THE STANDARD

DDI DOCTOR'S DATA INC. SCIENCE + INSIGHT

News and scientific learning from Doctor's Data, Inc., a clinical laboratory providing accurate, innovative specialty testing for over 40 years.

Assessment of Genetic Variability through SNP Testing

BY ANDREA GRUSZECKI, ND

Identifying SNPs that influence health and disease risk allows clinicians to support their patients with appropriate lifestyle changes to maximize health and wellness. DDI's new DNA Methylation Pathway Profile allows clinicians to screen their patients for a variety of genetic changes (single nucleotide polymorphisms, or SNPs) that may impact the function of important biochemical processes such as methionine metabolism, detoxification, hormone balance and Vitamin D function.

Identifying SNPs that influence health and disease risk allows clinicians to support their patients with appropriate lifestyle changes to maximize health and wellness.

What is a SNP?

All humans carry mutations. Between inheritance and environment, we each carry between 100-200 mutations in our genetic code. Deoxyribonucleic acid (DNA) is the code of inheritance. DNA stores information as a code of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T). The order, or *sequence* of the bases provides the information needed to build proteins and maintain the body.

If the sequence of DNA bases is changed, the encoded information may change, too. If a change in the DNA sequence is not repaired, it becomes permanent and a mutation occurs in the genetic code. A mutation is not considered a SNP unless it is present in at least one percent of the population. SNPs are far more common than any known disease mutations. In addition, SNPs may or may not be located inside a gene, and some SNPs may be "silent" and cause no change in proteins or function. *Linked* SNPs act as indicators for active SNPs near their location. Linked SNPs may be associated with disease risk and drug responses. *Causitive* SNPs affect protein functions and are associated with disease process, risk or unusual drug responses. SNPs may change medication response either by changing the conformation of a receptor or by affecting detoxification or metabolic pathway functions. (SNPs are only one of several factors that may influence drug metabolism.)

The identification of SNPs and their impact on health and physiology is an ongoing area of research – the hope is that finding and studying these small variations in DNA will lead to better and more specialized medical interventions.

How are SNPs assessments used in practice?

In many cases the environment – diet, nutrition, toxicant exposures, stress – may modify the expression of genes and SNPs. Knowing that a patient carries certain SNPs helps the clinician help the patient. Disease risk may be modified by lifestyle changes and sluggish, inefficient biochemical pathways can be supported by diet and nutritional supplements to maximize enzyme efficiency and pathway functions. Supporting detoxification processes and reduction of toxicant exposures will further promote health and reduce disease risk.

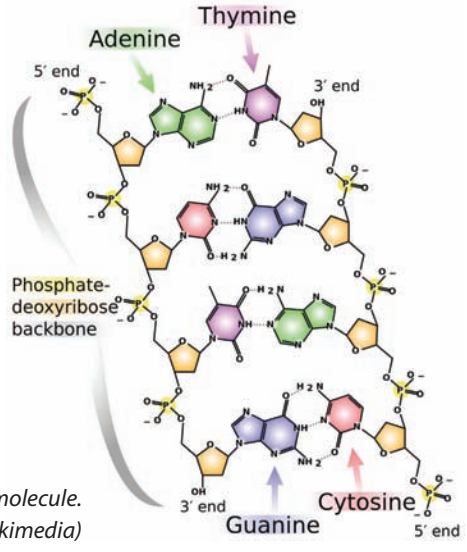

Figure 1. *The DNA molecule. (Courtesy of Wikimedia)*

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KNOWLEDGE SPOT

How does DNA oxidative damage (Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG)) relate to SNPs?

Assessment of Genetic Variabilty through SNP Testing

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Any SNP that affects protein function may have an impact; the impact may be very minor or very significant, depending on the functions and conformation of the proteins impacted. For example, a SNP that makes a change in the vitamin D receptor (VDR) may influence any of the following (and when combined with epigenetic factors result in health status):

- **DNA** (RNA translation accuracy)
- **mRNA** (molecular stability, splicing, conformation)
- **Protein** (protein stability and conformation, protein/protein interactions and functions
- **Cells** (transcriptional activity, cell growth inhibition, receptor function)
- **Phenotype** (serum parameters such as osteocalcin levels; intestinal calcium absorption, bone density, optimum vitamin D level, etc.)

+ Epigenetics (environment, diet, nutrition, toxicants, lifestyle)

= Health Status

Figure 2. *A SNP may impact function at molecular, cellular, tissue, organ or system levels of function.*

SNPS do not cause disease, but they may influence a patient's biochemistry and increase (or decrease) disease **risk**. Epigenetic factors, such as diet, nutrition, environment, toxicant exposures and lifestyle also affect disease risk, and may affect gene expression and the influence a SNP might have in an individual.

Which SNPs are important?

The new DDI DNA Methylation Pathway Profile includes a variety of SNPS known to influence many aspects of health including insulin sensitivity, bone health, cancer risk, cardiovascular health, detoxification capacity, fertility, mitochondrial function and metabolism, methylation capacity and neurotransmitter balance.

Any SNP that affects protein function may have an impact; the impact may be very minor or very significant, depending on the functions and conformation of the proteins impacted.

VDR (the vitamin D receptor) is a nuclear receptor that binds 1,25-dihydroxy vitamin D to form a functional signaling molecule that may influence up to a third of the human genome – genes, including those for xenobiotic detoxification, are regulated by vitamin D levels and VDR function.

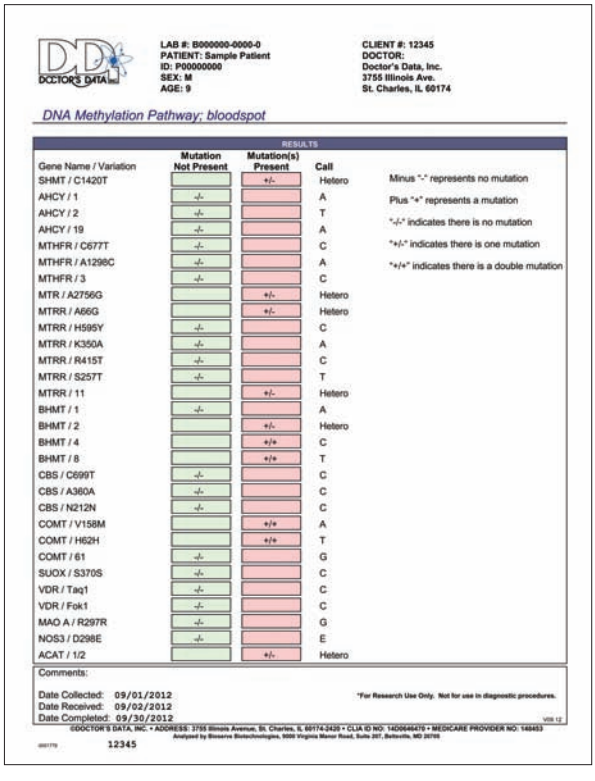
Detoxification is further influenced by:

BHMT - betaine-homocysteine methylatransferase SNPs affect an enzyme that catalyzes the transfer of a methyl group from betaine to homocysteine, which regenerates methionine. BHMT is also involved in choline oxidation processes, necessary to recycle betaine. The enzyme is found almost exclusively in liver and kidney tissues.

COMT – catechol-O-methyltransferase is found in nerve cells, and in the liver, kidneys and red blood cells. In the liver, COMT helps inactivate 2- and 4-hydroxyestradiols, along with other catecholamine hormones, prior to excretion in bile. COMT is also present in the CNS, where it degrades catecholamine neurotransmitters.

MAO A – monoamine oxidase type A detoxifies biological and xenobiotic amines (ammonia derivatives) through oxidative deamination. MAO A degrades neurotransmitters such as serotonin and dopamine, and the catecholamines epinephrine and norepinephrine in both the central and peripheral nervous systems. Exogenous amines from the diet may be toxic and must be metabolized; MAO enzymes are part of this process.

Normal methionine metabolism is a critical component of Phase II detoxification processes. The B-12 and folate-dependent transmethylation and B-6 dependent transsulfuration pathways convert homocysteine to cysteine. Cysteine is an important precursor in glutathione synthesis. In addition to BHMT, other enzymes required for normal methionine metabolism include:



LAB #: B000000-0000-0
PATIENT: Sample Patient
ID: P00000000
SEX: M
AGE: 9

CLIENT #: 12345
DOCTOR:
Doctor's Data, Inc.
3755 Illinois Ave.
St. Charles, IL 60174

DNA Methylation Pathway; bloodspot

RESULTS

Gene Name / Variation	Mutation Not Present	Mutation(s) Present	Call
SHMT / C1420T		+/-	Hetero
AHCY / 1	-/-		A
AHCY / 2	-/-		T
AHCY / 19	-/-		A
MTHFR / C677T	-/-		C
MTHFR / A1298C	-/-		A
MTHFR / 3	-/-		C
MTR / A2756G		+/-	Hetero
MTRR / A66G		+/-	Hetero
MTRR / H595Y	-/-		C
MTRR / K350A	-/-		A
MTRR / R415T	-/-		C
MTRR / S257T	-/-		T
MTRR / 11		+/-	Hetero
BHMT / 1	-/-		A
BHMT / 2		+/-	Hetero
BHMT / 4		+/+	C
BHMT / 8		+/+	T
CBS / C699T	-/-		C
CBS / A360A	-/-		C
CBS / N212N	-/-		C
COMT / V158M		+/+	A
COMT / H62H		+/+	T
COMT / 61	-/-		G
SUOX / S370S	-/-		C
VDR / Taq1	-/-		C
VDR / Fok1	-/-		C
MAO A / R297R	-/-		G
NOS3 / D298E	-/-		E
ACAT / 1/2		+/-	Hetero

Minus "-" represents no mutation
Plus "+" represents a mutation
"-/-" indicates there is no mutation
"+/-" indicates there is one mutation
"+/+" indicates there is a double mutation

Comments:

Date Collected: 09/01/2012
Date Received: 09/02/2012
Date Completed: 09/30/2012

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Figure 3. *DDI's new DNA Methylation Pathway Profile Results Report*

SNPS do not cause disease, but they may influence a patient's biochemistry and increase (or decrease) disease risk.

AHCY – adenosylhomocysteinase converts S-adenosylhomocysteine to homocystiene

CBS – cystathionine beta-synthase catalyzes the transsulfuration reaction between serine and homocysteine, which produces the intermediate product cystathionine.

MTHFR – methylenetetrahydrofolate reductase converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate a necessary cofactor for the retroconversion of homocysteine to methionine

MTR – methionine synthase catalyzes the re-methylation of homocysteine to methionine using methyl-B-12 as a cofactor

MTRR – methionine synthase reductase supports MTR function by regenerating methyl-B-12

SHMT – serine hydroxymethyltransferase catalyzes the interconversion of glycine and serine (CBS requires serine to transform homocysteine) [see Figure 5]

SUOX – sulfite oxidase is found in the mitochondria; it oxidizes sulfite (SO_3^{2-}) from the transsulfuration cycle or from the diet, to sulfate (SO_4^{2-}).

SNPs affecting detoxification and methylation become even more important if a patient has been exposed to the toxicants such as mercury, lead and bisphenol A (BPA). Lead and BPA inhibit the function of methyltransferases, and mercury inhibits MTR. Methylation is an essential step in the detoxification and elimination of arsenic and other xenobiotics. Disruption of methionine metabolism may also affect the amount of glutathione available in cells and blood.

Follow-up Tests – *Methylation Profile; plasma* and *Vitamin D (25OH D2 & D3)*

The greatest difficulty in interpreting a SNPs test is knowing how the DNA genotype is expressing through the *phenotype* – the physical form and function. For example a study of genetic markers and their effects on educational achievement found that the genetic markers with the strongest known effects could each only explain 0.02% of the difference in achievement. Another study found that the SNP with the largest effect on human height accounts for only 0.40% of the variation. However, a SNP like MTHFR may influence methylation by 30-60%. Functional tests, combined with evaluation of the patient's symptoms and responses to intervention, are also necessary to assess the influence of known SNPs. DDI's Methylation Profile is one such test; it provides a direct assessment of several major metabolites that may indicate SNP influence. The Methylation Profile is a great follow-up test if SNPs affecting detoxification or methionine metabolism are identified.

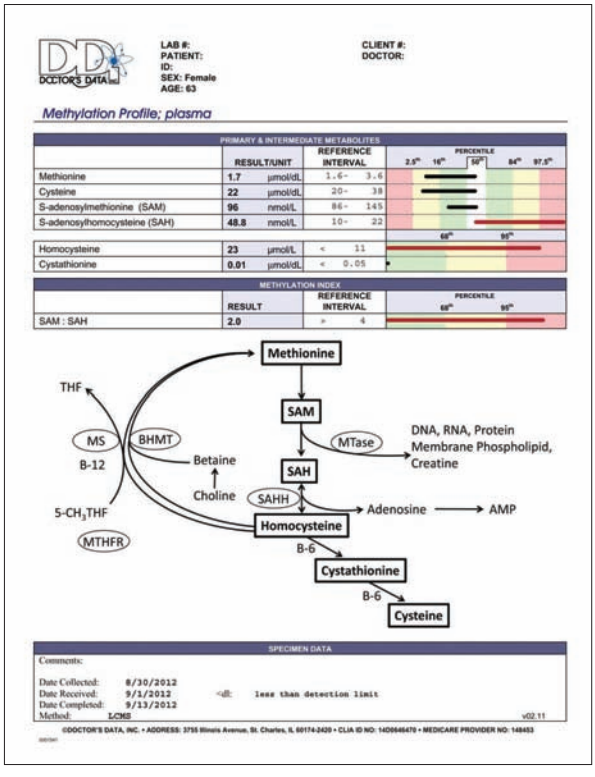

Figure 5. *DDI's Methylation Profile is a direct assessment of SNP influence.*

VDR (the vitamin D receptor) SNPs decrease receptor efficiency, which may result in "sluggish" detoxification processes. An inefficient receptor requires a sufficiency of substrate. Vitamin D levels may be monitored with DDI's Vitamin D (25OH D2 & D3) test.

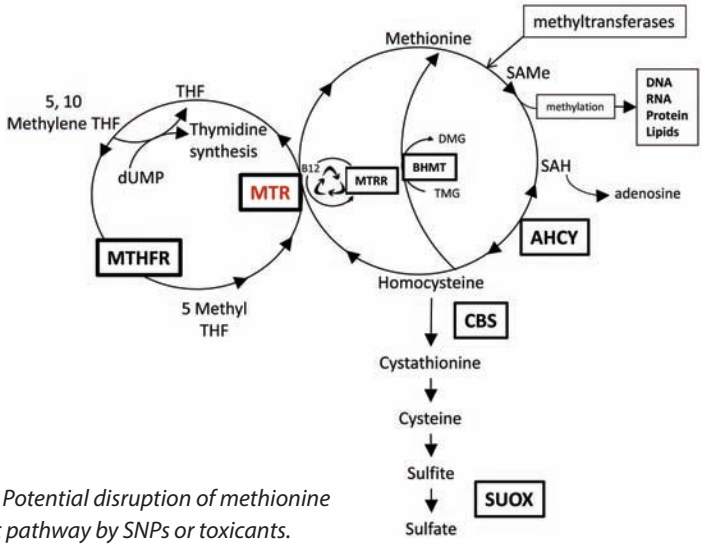

Figure 4. *Potential disruption of methionine metabolic pathway by SNPs or toxicants.*

Conclusion

DDI's new DNA Methylation Pathway Profile allows clinicians to screen their patients for a variety of genetic changes (single nucleotide polymorphisms, or SNPs) that may impact the function of important biochemical processes such as methionine metabolism, detoxification, hormone balance and Vitamin D function. Identifying SNPs that influence health and disease risk allows clinicians to support their patients with appropriate lifestyle changes to maximize health and wellness.

KNOWLEDGE SPOT

How does DNA oxidative damage (Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG)) relate to SNPs?

SNPs that decrease the efficiency of detoxification pathways may increase oxidative stress. Further, methylation of DNA is one of the ways that gene expression is regulated; a gene may be turned on or off via DNA methylation. Methionine metabolism (methylation) and detoxification pathways (transsulfuration) may be compromised by SNPs such as MTHFR, MTR, MTRR, SHMT, etc. (See the article "Accessing Genetic Variability through SNP Testing" in this issue.)

SNP-associated changes in gene expression may result in excessive oxidative stress. Increased oxidative stress causes damage to mitochondrial and nuclear DNA. Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) measured as a first morning void sample, is an excellent biomarker for oxidative stress and the DNA damage it causes. 8-OHdG levels have been associated with numerous pathological processes in the body; it may be used to monitor the effects of comprehensive detoxification and anti-oxidant therapies.

How does the Hepatic Detox Profile relate to SNPs?

The Hepatic Detox Profile may provide important information regarding the phenotypic expression of SNPs associated with detoxification processes. The efficient detoxification of toxicants and toxins is a vital component of good health. If the formation of functionalized xenobiotics (phase I products) exceeds phase II capacity, more potent toxins may accumulate in the body and impair health.

There is an enormous variety of enzymes involved in hepatic phase I detoxification; one study cataloged 906 SNPs in the 27 genes that encode the cytochrome P450 enzymes and aldehyde dehydrogenases. Many of these SNPs are being evaluated for their effects on physiology, detoxification and disease risk. Methionine metabolism and phase II detoxification processes may be influenced by other SNPs such as MTHFR, MTR, MTRR, SHMT, etc. (See the article "Accessing Genetic Variability through SNP Testing" in this issue.)

The Hepatic Detox Profile provides a functional assessment of phase I and phase II liver detoxification activities. Elevated levels of urinary D-glucaric acid indicate increased phase I induction, which indicates exposure to toxicants or toxins. Urinary mercapturic acids indicate phase II activity and capacity. An accurate assessment of phase I/phase II activities facilitates clinical decisions regarding nutrition and other liver supportive interventions.

BEHIND THE SCENES AT DDI

Meet Jason Lockhart Sales Manager

Jason Lockhart joined the team at Doctor's Data in May 2013, and currently serves as the Sales Manager. He brings to DDI an extensive scientific background that allows a much more consultative approach to his salesmanship. Jason received his Bachelor's of Science Degree in Biology at the University of Alabama at Birmingham (UAB). While pursuing his bachelor's degree, he was awarded an NIH fellowship and placement in the Department of Behavioral Neurobiology where he focused on transglutaminase activity in Alzheimer's disease. His work was later published in the peer-reviewed journal Brain Research. Upon college graduation, Jason further expanded his scientific expertise in the Department of Physiology and Biophysics at UAB where he studied the sensitivity of epithelial sodium channels in cystic fibrosis. This work was published in both The Journal of Biological Chemistry and The American Journal of Physiology and Cell. Jason eventually moved from academia to industry research where he led a team of scientists at Invitrogen™ that provided microarray gene expression analysis via the Affymetrix® GeneChip. He transitioned into sales at Invitrogen™ where he supported scientists in the areas of PCR cloning, transfection assays, DNA/RNA purification, and cell culture. Jason comes to Doctor's Data after serving five years as sales manager at Wellness Pharmacy®, a nationally accredited compounding pharmacy located in Birmingham, AL. Jason is an active member of the Autism Society of Alabama Junior Board, and is also a founding member of the Alabama Autism Assistance Program's Board of Directors. Doctor's Data is especially pleased to be able to associate itself with the background and talent that Jason brings to our endeavors.

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