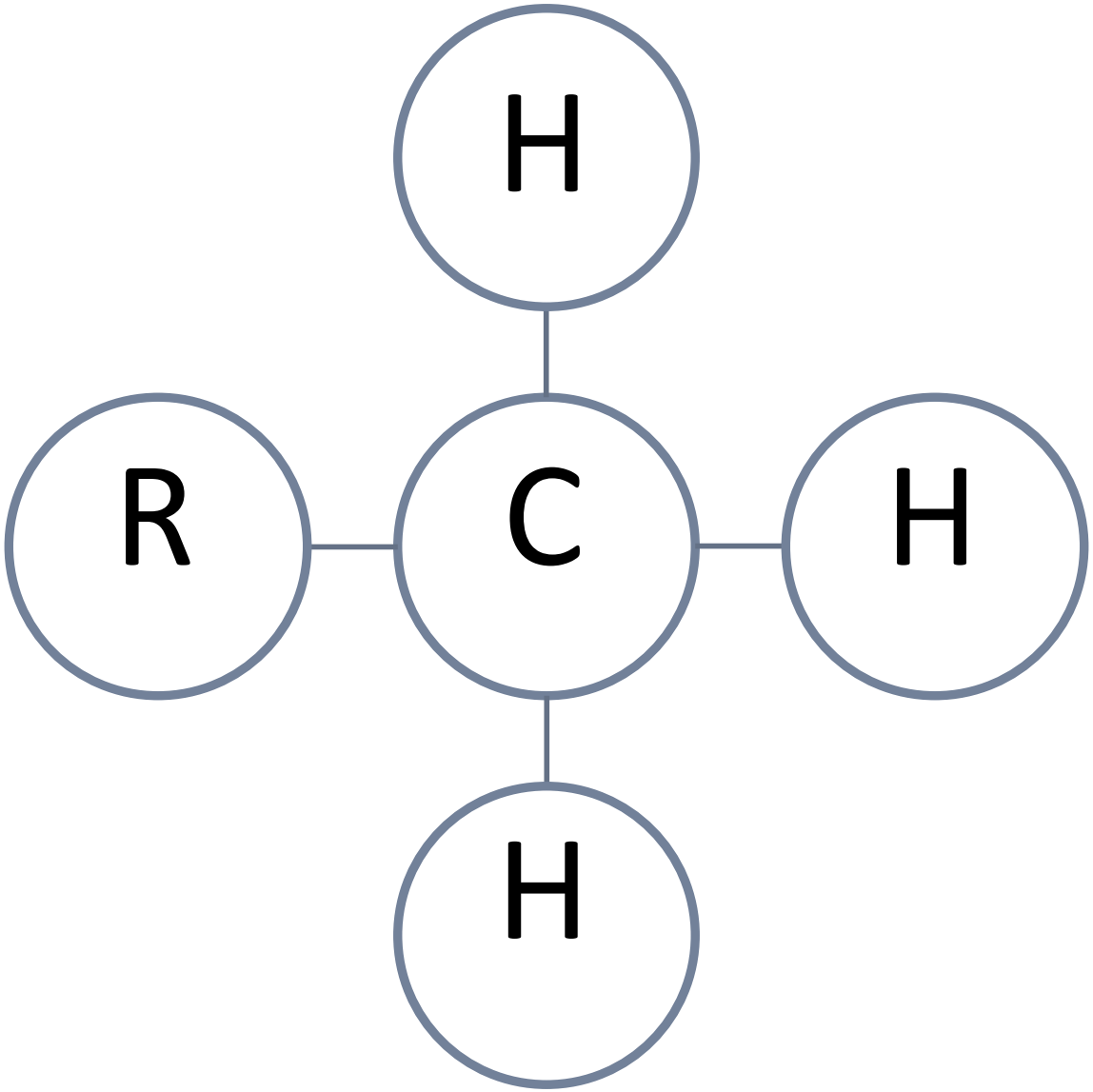
H
R
C
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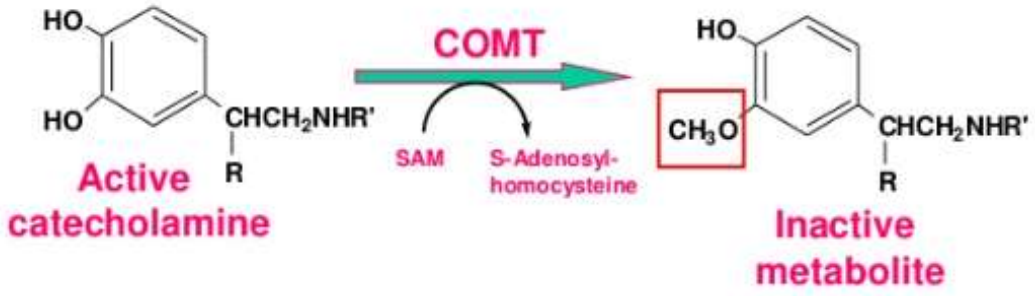
H

# Methylation Reactions

- “One-carbon metabolism” = transfer or formation of methyl (CH3) groups
- CH3 formation by additional hydrogen: e.g. MTHFR
- CH3 transfer from a methyl donor, commonly SAMe: e.g. COMT

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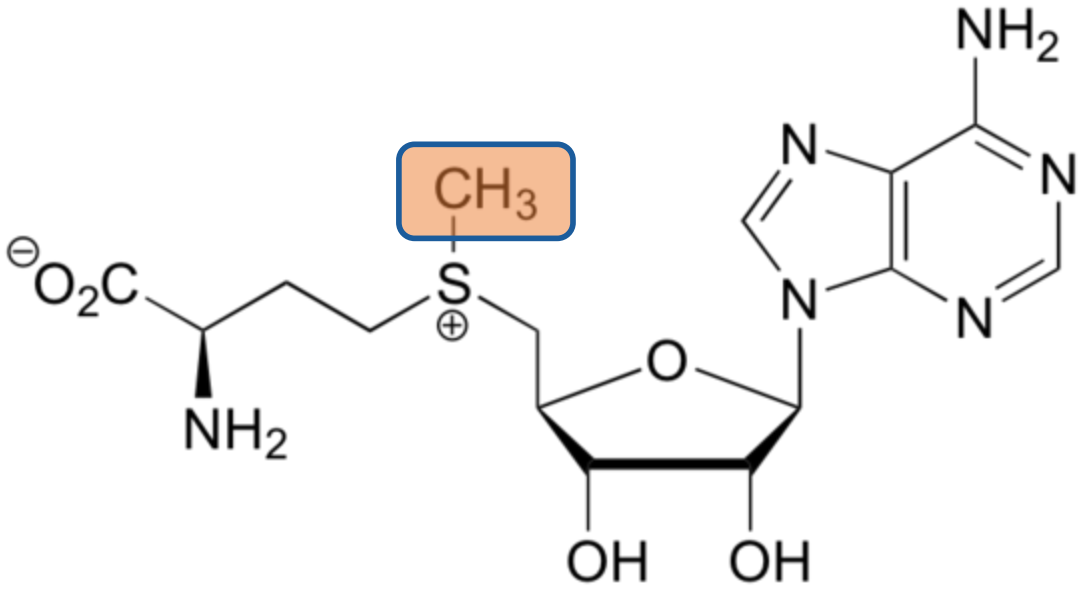
# Discovered by Wilhelm His

- German physician, Wilhelm His, discovered the body's ability to methylate in 1887
- **The mechanism of methylation** (role of S-adenosyl methionine as active co-factor) in methylation reactions wasn't discovered until much later, in **1953 by Cantoni**


Photo: http://www.issx.org/page/Methylation.

# SAMe = Major Methyl Donor




S-adenosylmethionine

Photo: https://en.wikipedia.org/wiki/S-Adenosyl_methionine.

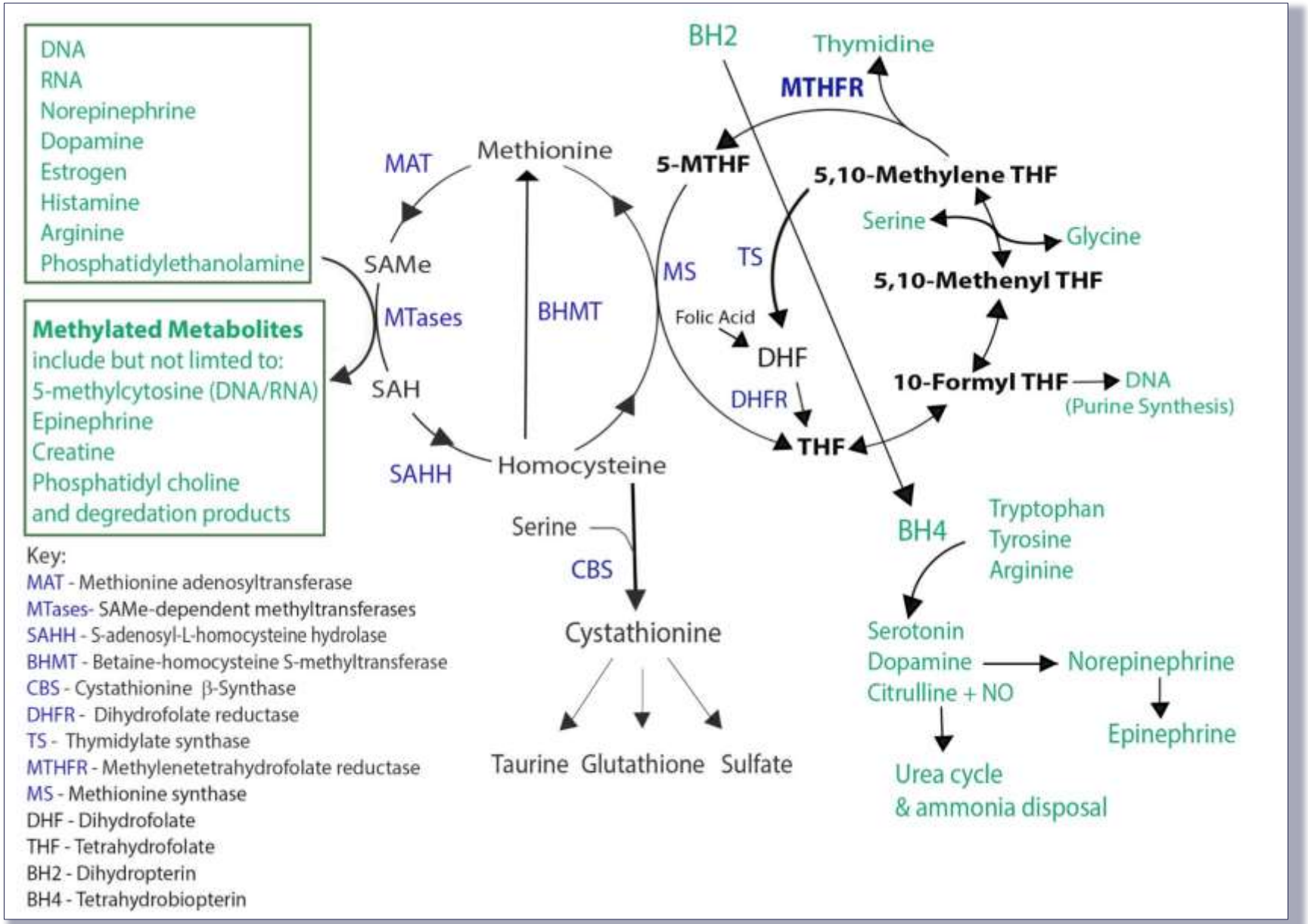



Photo: https://wellnessdoctorrx.com/introduction-to-dna-methylation-status-activity/.

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# 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 | | UPREGULATING SNPS | |
|---|---|---|---|
| **MTHFR** | | **MTR** | |
| C677T | −− | A2756G | −− |
| A1298C | ++ | **CBS** | |
| **COMT** | | C699T | −+ |
| V158M | −+ | **BHMT** | |
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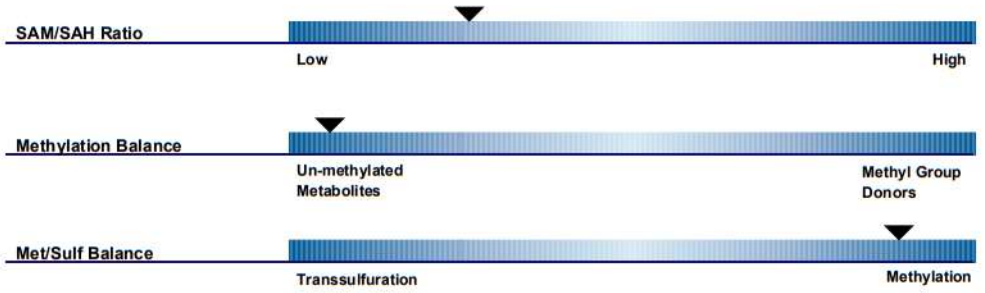
| A66G | ++ | **GNMT** | |
| **MAT1A** | | C1289T | −+ |
| D18777A | −+ | | |
| **SHMT1** | | | |
| C1240T | −+ | | |

### Transsulfuration

- Glutathione ▼
- Cystathionine ▲
- Cysteine ▲

## Methylation Status





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**3534 Methylation Panel** - Plasma & Whole Blood

*Methodology: LCMSMS & Colorimetric*

| | | | QUINTILE DISTRIBUTION (1st / 2nd / 3rd / 4th / 5th) | Reference Range |
|---|---|---|---|---|
| | Results micromol/L | | | |
| **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.*





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# 208 Human Methyltransferase Enzymes Identified

**Including:**

- Histamine N-methyltransferase
- Catechol O-methyltransferase
- DNA methyltransferases
- RNA methyltransferases
- Glycine N-methyltransferase
- Indolethylamine N-methyltransferase
- Leucine carboxyl methyltransferases
- Nicotinamide N-methyltransferase
- Methionine synthase
- Betaine-homocysteine S-methyltransferases
- Protein arginine methyltranferases

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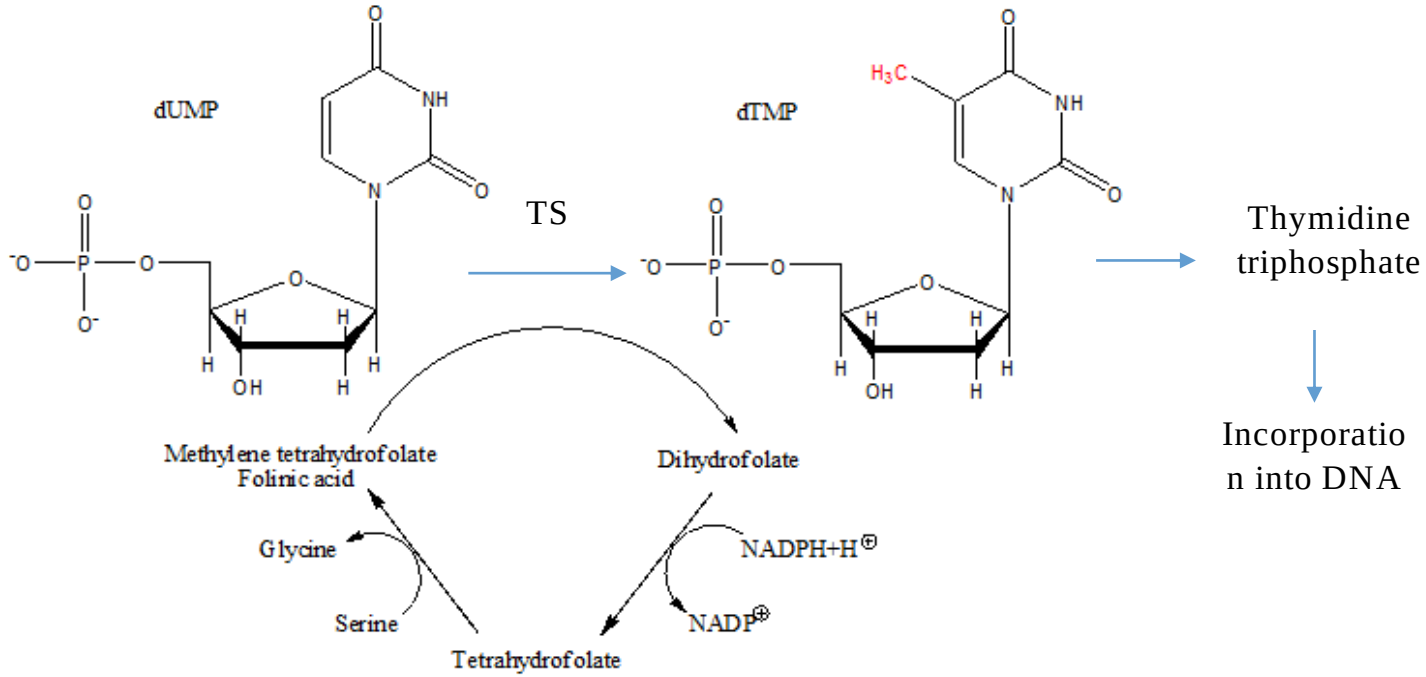
Petrossian TC et Clarke SG. *Mol Cell Proteomics.* 2011;10(1):M110.000976.

# Methylation Uses

- **Cell division (DNA, RNA synthesis and repair)**
- Early CNS development (neural tube defects)
- Immune cell differentiation
- Neurotransmitter biosynthesis and metabolism (dopamine, norepinephrine, epinephrine, acetylcholine, and melatonin)
- Histamine clearance
- Detoxification and hormone biotransformation
- Cellular energy metabolism/phosphocreatine synthesis
- Phospholipid synthesis
- Myelination of peripheral nerves
- **Epigenetic regulation of gene expression (esp gene silencing)**

# Formation of DNA Bases

*note methylene THF as methyl donor for this reaction




dTMP = deoxythymidine monophosphate
TS = thymidylate synthase

Photo Adapted: https://www.pharmacorama.com/en/Sections/DNA-RNA-biosynthesis-6.php.

# Epigenetic (DNA) Methylation

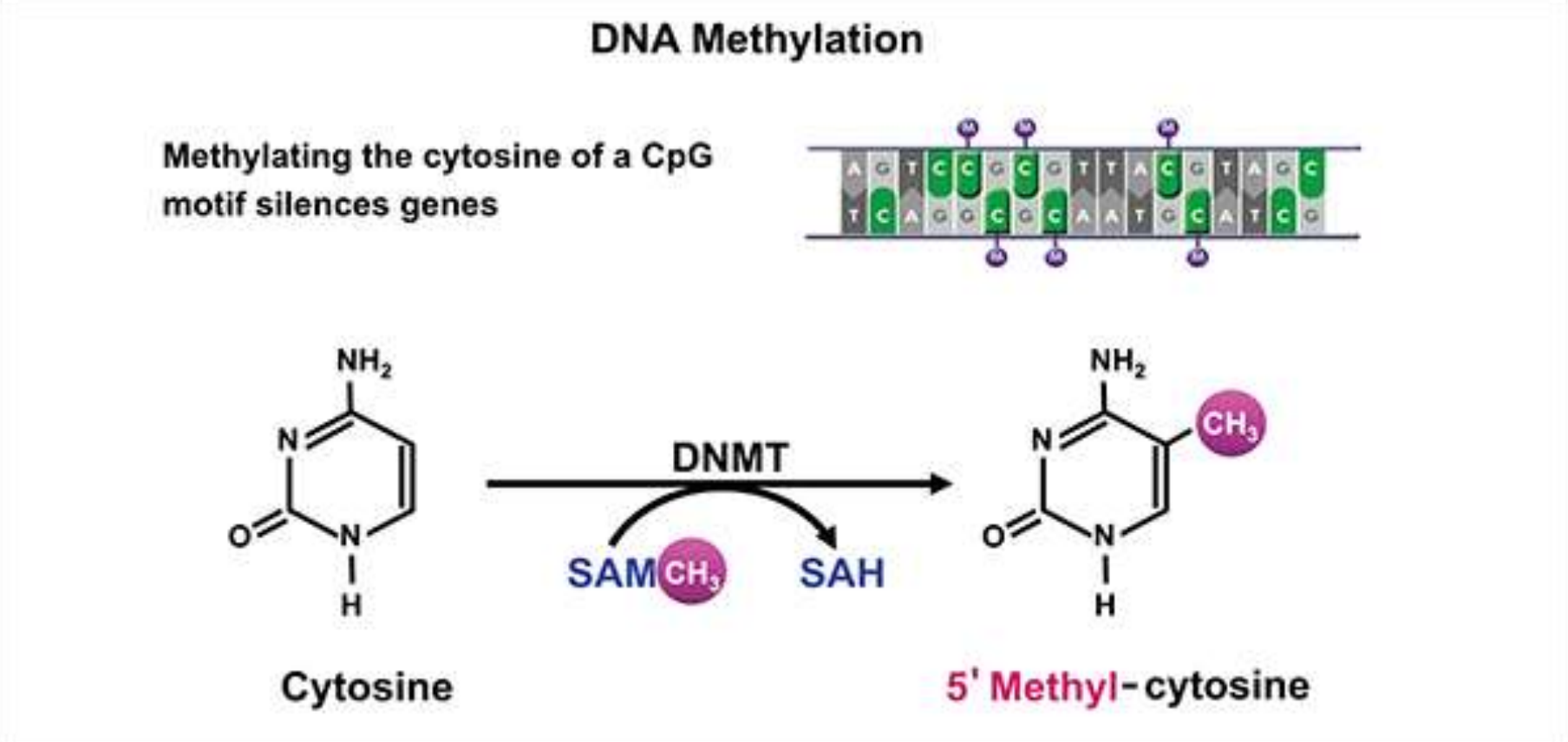
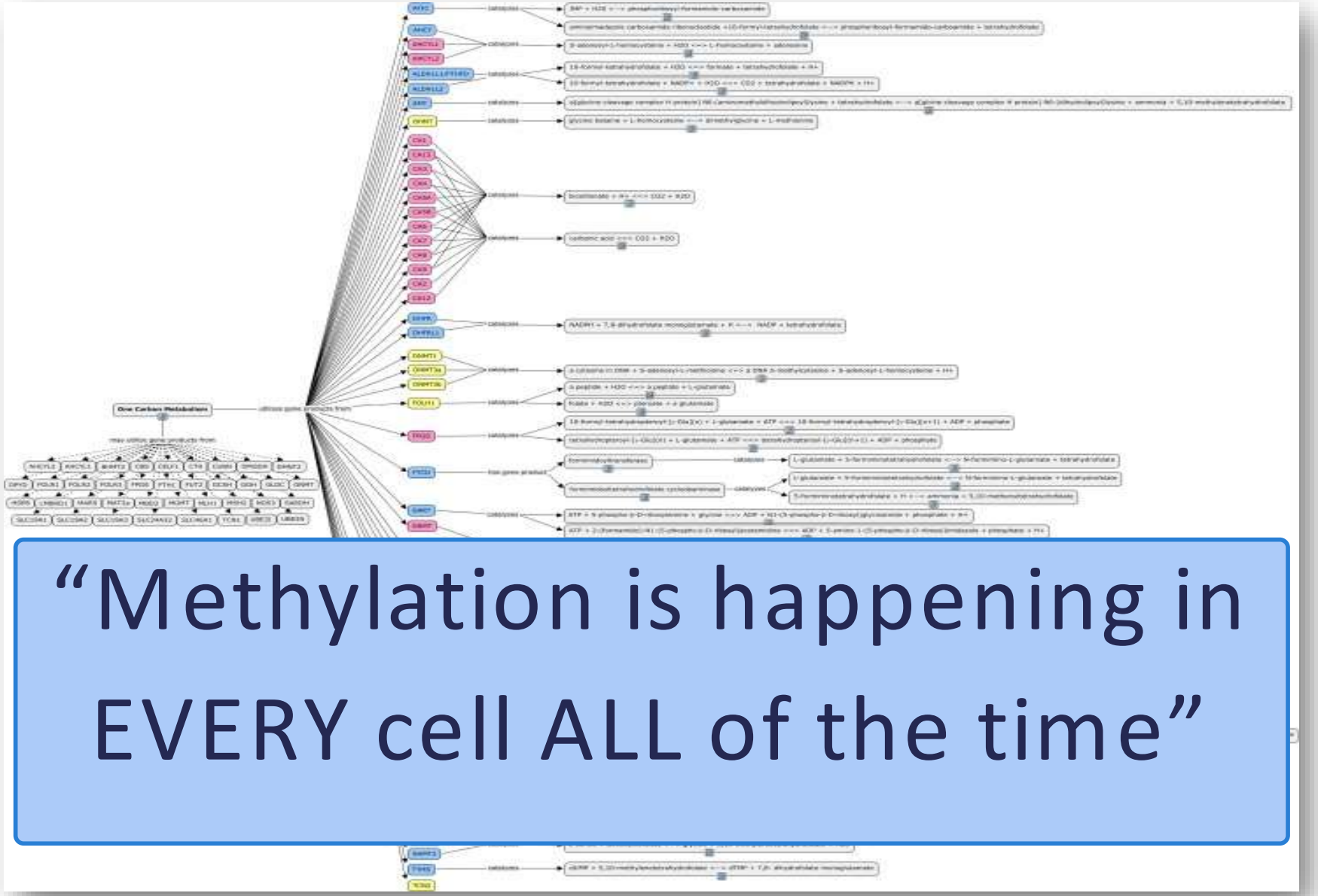



Photo: https://pubs.niaaa.nih.gov/publications/arcr351/6-16.htm.

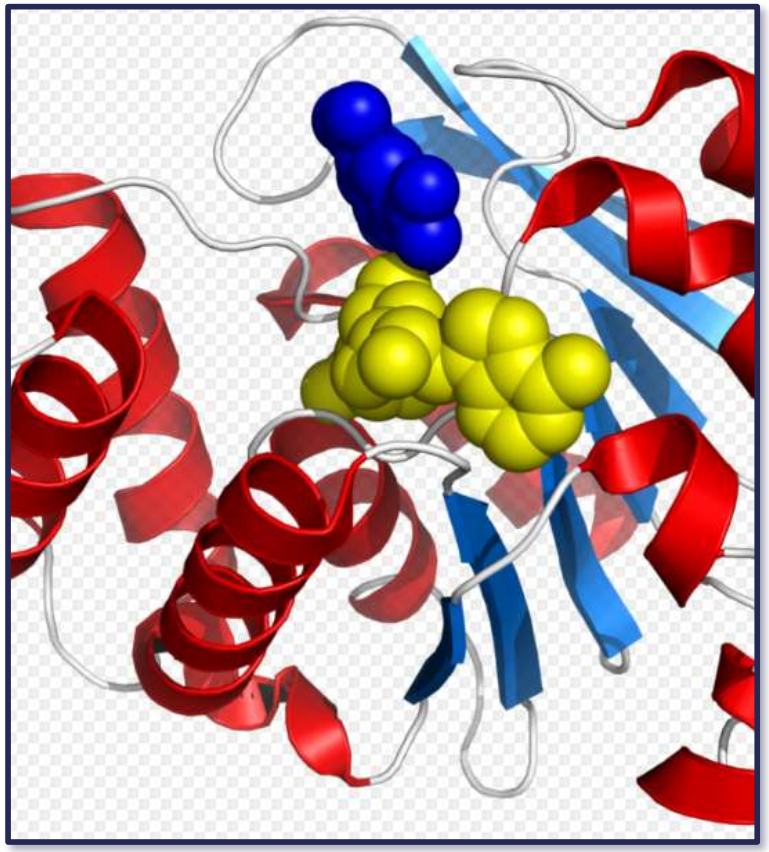



"Methylation is happening in EVERY cell ALL of the time"

Joslin AC, et al. *Genes Nutr.* 2014;9(5);419-36.

# “Two Areas” of Methylation Activity

## Metabolic (e.g. COMT)



## Epigenetic (DNA)

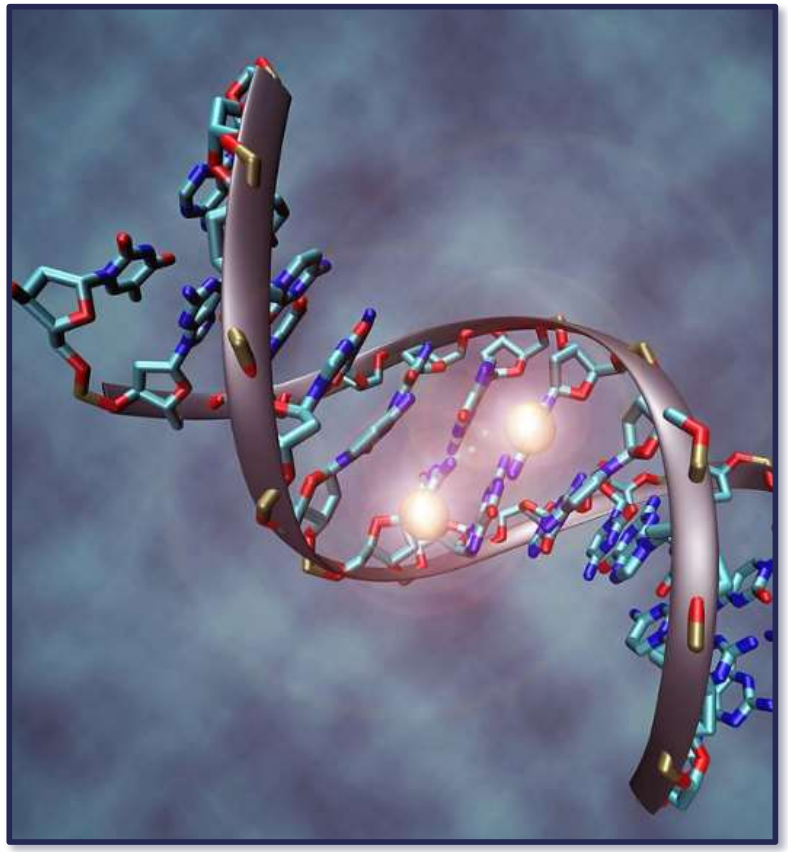


Photo: https://en.wikipedia.org/wiki/Catechol-O-methyltransferase

Photo: https://en.wikipedia.org/wiki/DNA_methylation

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ORIGINAL ARTICLE

Aging Cell WILEY

# Reversal of epigenetic aging and immunosenescent trends in humans

Gregory M. Fahy[1] | Robert T. Brooke[1] | James P. Watson[2] | Zinaida Good[3] | Shreyas S. Vasanawala[4] | Holden Maecker[5] | Michael D. Leipold[5] | David T. S. Lin[6] | Michael S. Kobor[6] | Steve Horvath[7]

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**Present address**
Zinaida Good, Stanford Cancer Institute, Stanford University, Stanford, CA, USA

**Funding information**
Intervene Immune, Inc

**Abstract**

Epigenetic "clocks" can now surpass chronological age in accuracy for estimating biological age. Here, we use four such age estimators to show that epigenetic aging can be reversed in humans. Using a protocol intended to regenerate the thymus, we observed protective immunological changes, improved risk indices for many age-related diseases, and a mean epigenetic age approximately 1.5 years less than baseline after 1 year of treatment (−2.5-year change compared to no treatment at the end of the study). The rate of epigenetic aging reversal relative to chronological age accelerated from −1.6 year/year from 0–9 month to −6.5 year/year from 9–12 month. The GrimAge predictor of human morbidity and mortality showed a 2-year decrease in epigenetic vs. chronological age that persisted six months after discontinuing treatment. This is to our knowledge the first report of an increase, based on an epigenetic age estimator, in predicted human lifespan by means of a currently accessible aging intervention.

KEYWORDS

c-reactive protein, lymphocyte-to-monocyte ratio, naive T cells, PD-1, PSA, thymic regeneration

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Fahy GM, et al. *Aging Cell.* 2019;00:e13028.

# Advancing Our Understanding of the Human Methylome:

Optimising epigenetic expression has the potential to modify disease, health span and lifespan.

Broad, upstream dietary and lifestyle interventions appear essential for optimising epigenetic expression.

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# Genetic Methylation

- Highly regulated – DNA methylation marks very stable, but malleable (continuum)
- DNA methylation favored at embryogenesis
- Methylation at CpG islands by DNMT enzymes (associated with gene repression)
  - Histone methylation (can induce or inhibit expression)
  - RNA methylation
  - Mitochondrial DNA methylation (miDNMT)
  - Demethylation (active and passive)



Bagga S. *BioFiles. 2012;7(3):1-20.*

# DNA Methylation and Demethylation

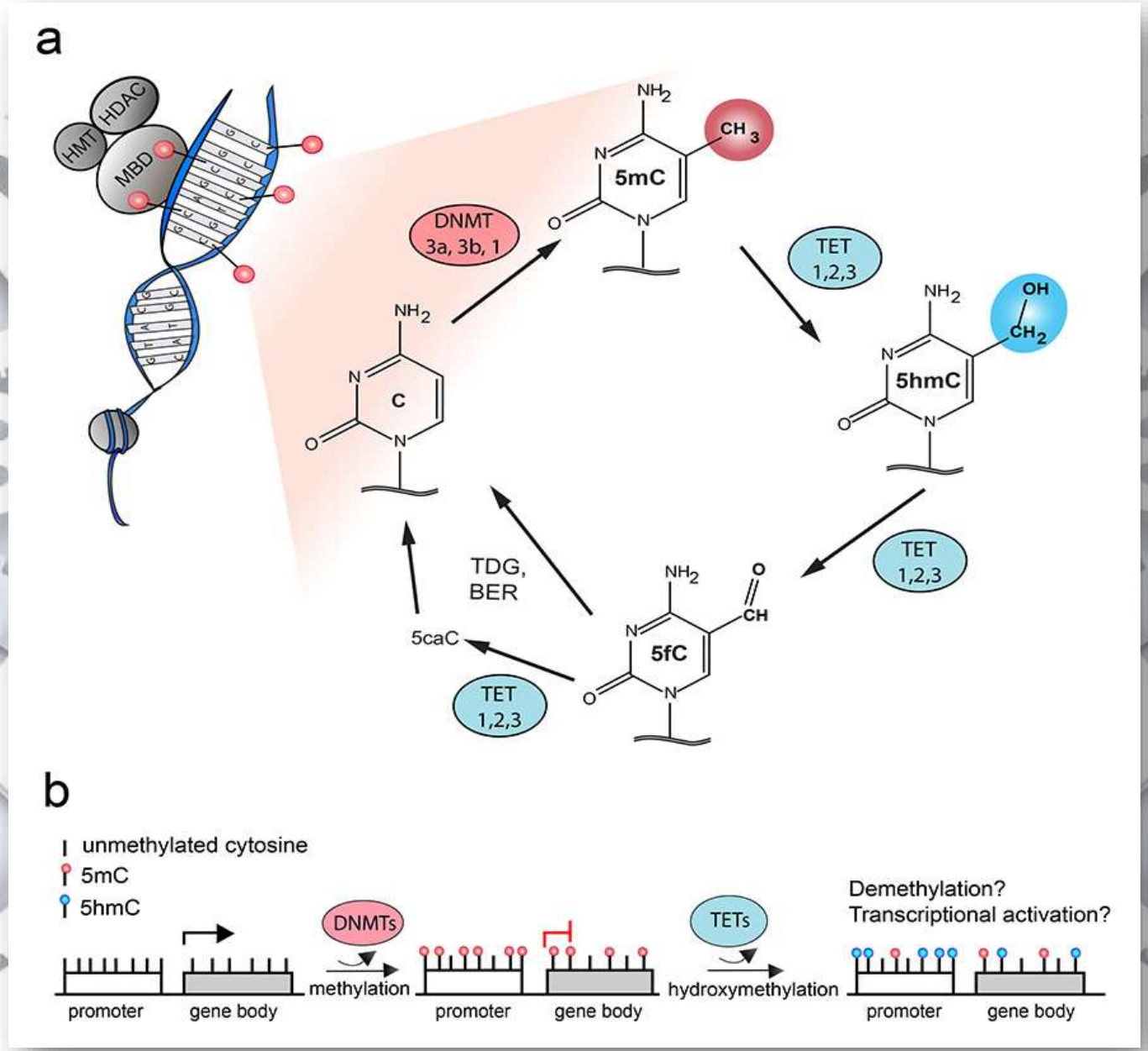
- DNMT1 responsible for maintenance of DNA methylation patterns
- DNMT3A and DNMT3B carry out *de novo* methylation activity.
  - DNMT3 role in genomic imprinting during embryonic development.

Stefanska B, et al. *Br J Pharmacol.* 2012;167(2):279-97.
H Yang, et al. *Oncogene.* 2013;32(5)663-69.
Rampal R, et al. *Cell Reports.* 2014;9(5):1841-55.

# DNA Methylation and Demethylation

- Ten-eleven translocation enzymes 1-3
  - Demethylation- “part of the clean-up crew that sanitizes DNA methylation imprints for the next generation”
  - Oxidizes 5mC to 5hmC
  - Major players in genomic imprinting during embryonic development
  - High quantity in CNS
  - TET2- known tumor suppressor, often inhibited in cancer cells
  - Other players in demethylation: enzymes and ROS

Stefanska B, et al. *Br J Pharmacol.* 2012;167(2):279-97.
H Yang, et al. *Oncogene.* 2013;32(5)663-69.
Rampal R, et al. *Cell Reports.* 2014;9(5):1841-55.




Leoni C, et al. *Swiss Med Wkly.* 2015;145:w14191.

# DNA Methylation Changes as Cause, Mechanism and Consequence of Disease

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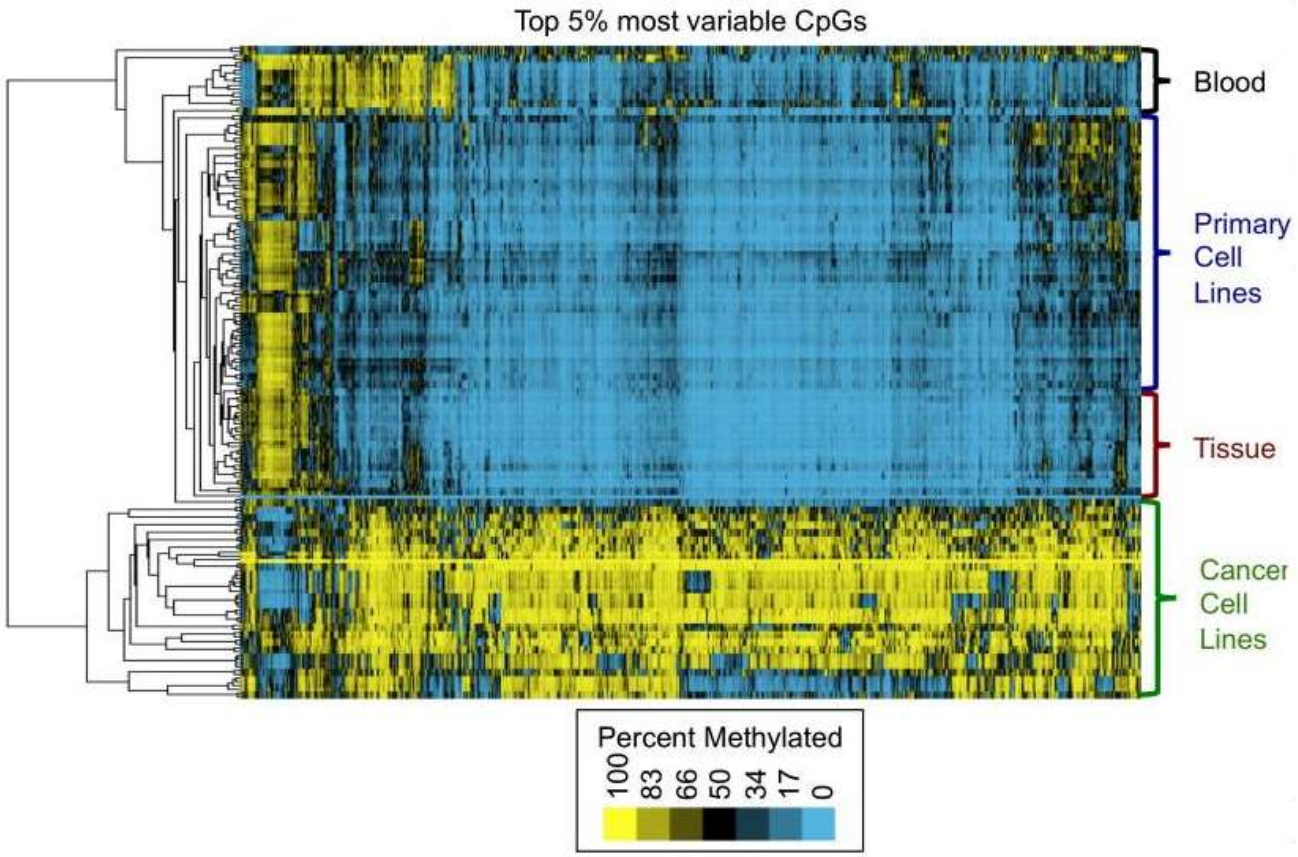
# DNA Methylation and Cancer

| Study | Study Outcomes |
|---|---|
| Mijnes et al., 2018<br>Shalaby et al., 2018<br>Wojtczyk-Miaskowska et al., 2017<br>Takeda et al., 2016<br>Guo et al., 2015<br>Bhagat & Krishnamoorthy, 2014 | Promoter methylation of DNA repair genes inhibits expression in several cancers, including bladder, rectal, endometrial, non-small-cell lung carcinoma (NSCLC) and epithelial ovarian cancer. |
| Karpf (ed.), 2013 | Epigenetic inactivation of BRCA1 via promoter hypermethylation plays an important role in tumorigenesis in a wide array of cancers including breast, ovarian, gastric, bladder, NSCLC (hereditary and sporadic forms). |
| Yang & Park, 2012 | Aberrant promoter methylation (hypo/hyper) of critical pathway genes could be potential biomarkers and therapeutic targets for prostate cancer. Genes involved include androgen receptors (AR), retinoid acid receptor beta (RARβ, tumor suppressor gene), glutathione S tranferase P1 (GSTP1), multidrug resistance 1 (MDR1), methylguanine-methyltransferase (MGMT, DNA repair gene). |

# DNA Hypermethylation and Cancer




- Pooled cancer cell lines from breast, prostate, lung, ovarian, endometrial, liver and pancreatic cancer cells, as well as neuro-blastoma and leukaemias

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Varley KE, et al. *Genome Res.* 2013;23(3):555-67.

# Demethylating Agents are Used In Cancer Therapy

Adv Genet. 2010;70:327-40. doi: 10.1016/B978-0-12-380866-0.60012-5.

ELSEVIER
FULL-TEXT ARTICLE

## DNA demethylating agents and epigenetic therapy of cancer.

Mani S[1], Herceg Z.

Author information

**Abstract**

Epigenetic events have been associated with virtually every step of tumor development and progression, and epigenetic alterations are believed to occur early in tumor development and may precede the malignant process. In contrast to genetic changes, epigenetic alterations arise in a gradual manner, leading to a progressive silencing of specific genes. An important distinction between epigenetic and genetic alterations is intrinsic reversibility of the former, making cancer-associated changes in DNA methylation, histone modifications, and expression of noncoding RNAs attractive targets for therapeutic intervention. This realization has triggered an impressive quest for the development of "epigenetic drugs" and epigenetic therapies. A number of agents have been subjected to an intensive investigation, many of which have been found capable of altering epigenetic states, including DNA methylation patterns and histone modification states. Many of these agents are currently being tested in clinical trials, while several of them are already used in clinics. This review will focus on the recent advances in the development of epigenetic drugs based on the inhibition of DNA methylation. Combinatorial therapies that couple DNA demethylating agents with histone deacetylase inhibitors will also be discussed.



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Mani S, et al. *Adv Genet.* 2010;70:327-40.

# DNA Methylation and Other Diseases

| Study | Study Outcomes |
|---|---|
| Qazi et al., 2017 | Marked loss of DNA methylation takes place in brains affected by **Alzheimer's disease**. Hypermethylation of ApoE4 promoter is a high risk marker associated with late-onset Alzheimer's Disease (LOAD) |
| Demura & Saijoh 2017 | "Increasing evidence from both human and animal studies indicates that DNA methylation exerts a causal impact on the development of **hypertension**." |
| Aberg et al., 2018 | Identified and replicated methylated CpG loci associated with **major depressive disorder** (MDD) that are "involved in biological functions of likely importance to MDD etiology. |
| Dunn et al., 2014 | Disturbed blood flow in carotid artery results in hypermethylation with promoters of 11 mechanosensitive genes.. which alter endothelial gene expression and induce **atherosclerosis**. |

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**Table 2** Epigenetic modifications and corresponding metabolites in autoimmune diseases

| Autoimmune disease | Metabolites | Changes | Corresponding epigenetic modifications | Functions and roles in disease | References |
|---|---|---|---|---|---|
| Systemic lupus erythematosus (SLE) | SAM | Decreased | Decreased methylation of DNA and histone | Inhibits lymphocyte trans-methylation level and T cell activation | [22, 88, 89] |
| | GSH | Decreased | Decreased methylation of DNA and histone | Suppresses methyltransferase activity, inhibits oxidative stress | [22, 93–96] |
| | SAH | Increased | Decreased methylation of DNA and histone | Suppresses methyltransferase activity | [22, 97] |
| | Adenosine | Not determined | Decreased methylation of DNA and histone | Inhibits arachidonic acid, which is an indicator of lupus progression, and regulates immune responses | [98, 99] |
| | Homocysteine | Increased | Decreased methylation of DNA and histone | Regulates T cell activation, differentiation, and cell viability Increased in children with SLE and correlated with renal involvement | [100–102] |
| | α-KG, $Fe^{2+}$ | Decreased | Histone demethylation | Overactive lymphocyte response | [103] |
| | Nitric oxide | Increased | Histone methylation | T cell auto-activation | [105] |
| | Acetyl-CoA | Decreased | Histone acetylation | Lupus disease-related bioenergetic reactions | [109] |
| | $NAD^+$ | Increased | Histone deacetylation | Lupus disease-related bioenergetic reactions | [115] |
| Multiple sclerosis (MS) | SAM | Decreased | Decreased methylation of DNA and histone | Alters methylation levels | [125, 126] |
| | Betaine | Decreased | Histone deacetylation | Neurological pathology | [125] |
| | $NAD^+$ | Increased | Histone deacetylation | Enhances brain volume loss | [131] |
| Type 1 diabetes mellitus (T1DM) | SAM | Decreased | Decreased methylation of DNA and histone | Inhibits T cell responses | [90, 139] |
| Rheumatoid arthritis (RA) | Polyamine | Increased | DNA hypomethylation | Reduces SAM | [166] |
| | SAM | Decreased | Decreased methylation of DNA and histone | Inhibits T cell responses? | [127, 166] |



Wang Z, et al. *Cell Mol Life Sci.* 2018;75(18):3353-369.

# Establishing Non-Genetic Transgenerational & Fetal Origins of Disease

| Study Populations | Study Outcomes | Evidence Offered |
|---|---|---|
| **Överkalix cohort** 1890-1995 (Kaati et al., 2002; Bygren et al., 2014) | Grandparent food supply during prepubertal 'slow growth period' associated with first & second generation disease/mortality. Low food availability correlated with low CVD mortality. Surfeit of food correlated with increased diabetes mortality. | Transgenerational environmental (non-genetic) origins of health and disease |
| **Dutch Hunger Winter** of 1944-45 (Schulz, 2010) | Maternal exposure to famine during gestation associated with later disease including obesity, CVD | Foetal environmental (non-genetic) origins of health and disease |
| **Chinese famine** of 1959-61 (Wang et al., 2016; Xin et al., 2018) | Maternal exposure to famine during gestation associated with later disease including dyslipidemia and cognitive impairment | Foetal environmental (non-genetic) origins of health and disease |
| **Rural Gambia** (Waterland, et al, ) | Season of conception (associated with marked dietary differences) in rural Gambia affects DNA methylation at metastable epialleles. | Foetal environmental (non-genetic) origins of health and disease |

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# DNA Methylation and Other Epigenetic Imprints as Mechanism of Inheritance – Human Studies

| Year/Author | Study Outcomes | Evidence Offered |
|---|---|---|
| **Project Ice Storm** 1998 – 2011<br>(Cao-Lei et al., 2014) | Prenatal maternal **objective** hardship correlated with DNA methylation changes in offspring | Human transgenerational origins of altered DNA methylation. |
| 2018 Sun et al.<br>(**Human and animal study**) | Retrospective human study showed **paternal** pre-conception **cold temperature exposure** increased brown adipose tissue formation in offspring. Supporting mouse study showed associated changes in DNA methylome | Human transgenerational environmental origin of physiological changes, likely via DNA methylation including on *paternal* side |

# Formation of SAMe - Universal Methyl Donor

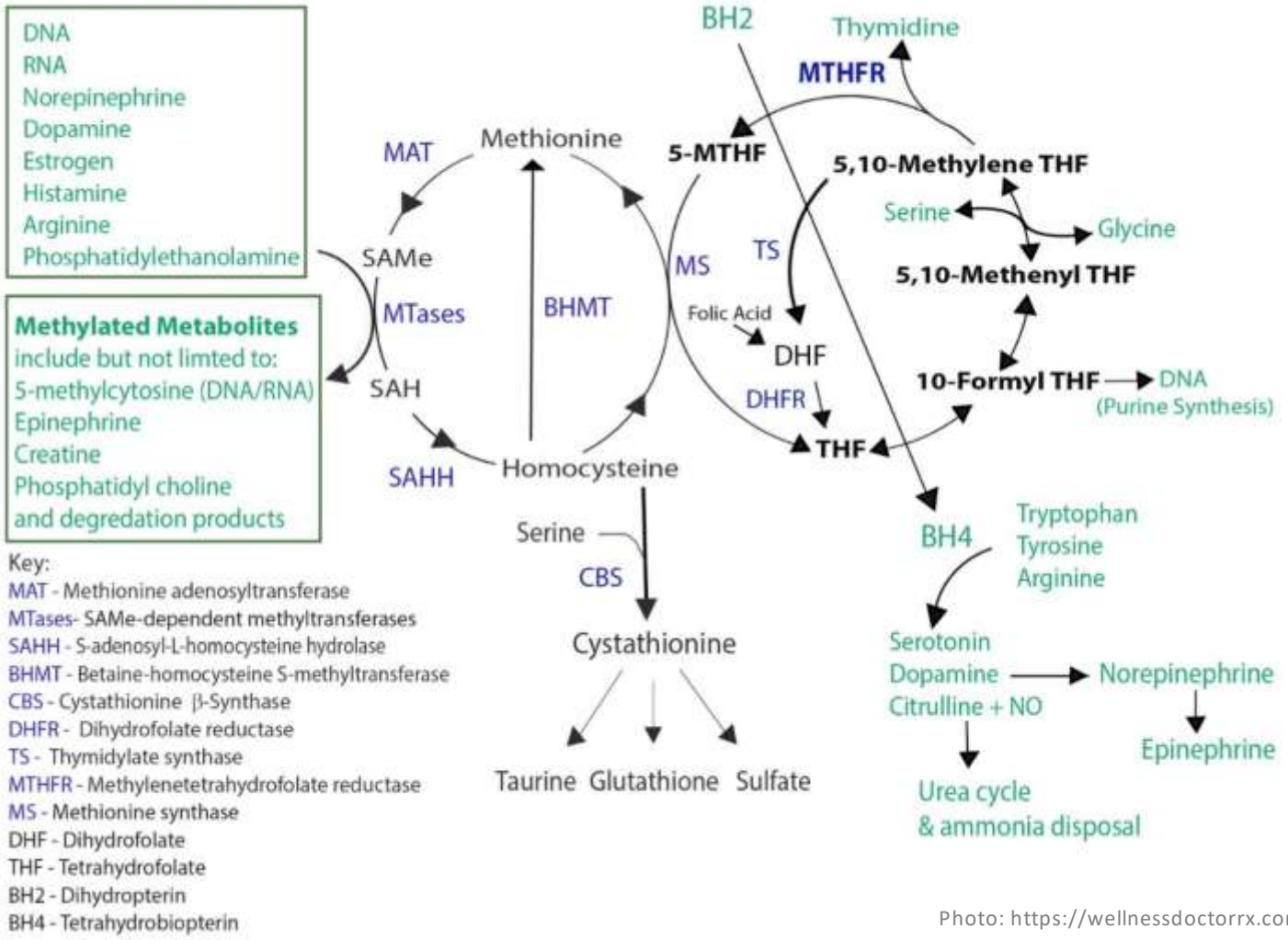
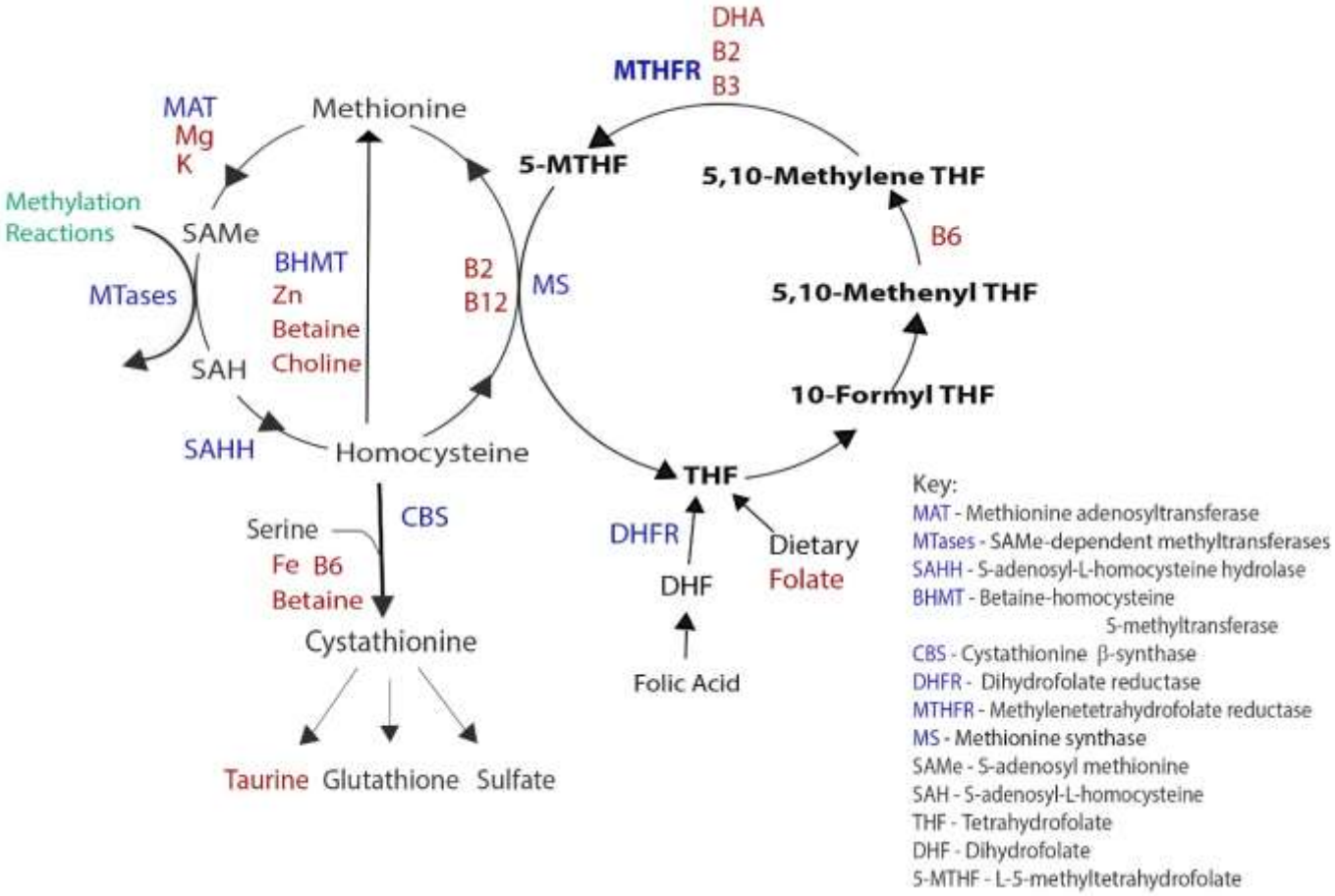



Photo: https://wellnessdoctorrx.com/introduction-to-dna-methylation-status-activity/.

# Formation of SAMe: Nutrient Cofactors and Allosteric Activators

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- Choline ▲
- Betaine ▲
- DMG ▲
- Sarcosine ▲

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| V158M | −+ | **BHMT** | |
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| A66G | ++ | **GNMT** | |
| **MAT1A** | | C1289T | −+ |
| D18777A | −+ | | |
| **SHMT1** | | | |
| C1240T | −+ | | |

### Transsulfuration

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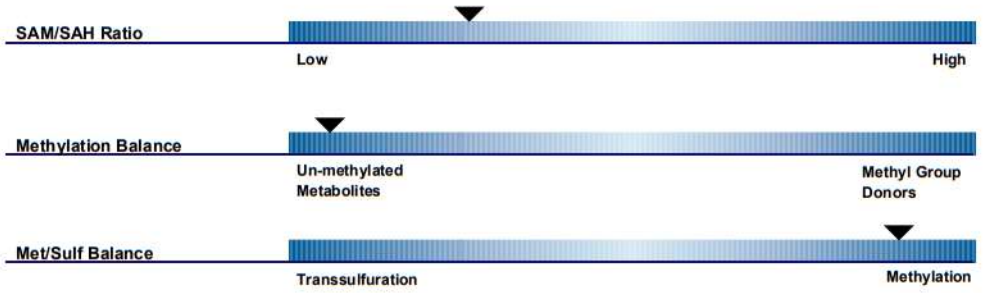
- Cystathionine ▲
- Cysteine ▲

## Methylation Status





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**3534 Methylation Panel** - Plasma & Whole Blood

*Methodology: LCMSMS & Colorimetric*

| | | | QUINTILE DISTRIBUTION (1st / 2nd / 3rd / 4th / 5th) | Reference Range |
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| **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.*





GENOVA DIAGNOSTICS

# Changing DNA Methylation Status in Offspring Using Maternal Diet – ANIMAL Data

| Study | Outcome | Evidence Offered |
|---|---|---|
| 2003 Waterland & Jirtle | Agouti mouse study used maternal **FA, B12, choline, betaine** to shut down Agouti gene expression in offspring via DNA methylation | Supplemental maternal **methylation cofactor nutrients** alter gene expression in offspring |
| 2006 Dolinoy et al. | Agouti mouse maternal **genistein supplementation** induces DNA hypermethylation that silences Agouti gene in offspring | Foetal exposure to non-methyl donor ('**adaptogen**') has lasting epigenetic effects |
| 2007 Dolinoy et al. | Agouti mouse maternal **methyl donors (FA, B12, choline, betaine)** or **genistein supplementation** reverses BPA-driven hypomethylation of Agouti gene | Fetal exposure to methyl donors and non-methyl donor ('**adaptogen**') prevents negative epigenetic effects of toxin |
| 2018 Yang et al. | Maternal **betaine** supplementation induced DNA methylation changes and altered IGF2 gene expression in offspring (rats) | Supplemental maternal **methylation cofactor nutrients** alter gene expression in offspring |

## These Two Mice are Genetically Identical and the Same Age

**While pregnant, both of their mothers were fed Bisphenol A (BPA) but DIFFERENT DIETS:**

The mother of this mouse received a **normal mouse diet**

The mother of this mouse received a diet **supplemented** with choline, folic acid, betaine and vitamin B12

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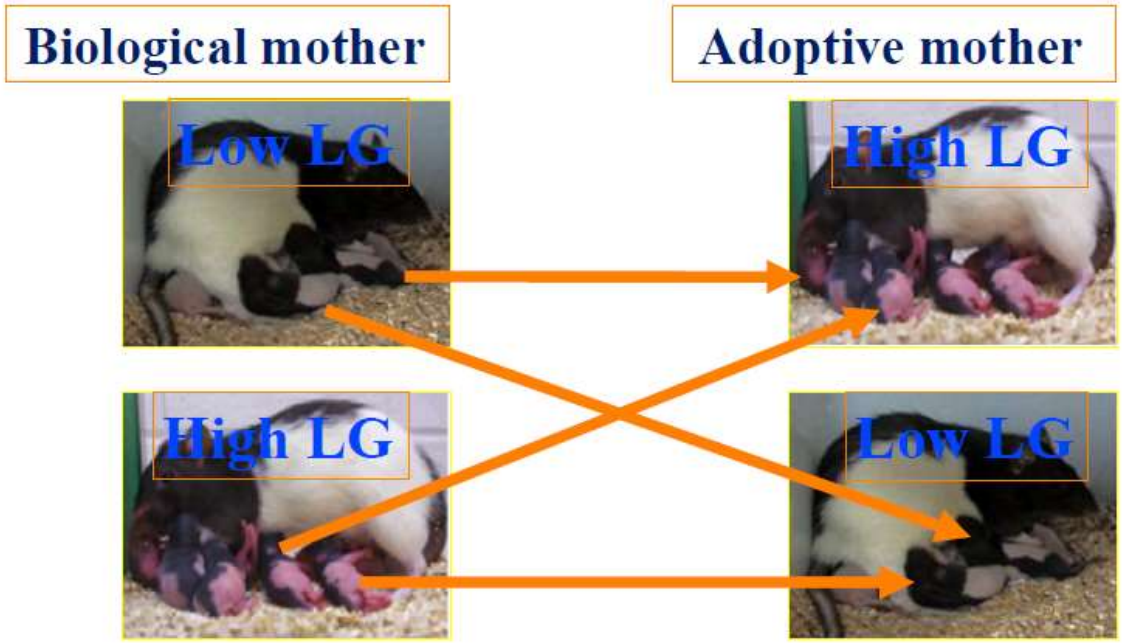
Photo curtesy Randy L. Jirtle, PhDhttps://learn.genetics.utah.edu/content/epigenetics/nutrition/.

# Behavioral Programming (Dr. Szyf)

Behavioral programming by the mother is epigenetic not genetic;
The fostering mother and not the biological mothers transfers the phenotype




Cross-fostering /adoption experiment



Szyf M, et al. *Front Neuroendocrinol.* 2005;26(3-4):139-62.

# Maternal Epigenome

- Tactile contact between mother and infant associated with altered epigenetic signatures in children even 4-5 years later
- For low contact group, significant, negative association between infant distress and older child epigenetic age deviation

Moore SR, et al. *Dev Psychopathol.* 2017;29(5):1517-538.
Horvath S. *Genome Biol.* 2013;14(10):R115.

GENOVA DIAGNOSTICS

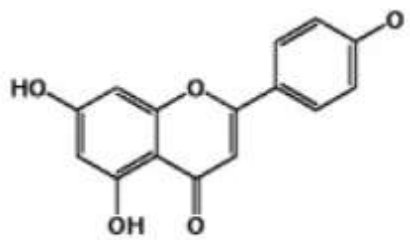
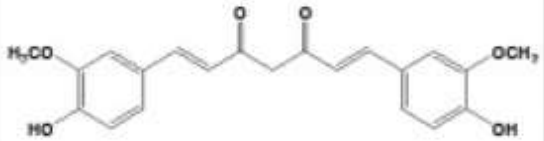
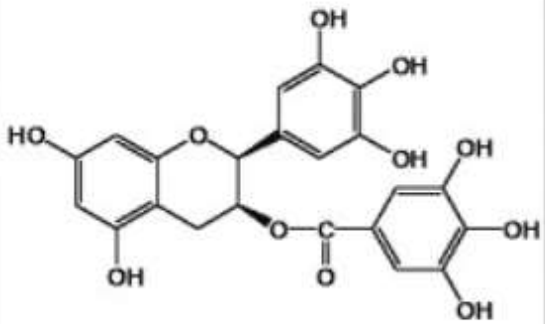
# Plasticity of DNA Methylation
# At Other Life Stages – HUMAN Data

| Study | Outcome | Evidence Offered |
|---|---|---|
| 2003 Ingrosso et al. | Human, controlled study of males with hyperhomocysteinemia used **folate** to restore global DNA methylation to that of healthy controls | Supplemental **methylation cofactor nutrients** alter gene expression outside of foetal developmental window |
| 2012 Tyrka et al. | **Human childhood adverse experiences** associate with epigenetic changes on glucocorticoid receptor gene | Early life **psychological stress experiences** alter gene expression which persists into adulthood |
| 2017 Arpón et al. | Data subset from PREDIMED-Navarra clinical trial showed that intervention with **Mediterranean Diet + nuts, and separately + extra-virgin olive oil** resulted in methylation changes in several genes relating to insulin sensitivity, energy regulation, CVD risk | Supplementation with non-methyl donor (‘**adaptogen**’) alters gene expression outside of fetal developmental window |
| 2017 Karimi et al. | 1.7g **DHA** + 0.6 g **EPA** for 6 months resulted in global DNA hypomethylation in peripheral blood leukocytes of Alzheimer’s disease patients | Supplementation with non-methyl donor (‘**adaptogen**’) alters gene expression outside of fetal developmental window |

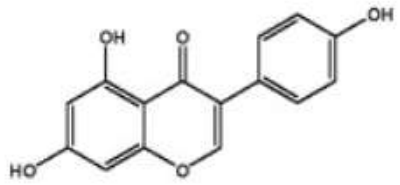
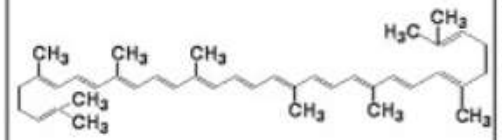
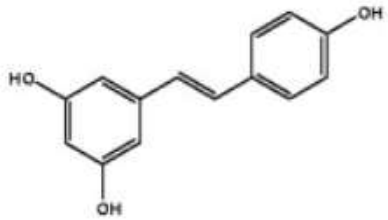
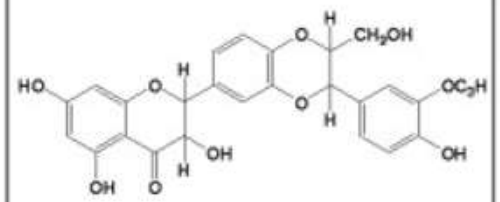
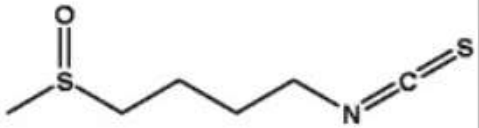
GENOVA DIAGNOSTICS

# Plasticity of DNA Methylation
# At Other Life Stages – HUMAN Data

| Study | Outcome | Evidence Offered |
|---|---|---|
| 2017 Bilir et al. | 30 mg/d **genistein** for 3-6 weeks resulted in differentially methylated sites and expressed genes compared with placebo | Supplementation with non-methyl donor (‘**adaptogen**’) alters gene expression outside of foetal developmental window |
| 2017 Shorey-Kendrick et al. | Maternal smoker supplementation of 500 mg/d **vitamin C** improved offspring pulmonary function at age 1 yr and normalized 69.03% of maternal CpGs with at the most methylation difference between placebo and nonsmoker groups | Supplementation with non-methyl donor (‘**adaptogen**’) alters gene expression outside of foetal developmental window |

| Dietary agents | Structure | Epigenetic effect on Cancer* | Picture of the sources |
|---|---|---|---|
| Apigenin (Parsley)[1] (*Petroselinum*)[2] |  | DNMT inhibitor *(Fang M et al 2007)* |  |
| Allyl Mercaptan (Garlic)[1] (*Allium sativum*)[2] |  | HDAC inhibitor *(Lea et al 2001; Druesne et al 2004)* |  |
| Curcumin (Turmeric)[1] (*Curcuma longa*)[2] |  | DNMT inhibitor *(Liu et al 2009; Fang et al 2007; Fu and Kurzrock 2010)*<br><br>HDAC and HAT inhibitor *(Chen et al 2007; Liu et al 2005; Kang et al 2006; Cui et al 2007; Balasubramanyam et al 2004)* |  |
| Epigallocatechin-3-gallate (EGCG) (Green tea)[1] (*Camellia sinensis*)[2] |  | DNMT inhibitor *(Fang et al 2003; Kato et al 2008; Pandey et al 2010; Lee et al 2005)*<br><br>HAT inhibitor *(Choi et al 2009)* |  |

Meeran et al. *Clin Epigenetics.* 2010;1(3-4):101-116.

| | | | |
|---|---|---|---|
| Genistein (Soybean)[1] (*Glycine max*)[2] |  | DNMT inhibitor (*Majid et al 2008; Kikuno et al 2008; Fang et al 2005; Li et al 2009*)<br><br>HDAC inhibitor and HAT activator (*Fang et al 2005; Li et al 2009; King-Batoon et al 2008; Majid et al 2008*) |  |
| Lycopene (Tomatoes)[1] (*Solanum lycopersicum*)[2] |  | Demethylates the *GSTP1, RARβ2 and HIN-1* genes in breast cancer cells (MDA-MB-231 and MCF10A)<br><br>(*King-Batoon et al 2008*) |  |
| Resveratrol (Red grapes)[1] (*Vitis vinifera*)[2] |  | DNMT inhibitor (*Papoutsis et al 2010; Stefanska et al 2010*)<br><br>SIRT1 activator (*Kaeberlein et al 2008; Wang et al 2008; Boily et al 2009*) |  |
| Silymarin (Milk thistle)[1] (*Silybum marianum L.*)[2] |  | SIRT1 activator (*Li et al 2007*) |  |
| Sulforaphane (Cruciferous vegetables)[1] (*Brassicaceae*)[2] |  | DNMT inhibitor (*Meeran et al 2010; Traka et al 2005*)<br><br>HDAC inhibitor (*Myzak et al 2007; Dashwood and Ho 2007; Ho et al 2009; Myzak et al 2004*) |  |

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Meeran et al. *Clin Epigenetics.* 2010;1(3-4):101-116.

# Epigenetic Modifications of Nrf2 by 3,3′-diindolylmethane *In Vitro* in TRAMP C1 Cell Line and *In Vivo* TRAMP Prostate Tumors

Tien-Yuan Wu,[1] Tin Oo Khor,[1] Zheng-Yuan Su,[1] Constance Lay Lay Saw,[1] Limin Shu,[1] Ka Lung Cheung,[1] Ying Huang,[1] Siwang Yu,[2] and Ah-Ng Tony Kong[1,3]

*Received 20 January 2013; accepted 17 April 2013*

***Abstract.*** 3,3′-diindolylmethane (DIM) is currently including prostate, breast, and cervical cancers and h in several *in vivo* and *in vitro* models. Previously, chemopreventive effects in prostate carcinogenesis mechanism is unclear. The present study aims to inv modulation of DIM in TRAMP-C1 cells and in TR TRAMP-C1 cells showed that DIM suppressed DNM status of Nrf2 resulting in enhanced expression of Nrf TRAMP mice fed with DIM-supplemented diet showe metastasis than the untreated control group simila increased apoptosis, decreased cell proliferation and expression in prostate tissues. Importantly, immun reduced the global CpG 5-methylcytosine methylat chemopreventive target gene *Nrf2*, bisulfite genomic sequencing showed that DIM decreased the methylation status of the first five CpGs of the Nrf2 promoter region, corroborating with the results of *in vitro* TRAMP-C1 cells. In summary, our current study shows that DIM is a potent cancer chemopreventive agent for prostate cancer and epigenetic modifications of the CpG including Nrf2 could be a potential mechanism by which DIM exerts its chemopreventive effects.

**KEY WORDS:** 3,3′-diindolylmethane (DIM); epigenetic; methylation; Nrf2; prostate cancer.

- In vitro study utilizing TRAMP-C1 cells showed that **DIM suppressed DNMT expression and reversed CpG methylation status of Nrf2 resulting in enhanced expression of Nrf2** and Nrf2-target gene NQO1
- **In vivo study, TRAMP mice fed with DIM-supplemented diet showed much lower incidence of tumorigenesis and metastasis than the untreated control group** similar to what was reported previously

GENOVA DIAGNOSTICS

Wu TY, et al . *AAPS J.* 2013;15(3):864-74.

*Curr Pharmacol Rep*. 2015 April 1; 1(2): 112–120. doi:10.1007/s40495-014-0004-8.

# DNA Methylation and Flavonoids in Genitourinary Cancers

**Neelam Mukherjee**[1], **Addanki P Kumar**[1,2,3,4], and **Rita Ghosh**[1,2,3,*]

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[2]Department of Pharmacology, School of Medicine, University of Texas Health Science Center at San Antonio

[3]Department of Molecular Medicine, School of Medicine, University of Texas Health Science Center at San Antonio

[4]South Texas Veterans Health Care System, San Antonio, TX 78229

## Abstract

Malignancies of the genitourinary system have some of the highest cancer incidence and mortality rates. For example prostate cancer is the second most common cancer in men and ovarian cancer mortality and incidence are near equal. In addition to genetic changes modulation of the epigenome is critical to cancer development and progression. In this regard epigenetic changes in DNA methylation state and DNA hypermethylation in particular has garnered a great deal of attention. While hypomethylation occurs mostly in repeated sequence such as tandem and interspersed repeats and segment duplications, hypermethylation is associated with CpG islands. Hypomethylation leads to activation of cancer-causing genes with global DNA hypomethylation being commonly associated with metastatic disease. Hypermethylation-mediated silencing of tumor suppressive genes is commonly associated with cancer development. Bioactive phytochemicals such as flavonoids present in fruits, vegetables, beverages etc. have the ability to modulate DNA methylation status and are therefore very valuable agents for cancer prevention. In this review we discuss several commonly methylated genes and flavonoids used to modulate DNA methylation in the prevention of genitourinary cancers.

# Additional Environmental Factors with Effects on the DNA Methylome

| Study | Outcome | Evidence Offered |
|---|---|---|
| Hodge et al. 2005 (*In vitro* study) | IL-6 regulates activity of DNMTs, mRNAs and histone methyltransferases, *in vitro* | Mediators of **inflammation** operate via epigenetic mechanisms |
| Hou et al. 2012 (*In vivo* and *in vitro* study) | Compounds show *in vitro* and *in vivo* to induce DNA methylation and miRNA changes that are associated with human diseases including CVD, diabetes, cancer: arsenic, nickel, cadmium, chromium, aluminum, mercury, lead, pesticides, air pollution, benzene, BPA, dioxin, RDX, DES | **Environmental toxins** may increase risk for chronic disease via epigenetic mechanisms |
| Ren et al., 2012 (Human study, 500 women) | **Long-term tai-chi** practice (1+ hours per week, 3+ years) associated with slowing of age-related DNA methylation losses, of between 5-70% compared with controls. Most beneficial from ages 50-55+ | **Long-term, regular exercise** may preserve age-related global DNA methylation losses |

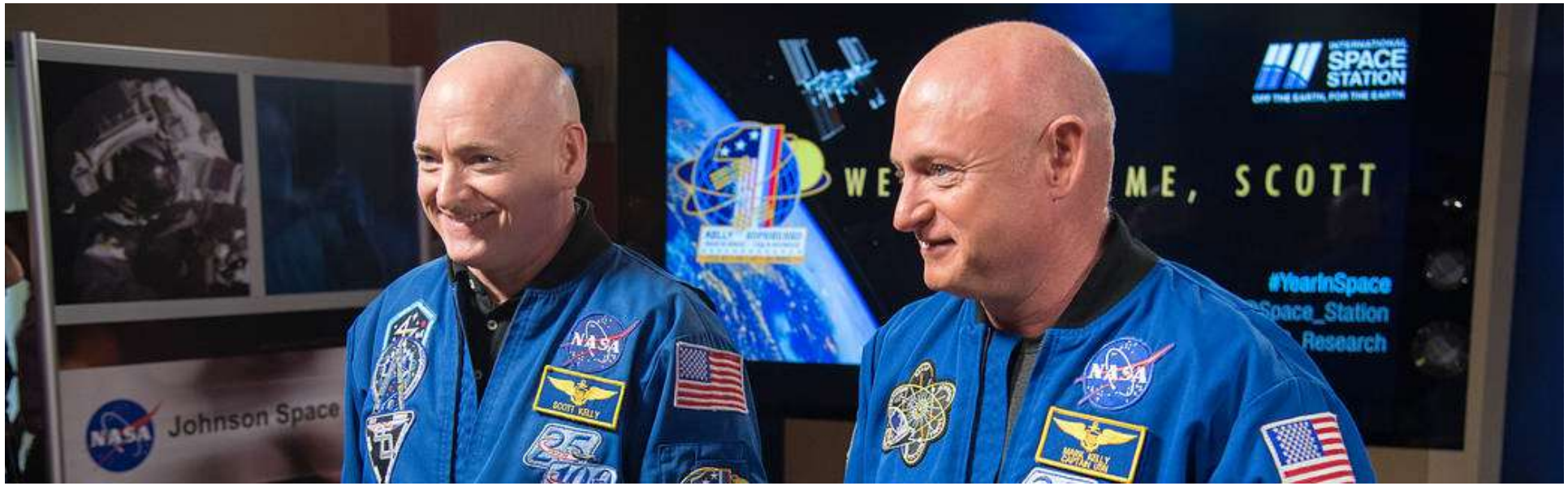
# Additional Environmental Factors with Effects on the DNA Methylome

| Study | Outcome | Evidence Offered |
|---|---|---|
| White et al., 2013 (Human study, 647 women) | Regular **exercise** on or above the median throughout a lifetime acts to preserve age-related depletion of global methylation status | **Regular exercise over a lifetime** may preserve age-related global DNA methylation losses |
| Neuman et al., 2013 (Rodent study) | **Exercise** prevents folate deficiency-induced hyperhomocysteinemia at least in part via increased BHMT expression (two-fold) in kidney | **Exercise** alters expression of genes involved in SAMe recycling pathway |
| Massart et al. 2014 (Rodent study) | Acute **sleep deprivation** altered cortical genome-wide distribution of DNA methylation and hydroxymethylation, especially in pathways related to neuritogenesis, synaptic plasticity, cytoskeleton, signaling and neurotransmission | **Sleep status** affects epigenetic imprinting |
| De Souza e Silva, 2014 (Systematic review, human studies) | Daily **physical activity** is consistently associated with lower plasma homocysteine levels in a dose-dependent manner | **Exercise** affects levels of intermediates in methylation pathways |

# Additional Environmental Factors with Effects on the DNA Methylome

| Study | Outcome | Evidence Offered |
|---|---|---|
| Lindholm et al., 2014 (Human study, 23 M/F) | Single-leg **training** (cycling) for 45 minutes, 4 times per week, for 3 mo resulted in >5000 myocyte DNA methylation changes in genes related to energy metabolism, insulin response and inflammation in active leg but not in control (other leg) | **Regular, moderate-intensity exercise** produces localized DNA methylation changes in muscle cells (tissue specific) |
| Rowlands et al. 2014 (Human study, 17 M/F) | 16 weeks of endurance or resistance **training** in obese adults led to multi-omic changes observed including miRNA, proteome, methylome, transcriptome. | Short-term aerobic **exercise programs** alter DNA methylation |
| Cedernaes et al., 2015 (Human study, 15 M) | Acute **sleep** deprivation (1 night) increased methylation in specific genes in adipose and skeletal muscle core clock genes. | Rapid epigenetic changes can occur due to **acute sleep deprivation**. |

# NASA Twins Study




**NASA Twins Study "NASA twins no longer identical after space flight alters DNA" (Independent)**

- Scott Kelly spent 1 year living on the International Space Station. His identical twin brother, Mark, remained on Earth as control subject
- Epigenetic changes were observed during spaceflight that returned to baseline upon return
- Preflight folate was low, consumption increased with space food system choices
- Scotts telomeres increased significantly while in space. They then shortened within about 48 hours of landing and stabilized at near preflight levels. Mark's remained relatively stable

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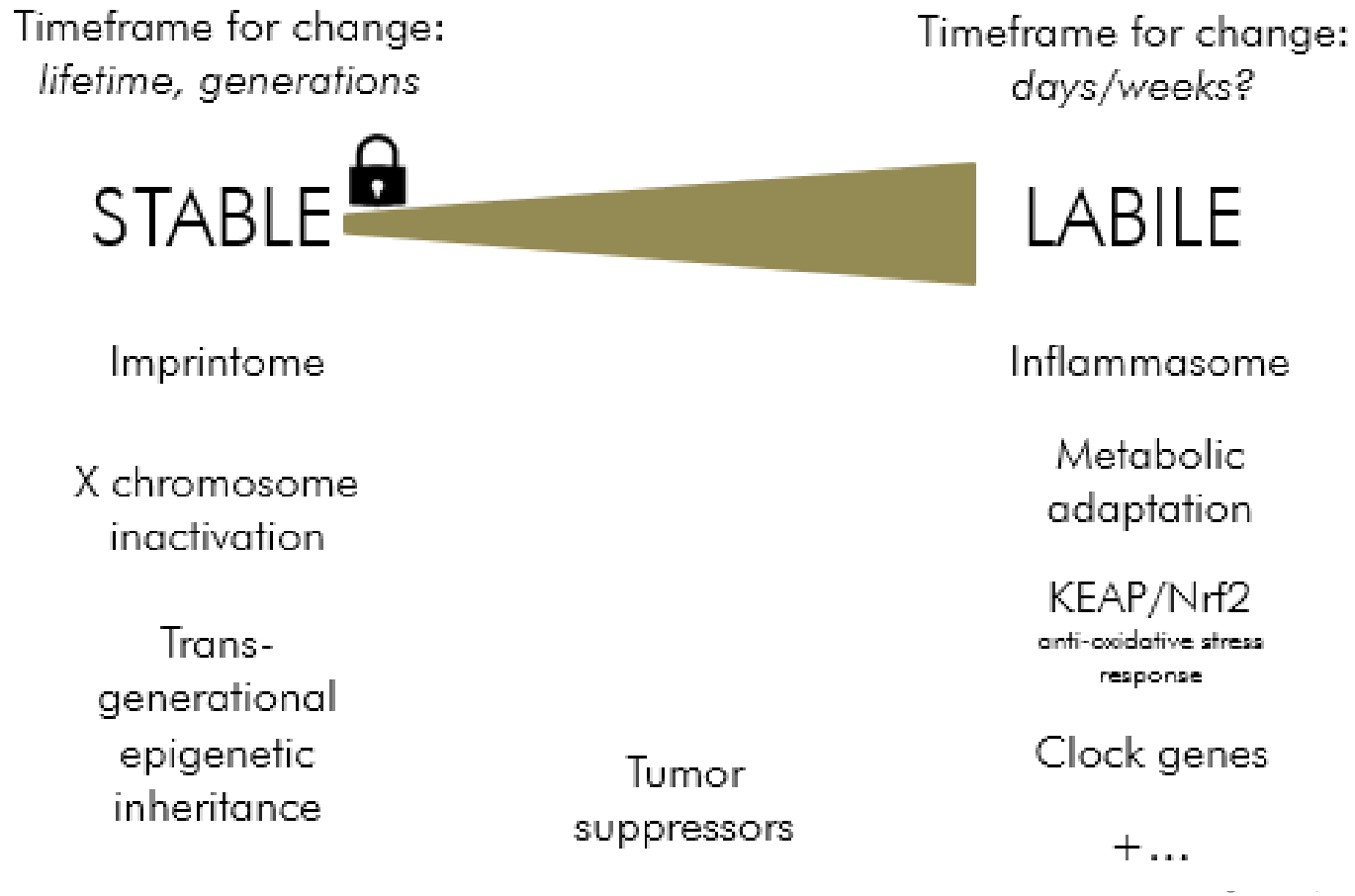
https://www.nasa.gov/feature/nasa-twins-study-investigators-to-release-integrated-paper-in-2018.

# Variability in Epigenome Lability




*Clin Epigenetics.* 2018;10(1):99-109.
Horsburgh S, et al. *Exerc Immunol Rev*. 2015;21:26–41.
Kim H, et al. *J Nutr Biochem*. 2016;33:54–62.
Phillips T et al. *Nature Education.* 2008;1(1):117.
Rowlands DS, et al. *Physiol Genomics*. 2014;46(20):747-65.

GENOVA DIAGNOSTICS

# Continuum of Methylation of the Agouti Gene

B


Dolinoy DC. *Nutr Rev.* 2008;66(1):S7-11.

Al-Moghrabi *et al. Clinical Epigenetics* (2018) 10:99
https://doi.org/10.1186/s13148-018-0529-5

Clinical Epigenetics

RESEARCH Open Access

# Methylation of *BRCA1* and *MGMT* genes in white blood cells are transmitted from mothers to daughters

Nisreen Al-Moghrabi[1*], Maram Al-Showimi[2], Nujoud Al-Yousef[1], Bushra Al-Shahrani[2], Bedri Karakas[1], Lamyaa Alghofaili[3], Hannah Almubarak[1], Safia Madkhali[4] and Hind Al Humaidan[5]

**Abstract**

**Background:** Constitutive methylation of tumor suppressor genes are associated with increased cancer risk. However, to date, the question of epimutational transmission of these genes remains unresolved. Here, we studied the potential transmission of *BRCA1* and *MGMT* promoter methylations in mother-newborn pairs.

**Methods:** A total of 1014 female subjects (cancer-free women, $n = 268$; delivering women, $n = 295$; newborn females, $n = 302$; breast cancer patients, $n = 67$; ovarian cancer patients, $n = 82$) were screened for methylation status in white blood cells (WBC) using methylation-specific PCR and bisulfite pyrosequencing assays. In addition, *BRCA1* gene expression levels were analyzed by quantitative real-time PCR.

**Results:** We found similar methylation frequencies in newborn and adults for both *BRCA1* (9.9 and 9.3%) and *MGMT* (12.3 and 13.1%). Of the 290 mother-newborn pairs analyzed for promoter methylation, 20 mothers were found to be positive for *BRCA1* and 29 for *MGMT*. Four mother-newborn pairs were positive for methylated *BRCA1* (20%) and nine pairs were positive for methylated *MGMT* (31%). Intriguingly, the delivering women had 26% lower *BRCA1* and *MGMT* methylation frequencies than those of the cancer-free female subjects. *BRCA1* was downregulated in both cancer-free woman carriers and breast cancer patients but not in newborn carriers. There was a statistically significant association between the *MGMT* promoter methylation and late-onset breast cancers.

**Conclusions:** Our study demonstrates that *BRCA1*and *MGMT* epimutations are present from the early life of the carriers. We show the transmission of *BRCA1* and *MGMT* epimutations from mother to daughter. Our data also point at the possible demethylation of *BRCA1*and *MGMT* during pregnancy.

**Keywords:** *BRCA1*, *MGMT*, Methylation, Transmission, Blood, Breast cancer, Ovarian cancer

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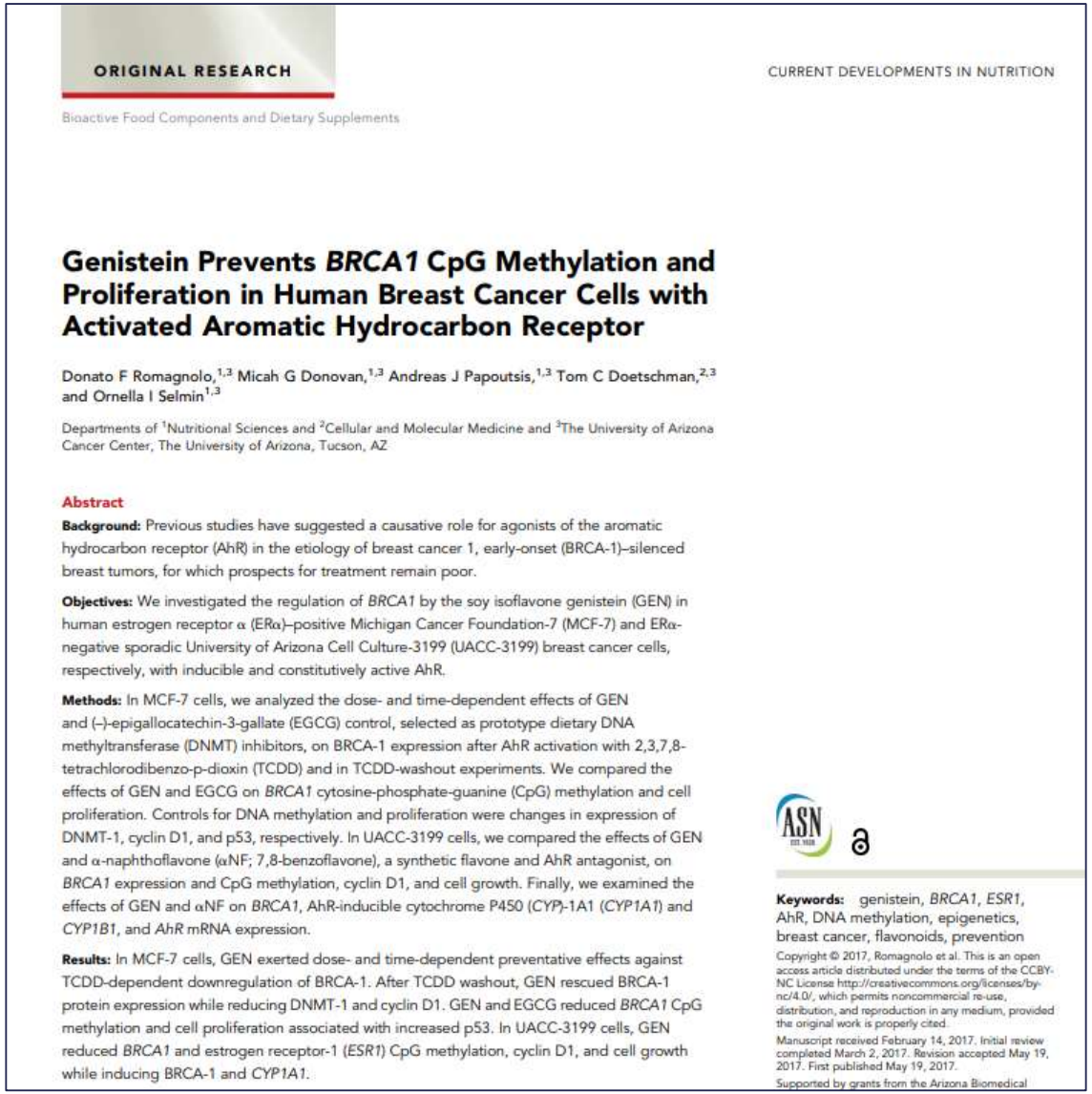

ORIGINAL RESEARCH

CURRENT DEVELOPMENTS IN NUTRITION

Bioactive Food Components and Dietary Supplements

# Genistein Prevents *BRCA1* CpG Methylation and Proliferation in Human Breast Cancer Cells with Activated Aromatic Hydrocarbon Receptor

Donato F Romagnolo,[1,3] Micah G Donovan,[1,3] Andreas J Papoutsis,[1,3] Tom C Doetschman,[2,3] and Ornella I Selmin[1,3]

Departments of [1]Nutritional Sciences and [2]Cellular and Molecular Medicine and [3]The University of Arizona Cancer Center, The University of Arizona, Tucson, AZ

## Abstract

**Background:** Previous studies have suggested a causative role for agonists of the aromatic hydrocarbon receptor (AhR) in the etiology of breast cancer 1, early-onset (BRCA-1)–silenced breast tumors, for which prospects for treatment remain poor.

**Objectives:** We investigated the regulation of *BRCA1* by the soy isoflavone genistein (GEN) in human estrogen receptor α (ERα)–positive Michigan Cancer Foundation-7 (MCF-7) and ERα-negative sporadic University of Arizona Cell Culture-3199 (UACC-3199) breast cancer cells, respectively, with inducible and constitutively active AhR.

**Methods:** In MCF-7 cells, we analyzed the dose- and time-dependent effects of GEN and (–)-epigallocatechin-3-gallate (EGCG) control, selected as prototype dietary DNA methyltransferase (DNMT) inhibitors, on BRCA-1 expression after AhR activation with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and in TCDD-washout experiments. We compared the effects of GEN and EGCG on *BRCA1* cytosine-phosphate-guanine (CpG) methylation and cell proliferation. Controls for DNA methylation and proliferation were changes in expression of DNMT-1, cyclin D1, and p53, respectively. In UACC-3199 cells, we compared the effects of GEN and α-naphthoflavone (αNF; 7,8-benzoflavone), a synthetic flavone and AhR antagonist, on *BRCA1* expression and CpG methylation, cyclin D1, and cell growth. Finally, we examined the effects of GEN and αNF on *BRCA1*, AhR-inducible cytochrome P450 (*CYP*)-1A1 (*CYP1A1*) and *CYP1B1*, and *AhR* mRNA expression.

**Results:** In MCF-7 cells, GEN exerted dose- and time-dependent preventative effects against TCDD-dependent downregulation of BRCA-1. After TCDD washout, GEN rescued BRCA-1 protein expression while reducing DNMT-1 and cyclin D1. GEN and EGCG reduced *BRCA1* CpG methylation and cell proliferation associated with increased p53. In UACC-3199 cells, GEN reduced *BRCA1* and estrogen receptor-1 (*ESR1*) CpG methylation, cyclin D1, and cell growth while inducing BRCA-1 and *CYP1A1*.

**Keywords:** genistein, *BRCA1*, *ESR1*, AhR, DNA methylation, epigenetics, breast cancer, flavonoids, prevention



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Supported by grants from the Arizona Biomedical

GENOVA DIAGNOSTICS

Romagnolo DF, et al. *Curr Dev Nutr*. 2017;1(6):e000562.

# Microbial Impact on Host Methylation Activity

| Study | Outcome | Evidence Offered |
|---|---|---|
| Taki et al. 2005 (Human study) | Administration of *B. longum* to hemodialysis patients reduced serum homocysteine. Attributed to increased supply of folate produced by this species in the gut | Administration of folate-producing probiotics can alter host methylation pathway intermediates |
| Pompei et al. 2007 (Rodent study) | In folate-deficient rats, administration of *B. adolescentis* and *B. pseudocatenulatum* raised serum folate levels, even moreso with coadministration of prebiotic fructans | Administration of folate-producing probiotics can alter host serum folate levels |
| Rossi et al. 2011 (*In vitro)* | Species capable of producing folate in presence of PABA: *L. plantarum, B bifidum, B. infantis, B. breve, B. longum, B. adolescentis, B. pseudocatenulatum* | Gut microbes can produce methyl donor nutrients |
| D'Aimmo et al. 2014 (*In vitro)* | Most bacteria produce both THF and 5mTHF, with *B. adolescentis* producing highest levels of 5mTHF | Gut microbes can produce methylated forms of folate as well as THF |

# Microbial Impact on Host Methylation Activity

| Study | Outcome | Evidence Offered |
|---|---|---|
| Kumar et al. 2014 (Human pilot study) | Higher levels of Bacteroidetes (vs firmicutes) associated with specific increases/decreases in promoter methylation on genes related to CVD, inflammation, metabolic pathways, cancer | Microbes may alter the course of chronic host/human disease via epigenetic regulation |
| Yu et al. 2015 (Rodent study) | DNA methylation of intestinal stem cells and epithelial cells is significantly dysregulated and reduced in germ-free mice. FMT to reestablish commensal populations correlates with significant increases in CpG methylation. | Microbes play a directive role in host epigenetic regulation, beyond simple facilitation |

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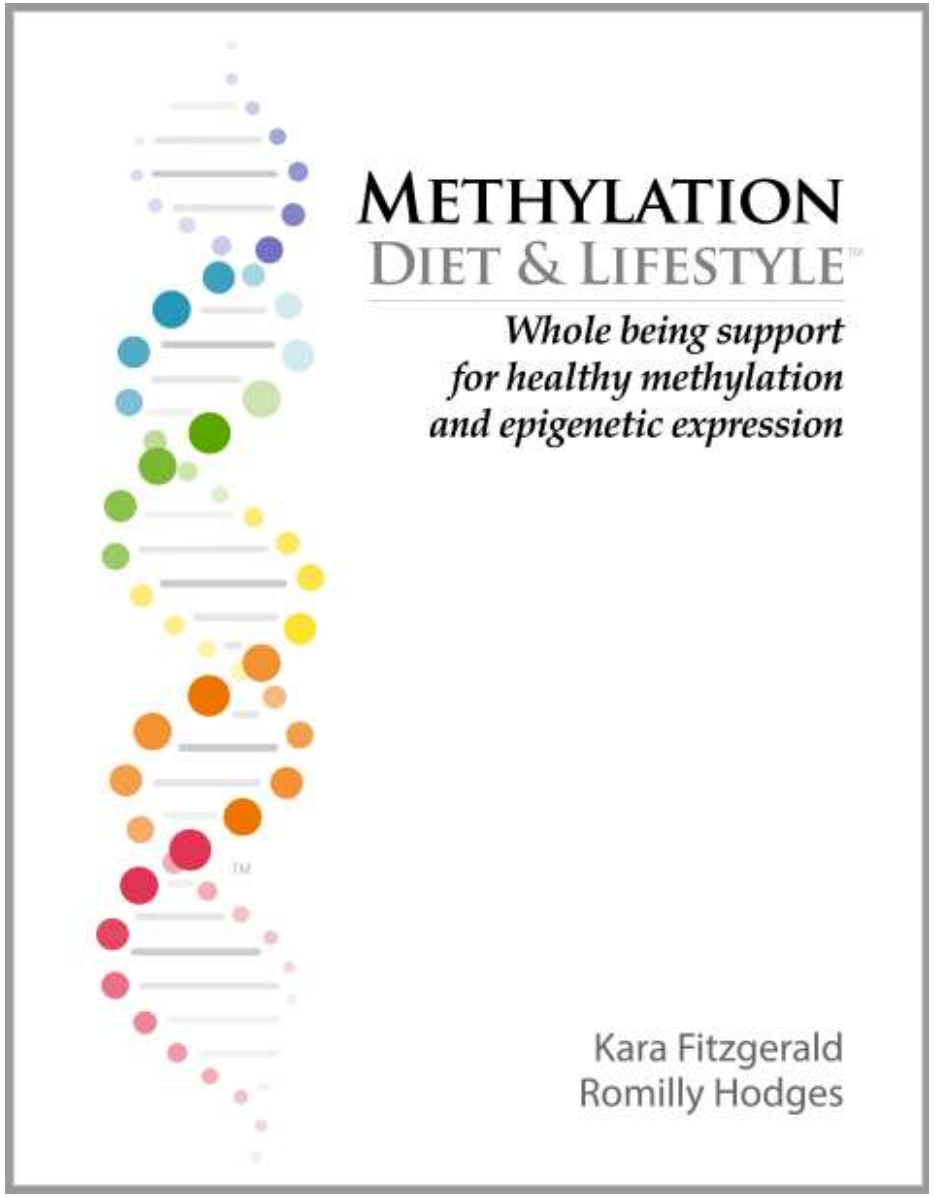
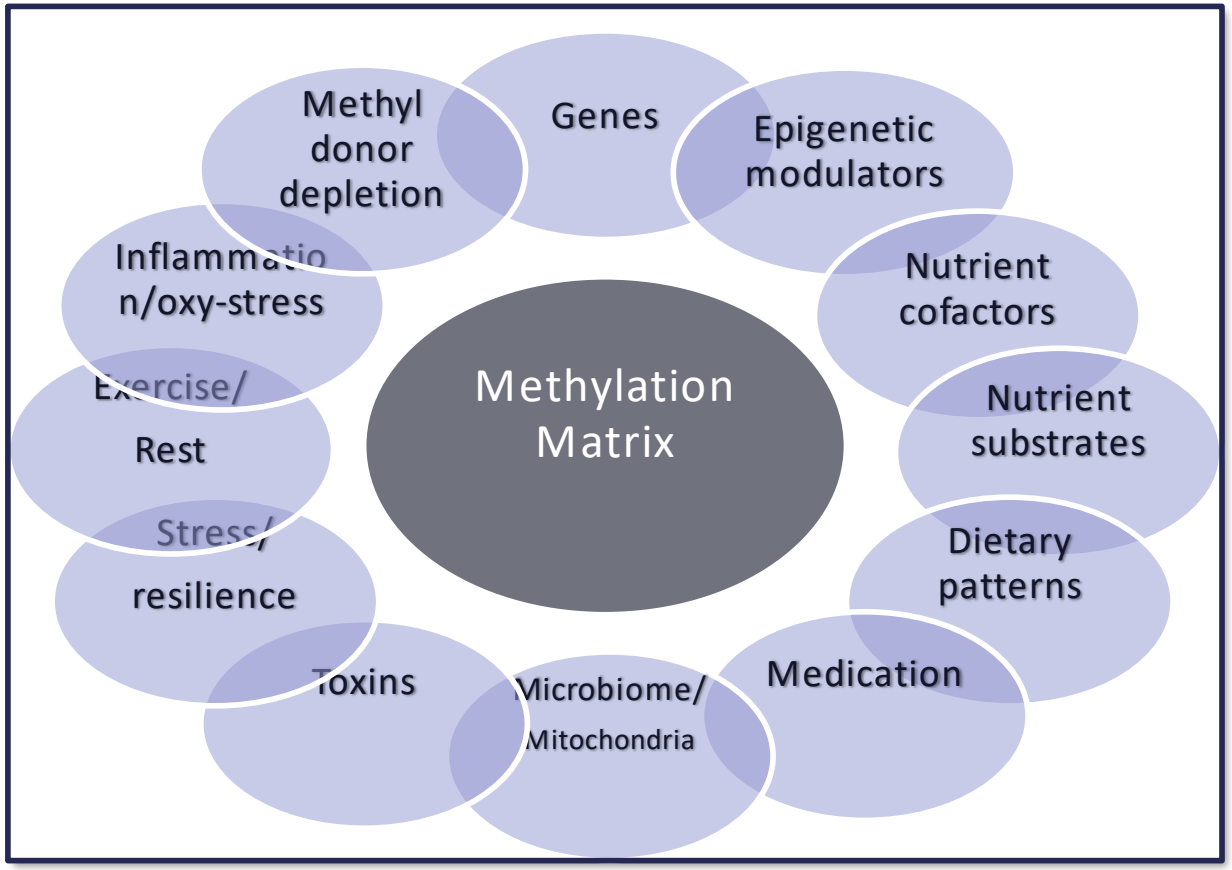



GENOVA DIAGNOSTICS

# Comprehensive Approach to Optimizing Epigenetic Methylation

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# Pilot Study: The Effects of Diet and Lifestyle on Quality of Life and Methylation-related Biomarkers in Vivo

- Run by the National University of Natural Medicine/Helfgott Institute, Dr. Kara Fitzgerald. Sponsored by Metagenics.
- Estimated enrollment 40 participants from Portland OR area, parallel assignment to intervention/ control groups
- Study population: Male, 50-72 years, healthy
- Study collection February 2018 – December 2018
- https://clinicaltrials.gov/ct2/show/NCT03472820


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# Study Intervention

- **Diet:** Specific dietary guidelines outlined for participants. Rich in methylation nutrients/adaptogens, low glycemic, anti-inflammatory, (intermittent fasting/ketosis) etc…
- **Sleep:** Participants are encouraged to average a minimum of 7 hours of sleep per night
- **Exercise:** Participants are encouraged to exercise a minimum of 30 minutes at least 5 days per week at an intensity of 60-80% of maximum perceived exertion
- **Stress Management:** Participants utilize the Cleveland Clinic's Stress Free Now application to engage in a variety of guided stress reduction techniques, including meditation and mindful breathing
  - Recommended frequency is twice daily, preferably morning and evening.
- **Supplemental Methylation Adaptogens:** a food-based phytonutrient blend containing organic fruit and vegetables, prebiotics, probiotics, milk thistle, turmeric, green tea, reishi, shitake, quercetin, maca root powder, cassia bark powder, dong quai, ginger root, garlic, plant enzymes and vitamin C
  - Participants took 2 servings daily in divided doses
- **Supplemental Probiotic:** *Lactobacillus plantarum* (299v)

# Why No Supplemental Methyl Donors?

- Bruce Ames – pushing pathways/reactions forward using high dose cofactor supplementation is possible
- Karpf 2013 - Epigenetic inactivation of BRCA1 via promoter hypermethylation associated with many cancers.
- JAMA/Cole et al. 2007 – 1 mg/d folic acid (FA) for colorectal ca prevention failed to reduce recurrence risk and risk for other cancers increased
- Kim 2006 - >400 mcg/d FA associated with 20% increase in breast cancer
- Lubecka-Pietruszewska et al 2012. High FA concentration suppresses transcription of tumor suppressor genes *in vitro*
- Raghavan et al. 2017 – highest levels of maternal folate and B12 associated with increased risk for Autism Spectrum Disorder

**Conclusion: Supraphysiological dosing may have unknown/ undesirable effects**

# Supporting Methylation Through Diet and Lifestyle

- Potentially a safer, more nuanced way to support the homeodynamic balance of methylation activity
- How we use dietary and lifestyle support in clinical practice:
  - Alongside cautious/cyclical folate and methylation nutrient supplementation to enhance efficacy
  - As an alternative intervention for individuals who do not tolerate methyl donor supplementation
  - As a stand-alone intervention
  - Integrated with other needed non-methylation interventions
- How we use supplemental methyl donors in clinical practice:
  - As therapeutic probes, tied to clinical outcome
  - With endpoints in mind
  - Concurrently with methylation adaptogens

# Dietary Principles

- Nutritionally replete, especially in methylation nutrients
- Enzyme modulators, adaptogens
- Anti-inflammatory and low-glycemic
  - Minimizes added sugars
  - Minimizes AGE formation (advanced glycation end products)
- Antioxidants
- Prebiotics and probiotics
- Optimal hydration
- Avoid caloric excess, considers intermittent caloric restriction

# Dietary Principles, continued…

- Avoid folic acid-fortified foods
- Avoid/minimize alcohol
- Supportive of detox processes (hydration, fiber, detox nutrients)
- Avoid foods from animals raised with antibiotics and hormones
- Avoids high-mercury fish including tuna, king mackerel, shark, and swordfish
- Minimize plastic food and beverage containers

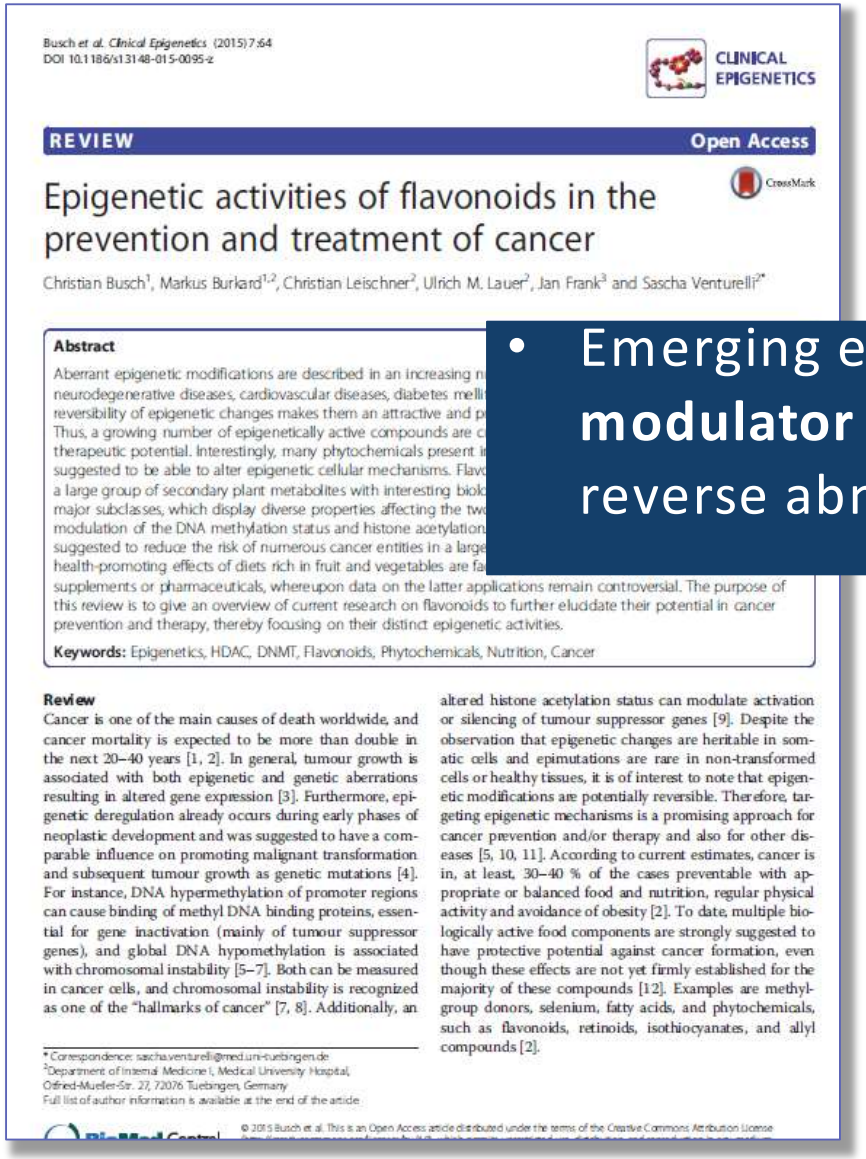
# Methylation Super Foods

- Beets
- Spinach
- Sprouts
- Sea vegetables
- Daikon radish
- Shiitake
- Salmon (wild)
- Fish roe
- Oysters
- Eggs
- Pumpkin seeds
- Sesame seeds
- Sunflower seeds
- Liver

# Primary Nutrients in Methylation Superfoods

- **Beets:** betaine (129 mg/100g)
- **Spinach:** potassium, folate, betaine (550 mg/100g)
- **Sprouts:** >3x increased nutrient concentration including folate
- **Sea vegetables:** zinc, magnesium, folate
- **Daikon:** magnesium, potassium, B2, B6, folate, methylation adaptogens
- **Shiitake:** zinc, potassium, B2, B3, B6, folate, methylation adaptogens

# Methylation Adaptogens



Busch et al. Clinical Epigenetics (2015) 7:64
DOI 10.1186/s13148-015-0095-z

CLINICAL EPIGENETICS

REVIEW Open Access

## Epigenetic activities of flavonoids in the prevention and treatment of cancer

Christian Busch[1], Markus Burkard[1,2], Christian Leischner[2], Ulrich M. Lauer[2], Jan Frank[3] and Sascha Venturelli[2*]

**Abstract**

Aberrant epigenetic modifications are described in an increasing n
neurodegenerative diseases, cardiovascular diseases, diabetes melli
reversibility of epigenetic changes makes them an attractive and p
Thus, a growing number of epigenetically active compounds are c
therapeutic potential. Interestingly, many phytochemicals present i
suggested to be able to alter epigenetic cellular mechanisms. Flavo
a large group of secondary plant metabolites with interesting biolo
major subclasses, which display diverse properties affecting the tw
modulation of the DNA methylation status and histone acetylation
suggested to reduce the risk of numerous cancer entities in a large
health-promoting effects of diets rich in fruit and vegetables are fa
supplements or pharmaceuticals, whereupon data on the latter applications remain controversial. The purpose of this review is to give an overview of current research on flavonoids to further elucidate their potential in cancer prevention and therapy, thereby focusing on their distinct epigenetic activities.

**Keywords:** Epigenetics, HDAC, DNMT, Flavonoids, Phytochemicals, Nutrition, Cancer

**Review**

Cancer is one of the main causes of death worldwide, and cancer mortality is expected to be more than double in the next 20–40 years [1, 2]. In general, tumour growth is associated with both epigenetic and genetic aberrations resulting in altered gene expression [3]. Furthermore, epigenetic deregulation already occurs during early phases of neoplastic development and was suggested to have a comparable influence on promoting malignant transformation and subsequent tumour growth as genetic mutations [4]. For instance, DNA hypermethylation of promoter regions can cause binding of methyl DNA binding proteins, essential for gene inactivation (mainly of tumour suppressor genes), and global DNA hypomethylation is associated with chromosomal instability [5–7]. Both can be measured in cancer cells, and chromosomal instability is recognized as one of the "hallmarks of cancer" [7, 8]. Additionally, an altered histone acetylation status can modulate activation or silencing of tumour suppressor genes [9]. Despite the observation that epigenetic changes are heritable in somatic cells and epimutations are rare in non-transformed cells or healthy tissues, it is of interest to note that epigenetic modifications are potentially reversible. Therefore, targeting epigenetic mechanisms is a promising approach for cancer prevention and/or therapy and also for other diseases [5, 10, 11]. According to current estimates, cancer is in, at least, 30–40 % of the cases preventable with appropriate or balanced food and nutrition, regular physical activity and avoidance of obesity [2]. To date, multiple biologically active food components are strongly suggested to have protective potential against cancer formation, even though these effects are not yet firmly established for the majority of these compounds [12]. Examples are methyl-group donors, selenium, fatty acids, and phytochemicals, such as flavonoids, retinoids, isothiocyanates, and allyl compounds [2].

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- Emerging evidence strongly suggests that **diet is a major modulator of the epigenetic state** of cells and is able to reverse abnormal or altered gene expression patterns

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Busch C, et al. *Clin Epigenetics. 2015;7(1):64-82.*

# Methylation Adaptogens

- Vitamins, minerals and PHYTONUTRIENTS that modulate epigenetic methylation activity
  - Includes selective de-methylation (epigenetic ‘cleaning’)
- Some also modulate enzymes in methylation pathways, altering SAMe and homocysteine status

# Methylation Adaptogens

- Anthocyanins
- Curcumin
- DHA
- Diindolylmethane (DIM)
- EGCG
- Ellagic acid
- Equol
- Genistein
- Lycopene
- Parthenolide
- Quercetin
- Resveratrol
- Rosmarinic acid
- Shiitake mushroom
- Sulforaphane
- Butyrate
- Vitamin A
- Vitamin D3
- Vitamin E
- Selenium
- Zinc

Fang MZ, et al. *Clin Cancer Res*. 2005;11(19 Pt 1):7033-41.
Stefanska B, et al. *Br J Pharmacol.* 2012;167(2):279-97.
Szarc vel Sk, et al. *Biochem Pharmacol.* 2010;80(12):1816-32.
Lu Q, et al. *Ageing Res rev*. 2006;5(4):449-67.

# Methylation Adaptogens

- Anthocyanins
- Curcumin
- DHA
- Diindolylmethane (DIM)
- Quercetin
- Resveratrol
- Rosmarinic acid
- Shiitake mushroom
- Zinc

Provide INFORMATION
to cellular genetic machinery

Fang MZ, et al. *Clin Cancer Res*. 2005;11(19 Pt 1):7033-41.
Stefanska B, et al. *Br J Pharmacol.* 2012;167(2):279-97.
Szarc vel Sk, et al. *Biochem Pharmacol.* 2010;80(12):1816-32.
Lu Q, et al. *Ageing Res rev*. 2006;5(4):449-67.

# Methylation Adaptogens

## Epigenetic Modifying Agents in Cancer Therapy

| NATURAL COMPOUNDS | | | |
|---|---|---|---|
| Genistein | Decreases DNMT1, DNMT3A, and DNMT3B concentration in prostate cancer cells, but the extent of altered DNA methylation is unclear | Phase III | (22) |
| Equol | Isolated from soy beans, equol has been shown to have some hypomethylating effect; however, its role in cancer is controversial, and it may even increase the viability of metastatic cancer cells | Phase III | (23) |
| Curcumin | Binds DNMT1 and blocks its catalytic function with potency similar to some synthetic, non-nucleoside DNMT inhibitors | Phase III | (24) |
| EGCG | A component of green tea that is shown to have chemopreventive characteristics. Functions as a DNMT inhibitor by depleting the amount of SAM available, leading to decreased DNMT activity | Phase III | (25) |
| Resveratrol | Found in grapes, resveratrol may function by blocking acetylation of STAT3 and preventing STAT3-mediated targeting of DNMT1 to promoter CpG islands | Phase II | (26) |
| Parthenolide | Binds the catalytic cysteine of DNMT1 with low potency | Pre-clinical | (27) |

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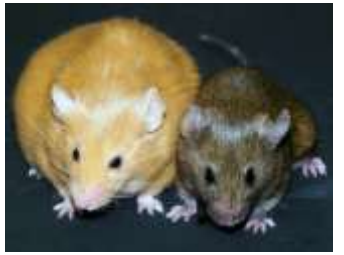
Heninger, et al. *Frontiers in Immunol.* 2015;6(29):1-14.

# Genistein



## Maternal Genistein Alters Coat Color and Protects $A^{vy}$ Mouse Offspring from Obesity by Modifying the Fetal Epigenome

*Dana C. Dolinoy,[1,2,3] Jennifer R. Weidman,[1,2] Robert A. Waterland,[4,5] and Randy L. Jirtle[1,2,3]*

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Genistein, the major phytoestrogen in soy, is linked to diminished female reproductive performance and to cancer chemoprevention and decreased adipose deposition. Dietary ge role in the decreased incidence of cancer in Asians compared with Westerne cancer incidence in Asians immigrating to the United States. Here, we repor genistein supplementation of mice during gestation, at levels comparable wi high-soy diets, shifted the coat color of heterozygous viable yellow agouti ( pseudoagouti. This marked phenotypic change was significantly associated w tion of six cytosine–guanine sites in a retrotransposon upstream of the transc *Agouti* gene. The extent of this DNA methylation was similar in endoder ectodermal tissues, indicating that genistein acts during early embryonic de this genistein-induced hypermethylation persisted into adulthood, decreasing sion and protecting offspring from obesity. Thus, we provide the first evidence that *in utero* dietary genistein affects gene expression and alters susceptibility to obesity in adulthood by permanently altering the epigenome. *Key words:* developmental origins of adult disease, DNA methylation, epigenetics, viable yellow agouti ($A^{vy}$) mouse. *Environ Health Perspect* 114:567–572 (2006). doi:10.1289/ehp.8700 available via *http://dx.doi.org/* [Online 26 January 2006]

breast and prostate cancer chemoprevention (Lamartiniere et al. 2002) and decreased adi

early exposure remain largely unknown (Badger et al. 2002). Limited evidence suggests that exposure to phytoestrogens postnatally alters the epigenome (Day et al. 2002; Lyn-Cook et al. 1995). Neonatal exposure to high doses of the phytoestrogens equol and

Maternal dietary genistein (soy isoflavone), at levels comparable with humans consuming high-soy diets, “silences” the Agouti(vy) gene via increased methylation, without methyl donor supplementation.

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Dolinoy DC, et al. *Environ Health Perspect*. 2006;114(4):567–72.

# Genistein

- Can change DNA methylation at gene promoters in the prostate of C57BL/6J mice
- Decreases DNMT activity resulting in transcriptional activation of genes such as p16INK4a, RAR β, MGMT, phosphatase and tensin homolog (PTEN) and CYLD in prostate cancer and RAR β2 in cervical cancer
- Genistein-mediated modulation of methylation of pro-tumorigenic miRNA-1260b, its targets sFRP1 and Smad4, inhibits prostate cancer cell proliferation, invasion and TCF reporter activity



Mukherjee, et al. 2015. *Curr Pharmacol Rep.* 1(2):112-120.

# Genistein

PNAS

## Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development

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Edited by R. Michael Roberts, University of Missouri, Columbia, MO, and approved June 25, 2007 (received for review April 23, 2007)

**The hypothesis of fetal origins of adult disease posits that early developmental exposures involve epigenetic modifications, such as DNA methylation, that influence adult disease susceptibility. *In utero* or neonatal exposure to bisphenol A (BPA), a high-production-volume chemical used in the manufacture of polycarbonate plastic, is associated with higher body weight, increased breast and prostate cancer, and altered reproductive function. This study shows that maternal exposure to this endocrine-active compound shifted the coat color distribution of viable yellow agouti ($A^{vy}$) mouse offspring toward yellow by decreasing CpG (cytosine-guanine dinucleotide) methylation in an intracisternal A particle retrotransposon upstream of the *Agouti* gene. CpG methylation also was decreased at another metastable locus, the CDK5 activator-binding protein ($Cabp^{IAP}$). DNA methylation at the $A^{vy}$ locus was similar in tissues from the three germ layers, providing evidence that epigenetic patterning during early stem cell development is sensitive to BPA exposure. Moreover, maternal dietary supplementation, with either methyl donors like folic acid or the phytoestrogen genistein, negated the DNA hypomethylating effect of BPA. Thus, we present compelling evidence that early developmental exposure to BPA can change offspring phenotype by stably altering the epigenome, an effect that can be counteracted by maternal dietary supplements.**

DNA methylation | environmental epigenomics | viable yellow agouti | fetal origins of adult disease

There is now significant evidence that the risk of many chronic adult diseases and disorders results from exposure to environmental factors early in development (1, 2). Moreover, it seems that there is a link between what we are exposed to *in utero* and disease formation in adulthood that involves epigenetic modifications such as DNA methylation of transposable elements and cis-acting, imprinting regulatory elements (3). Many xenobiotics, ubiquitously present in the environment, have estrogenic properties and function as endocrine disruptors; however, their potential to modify the epigenome remains largely unexplored (4). The epigenome is particularly susceptible to dysregulation during gestation, neonatal development, puberty, and old age. Nevertheless, it is most vulnerable to environmental exposures during embryogenesis because the elaborate DNA methylation and chromatin patterning required for normal tissue development is programmed during early development.

Most regions of the mammalian genome exhibit little variability among individuals in tissue-specific DNA methylation levels. In contrast, DNA methylation is determined stochastically at some transposable element insertion sites. This potentially can affect the expression of neighboring genes, resulting in the formation of loci with metastable epialleles (3). Cellular epigenetic mosaicism and individual phenotypic variability then can occur even in genetically identical individuals. These sites are also particularly vulnerable to environmentally induced epigenetic alterations (5–7).

The *Agouti* gene in the viable yellow agouti ($A^{vy}$) the most extensively studied metastable epiallele, is expressed variably in genetically identical indivi of epigenetic modifications established during e ment (9). The wild-type murine *Agouti* gene encod signaling molecule that produces either black eum yellow phaeomelanin (*A*). Both *A* and *a* transcripts from a hair cycle-specific promoter in exon 2. expression in hair follicles during a specific stage o results in a subapical yellow band on each blac causing the brown (agouti) coat color of wild-type $A^{vy}$ allele resulted from the insertion of a murine A particle (IAP) retrotransposon into the 5′ end gene (6, 8). A cryptic promoter in the proximal e IAP promotes constitutive ectopic *Agouti* transcri to yellow fur, obesity, diabetes, and tumorigen Methylation of cytosines in cytosine-guanine (C otide sites in and near the $A^{vy}$ IAP correlates i ectopic *Agouti* expression and varies dramatically vidual isogenic $A^{vy}/a$ mice. This results in a wide var color, ranging from yellow (unmethylated) to (methylated).

The present study uses this model to evaluate epigenome is affected by maternal exposure to t xenobiotic chemical bisphenol A (BPA). BPA is a tion volume chemical used in the manufacture of p plastic and epoxy resins. It is present in many co products including food and beverage containers, and dental composites. The detection of BPA in 9 urine samples (12) clearly attests to the widespre and widespread human exposure to BPA. Rodent associated pre- or perinatal BPA exposure with weight, increased breast and prostate cancer, alter tive function, and other chronic health effects (re 13). BPA also enters the placenta and accumula after rodent maternal oral exposure (14). Herein, effect of maternal BPA exposure alone or in com nutritional supplements on the epigenome of the

### Results

To evaluate the effects of maternal BPA exposur epigenome, female *a/a* mice received a phytoestrog 93G diet ($n = 16$ litters, 120 total offspring, 60 $A^{vy}$

Author contributions: D.C.D. and R.L.J. designed the research; D.C.D. a the research; D.C.D. and R.L.J. analyzed data; and D.C.D. and R.L.J. wr

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Abbreviations: $A^{vy}$, viable yellow agouti; BPA, bisphenol A; IAP, intrac Cabp, CDK5 activator-binding protein.

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- BPA exposure in utero or neonatal
- Decreased CpG methylation at metastable sites related to the Agouti gene
- Shifted coat color toward yellow
- **Both folic acid and genistein negated this effect**

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Dolinoy DC, et al. *Proc Natl Acad Sci USA*. 2007;104(32):13056–61.

# EGCG

- Inhibits tumorigenesis partially by affecting DNA methylation through **inhibition of DNMTs**
- Can re-express many transcriptionally silenced genes through **inhibition of DNMT1** enzymatic activity in prostate cancer cell lines
- Decreases growth and induces apoptosis in renal cell carcinoma by re-expressing tissue factor pathway inhibitor-2 (TFPI-2), a member of the Kunitz-type serine proteinase inhibitor family, and **decreases its promoter hypermethylation**
- **Decreases the promoter methylation** of other genes such as hTERT and CDX2 thereby helping in tumor suppression

Mukherjee, et al. 2015. Curr Pharmacol Rep. 1(2):112-120.

# EGCG

- EGCG is found in green tea and (to a lesser extent) in white and oolong tea.
- To increase EGCG content in your tea, steep fresh tea leaves or tea bags for 10 minutes.

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Lee WJ, et al. *Mol Pharmacol.* 2005;68(4):1018-30.

# Curcumin

- **Induces global hypomethylation** in MV4–11 leukemia cell line by inhibiting DNMT.
- **Negatively regulates DNMT1** in ovarian and melanoma.
- **Decreases CpG promoter methylation** of Neurog1, a highly hypermethylated marker in prostate cancer and also the hypermethylation at the promoter region of tumor suppressor retinoic acid receptor 2 gene in cervical cancer.
- Involved in the re-expression of Nrf2, a critical regulator of the antioxidant response by **reducing promoter hypermethylation** in TRAMP prostate cancer cells.
- **Reduces hypermethylation** of Fanconi anemia (FANCF) promoter, thereby regulating growth and proliferation in cervical cancer.

Mukherjee, et al. 2015. Curr Pharmacol Rep. 1(2):112-120.

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PLOS ONE

# Curcumin Modulates DNA Methylation in Colorectal Cancer Cells

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## Abstract

***Aim:*** Recent evidence suggests that several dietary polyphenols may exert their chemopreventive effect through epigenetic modifications. Curcumin is one of the most widely studied dietary chemopreventive agents for colon cancer prevention, however, its effects on epigenetic alterations, particularly DNA methylation, remain unclear. Using systematic genome-wide approaches, we aimed to elucidate the effect of curcumin on DNA methylation alterations in colorectal cancer cells.

***Materials and Methods:*** To evaluate the effect of curcumin on DNA methylation, three CRC cell lines, HCT116, HT29 and RKO, were treated with curcumin. 5-aza-2'-deoxycytidine (5-aza-CdR) and trichostatin A treated cells were used as positive and negative controls for DNA methylation changes, respectively. Methylation status of LINE-1 repeat elements, DNA promoter methylation microarrays and gene expression arrays were used to assess global methylation and gene expression changes. Validation was performed using independent microarrays, quantitative bisulfite pyrosequencing, and qPCR.

***Results:*** As expected, genome-wide methylation microarrays revealed significant DNA hypomethylation in 5-aza-CdR-treated cells (mean β-values of 0.12), however, non-significant changes in mean β-values were observed in curcumin-treated cells. In comparison to mock-treated cells, curcumin-induced DNA methylation alterations occurred in a time-dependent manner. In contrast to the generalized, non-specific global hypomethylation observed with 5-aza-CdR, curcumin treatment resulted in methylation changes at selected, partially-methylated loci, instead of fully-methylated CpG sites. DNA methylation alterations were supported by corresponding changes in gene expression at both up- and down-regulated genes in various CRC cell lines.

***Conclusions:*** Our data provide previously unrecognized evidence for curcumin-mediated DNA methylation alterations as a potential mechanism of colon cancer chemoprevention. In contrast to non-specific global hypomethylation induced by 5-aza-CdR, curcumin-induced methylation changes occurred only in a subset of partially-methylated genes, which provides additional mechanistic insights into the potent chemopreventive effect of this dietary nutraceutical.

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Link A, et al. *PLoS One. 2013;8(3=2):e57709.*

# Quercetin

- Quercetin was shown to decrease bladder cancer cell growth and to induce apoptosis by decreasing the DNA methylation of the estrogen receptor (ER-β), p16INK4a and RASSF1A (84).
- Quercetin is present in red onions, buckwheat, red grapes, apple skin, green tea
- Note, quercetin is metabolized via COMT and exhibits competitive inhibition for COMT (tea catechins also)



Mukherjee, et al. 2015. Curr Pharmacol Rep. 1(2):112-120.

# Glucosinolate Derivatives

## Epigenetic Modifications of Nrf2 by 3,3′-diindolylmethane *In Vitro* in TRAMP C1 Cell Line and *In Vivo* TRAMP Prostate Tumors

Tien-Yuan Wu,[1] Tin Oo Khor,[1] Zheng-Yuan Su,[1] Constance Lay-Lay Saw,[1] Limin Shu,[1] Ka-Lung Cheung,[1] Ying Huang,[1] Siwang Yu,[2] and Ah-Ng Tony Kong[1,3]

*Received 20 January 2013; accepted 17 April 2013*

***Abstract.*** 3,3′-diindolylmethane (DIM) is currently
including prostate, breast, and cervical cancers and
in several *in vivo* and *in vitro* models. Previously,
chemopreventive effects in prostate carcinogenesi
mechanism is unclear. The present study aims to in
modulation of DIM in TRAMP-C1 cells and in T
TRAMP-C1 cells showed that DIM suppressed DN
status of Nrf2 resulting in enhanced expression of Nr
TRAMP mice fed with DIM-supplemented diet show
metastasis than the untreated control group simi
increased apoptosis, decreased cell proliferation and
expression in prostate tissues. Importantly, immunohistochemical analysis showed that DIM reduced the global CpG 5-methylcytosine methylation. Focusing on one of the early cancer chemopreventive target gene *Nrf2*, bisulfite genomic sequencing showed that DIM decreased the methylation status of the first five CpGs of the Nrf2 promoter region, corroborating with the results of *in vitro* TRAMP-C1 cells. In summary, our current study shows that DIM is a potent cancer chemopreventive agent for prostate cancer and epigenetic modifications of the CpG including Nrf2 could be a potential mechanism by which DIM exerts its chemopreventive effects.

**KEY WORDS:** 3,3′-diindolylmethane (DIM); epigenetic; methylation; Nrf2; prostate cancer.

- In vitro study utilizing TRAMP-C1 cells showed that **DIM suppressed DNMT expression and reversed CpG methylation status of Nrf2 resulting in enhanced expression of Nrf2** and Nrf2-target gene NQO1.
- **In vivo study, TRAMP mice fed with DIM-supplemented diet showed much lower incidence of tumorigenesis and metastasis than the untreated control group** similar to what was reported previously

Wu TY, et al . AAPS J. 2013;15(3):864-74.

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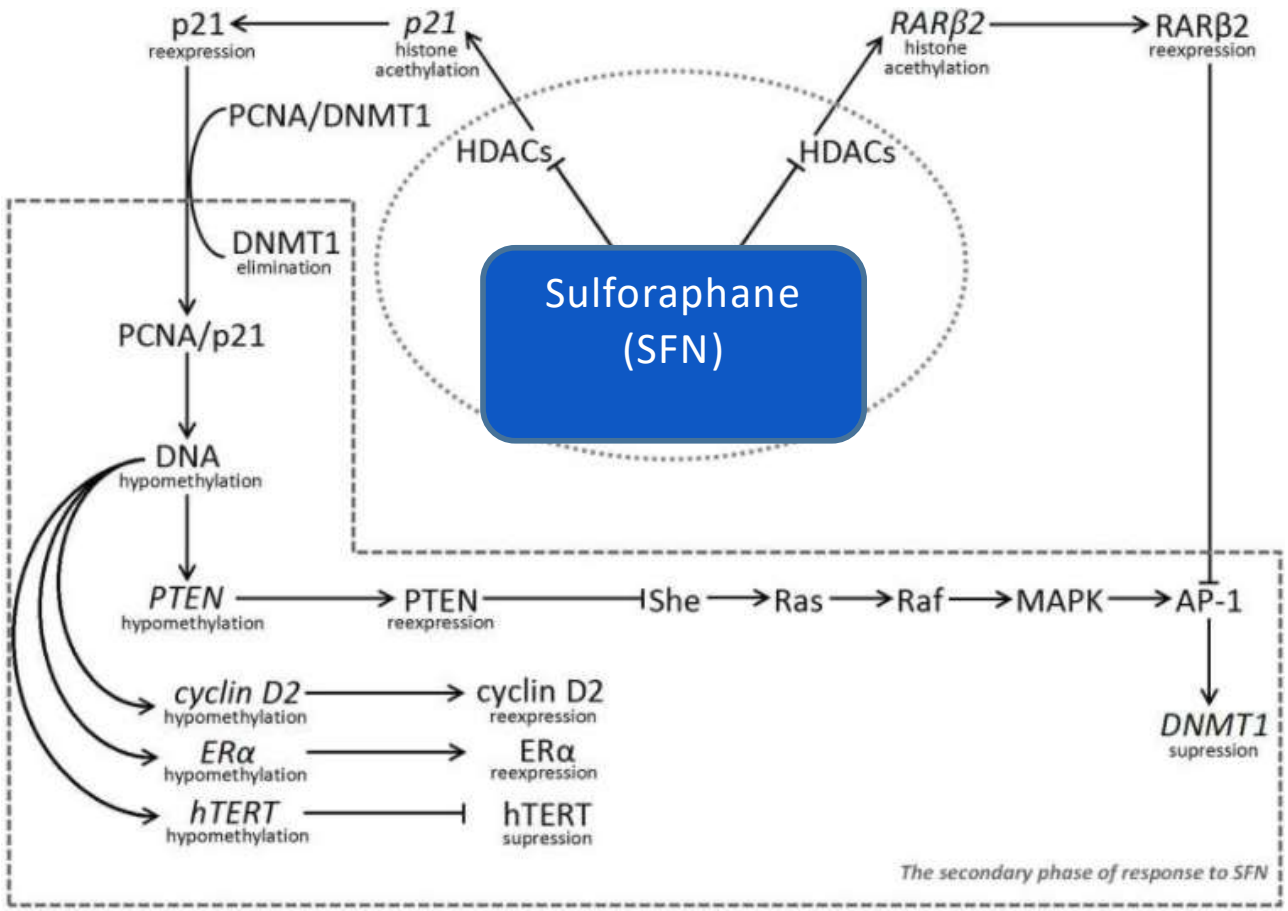
# Glucosinolate Derivatives




Kaufman-Szymczyk, et al. *Int J Mol Sci.* 2015;16(12):29732-43.

# Glucosinolate Derivatives

Modulation of genes involved in various mechanisms by the influence of I3C and DIM.

| Mechanisms involved | Upregulated genes (↑) modulated by I3C and DIM | Downregulated genes (↓) modulated by I3C and DIM | References |
|---|---|---|---|
| Apoptosis | JNK/SAPK and Bax | Bcl-xl, Bcl-2, surviving, and NF-κB | [14–16] |
| Xenobiotic metabolism | CYP, CYP 1A1, CYP 1A2, and CYP 1B1 | — | [17] |
| Antioxidant | GSH and GST | — | [4] |
| Transcription factor | Nrf2 and ATF3 | NF-κB and STAT3 | [4, 18] |
| Cell cycle | p21 WAF1, and p27 KIP1 | Cyclin D1, E, CDK2, and CDK6 | [19] |
| Inflammation | NAG-1 | NF-κB and MMP-9 | [16, 20] |
| Angiogenesis | VEGF, IL-6, and MMP-9 | — | [21] |

Maruthanila VL, et al. *Adv Pharmacol Sci*. 2014;2014:832161.

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# Glucosinolates, Gastric Acid Conversion and Gut Microbiome

- Cruciferous vegetables contain glucosinolates: arugula, broccoli, bok choy, Brussels sprouts, cabbage, cauliflower, horseradish, kale, kohlrabi, radish, rutabaga, wasabi and watercress
- I3C and sulforaphane formed via myrosinase enzyme in raw cruciferous upon mastication, or if cooked via bacterial myrosinase in the gut
- Diindolylmethane (DIM) formed in low pH (acid) from I3C

Megna, et al. *World J Gastrointest Surg.* 2016;8(2):115-123.
Barba FJ, et al. *Front Nutr*. 2016;3:24-36.
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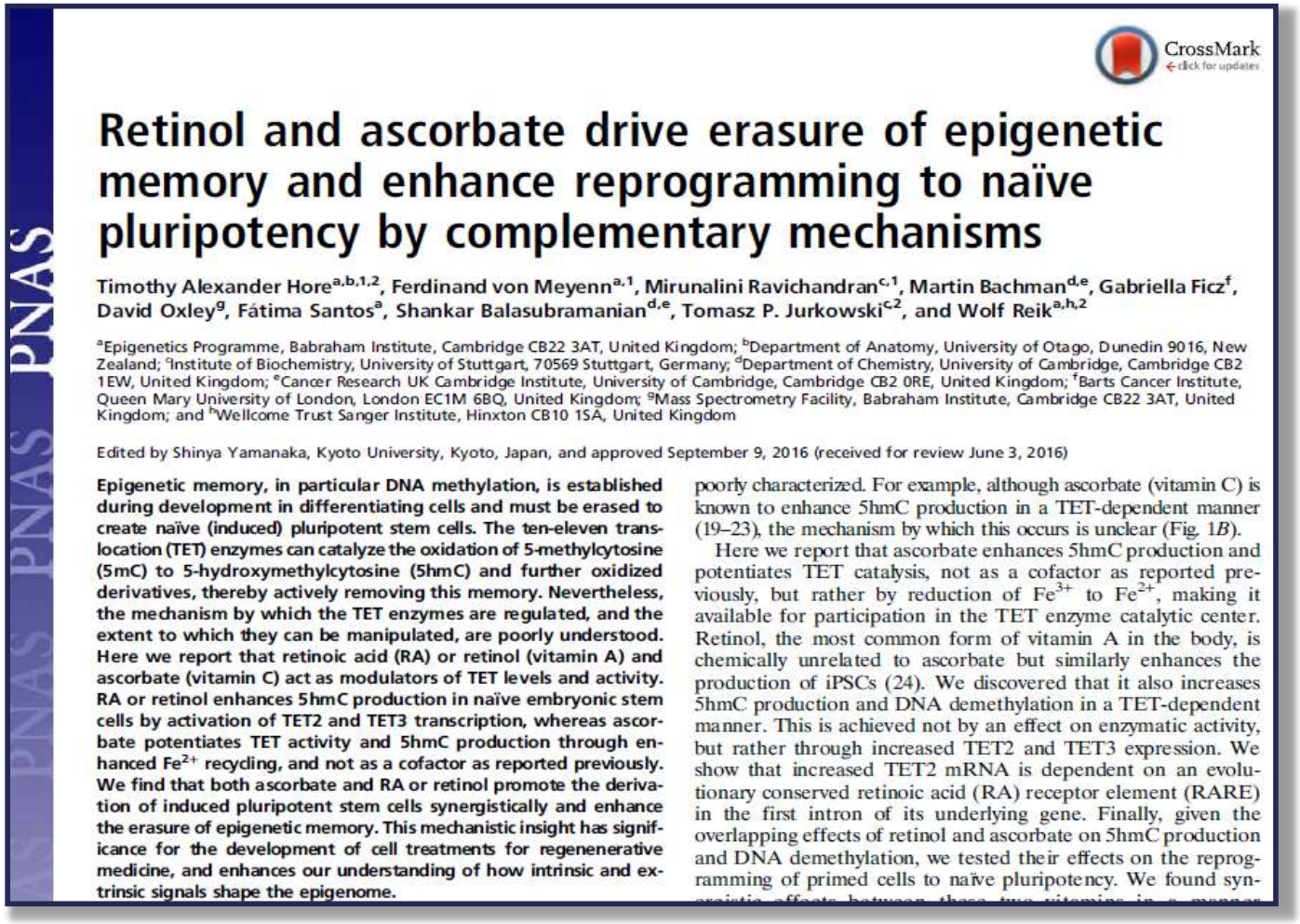
# Vitamin C and Vitamin A



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## Retinol and ascorbate drive erasure of epigenetic memory and enhance reprogramming to naïve pluripotency by complementary mechanisms

Timothy Alexander Hore[a,b,1,2], Ferdinand von Meyenn[a,1], Mirunalini Ravichandran[c,1], Martin Bachman[d,e], Gabriella Ficz[f], David Oxley[g], Fátima Santos[a], Shankar Balasubramanian[d,e], Tomasz P. Jurkowski[c,2], and Wolf Reik[a,h,2]

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Edited by Shinya Yamanaka, Kyoto University, Kyoto, Japan, and approved September 9, 2016 (received for review June 3, 2016)

**Epigenetic memory, in particular DNA methylation, is established during development in differentiating cells and must be erased to create naïve (induced) pluripotent stem cells. The ten-eleven translocation (TET) enzymes can catalyze the oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and further oxidized derivatives, thereby actively removing this memory. Nevertheless, the mechanism by which the TET enzymes are regulated, and the extent to which they can be manipulated, are poorly understood. Here we report that retinoic acid (RA) or retinol (vitamin A) and ascorbate (vitamin C) act as modulators of TET levels and activity. RA or retinol enhances 5hmC production in naïve embryonic stem cells by activation of TET2 and TET3 transcription, whereas ascorbate potentiates TET activity and 5hmC production through enhanced $Fe^{2+}$ recycling, and not as a cofactor as reported previously. We find that both ascorbate and RA or retinol promote the derivation of induced pluripotent stem cells synergistically and enhance the erasure of epigenetic memory. This mechanistic insight has significance for the development of cell treatments for regenenerative medicine, and enhances our understanding of how intrinsic and extrinsic signals shape the epigenome.**

poorly characterized. For example, although ascorbate (vitamin C) is known to enhance 5hmC production in a TET-dependent manner (19–23), the mechanism by which this occurs is unclear (Fig. 1*B*).

Here we report that ascorbate enhances 5hmC production and potentiates TET catalysis, not as a cofactor as reported previously, but rather by reduction of $Fe^{3+}$ to $Fe^{2+}$, making it available for participation in the TET enzyme catalytic center. Retinol, the most common form of vitamin A in the body, is chemically unrelated to ascorbate but similarly enhances the production of iPSCs (24). We discovered that it also increases 5hmC production and DNA demethylation in a TET-dependent manner. This is achieved not by an effect on enzymatic activity, but rather through increased TET2 and TET3 expression. We show that increased TET2 mRNA is dependent on an evolutionary conserved retinoic acid (RA) receptor element (RARE) in the first intron of its underlying gene. Finally, given the overlapping effects of retinol and ascorbate on 5hmC production and DNA demethylation, we tested their effects on the reprogramming of primed cells to naïve pluripotency. We found syn-

- Modulators of TET enzyme levels and activity

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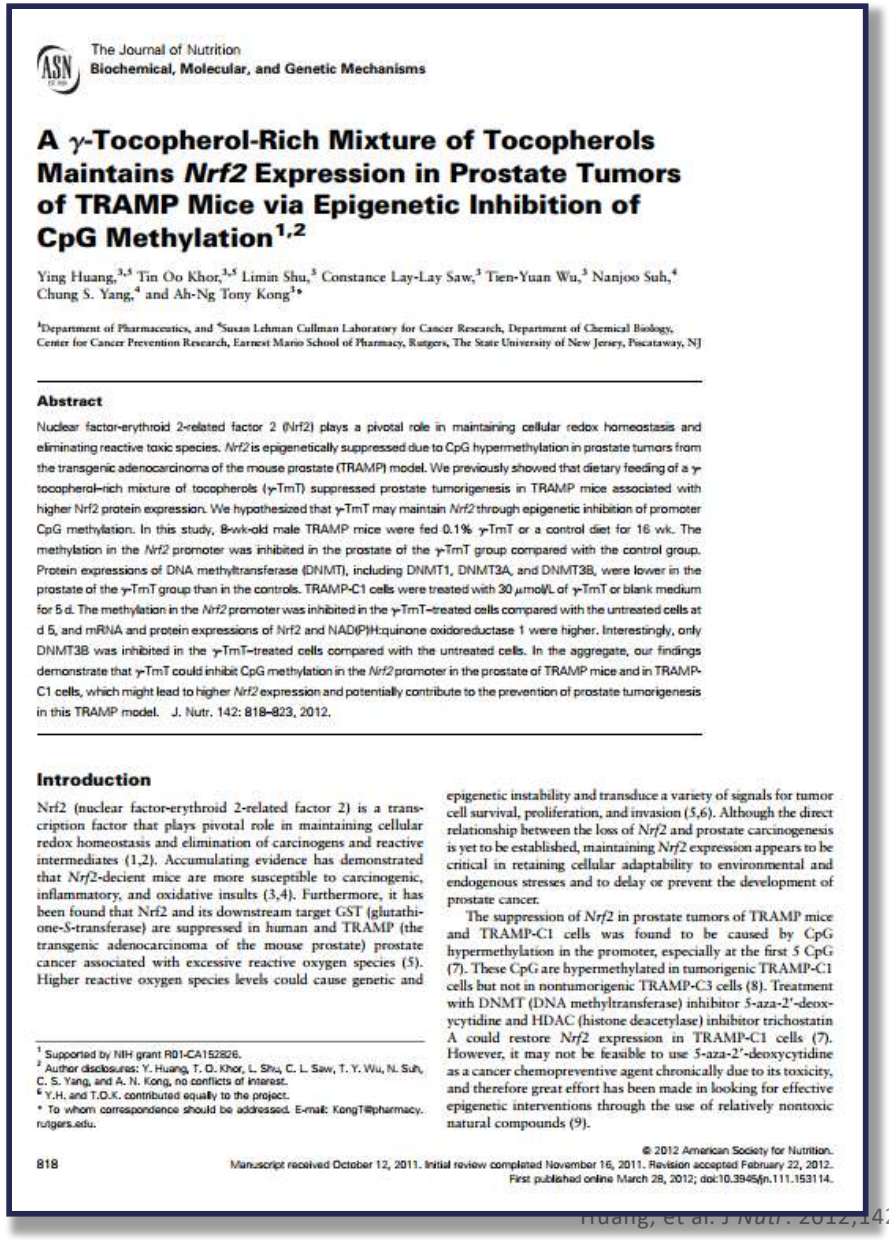
Hore TA, et al. *Proc Natl Acad Sci USA.* 2016;113(43):12202-12207.

# Vitamin E

- Gamma-tocopherol inhibits methylation in the Nfr2 promoter
- Increases expression of Nrf2 (master antioxidant and Phase II detox enzyme regulator)



The Journal of Nutrition
Biochemical, Molecular, and Genetic Mechanisms

## A γ-Tocopherol-Rich Mixture of Tocopherols Maintains *Nrf2* Expression in Prostate Tumors of TRAMP Mice via Epigenetic Inhibition of CpG Methylation[1,2]

Ying Huang,[3,5] Tin Oo Khor,[3,5] Limin Shu,[3] Constance Lay-Lay Saw,[3] Tien-Yuan Wu,[3] Nanjoo Suh,[4] Chung S. Yang,[4] and Ah-Ng Tony Kong[3]*

[3]Department of Pharmaceutics, and [4]Susan Lehman Cullman Laboratory for Cancer Research, Department of Chemical Biology, Center for Cancer Prevention Research, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ

**Abstract**

Nuclear factor-erythroid 2-related factor 2 (Nrf2) plays a pivotal role in maintaining cellular redox homeostasis and eliminating reactive toxic species. *Nrf2* is epigenetically suppressed due to CpG hypermethylation in prostate tumors from the transgenic adenocarcinoma of the mouse prostate (TRAMP) model. We previously showed that dietary feeding of a γ-tocopherol–rich mixture of tocopherols (γ-TmT) suppressed prostate tumorigenesis in TRAMP mice associated with higher Nrf2 protein expression. We hypothesized that γ-TmT may maintain *Nrf2* through epigenetic inhibition of promoter CpG methylation. In this study, 8-wk-old male TRAMP mice were fed 0.1% γ-TmT or a control diet for 16 wk. The methylation in the *Nrf2* promoter was inhibited in the prostate of the γ-TmT group compared with the control group. Protein expressions of DNA methyltransferase (DNMT), including DNMT1, DNMT3A, and DNMT3B, were lower in the prostate of the γ-TmT group than in the controls. TRAMP-C1 cells were treated with 30 μmol/L of γ-TmT or blank medium for 5 d. The methylation in the *Nrf2* promoter was inhibited in the γ-TmT–treated cells compared with the untreated cells at d 5, and mRNA and protein expressions of Nrf2 and NAD(P)H:quinone oxidoreductase 1 were higher. Interestingly, only DNMT3B was inhibited in the γ-TmT–treated cells compared with the untreated cells. In the aggregate, our findings demonstrate that γ-TmT could inhibit CpG methylation in the *Nrf2* promoter in the prostate of TRAMP mice and in TRAMP-C1 cells, which might lead to higher *Nrf2* expression and potentially contribute to the prevention of prostate tumorigenesis in this TRAMP model. J. Nutr. 142: 818–823, 2012.

**Introduction**

Nrf2 (nuclear factor-erythroid 2-related factor 2) is a transcription factor that plays pivotal role in maintaining cellular redox homeostasis and elimination of carcinogens and reactive intermediates (1,2). Accumulating evidence has demonstrated that *Nrf2*-decient mice are more susceptible to carcinogenic, inflammatory, and oxidative insults (3,4). Furthermore, it has been found that Nrf2 and its downstream target GST (glutathione-*S*-transferase) are suppressed in human and TRAMP (the transgenic adenocarcinoma of the mouse prostate) prostate cancer associated with excessive reactive oxygen species (5). Higher reactive oxygen species levels could cause genetic and epigenetic instability and transduce a variety of signals for tumor cell survival, proliferation, and invasion (5,6). Although the direct relationship between the loss of *Nrf2* and prostate carcinogenesis is yet to be established, maintaining *Nrf2* expression appears to be critical in retaining cellular adaptability to environmental and endogenous stresses and to delay or prevent the development of prostate cancer.

The suppression of *Nrf2* in prostate tumors of TRAMP mice and TRAMP-C1 cells was found to be caused by CpG hypermethylation in the promoter, especially at the first 5 CpG (7). These CpG are hypermethylated in tumorigenic TRAMP-C1 cells but not in nontumorigenic TRAMP-C3 cells (8). Treatment with DNMT (DNA methyltransferase) inhibitor 5-aza-2′-deoxycytidine and HDAC (histone deacetylase) inhibitor trichostatin A could restore *Nrf2* expression in TRAMP-C1 cells (7). However, it may not be feasible to use 5-aza-2′-deoxycytidine as a cancer chemopreventive agent chronically due to its toxicity, and therefore great effort has been made in looking for effective epigenetic interventions through the use of relatively nontoxic natural compounds (9).

[1] Supported by NIH grant R01-CA152826.
[2] Author disclosures: Y. Huang, T. O. Khor, L. Shu, C. L. Saw, T. Y. Wu, N. Suh, C. S. Yang, and A. N. Kong, no conflicts of interest.
[5] Y.H. and T.O.K. contributed equally to the project.
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Huang, et al. J Nutr. 2012;142(5):818-823.

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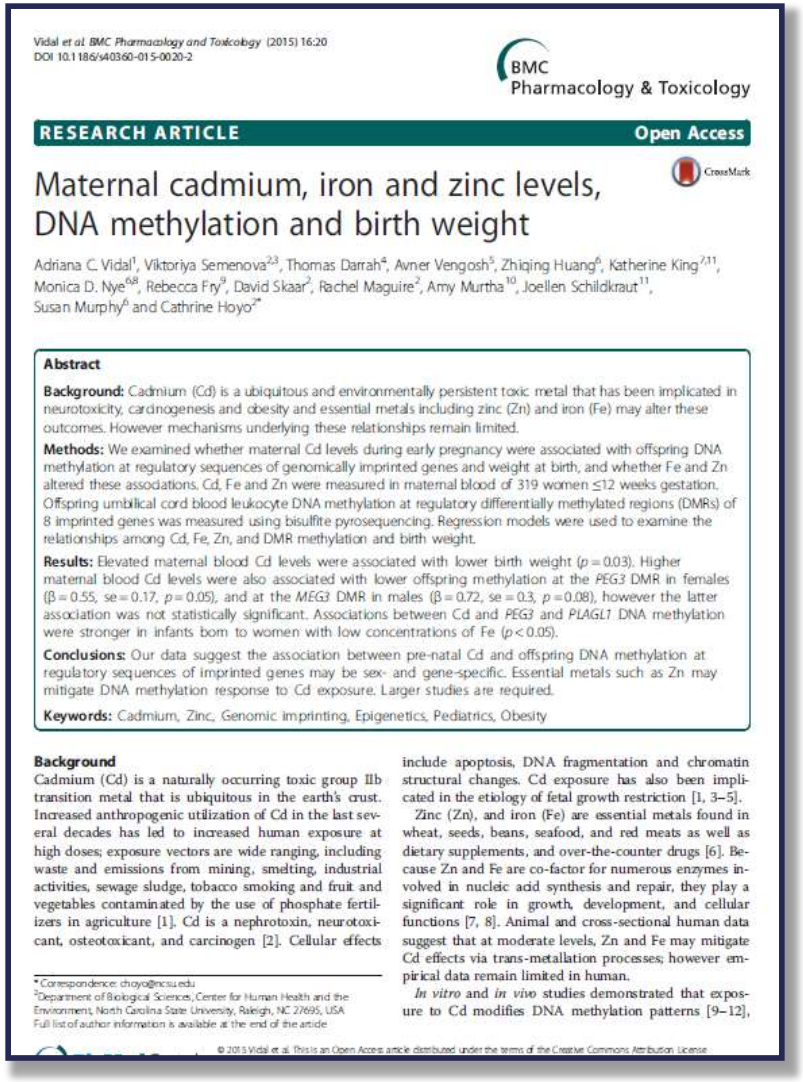
# Zinc Protective Against Cd-Induced DNA Methylation Changes

- Maternal Cd exposure in early pregnancy alters DNA methylation in offspring
- Associated with lower birth weight
- Higher maternal Zn concentrations may mitigate DNA methylation effects of Cd



Vidal et al. BMC Pharmacology and Toxicology (2015) 16:20
DOI 10.1186/s40360-015-0020-2

BMC Pharmacology & Toxicology

RESEARCH ARTICLE Open Access

## Maternal cadmium, iron and zinc levels, DNA methylation and birth weight

Adriana C. Vidal[1], Viktoriya Semenova[2,3], Thomas Darrah[4], Avner Vengosh[5], Zhiqing Huang[6], Katherine King[7,11], Monica D. Nye[6,8], Rebecca Fry[9], David Skaar[2], Rachel Maguire[2], Amy Murtha[10], Joellen Schildkraut[11], Susan Murphy[6] and Cathrine Hoyo[2*]

**Abstract**

**Background:** Cadmium (Cd) is a ubiquitous and environmentally persistent toxic metal that has been implicated in neurotoxicity, carcinogenesis and obesity and essential metals including zinc (Zn) and iron (Fe) may alter these outcomes. However mechanisms underlying these relationships remain limited.

**Methods:** We examined whether maternal Cd levels during early pregnancy were associated with offspring DNA methylation at regulatory sequences of genomically imprinted genes and weight at birth, and whether Fe and Zn altered these associations. Cd, Fe and Zn were measured in maternal blood of 319 women ≤12 weeks gestation. Offspring umbilical cord blood leukocyte DNA methylation at regulatory differentially methylated regions (DMRs) of 8 imprinted genes was measured using bisulfite pyrosequencing. Regression models were used to examine the relationships among Cd, Fe, Zn, and DMR methylation and birth weight.

**Results:** Elevated maternal blood Cd levels were associated with lower birth weight ($p = 0.03$). Higher maternal blood Cd levels were also associated with lower offspring methylation at the *PEG3* DMR in females ($\beta = 0.55$, se = 0.17, $p = 0.05$), and at the *MEG3* DMR in males ($\beta = 0.72$, se = 0.3, $p = 0.08$), however the latter association was not statistically significant. Associations between Cd and *PEG3* and *PLAGL1* DNA methylation were stronger in infants born to women with low concentrations of Fe ($p < 0.05$).

**Conclusions:** Our data suggest the association between pre-natal Cd and offspring DNA methylation at regulatory sequences of imprinted genes may be sex- and gene-specific. Essential metals such as Zn may mitigate DNA methylation response to Cd exposure. Larger studies are required.

**Keywords:** Cadmium, Zinc, Genomic imprinting, Epigenetics, Pediatrics, Obesity

**Background**

Cadmium (Cd) is a naturally occurring toxic group IIb transition metal that is ubiquitous in the earth's crust. Increased anthropogenic utilization of Cd in the last several decades has led to increased human exposure at high doses; exposure vectors are wide ranging, including waste and emissions from mining, smelting, industrial activities, sewage sludge, tobacco smoking and fruit and vegetables contaminated by the use of phosphate fertilizers in agriculture [1]. Cd is a nephrotoxin, neurotoxicant, osteotoxicant, and carcinogen [2]. Cellular effects include apoptosis, DNA fragmentation and chromatin structural changes. Cd exposure has also been implicated in the etiology of fetal growth restriction [1, 3–5].

Zinc (Zn), and iron (Fe) are essential metals found in wheat, seeds, beans, seafood, and red meats as well as dietary supplements, and over-the-counter drugs [6]. Because Zn and Fe are co-factor for numerous enzymes involved in nucleic acid synthesis and repair, they play a significant role in growth, development, and cellular functions [7, 8]. Animal and cross-sectional human data suggest that at moderate levels, Zn and Fe may mitigate Cd effects via trans-metallation processes; however empirical data remain limited in human.

*In vitro* and *in vivo* studies demonstrated that exposure to Cd modifies DNA methylation patterns [9–12],

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Vidal AC, et al. *BC Pharmacol Toxicol.* 2015;16:20.

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# Study: 2-Week Rotation Menu Option

| Nutrient | Week 1 Menu (Daily Average) | Week 2 Menu (Daily Average) | RDA (adult male/female) |
|---|---|---|---|
| **Folate** | 626 mcg | 644 mcg | 400/400 mcg DFE[1] |
| **Folic acid** | 0 mcg | 0 mcg | 400/400 mcg DFE[1] |
| **Vitamin B12** | 5.6 mcg | 10.15 mcg | 2.4/2.4 mcg |
| **Choline** | 414 mg[2] | 601 mg[2] | 550/425 mg |
| **Riboflavin (Vitamin B2)** | 1.9 mg | 2.4 mg | 1.3/1.1 mg |
| **Niacin (Vitamin B3)** | 22 mg | 26.8 mg | 16/14 mg |
| **Vitamin B6** | 2.8 mg | 3.1 mg | 1.3/1.3 mg |
| **Zinc** | 13.9 mg | 14 mg | 11/8 mg |
| **Magnesium** | 569 mg | 534 mg | 420/320 mg |
| **Omega-3 fatty acids** | 4.9 g | 5.7 g | |
| **Total calories** | 1743 kcal | 1792 kcal | - |
| **% calories from carbohydrates** | 30% | 27% | - |
| **% calories from fats** | 52% | 53% | - |
| **% calories from protein** | 18% | 20% | - |

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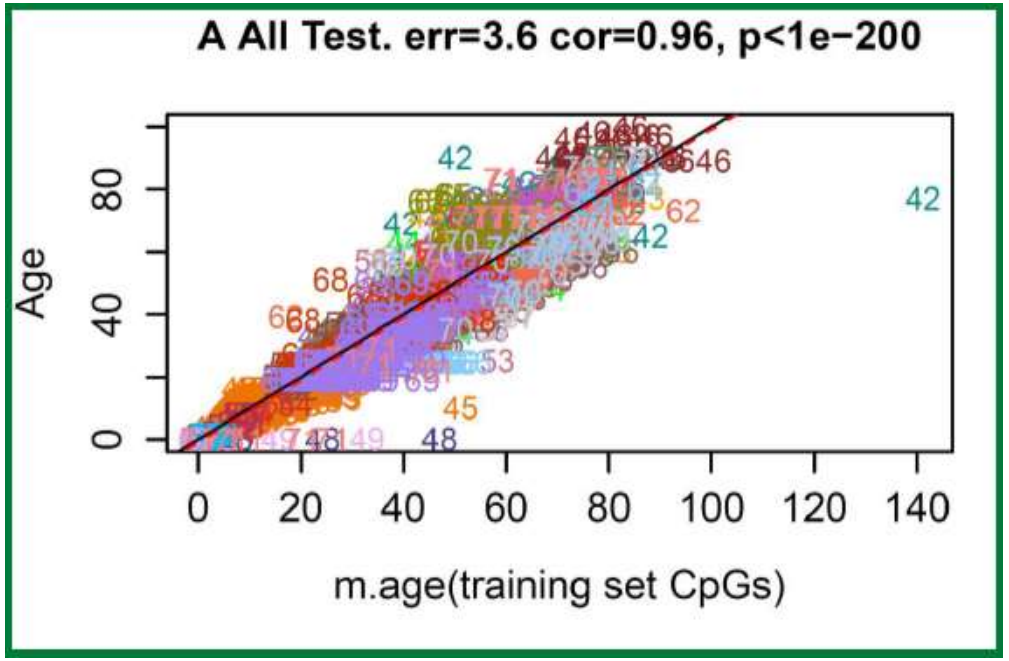
# Study Outcome Measures

- Patient-Reported Outcomes Measurement Information System-29 (PROMIS-29)
- Measure Yourself Medical Outcome Profile (MYMOP)
- National University of Natural Medicine Multi-system Symptom/Adverse Event Questionnaire
- Infinium Methylation EPIC Index by Illumina (850,000 CpG DNA sites/methylation)
- Methylation Profile, including SAMe:SAH ratio
- Folate Vitamer Panel
- Medical Symptom Questionnaire MSQ
- Methylation Pathway Profile (SNPs)

# Epigenetic Youthfulness – Can We Turn Back the Clock?

- Several epigenetic clocks, predicting biological age with strong accuracy
  - **Multi-tissue** 353 CpGs
  - **71 CpGs** for blood
  - **DNAm PhenoAge** 513 CpGs




"Human multi-tissue DNAm age estimator is the most accurate molecular biomarker of age across tissues" – Horvath 2018


Horvath S. *Genome Biol.* 2013;14(10):R115.
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Levine ME, et al. *Aging.* 2018;10(4):573-591.


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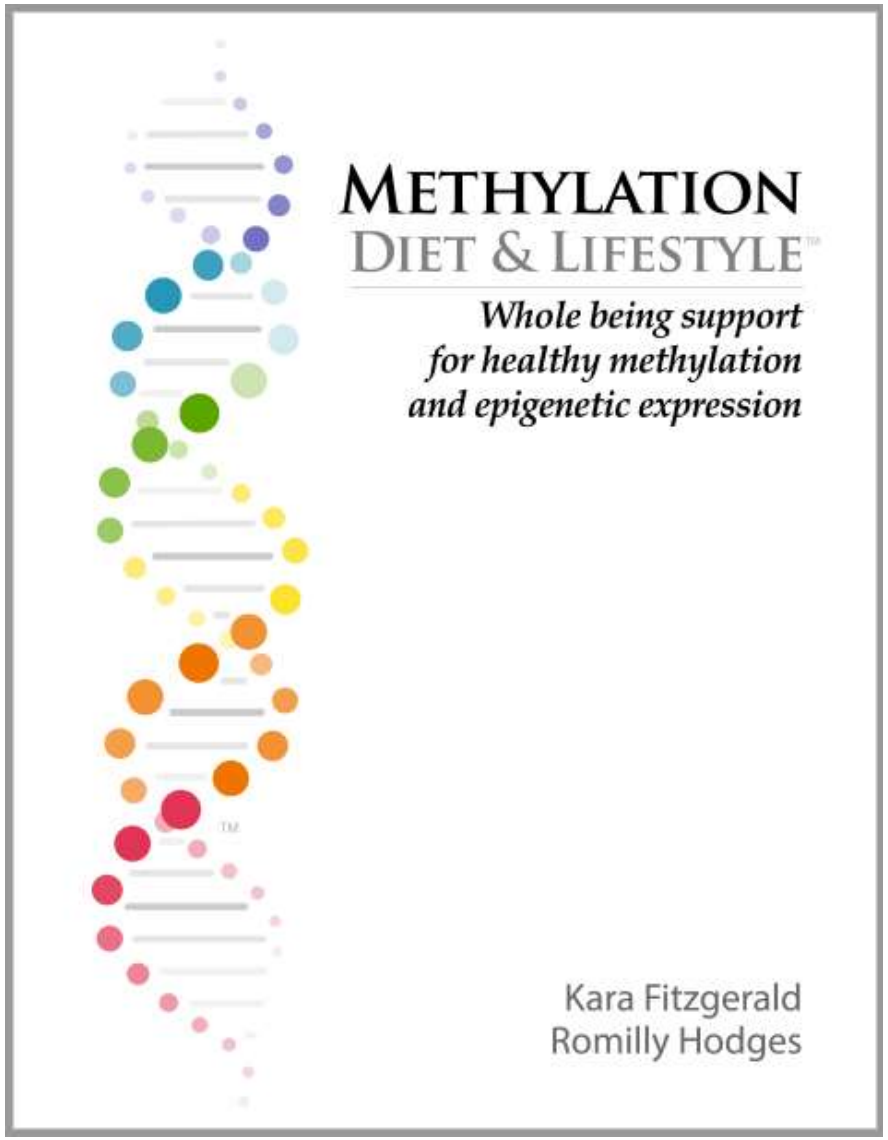
# Study Credits and Appreciation


- Metagenics: Brent Eck, Kirti Salunke, Kim Koch
- NUNM Helfgott Institute: Ryan Bradley, Emily Stack, Trina Soileau
- My team: Romilly Hodges, Samantha Bucur, Sophie Wallas-Rassmusen
- Study coaches: Janine Henkel, Despina Giannapoulou, Sally Logan, Melissa Twedt, Josette Herdell
- MDL study app: MBody360, Kari Thorstensen
- Advisors: Moshe Szyf, Terry Wahls
- Spiritual and scientific support: Jeff Bland, Michael Stone

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