

## **Genetic Testing for MTHFR and Beyond - An Introduction and Discussion for Herbalists**

The future of medicine is soon to look very different than it has ever looked before. The horizon of genetic research and genetic medicine is evolving at an incredible pace. This review of genetic testing is intended to summarize the past, present, and future of genomic medicine as it is being used by researchers, clinicians, and individuals seeking to learn more about genetic influences on disease. One area of genetic testing that has gained popular public interest has been looking at the Methylation cycle involving certain genes such as MTHFR. We will discuss this area of genetics and more as well continue to understand how the future of genetic testing may transform the healthcare system and how diseases will be prevented, diagnosed, and treated.

### **What is genetic testing?**

It is an evaluation of a person's genetic blueprint to identify areas that may help health practitioners more effectively diagnose, evaluate, or prepare treatment protocols for their patients. It also informs patients about various options that they may have in making healthcare choices. Genetic testing has existed since the 1960s and has evolved from testing for chromosomal abnormalities and mutations in single genes linked with developmental diseases to now being able to map the whole human genome and look at over 600,000 genetic single-nucleotide polymorphisms (SNPs). Currently, a major trend is to assess a limited collection of genetic variants for analysis of potential current or future health risk factors.

### **Regulation of Genetic Testing:**

Genetic Testing is broadly regulated by the FDA. The United States Department of Energy has guided the progress of genetic research through the Human Genome Project, which was completed in 2003 and is now being developed by the National Human Genome Research Institute (NHGRI). Genetic research is a booming area of global research and therefore has little collective oversight and agreement on the research, ethics, and development of the genome research.

### **Why is genetic testing being used?**

It can be used to help diagnose disease, identify risk factors for developing a disease or passing it onto children, and inform doctors on specific treatments (including drugs and nutraceuticals) best suited for individuals with certain genetic traits. There are many types of genetic tests used.

#### **Types of genetic testing outlined by the National Human Genome Research Institute:**

**Diagnostic testing** – Used to identify a specific disease that is making you ill based on a specific gene or chromosomal condition. Often used to confirm a suspected disease.

**Predictive and pre-symptomatic genetic tests** – Identifies genetic polymorphisms (SNPs) to evaluate potential risk for developing certain diseases or deficiencies.

**Carrier testing** – This testing is used to identify people who carry a gene for a certain disease that is known to be hereditary. Carriers may have no signs of the disease but could have the ability to pass that gene onto their children.

**Prenatal testing** – This form of testing is used during pregnancy to identify fetuses with genetic risk factors or diseases.

**Newborn screening** – Babies are tested 1-2 days after birth for genetic traits that are linked with specific diseases that may influence their development (i.e. phenylketonuria).

**Pharmacogenomic testing** – This is a new form of testing being used to assess how various drugs may be metabolized by a person based on their genetic make-up. It is intended to help guide health care providers in choosing drugs that will have a reduced risk of side effects for that individual.

**Research genetic testing** – It is used to understand how genes may contribute to health and disease so that in the future, others with that disease may benefit from more accurate diagnostic and therapeutic treatments. Participants must sign informed consent and may or may not receive results about their genetic information.

### **The Current State of Genetic Testing Counseling:**

There are many companies offering genetic testing assessment. Saliva Spit-Kits are sold in a “Direct-to-Consumer” market that does not require a doctor’s order. The popularity of these kits have sky rocketed in the last 10 years due to affordability and public interest both to determine ancestral roots but more especially in regards to health risk factors. In response to the massive influx of companies offering testing and counseling based on the results, the FDA and the GAO (Government Accountability Office) have cracked down on many illegal practices. In 2010, the GAO published a report that took an undercover look at 4 testing companies and 15 companies offering assessment (including the 4 testing companies) of individual genomes. Here is a summary from their report titled: “DIRECT-TO-CONSUMER GENETIC TESTS: Misleading Test Results Are Further Complicated by Deceptive Marketing and Other Questionable Practices”

“GAO purchased 10 tests each from four companies, for \$299 to \$999 per test. GAO then selected five donors and sent two DNA samples from each donor to each company: one using factual information about the donor and one using fictitious information, such as incorrect age and race or ethnicity. After comparing risk predictions that the donors received for 15 diseases, GAO made undercover calls to the companies seeking health advice. GAO did not conduct a scientific study but instead documented observations that could be made by any consumer. To assess whether the tests provided any medically useful information, GAO consulted with genetics experts. GAO also interviewed representatives from each company. To investigate advertising methods, GAO made undercover contact with 15 DTC companies, including the 4 tested, and asked about supplement sales, test reliability, and privacy policies. GAO again consulted with experts about the veracity of the claims.

GAO’s fictitious consumers received test results that are misleading and of little or no practical use. For example, GAO’s donors often received disease risk predictions that varied across the four companies, indicating that identical DNA samples yield contradictory results. As shown below, one donor was told that he was at below-average, average, and above-average risk for prostate cancer and hypertension. GAO’s donors also received DNA-based disease predictions that conflicted with their actual medical conditions—one donor who had a pacemaker implanted years ago to treat an irregular heartbeat was told that he was at

decreased risk for developing such a condition. Also, none of the companies could provide GAO's fictitious African American and Asian donors with complete test results, but did not explicitly disclose this limitation prior to purchase. Further, follow-up consultations offered by three of the companies failed to provide the expert advice that the companies promised. In post-test interviews with GAO, each of the companies claimed that its results were more accurate than the others'. Although the experts GAO spoke with believe that these tests show promise for the future, they agreed that consumers should not rely on any of the results at this time. As one expert said, "the fact that different companies, using the same samples, predict different directions of risk is telling and is important. It shows that we are nowhere near really being able to interpret [such tests]."

GAO also found 10 egregious examples of deceptive marketing, including claims made by four companies that a consumer's DNA could be used to create personalized supplement to cure diseases. Two of these companies further stated that their supplements could "repair damaged DNA" or cure disease, even though experts confirmed there is no scientific basis for such claims. One company representative even fraudulently used endorsements from high-profile athletes [Lance Armstrong and Michael Phelps] to convince GAO's fictitious consumer to purchase such supplements. Two other companies asserted that they could predict in which sports children would excel based on DNA analysis, claims that an expert characterized as "complete garbage." Further, two companies told GAO's fictitious consumer that she could secretly test her fiancé's DNA to "surprise" him with test results—though this practice is restricted in 33 states. Perhaps most disturbing, one company told a donor that an above average risk prediction for breast cancer meant she was "in the high risk of pretty much getting" the disease, a statement that experts found to be "horrifying" because it implies the test is diagnostic."

By 2013, many companies came closer and closer to advertising their test kits as predictive for various health problems. Popularity grew especially among people wondering if they were for diseases such as breast cancer or metabolic diseases. After years of several warnings, the FDA cracked down on the company, 23andMe, advising them that their kits could not be used as a "medical device" intended to treat, cure, prevent, or diagnose disease. This gained a lot of press attention but with some simple adjustments to their marketing, a shift in FDA regulatory pathways (its now a "novel device"), as well as a reduction in the price of their kits from \$299 to \$99, the company has been one of the fastest growing genetic testing companies. Not only that, the company is co-founded by Anne Wojcicki, the wife of Sergei Brin who is the founder of Google and has strong backing from pharmaceutical companies. The company has become a massive collection resource for genetic research and pharmacogenomic research. Just this year, 23andMe has hired several leading drug company researchers and recently announced its collaboration with Pfizer Inc. in January 2015 to start conducting genetic research based on their bulk data. What else can we expect from this massive data mining in the future?

Critics of the fast evolving power 23andMe is gaining over personal genetics argue that many people who voluntarily signed up for direct-to consumer genetic tests would not want to have their genetic information used by 23andMe for research and passed onto other 3<sup>rd</sup> parties (such as Pfizer). Journalist, Charles Seife wrote a critique of 23andMe in *Scientific American* in 2013 stating, "For 23andMe's Personal Genome Service is much more than a medical device; it is a one-way portal into a world where corporations have access to the innermost contents of your cells and where insurers and pharmaceutical firms and marketers might know more about your

body than you know yourself.” If his statement sounds overly paranoid, I believe it is better to be overly cautious in proceeding with too much trust in the goodwill and honesty of corporate promises and interests to protect the consumer. It is clear that the initial intentions of these corporations and institutes is to further the good of humankind but how that plays out 5, 10, 100 years from now is yet to be determined.

### **The future of genetic testing:**

In 2011, the NHGRI published the article, “Charting a course for genomic medicine from base pairs to bedside” in the journal, *Nature*, detailing their vision for how genetic research would evolve into genetic medical treatments. The goals were as follows:

- 1) Make genomic-based diagnostics routine so that within the next decade, complete genome diagnostic panels will be as routine as blood panels have become.
- 2) Define the genetic components of disease to understand the genetic variation involved in both rare genetic disorders and common diseases by studying over 1 million patients (the testing company 23 and Me announced this year they sold their one millionth test kit).
- 3) Create a comprehensive characterization of all cancer genomes to create stronger diagnostic and therapeutic treatments.
- 4) Develop practical systems for clinical genomic information interpretation making it easier for patient education and healthcare provider interpretation of the results as the research evolves.
- 5) Understand and evaluate the role of the human microbiome in health and disease to create correlations between specific diseases and the composition of the bacteria in the gut.

It is anticipated by some that future visits to the doctor will include regular saliva and stool samples to evaluate the individual patient’s genome and microbiome composition. This new generation of medicine is intended to benefit all of human kind by transforming the way that humans will be diagnosed and treated, and especially how diseases are prevented.

### **Genetically Modifying the Human Genome: “Epigenome Editing”**

A new technology called CRISPR, developed by Dr. Gersbach and colleagues, has the ability to directly target specific histones on genes to turn on and off specific genetic expressions. While the technology has promising potential, currently it has gained a lot of controversy as it has become internationally used without regulation. Gersbach and others have issued a worldwide moratorium on the use of CRISPR for human genome modifications until the technology is more precise and regulated by scientific and governmental organizations. In April 2015, scientists from China reported in the journal *Protein and Cell* that they used CRISPR to attempt to alter and correct the DNA of a non-viable human embryo with a mutation that causes beta thalassemia, a inherited blood disorder that can lead to death in the first 2 years of life if left untreated. In an interview with the scientists, *Nature* reported that the work was halted due to an inability to be close to 100% precise and that the technology is still developing.

## **INTRODUCTION TO GENETICS**

DNA, genes, and chromosomes are like a blueprint of life. Long molecules of DNA containing our genes are organized within chromosomes. Chromosomes are found in the nucleus of every cell and humans have 23 pairs of chromosomes. DNA has a spiral ladder type structure that has

nucleotides, called Single Nucleotide Polymorphisms (SNPs). These SNPs are the rung in the ladder structure. Variations in our genetic blueprints occur because of variations in our SNPs. The term “epigenetics” refers to how these genes are turned on or off based on external factors that influence genetic expression. How the DNA is read by a cell is influenced by its epigenetic expression.

### **Why are SNP's important:**

We receive genetic coding from our parents. Our genetic blueprint is based on the genetic information and variants passed down from. In the formation of the embryo, genetic differentiation occurs in such a way that accounts for why hair and eye color manifest. Much more than that, genetic variations occur that influence our response to chemicals, how our cells methylate, and how sensitive we may be to gluten, to name a few. The SNP can have either consistent genetic coding as both parents are it can have a variation in 1 or 2 ways. These variations are how geneticists account for risk factors in health.

### **Understanding Genetic Variation:**

Genetic variants are defined by being either homozygous (2 variations in the SNP) and heterozygous polymorphisms (1 variation in the SNP).

#### Heterozygous:

If both parents are heterozygous for the variant, then there is a:

- 25% chance child will be homozygous (2 variants)
- 25 % chance homozygous **normal**
- 50% chance heterozygous (1 variant)

#### Homozygous

- If both parents are homozygous for a variant then all children will be homozygous defective as well.
- If both parents are homozygous normal, then all children will be normal as well.

### **AN EXAMPLE OF A SNP: The MTHFR gene**

Remember a gene is like a ladder with many rungs (SNPs). On the MTHFR gene, the SNP at the position called C677 (a rung on the DNA ladder) For normal genetic expression, the SNP presentation includes two cytosine (C) nucleotides. With a variation in the SNP, there may be 1 cytosine (C) and 1 thymine (T) (this is called heterozygous) or 2 thymine (T) in place of the 2 normally occurring cytosine nucleotides (this is homozygous).

The gene MTHFR stands for Methyltetrahydrofolate Reductase which is the enzyme that converts folate (in the form of methyltetrahydrofolate) in the methylation cycle. Research shows that different MTHFR SNP variations can result in decreased function of those enzymes (Lynch, 2011):

- MTHFR A1298C heterozygous (1 copy): 20% reduction in function
- MTHFR A1298C homozygous (2 copies): 40% reduction in function
- MTHFR C677T heterozygous (1 copy): 30 to 40% reduction in function
- MTHFR C677T homozygous (2 copies): 60 to 80% reduction in function

- MTHFR A1298 heterozygous and C677T heterozygous (one copy of each): 70% reduction in function

SNP variations (or mutations) can result in a change of enzyme, receptor, or carrier protein function. This could account for differences in hair/eye color but also how people develop diseases or respond to drugs. Yet many SNPs lead to no difference in the body. These SNPs are the mechanism by which epigenetic changes are determined. It is important to note that it is the net sum of all the genes, not just the variation in a few genes. This is where the research is expanding its current understanding of risk factors by looking at patterns, not just isolated genes.

## **INTRODUCTION TO THE METHYLATION CYCLE**

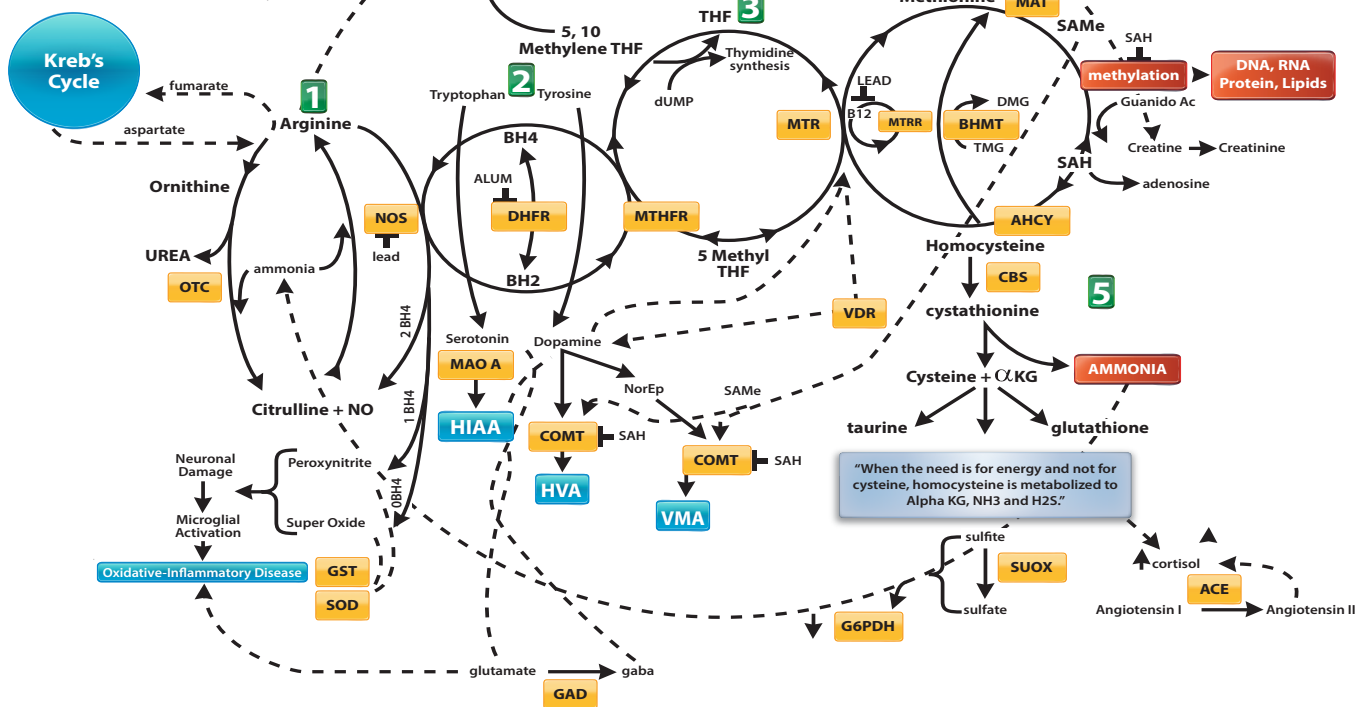
What is the Methylation Cycle?

This cycle is a scientific understanding of how biochemical pathways regulate cellular function through various enzymatic functions. It influences immune function, detoxification, DNA function, energy, mood, and overall health. Methylation refers to the addition of a methyl group (CH<sub>3</sub>) to another molecule (enzyme, RNA, DNA, toxin, protein).

## Methylation Pathway Cycles

- 1 Urea Cycle
- 2 Neurotransmitter (BH4) Cycle
- 3 Folate cycle
- 4 Methionine (Methylation) Cycle
- 5 Transulfuration Cycle

Mutations anywhere in this pathway can compromise critical functions in the body.



1. Urea Cycle
2. Neurotransmitter Cycle (BH4)
3. Folate Cycle
4. Methionine Cycle
5. Transulfuration Cycle

In regards to the methylation cycle, people can either be “OVER-METHYLATORS” (too much methyl) or “UNDER-METHYLATORS” (too little methyl). This is generally not based on one genetic variant but rather the net sum of several variants affecting the methylation cycle as a whole. Dr. William Walsh, considered to be the “father of methylation” collected a database of over 30,000 people and found that 60-70% of the people were considered to have “normal” methylation while 22% had “under-methylation” and 8% were “over-methylation”. There are many genetic factors that could contribute to this factor based on methylation cycle and also dietary excesses and deficiencies (such as histamine and folate). Interestingly in Walsh’s research, 70% of people with mental disorders had a disrupted methylation cycle (from either OVER and UNDER methylation). Psychiatrist, Courtney Snyder summarizes Dr. Walsh’s

findings on the symptoms, traits, and incidence of mental disorders in regards to methylation function here:

**UNDERMETHYLATION:**

Low methyl, high folate (or folic acid), and low neurotransmitter activity

Symptoms & Traits:

- Obsessive compulsive tendencies, ritualistic, perfectionistic, dietary inflexibility
- Very strong willed, competitive at sports
- Calm demeanor with high inner tension
- High accomplishment or family history of high accomplishment
- Seasonal allergies and high fluidity in eyes and mouth (remember - high histamine)
- Good response to serotonin reuptake inhibitors, ie. Prozac, Paxil, Zoloft, Celexa

The Incidence of Undermethylation:

- 98% of those on the Autism Spectrum
- 95% of those with Antisocial Personality Disorder
- 90% of those with Schizoaffective Disorder
- 85% of those with Oppositional - Defiance
- 62% of those with Anorexia
- 38% of those with Depression

**OVERMETHYLATION:**

High methyl, low folate, and high neurotransmitters

Symptoms and Traits:

- High artistic or musical ability
- Hyperactivity, high energy, verbose
- High empathy for others, good neighbor, noncompetitive in sports
- Food and chemical sensitivities, but absence of seasonal allergies and dry eyes and mouth
- Adverse reaction to SSRI's (Prozac, Paxil, Zoloft, Celexa, etc.) as well as Methionine and SAMe

The Incidence of Overmethylation:

- 64% of those with Panic Attacks
- 52% of those with Paranoid Schizophrenia
- 28% of those with ADHD
- 18% of those with Depression

These results indicate the important mechanisms involved in methylation and how this process is related to these mood disorders. It also gives us some insight into how various drug, supplement,



and herbal mechanisms might work to influence the neurotransmitters downstream (i.e. SAM-e). To test for methylation status, Whole Blood Histamine or SAME/SAH ratios can be measured.

Dr. Walsh also suggests the Niacin Flush Test. It can be done as follows:

1. Give 50mg of nicotinic acid on an empty stomach. If they flush, they are likely low in methyl and high in histamine.
2. Give 100mg of nicotinic acid on an empty stomach. If they slightly flush, they are likely balanced in histamine and methyl.
3. Give 150mg of nicotinic acid on an empty stomach. If no flush, they are likely over methylators.

### **Genetic Compensation**

It is well documented that based on individual genetic variability, dietary intervention can have important and sometimes dramatic effects, such as in the case of Celiac disease. Personalized medicine and nutraceutical supplementation is becoming a rapidly expanding field. The term, *Nutrigenomics* (or nutritional genomics) refers to the study of how genetic variants and dietary needs influence our health and how genetic variation influences nutrient function in the body. More specifically, *Nutrigenetics*, looks at how our genetics may influence our dietary preferences, habits, and how the body responds to food based on genetics. Understanding that humans have diverse genetic mechanisms affecting nutrient use in the body helps to explain why some people do really well on certain diets while others do not.

The personalized approach of nutrigenomics seeks to prevent and treat diseases with specific dietary protocols and supplementation based on that individual's genetics. Folate is a good example of how genetic variants can influence methylation function. This approach also focuses on informing people about foods they shouldn't eat such as gluten (HLA-DQ1 gene), lactose, and coffee. Studies examining people with variations in the CYP2A1 genotype found that coffee could influence the risk of hypertension and myocardial infarction because their ability to metabolize it was slower than others with normal genetic expression (Cornelis et al. 2006, Palantini et al. 2009). This is just one example of many more regarding how food may individually influence health. Evolving research is demonstrating that people are much more willing to comply with dietary recommendations based on these genetic findings when presented with the research correlations relating specifically to their genome.

Nutrigenomic approaches aim to identify patterns of genetic variants that may contribute to the acute or chronic symptomology that are presenting or could present. According to the NCMHD Center for Nutritional Genomics, "Dietary intervention based on knowledge of nutritional requirement, nutritional status, and genotype (i.e., "personalized nutrition") can be used to prevent, mitigate or cure chronic disease... Knowledge gained from comparing diet/gene interactions in different populations may provide information needed to address the larger problem of global malnutrition and disease." There are some practitioners and nutraceutical companies developing product lines that specifically target these genetic patterns based on DNA results and software, calculating the accumulation of risk factors in genetic variants. For example, some practitioners are taking into account how some of the genetic variants in genes such as MTHFR, MTRR, and CBS (see methylation map above) could effect the methylation cycle. The goal then is to "compensate" for those genetic variants with nutraceutical recommendations and formulations specific to these patterns.

As folate is a very important component in the methylation cycle, it is important to clarify the difference between the various forms of folate. Folic acid is a synthetic nutrient that is converted through a complex process in the body into folate. Unfortunately, some people lack the ability to convert this folic acid and the folic acid can accumulate in the body. High levels of unmetabolized folic acid have been associated with increased rates of cancer, prostate and colon cancer, ischemic heart disease, and could mask B12 deficiencies. Cognitive decline and anemia in older adults has been associated with high blood levels of folic acid and low B12 levels (Selhub et al. 2009). The form of folate that we get from our food is called Tetrahydrofolate (THF). Some of the best sources of THF are in chicken and calf's liver as well as in many legumes, dark green leafy vegetables, and brassicas. Supplements with folinic acid or 5-Methyltetrahydrofolate (5-MTHF) are also good sources of folate.

## PHYTOTHERAPY AND GENETICS

There are many options for how herbal medicine may play a role in the future of personalized genetic medicine. Through scientific reductionism, herbs can continue to be employed based on the various mechanisms involved in these genetic pathways. For example, many herbs are used for supporting SOD production, which is needed to inhibit the formation of Superoxide and eventually peroxynitrite.

SOD Support (Herbs known to increase endogenous SOD)

Jiaogulan (*Gynostemma pentaphyllum*)

Mulberry Fruit (*Morus alba*)

Burdock Root (*Arctium lappa*)

Cubeb Berries (*Piper cubeba*)

We also know many herbs that have support GABA production and may be useful for people with many variants in the GAD gene, which creates the enzyme that converts glutamate to GABA.

In the language of “compensation”, herbs could help to compensate for various genetic variations. Perhaps a person with allergies has many variables in their DAO and HNMT genes, which help with histamine regulation and breakdown. Maybe they need herbs that can help compensate for these variables. Unfortunately, there are many factors in this reductionist approach that limit the application of herbs. For one, genetic research regarding chronic disease is still in its infancy and so the web of complexity of genetic involvement is still being determined for actual pathological mechanisms. Additionally, herbs will not likely be tested in relationship to the web of genetic factors involved in contributing to that disease (at least in the near future).

The art of herbal medicine not only “treats the person and not the problem” but works to “match the plants with the person”. Identifying unique traditional assessment tools including pulse, tongue, lengthy intakes, family histories, diet, lifestyle, and more, help the herbalist to get the big picture and unite that with the indicated herbs. Genetic testing may be helpful as a diagnostic tool for identifying underlying issues affecting one's detoxification mechanisms or use of dietary

nutrients (i.e. folate). It is possible to unify with this new era of personalized genetic medicine. In fact, in a more technological way, it is trying to do what herbalists have been doing for centuries, treating people on a person to person basis.

## DISCUSSION

How will medicine evolve in the future? Will it truly become more democratized and altruistic, helping to save the lives of millions with the new era of personalized genetic medicine? Personalized medical technologies, like the FitBit, are the beginning of a trend that will continue to deepen our ability to assess risk of diseases. As medicine becomes more and more digitalized, how are herbalists adapting to this future in a way that preserves the resources of our planet and deepens our connection with all the elements of life on earth? How are we informing ourselves and training to understand these changes? What changes are needed within the community of herbal medicine? How do we maintain our traditional roots and continue to evolve in our diversity? Biologically speaking, a population of an organism with a more diverse gene pool (as well as gut microbiome gene pool) has a greater chance of surviving and flourishing than a population with limited variability in their genes. Let us remember our elders as well as our children seven generations from now and ask, what is required for the goodness of all beings during this evolution of change?

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