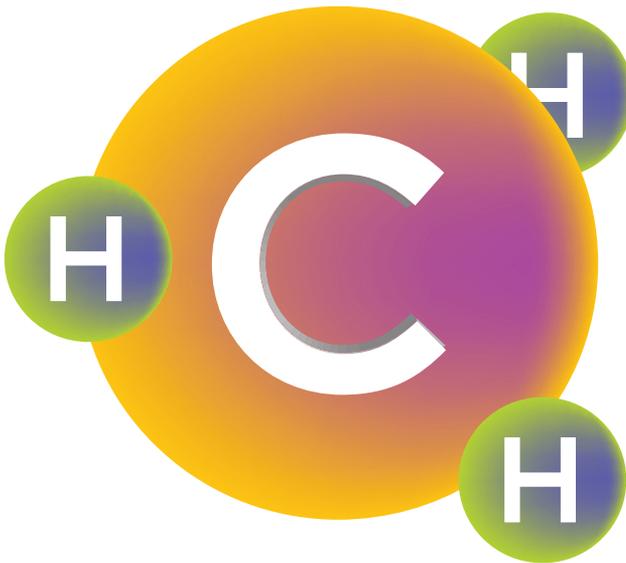


A Guide To Nutrigenomic Testing

Dr. Amy Yasko



Disclaimer: The information is presented by independent medical experts whose sources of information include studies from the world's medical and scientific literature, patient records and other clinical and anecdotal reports. The publisher, author and/or experts specifically cited in this publication are not responsible for any consequences, direct or indirect, resulting from any reader's action(s). This booklet is not intended to be a substitute for consultation with a health care provider. You, the reader, are instructed to consult with your personal health care provider prior to acting on any suggestions contained herein. The purpose of this guide is to educate the reader. The material in this booklet is for informational purposes only and is not intended for the diagnosis or treatment of disease.

© Copyright 2008 Genetic Profiling Systems, LLC

Advanced nutrigenomic testing

The nutrigenomic testing offered by Genetic Profiling Systems, LLC focus on genetic weaknesses in pathways that are involved in generating methyl groups in the body. These central pathways are particularly important to nutrigenomic screening. Genetic weaknesses which hinder the methylation cycle can lay the groundwork for further assault of environmental and infectious agents which may result in a wide range of increased risk for additional health conditions including diabetes, cardiovascular disease, thyroid dysfunction, neurological inflammation, chronic viral infection, neurotransmitter imbalances, atherosclerosis, cancer, aging, neural tube defects, Alzheimer's disease and autism.

As a result of decreased activity in the methylation pathway due to mutations, there is a shortage of methyl groups in the body for a variety of important functions. Methyl groups are "CH₃" groups that are moved around in the body to turn on or off genes. There are several particular sites in this pathway where a blockage can occur as a result of genetic weaknesses.

Supplementation with appropriate foods and nutrients can bypass these mutations to allow for restored function of the pathway.

By looking at diagrammatic representations of the methylation pathway and relating the effects of genetic polymorphisms (variations) to biochemical pathways, it is possible to draw a personalized map for each individual's imbalances which may impact upon their health. By identifying the precise areas of genetic fragility, it is then possible to target appropriate nutritional supplementation of these pathways to optimize the functioning of these crucial biochemical processes.

What is nutrigenomic testing?

UC Davis has an entire website (<http://nutrigenomics.ucdavis.edu/>) dedicated to promoting the new science of nutritional genomics. This site is a provider of news, information, and commentary on current developments and breakthroughs in fields of nutrition, genomics, and health. This website is intended for anyone and everyone who wants to learn more about the way diet and genetics can influence the delicate balance between human health and disease.

Much of the content for this site comes from the Center of Excellence for Nutritional Genomics grant (P60MD00222) sponsored by the National Center for Minority Health and Health Disparities (NCMHD) at the National Institutes of Health (NIH). The Center director is Dr. Raymond L. Rodriguez, professor of molecular biology at the University of California, Davis. Dr. Bertram Lubin of the Children's Hospital Oakland Research Institute, is the Center's associate director.

Developed as a multi-disciplinary research collaboration between Molecular/Cellular Biology at UC Davis, as well as the USDA Western Human Nutrition Research Center among others, the information on this site defines nutrigenomics as

“The science of nutrigenomics seeks to provide a molecular understanding for how common dietary chemicals (i.e., nutrition) affect health by altering the expression and/or structure of an individual's genetic makeup. The conceptual basis for this new branch of genomic research can best be summarized by the following Five Tenets of Nutrigenomics:

- Under certain circumstances and in some individuals, diet can be a serious risk factor for a number of diseases.
- Common dietary chemicals can act on the human genome, either directly or indirectly, to alter gene expression or structure.
- The degree to which diet influences the balance between healthy and disease states may depend on an individual's genetic makeup.
- Some diet-regulated genes (and their normal, common variants) are likely to play a role in the onset, incidence, progression, and/or severity of chronic diseases.
- 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.

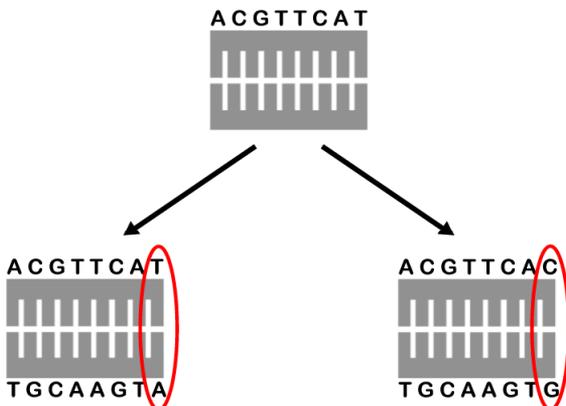
Just as pharmacogenomics has led to the concept of “personalized medicine” and “designer drugs”, so will the new field of nutrigenomics open the way for “personalized nutrition.” In other words, by understanding our nutritional needs, our nutritional status, and our genotype, nutrigenomics should enable individuals to manage better their health and well-being by precisely matching their diets with their unique genetic makeup.”

What are SNPs?

While the first step that emerged from the Human Genome Project has been to identify genes associated with a particular disease, the next step is to use this information to look for the presence of these identified disease causing genes in an individual person. Rather than looking at complete gene profiles, it is also possible to look at particular changes in the “spelling” of your DNA in only specific areas of interest. In this way, you can more quickly get a sense of known genetic weaknesses. Companies that offer this service enable you to look at genes of interest that may affect your susceptibility to heart disease, inflammation, detoxification or simply your ability to absorb nutrients. These tests are available using saliva samples, cheek swabs as well as blood samples.

In order to find relationships between genetic changes and the susceptibility to disease this testing is done utilizing single nucleotide polymorphisms, otherwise known as SNP's (pronounced snips). This process systematically compares genomes of those individuals with a disease to the corresponding DNA of a “normal” population. To identify a SNP is a very arduous and time consuming process as there may be 400 or more genes in a shared region, making it difficult to identify changes and trends. However, once it has been identified, making practical use of this information is quick and straightforward.

Single Base Difference SNP



Comprehensive Methylation Panel (30SNPs):

This Panel includes SNPs for a number of genes that are integral to the methylation pathway. There are multiple SNPs that will be analyzed for each of the relevant genes. By analyzing patterns that are seen as a function of SNPs on nutritional status, Genetic Profiling Systems, LLC has been able to discern relationships between genes that may not have been intuitively obvious. This was the case for the interaction between the VDR Taq SNPs and the COMT gene as well as the impact of the MTHFR A1298C mutation and its interaction with CBS C699T.

Comprehensive Methylation Panel

GENE NAME	VARIATION
ACE	Del 16
ACAT1-02	N/A*
AHCY-01	N/A*
AHCY-02	N/A*
AHCY-19	N/A*
BHMT-01	N/A*
BHMT-02	N/A*
BHMT-04	N/A*
BHMT-08	N/A*
CBS	A360A
CBS	Y233Y (C699T)
COMT	H62H
COMT	V158M
COMT-61	P199P
MAO A	R297R
MTHFR	A222V (C677T)
MTHFR	E429A (A1298C)
MTHFR-03	P39P
MTR	A919G (A2756G)
MTRR	H595Y
MTRR	K350A
MTRR	R415T
MTRR	S257T
MTRR	A919G (A66G)
MTRR	11
NOS	D298E
SHMT	C1420T
SUOX	S370S
VDR	Fok
VDR	Taq

How to Read the Nutrigenomic Test

There are two copies of each gene that we are looking at in the profile. One copy comes from each parent. When both copies have a particular SNP or mutation, in other words when both copies are identical, either + or - it is called “**homozygous**”. When you have one copy that is + for the change and the other is - for the change it is called “**heterozygous**.” The + and - designations themselves refer to whether or not the gene has a change from what is considered the **norm**. If there is a change from the **norm** then it is termed as +. No change is designated by a - sign. The definition of what is the **norm** can vary from lab to lab. It will depend in part on what the lab uses as a reference database. This is why you are also given the **call letter** for each SNP. The call letter tells you what base was seen by the lab at a precise location on the gene.

For instance, when we look at the MTHFR gene, and the particular SNP we are interested in is the C677T. The lab is looking at position 677 in the DNA for a change from a C to a T. If there is a change then the call letter will show a T and the designation will be +. If there is no change then the call letter will be C and the designation will be -. If a different lab considers the change to a T as the **norm**, then they might show a T in position 677 as a -, as their reference database may feel that it is normal to have a T in that position. This is why the call letter is so important. In cases where there is a discrepancy from one lab to another the actual call letter will let you know what base was seen at a precise location. This enables you to be certain that tests run from different labs gave the same actual experimental result even if their reference standard for a **norm** was different.

Assume the following scenario as an example, I am 5 feet 3 inches tall. That is equivalent to the call letter in this analogy. It is a precise measurement. If I compare my height to that of my children I am taller than one of my girls, the same height as one and shorter than one of them. Using my daughters as a reference base I would consider my height average. However if I compare my height to that of the rest of the population of Bethel I am actually quite short. Many of the individuals in our town are very tall. Using the + + and - - designations I might be + - if my reference norm was my own girls or + + if the reference database was the town of Bethel. In either event my height, by precise measurement is 5’ 3” and that will not change. Knowing the lab value allows me to compare my height to other databases in the future.

ACE (angiotensin converting enzyme):

Changes can occur that affect the activity of the ACE gene that can lead to elevated blood pressure. In animal studies imbalances in this pathway were also correlated with increased anxiety and decreases in learning and memory. Increased ACE activity can also throw off the essential mineral balance in the your system due to decreased excretion of sodium in the urine and increased excretion of potassium in the urine. This reaction is also tied to the stress response such that situations of chronic stress can result in additional sodium retention and increased potassium excretion. This excess potassium is excreted provided that the kidneys are functioning properly. In the event that kidney function is compromised, it can lead to the retention of potassium in the body. ACE is a deletion, it is not a SNP. As a consequence it does not associate in the same manner that the other single nucleotide polymorphisms (SNP) on this panel do, so the inheritance pattern of the ACE deletion may not distribute in the same manner as single base changes.

ACAT 102 (acetyl coenzyme A acetyltransferase):

ACAT plays a role in cholesterol and other lipid balance in the body, helping to prevent the accumulation of excess cholesterol in certain parts of the cells in the body. ACAT is also involved in energy generation in the body. IT is involved in helping to allow protein, fats and carbohydrates from food to be converted into an energy form that can be used by your body. In addition, lack of ACAT may also cause a depletion of B12, which is needed for the long route around the methylation cycle.

AHCY 1,2,19 (S adenosylhomocysteine hydrolase):

AHCY is the enzyme that converts s adenosyl homocysteine (SAH) to adenosine and homocysteine. Decreased AHCY activity should lead to lower levels of homocysteine. Studies using animals with no CBS function suggests that the relationship between CBS enzyme activity, homocysteine levels and SAH and SAME levels may not be as simple or predictable as one might expect from pathway diagrams. In addition, both SAH and SAME have been found to affect CBS activity and SAH is known to inhibit methyltransferase reactions. Also the level of homocysteine affects SAH levels such that higher levels of homocysteine can increase SAH. Clearly, the relationship between these intermediates appears to be complex. (PNAS 2008, 103:17; Theoretic Biology and Medical Modelling 2008, 5:8;

JBC 2002, 277:41; Jnutrition 2002,132) It may be especially important for those with AHCY mutations to monitor amino acid levels in order to balance the effects of AHCY mutations, CBS up regulations and other methylation cycle mutation on the system.

BHMT 1,2,4,8 (betaine homocysteine methyltransferase):

The product of this gene is central to the ‘short cut’ through the methylation cycle, again helping to convert homocysteine to methionine. The activity of this gene product can be affected by stress, by cortisol levels and may play a role in ADD/ADHD by affecting norepinephrine levels.

CBS C699T, A360A (cystathionine-beta-synthase):

The CBS enzyme basically acts as a gate between homocysteine and the downstream portion of the pathway that generates ammonia in the body. The types of CBS mutations that are identified on this SNP panel cause this “CBS gate” to be left open, this ‘open gate’ is not a neutral situation. The “open gate” can allow support that is added for the rest of the methylation pathway to be depleted, including any B12 that is used to address MTR and MTRR mutations. While there are some positive end products that are generated via the downstream portion of the pathway such as glutathione and taurine, there are also negative byproducts such as excess ammonia and sulfites. By virtue of increased CBS activity, these sulfur groups that were complexed as part of the methylation cycle can now be released into the system as sulfites which are toxic to the body and put an additional burden on the SUOX gene product.

COMT V158M, H62H, 61 (catechol-O-methyltransferase):

A primary function of this gene is to help to break down dopamine. Dopamine is a neurotransmitter that is recognized for its role in attention, as well as reward seeking behavior. Dopamine helps to cause pleasurable feelings that aid in reinforcing positive behaviors and motivating individuals to function in certain reward gaining activities. COMT is also involved in the breakdown of another neurotransmitter, norepinephrine. The balance between norepinephrine levels and dopamine levels has been implicated in ADD/ADHD; in addition, dopamine levels are important in conditions such as Parkinson’s disease. COMT is also involved in the proper processing of estrogen in the body. Sensitivity to pain has recently been found to be correlated with COMT activity, such that COMT + + individuals may be more sensitive to pain. The COMT V158M -- would indicate that a maximum level of support is needed for this SNP.

MAO A R297R (monoamine oxidase A):

MaoA is involved in the breakdown of serotonin in the body. Like dopamine, serotonin is another neurotransmitter in the body. It is involved with mood, and imbalances in serotonin levels have been associated with depression, aggression, anxiety and OCD behavior. Since Mao A is inherited with the X chromosome and is considered a dependent trait it may not show standard inheritance characteristics in males. Since the X chromosome in males can only come from the mother, this means that the fathers Mao A mutations (or lack there of) does not play a role in their son's Mao A status. For females, since one X chromosome is inherited from each parent, the genetics tend to reflect the Mao A status of both parents.

MTHFR A1298C, C677T, 3 (methylenetetrahydrofolate reductase):

The MTHFR gene product is at a critical point in the methylation cycle. It helps to pull homocysteine into the cycle, serving to aid in keeping the levels in a normal healthy range. Several mutations in the MTHFR gene have been well characterized as increasing the risk of heart disease, as well as cancer, and may play a role in the level of the neurotransmitters serotonin and dopamine.

MTR A2756G/MTRR A66G, H595Y, K350A, R415T, S257T, 11 (methionine synthase/ methionine synthase reductase):

These two gene products work together to regenerate and utilize B12 for the critical long way around the methylation pathway, helping to convert homocysteine to methionine. High levels of homocysteine have been implicated as risk factors in a number of health conditions including heart disease as well as Alzheimer's disease. As is the case for COMT and VDR Bsm/Taq, the MTR and MTRR composite status is also important. Mutations in MTR can increase the activity of this gene product so that it leads to a greater need for B12 as the enzyme is using up B12 at a faster rate. The MTRR helps to recycle B12 for use by the MTR. Mutations that affect its activity would also suggest a greater need for B12.

NOS D298E (nitric oxide synthase):

The NOS enzyme plays a role in ammonia detoxification as part of the urea cycle. Individuals who are NOS + + have reduced activity of this enzyme. NOS mutations can have additive effects with CBS up regulations due to the increased ammonia that is generated by the CBS up regulations

SHMT C1420T (serine hydroxymethyltransferase):

This gene product helps to shift the emphasis of the methylation cycle toward the building blocks needed for new DNA synthesis and away from the processing of homocysteine to methionine. While DNA building blocks are important, mutations which affect the ability to regulate this gene product and interfere with the delicate balance of the methylation cycle may cause accumulations in homocysteine as well as imbalances in other intermediates in the body.

SUOX S370S (sulfite oxidase):

This gene product helps to detoxify sulfites in the body. Sulfites are generated as a natural byproduct of the methylation cycle as well as ingested from foods we eat. Sulfites are sulfur based preservatives that are used to prevent or reduce discoloration of light-colored fruits and vegetables, prevent black spots on shrimp and lobster, inhibit the growth of microorganisms in fermented foods such as wine, condition dough, and maintain the stability and potency of certain medications. Sulfites can also be used to bleach food starches, to prevent rust and scale in boiler water that is used to steam food, and even in the production of cellophane for food packaging. The Food and Drug Administration estimates that one out of a hundred people is sulfite-sensitive, and five percent of those also suffer from asthma. A person can develop sulfite sensitivity at any point in life. Because many reactions have been reported, the FDA requires the presence of sulfites in processed foods to be declared on the label. Scientists have not pinpointed the smallest concentration of sulfites needed to trigger a reaction in a sulfite-sensitive person. Difficulty in breathing is the most common symptom reported by sulfite-sensitive people. Sulfites give off the gas sulfur dioxide, which can cause irritation in the lungs, and cause a severe asthma attack for those who suffer from asthma. Responses in the sulfite-sensitive person can vary. Sulfites can cause chest tightness, nausea, hives and in rare cases more severe allergic reactions. Mutations in SUOX may be a risk factor for certain types of cancer, including leukemia.

VDR/Taq and VDR/Fok (vitamin D receptor):

The panel looks at more than one portion of the vitamin D receptor, the Taq as well as the Fok sites. While the Fok change has been related to blood sugar regulation, changes at Taq can affect dopamine levels. For this reason it is important to look at the composite of the COMT and VDR/Taq status and make supplement suggestions based on the combined results at these two sites. The focus on changes in the Fok portion of the VDR is in

regard to supplements that support the pancreas and aid in keeping blood sugar in the normal healthy range.

Specific SNPs

Evaluated using Genetic Profiling Systems Nutrigenomic Panels

The information on specific genetic variants presented below is obtained from PubMed Central. This is the U.S. National Institutes of Health (NIH) free digital archive of biomedical and life sciences journal literature. In particular, abstracts are included from PubMed, a service of the National Library of Medicine, that includes over 15 million citations for biomedical articles dating back to the 1950's. These citations are from MEDLINE and additional life science journals. PubMed includes links to many sites providing full text articles and other related resources. The link to pubmed is <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>

Additional information included about genetic variants was obtained from OMIM. OMIM is the Online Mendelian Inheritance in Man database catalog of human genes and genetic disorders authored and edited by Dr. Victor A. McKusick and his colleagues at Johns Hopkins and elsewhere, and developed for the World Wide Web by NCBI, the National Center for Biotechnology Information. The database contains textual information and references. It also contains copious links to MEDLINE and sequence records in the Entrez system, and links to additional related resources at NCBI and elsewhere. More complete descriptions of genetic variations can be obtained by linking directly to this site:

Online Mendelian Inheritance in Man, OMIM (TM). McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), 2000. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

NOTE: OMIM is intended for use primarily by physicians and other professionals concerned with genetic disorders, by genetics researchers, and by advanced students in science and medicine. While the OMIM database is open to the public, users seeking information about a personal medical or genetic condition are urged to consult with a qualified physician for diagnosis and for answers to personal questions. OMIMTM and Online Mendelian

Inheritance in Man™ are trademarks of the Johns Hopkins University.

OMIM™ and Online Mendelian Inheritance in Man™ are trademarks of the Johns Hopkins University.

Specific SNPs

Evaluated using Genetic Profiling Systems Nutrigenomic Panels

ACE: ANGIOTENSIN I-CONVERTING ENZYME

Angiotensin I-converting enzyme (EC 3.4.15.1), or kininase II, is a dipeptidyl carboxypeptidase that plays an important role in blood pressure regulation and electrolyte balance by hydrolyzing angiotensin I into angiotensin II, a potent vasopressor, and aldosterone-stimulating peptide. The enzyme is also able to inactivate bradykinin, a potent vasodilator. Variability of plasma ACE concentration is determined by an insertion (I)/deletion (D) polymorphism in intron 16 of the ACE gene and known as the ACE/ID polymorphism; see 106180.0001.

ACAT1: ACETYL-CoA ACETYLTRANSFERASE 1

The ACAT1 gene encodes mitochondrial acetyl-CoA acetyltransferase, a short-chain-length-specific thiolase (EC 2.3.1.9). Cytosolic acetoacetyl-CoA thiolase is encoded by the ACAT2 gene (100678).

Fukao et al. (1990) cloned and sequenced cDNA encoding the precursor of hepatic mitochondrial acetoacetyl-CoA thiolase. The 427-amino acid precursor had a molecular mass of 45.2 kD. The sequence included a 33-residue leader peptide and a 394-amino acid subunit of the mature enzyme, which had a molecular mass of 41.4 kD. By Northern blotting, they analyzed the T2 gene expression in fibroblasts from 4 patients with 3-ketothiolase deficiency. In all 4 cell lines, the T2 mRNA had the same 1.7-kb transcript as that of the control; however, content was reduced in 2 cell lines and normal in the other 2. Human T2 is a homotetramer of 41-kD subunits.

Kano et al. (1991) determined that the ACAT gene spans approximately 27 kb and contains 12 exons. Using a plasmid clone of an EcoRI genomic fragment of the ACAT1 gene, containing exons 9 to 12, Masuno et al. (1992) assigned the ACAT1 locus to 11q22.3-q23.1 by in situ hybridization.

Matsuda et al. (1996) determined the chromosomal locations of the Atm (607585) and Acat1 genes in mouse, rat, and Syrian hamster by direct

R-banding fluorescence in situ hybridization. The 2 genes colocalized to mouse 9C-D, the proximal end of rat 8q24.1, and 12qa4-qa5 of Syrian hamster. The regions in the mouse and rat are homologous to human chromosome 11q. In the study of interspecific backcross mice, no recombinants were found among *Atm*, *Npat* (601448), and *Acat1*.

AHCY: S-ADENOSYLHOMOCYSTEINE HYDROLASE

Baric et al. (2004) noted that AdoHcy hydrolase catalyzes the hydrolysis of AdoHcy to adenosine and homocysteine. In eukaryotes, this is the major route for disposal of the AdoHcy formed as a common product of each of many S-adenosylmethionine (AdoMet)-dependent methyltransferases. The reaction is reversible, but under normal conditions the removal of both adenosine and homocysteine is sufficiently rapid to maintain the flux in the direction of hydrolysis. Physiologically, AdoHcy hydrolysis serves not only to sustain the flux of methionine sulfur toward cysteine, but is believed also to play a critical role in the regulation of biologic methylations.

Baric et al. (2004) pointed to the numerous AdoMet-dependent methyltransferases which are inhibited to a greater or lesser extent by AdoHcy. They pointed out that changes in DNA methylation patterns are heritable and could negatively affect tissue-specific gene expression during embryogenesis and after birth. Because the silencing of genes by inappropriate methylation is the functional equivalent of somatic mutations, the heritability of DNA methylation patterns suggests that restoration of 'normal' genomic methylation patterns may not occur.

Causes of hypermethioninemia include hereditary tyrosinemia (276700), cystathionine beta-synthase deficiency (236200), and methionine adenosyltransferase deficiency (250850).

BHMT: BETAINE-HOMOCYSTEINE METHYLTRANSFERASE

Homocysteine is a sulfur-containing amino acid that plays a crucial role in 1-carbon metabolism and methylation reactions. MTR (156570)-mediated transfer of the methyl group from methyltetrahydrofolate to homocysteine creates methionine, which donates the methyl group to methylate DNA, proteins, lipids, and other intracellular metabolites. Alternatively, homocysteine is metabolized to form cysteine through transsulfuration. Homocystinemia (603174) is associated with vascular disease and birth defects such as spina bifida.

Betaine-homocysteine methyltransferase (BHMT; EC 2.1.1.5) is a cytosolic enzyme that catalyzes the conversion of betaine and homo-

cysteine to dimethylglycine and methionine, respectively. This reaction is also required for the irreversible oxidation of choline cytosolic enzyme that catalyzes the conversion of betaine and homocysteine to dimethylglycine and methionine, respectively. This reaction is also required for the irreversible oxidation of choline

CBS ; CYSTATHIONEINE BETA SYNTHASE

The polymorphisms are a 68 bp insertion in exon 8, C699T in exon 8, C1080T in exon 11 and C1985T in the 3' untranslated region.

Cystathionine beta synthase (CBS) is a key enzyme in homocysteine metabolism. Four apparently non-functional polymorphisms in the CBS gene have been examined and determined their frequency, degree of linkage disequilibrium and association with plasma homocysteine levels. The polymorphisms are a 68 bp insertion in exon 8, C699T in exon 8, C1080T in exon 11 and C1985T in the 3' untranslated region. Examination of the association between these polymorphisms and plasma homocysteine levels revealed that the carriers of the rare alleles of the C699T, C1080T and C1985T polymorphisms had lower plasma homocysteine concentrations than those homozygous for the common alleles. PMID: 10363126

1,055 individuals with two silent polymorphisms, the 699C--> T and the 1080C-->T, of the cystathionine beta-synthase (CBS) gene were studied for the effects on plasma tHcy levels. Individuals who were heterozygous or homozygous for the T699 allele had lower post-methionine load (PML) tHcy levels when compared to individuals with the C/C genotype. This association was statistically significant ($p = 0.005$) for the T/T genotype compared to the C/C genotype and became even more significant ($p = 0.000002$) when individuals carrying the 68-bp insertion (844ins68) and the T1080 allele were excluded from the analysis. With regard to the 1080C-->T polymorphism, the T1080 allele was associated with significantly lower PML tHcy levels only when individuals carrying the 844ins68 and T699 allele were excluded from the study ($p = 0.01$ for 1080T/T genotype compared to 1080C/C genotype). We speculate that the 699C-->T and 1080C-->T polymorphisms may be in linkage disequilibrium with regulatory elements that upregulate CBS gene transcription. PMID: 11149614

COMT; CATECHOL-O-METHYLTRANSFERASE

Catechol-O-methyltransferase (COMT; EC 2.1.1.6) catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines, including the neurotransmitters dopamine, epinephrine, and norepinephrine. This O-methylation results in one of the major degradative pathways of the cat-

echolamine transmitters. In addition to its role in the metabolism of endogenous substances, COMT is important in the metabolism of catechol drugs used in the treatment of hypertension, asthma, and Parkinson disease. In blood COMT is found mainly in erythrocytes; in leukocytes it exhibits low activity. Weinshilbourn and Raymond (1977) found bimodality for red cell catechol-O-methyltransferase activity. Of a randomly selected population, 23% had low activity. Segregation analysis of family data suggested that low activity is recessive. Scanlon et al. (1979) found that homozygotes have a thermolabile enzyme. Thus, the site of the low COMT mutation is presumably the structural locus. Levitt and Baron (1981) confirmed the bimodality of human erythrocyte COMT. They further showed thermolability of the enzyme in 'low COMT' samples, suggesting a structural alteration in the enzyme. Autosomal codominant inheritance of the gene coding for erythrocyte COMT activity was adduced by Floderus and Wetterberg (1981) and by Weinshilbourn and Dunnette (1981). Gershon and Goldin (1981) concluded that codominant inheritance was consistent with the family data. Spielman and Weinshilbourn (1981) suggested that the inheritance of red cell COMT is intermediate, or codominant, there being 3 phenotypes corresponding to the 3 genotypes in a 2-allele system. The COMT of persons with low enzyme activity is more thermolabile than that of persons with high activity.

Comorbid panic disorder may define a subtype of bipolar disorder and may influence the strength of association between bipolar disorder and candidate genes involved in monoamine neurotransmission. Rotondo et al. (2002) studied the frequency of the V158M polymorphism, the 5-HTTLPR polymorphism of the serotonin transporter SLC6A4 (182138.0001), and a splice site polymorphism (IVS7+218C-A) of tryptophan hydroxylase (TPH; 191060) in a case-control association study of bipolar disorder patients with or without lifetime panic disorder. They compared results from DNA extracted from blood leukocytes of 111 unrelated subjects of Italian descent meeting DSM-III-R criteria for bipolar disorder, including 49 with and 62 without comorbid lifetime panic disorder, with those of 127 healthy subjects. Relative to the comparison subjects, subjects with bipolar disorder without panic disorder, but not those with comorbid bipolar disorder and panic disorder, showed significantly higher frequencies of the COMT met158 and the short 5-HTTLPR alleles.

Zubieta et al. (2003) examined the influence of the V158M polymorphism, which affects the metabolism of catecholamines, on the modulation of responses to sustained pain in humans. Individuals homozygous for the M158 allele showed diminished regional mu-opioid system (see

600018) responses to pain compared with heterozygotes. These effects were accompanied by higher sensory and affective ratings of pain and a more negative internal affective state. Opposite effects were observed in V158 homozygotes. Zubieta et al. (2003) concluded that the COMT V158M polymorphism influences the human experience of pain and may underlie interindividual differences in the adaptation and responses to pain and other stressful stimuli.

MAOA; MONOAMINE OXIDASE A

Because the monoamine oxidase A inhibitors are effective in the treatment of panic disorder, the MAOA gene is a prime candidate for involvement. Deckert et al. (1999) investigated a novel repeat polymorphism in the promoter of the MAOA gene for association with panic disorder in 2 independent samples, a German sample of 80 patients and an Italian sample of 129 patients. Two alleles (3 and 4 repeats) were most common and constituted more than 97% of the observed alleles. Functional characterization in a luciferase assay demonstrated that the longer alleles were more active than allele 3. The longer alleles were significantly more frequent among 209 females of both the German and the Italian samples of panic disorder patients than among 190 females of the corresponding control samples ($P = 0.001$).

Impairment in the central dopaminergic system has been suggested as a factor in the pathogenesis of restless legs syndrome (RLS; 102300). In 96 unrelated patients with RLS, Desautels et al. (2002) found that females with the high activity alleles (3.5, 4, and 5 repeats) in the promoter region polymorphism of the MAOA gene had a greater risk (odds ratio = 2.0) of being affected with RLS than females carrying the low activity allele (3 repeats). The association was not observed in males, and there were no differences for either group regarding the MAOB gene. Desautels et al. (2002) suggested that the MAOA gene may modulate the pathogenesis of RLS and that estrogen may interact with specific MAOA alleles.

Following up on the report of Fossella et al. (2002) in which polymorphisms in the dopamine receptor (DRD4; 126452) and MAOA genes showed significant associations with efficiency of handling conflict as measured by reaction time differences in the Attention Network Test (ANT), Fan et al. (2003) examined whether this genetic variation contributed to differences in brain activation within the anterior cingulate cortex. They genotyped the DRD4 and MAOA genes in 16 subjects who had been scanned during the ANT and identified in each of the 2 genes a polymorphism in which persons with the allele associated with better behavioral performance showed

significantly more activation in the anterior cingulate while performing the ANT than those with the allele associated with worse performance. The polymorphisms were the 3-repeat allele of the 30-bp repeat in the MAOA promoter (309850.0002) and the -1217G insertion/deletion polymorphism in the upstream region of the DRD4 gene.

Tadic et al conducted a study to detect a possible association of a T941G single nucleotide polymorphism (SNP) in the monoamine oxidase A (MAOA) gene with generalized anxiety disorder (GAD), panic disorder (PD), or major depression (MD). The 941T allele was over-represented in patients suffering from GAD ($\chi^2(2) = 6.757$; $df = 1$; $P < 0.01$, not corrected for multiple testing) when compared to healthy volunteers. PMID: 12555227

Monoamine oxidase A (MAO A) is located on the X chromosome and metabolizes biogenic amines including dopamine, norepinephrine and serotonin. A functional promoter-region polymorphism of this gene has been described that has been studied in a number of mental illnesses but not in attention deficit hyperactivity disorder (ADHD). In the current study, Manor, I. et al. examined the MAO A promoter-region polymorphism initially in 133 triads and observed preferential transmission of the long alleles from 74 heterozygote mothers to ADHD probands. All three complementary approaches employed (family-based, case-control and quantitative trait design) suggest a role for the MAO A promoter-region polymorphism in conferring risk for ADHD in our patient population. PMID: 12140786

MTHFR; 5,10-METHYLENETETRAHYDROFOLATE REDUCTASE

Methylenetetrahydrofolate reductase (EC 1.5.1.20) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a cosubstrate for homocysteine remethylation to methionine.

By RT-PCR of RNA from MTHFR-deficient (236250) patients, followed by single-strand conformation polymorphism (SSCP) analysis, Goyette et al. (1994) identified 3 substitutions in the MTHFR gene: 2 missense mutations (in residues conserved in the enzyme and bacteria) and 1 nonsense mutation. The nonsense mutation (607093.0001) and 1 of the missense mutations (thr to met) were identified in severe early-onset patients; the second missense mutation (arg to gln; 607093.0002) was identified in 2 patients with a thermolabile enzyme and late-onset neurologic disease. Goyette et al. (1995) described 7 additional mutations.

Frosst et al. (1995) identified a C-to-T substitution at nucleotide 677 (607093.0003) that converts an alanine to a valine residue and is responsible for the synthesis of a thermolabile form of MTHFR.

Goyette et al. (1996) reported an additional 5 mutations causing severe

MTHFR deficiency. They also reported results of analysis of the enzyme thermolability in 22 patients with MTHFR deficiency. Six of the 22 patients had 4 mutations in the MTHFR gene--2 rare mutations causing severe deficiency and 2 mutations for the common ala-to-val mutation associated with enzyme thermolability.

Rozen (1996) tabulated 9 point mutations that had been identified in cases of severe MTHFR deficiency.

Bagley and Selhub (1998) used a chromatographic method for folate analysis to test the hypothesis that the 677C-T mutation is associated with altered distribution of red blood cell (RBC) folates. An alteration was found as manifested by the presence of formylated tetrahydrofolate polyglutamates in addition to methylated derivatives in the RBCs from homozygous mutant individuals. 5-Methylenetetrahydrofolate polyglutamates were the only folate form found in RBCs from individuals with the wildtype genotype. Existence of formulated folates in RBCs only from individuals with the thermolabile MTHFR is consistent with the hypothesis that there is in vivo impairment in the activity of the thermolabile variant of MTHFR and that this impairment results in an altered distribution of RBC folates.

Friso et al. (2002) sought to determine the effect of folate status on genomic DNA methylation with an emphasis on interaction with the common 677C-T mutation in the MTHFR gene. They used the liquid chromatography/mass spectrometry method for the analysis of nucleotide bases to assess genomic DNA methylation in peripheral blood mononuclear cell DNA from 105 subjects homozygous for the TT genotype and 187 homozygous for the wildtype (CC) MTHFR genotype. The results showed the genomic DNA methylation directly correlates with folate status and inversely with plasma homocysteine levels (P less than 0.01). TT genotypes had a diminished level of DNA methylation compared with those with the CC wildtype. When analyzed according to folate status, however, only the TT subjects with low levels of folate accounted for the diminished DNA methylation. Moreover, in TT subjects DNA methylation status correlated with the methylated proportion of red blood cell folate and was inversely related to the formylated proportion of red blood cell folates that is known to be solely represented in those individuals. These results indicated that the MTHFR 677C-T polymorphism influences DNA methylation status through interaction with folate status.

The A1298C mutation has been mapped to the SAME regulatory region of the gene. Mutations in the A1298C do not lead to increased levels of homocysteine; as such it has been felt that this mutation may not be of serious consequence. Literature suggests that the MTHFR enzyme can

drive the reverse reaction leading to formation of BH₄. Dr. Yasko has suggested that the A1289C mutation is associated with a defect in the reverse reaction leading to the formation of BH₄. The A1298C mutation would then be associated with an inability to convert BH₂ to BH₄. This would impact levels of dopamine, serotonin, norepinephrine, and phenylalanine. The availability of BH₄ helps to determine whether nitric oxide, peroxy nitrite or super oxide are formed as a function of the urea cycle; two molecules of BH₄ are required for formation of nitric oxide, one molecule of BH₄ leads to the formation of peroxy nitrite and the absence of BH₄ leads to super oxide formation.

Selzer et al. (2003) reported the neurologic deterioration and death of a child anesthetized twice with nitrous oxide before the diagnosis of MTHFR deficiency was established. The patient carried a novel MTHFR mutation (1755G-A; 607093.0010) which caused a met581-to-ile amino acid substitution. This mutation was coinherited with 2 other, common MTHFR polymorphisms, 677C-T (607093.0003) and 1298A-C (607093.0004), each of which is associated with depressed enzyme function. Selzer et al. (2003) proposed that a nitrous oxide-induced defect of methionine synthase superimposed on an inherited defect of MTHFR caused the patient's death.

MTRR; METHIONINE SYNTHASE REDUCTASE

Methionine is an essential amino acid in mammals. It is required for protein synthesis and is a central player in one carbon metabolism. In its activated form, S-adenosylmethionine, it is the methyl donor in hundreds of biologic transmethylation reactions and the donor of propylamine in polyamine synthesis. The eventual product of the demethylation of methionine is homocysteine, and its remethylation is catalyzed by a cobalamin-dependent enzyme, methionine synthase. Methionine synthase catalyzes the remethylation of homocysteine to methionine via a reaction in which methylcobalamin serves as an intermediate methyl carrier. Over time, the cob(II)alamin cofactor of methionine synthase becomes oxidized to cob(III)alamin, rendering the enzyme inactive. Regeneration of functional enzyme requires reductive methylation via a reaction in which S-adenosylmethionine is utilized as a methyl donor. Patients of the cbl E complementation group of disorders of folate/cobalamin metabolism who are defective in reductive activation of methionine synthase exhibit megaloblastic anemia, developmental delay, hyperhomocysteinemia, and hypomethioninemia.

The cloning of the cDNA for MTRR (Leclerc et al., 1998) led to the identification of a polymorphism, 66A-G, that was shown by Wilson et al. (1999) to be associated with increased risk for spina bifida (182940). Hobbs et

al. (2000) evaluated the frequencies of the MTHFR 677C-T (236250.0003) and MTRR 66A-G polymorphisms in DNA samples from 157 mothers of children with Down syndrome (190685) and 144 control mothers. Odds ratios were calculated for each genotype separately and for potential gene-gene interactions. The results were consistent with the preliminary observations of James et al. (1999) that the MTHFR 677C-T polymorphism is more prevalent among mothers of children with Down syndrome than among control mothers, with an odds ratio of 1.91 (95% CI, 1.19-3.05). In addition, the homozygous MTRR 66A-G polymorphism was independently associated with a 2.57-fold increase in estimated risk (95% CI, 1.33-4.99). The combined presence of both polymorphisms was associated with a greater risk of Down syndrome than was the presence of either alone, with an odds ratio of 4.08 (95% CI, 1.94-8.56). The 2 polymorphisms appeared to act without a multiplicative interaction. The association between folate deficiency and DNA hypomethylation lent support to the possibility that the increased frequency of the MTHFR and MTRR polymorphisms observed in this study may be associated with chromosomal nondisjunction and Down syndrome.

Doolin et al. (2002) studied the potential involvement of both the maternal and embryonic genotypes in determining risk of spina bifida. Analysis of data on this polymorphism and the A2756G polymorphism of the methionine synthase gene (156570.0008) provided evidence that both variants influence the risk of spina bifida via the maternal rather than the embryonic genotype. For both variants the risk of having a child with spina bifida appeared to increase with the number of high-risk alleles in the maternal genotype.

Bosco et al. (2003) studied the influence of polymorphisms of methylenetetrahydrofolate reductase (MTHFR 677C-T and 1298A-C, 607093.0004), methionine synthase (MTR 2756A-G), and methionine synthase reductase (MTRR 66A-G) on the risk of being a Down syndrome (190685) case or of having a child with Down syndrome (case mother). Plasma homocysteine and other factors were likewise studied. They found that after adjustment for age, total homocysteine and MTR 2756 AG/GG genotype were significant risk factors for having a Down syndrome child, with odds ratio (OR) of 6.7 and 3.5, respectively. The MTR 2756 AG/GG genotype increased significantly the risk of being a Down syndrome case, with an OR of 3.8. Double heterozygosity for MTR 2756 AG/MTRR 66 AG was the single combined genotype that was a significant risk factor for having a Down syndrome child, with an OR estimated at 5.0, after adjustment for total homocysteine level.

MTR; 5-METHYLTETRAHYDROFOLATE-HOMOCYSTEINE S-METHYLTRANSFERASE

The remethylation of homocysteine to form methionine is catalyzed by the cytoplasmic enzyme 5-methyltetrahydrofolate-homocysteine S-methyltransferase (EC 2.1.1.13), which is also called methionine synthase. This enzyme requires methylcobalamin (MeCbl), a derivative of cobalamin, or vitamin B12, for activity.

Evaluation of the relationship between variation in genes that are involved in the folate-homocysteine metabolic axis and the risk of spina bifida (182940) is complicated by the potential involvement of both the maternal and embryonic genotypes in determination of disease risk. Doolin et al. (2002) designed a study to address questions regarding both maternal and embryonic genetic risk factors for spina bifida by use of the 2-step transmission/disequilibrium test. Analysis of data on variants of 2 genes involved in homocysteine remethylation/methionine biosynthesis, viz., the MTR 2756A-G (156570.0008) and methionine synthase reductase (MTRR) 66A-G (602568.0003) polymorphisms, provided evidence that both variants influence the risk of spina bifida via the maternal rather than the embryonic genotype. For both variants, the risk of having a child with spina bifida appeared to increase with the number of high-risk alleles in the maternal genotype. The findings highlighted the importance of considering both the maternal and embryonic genotypes when evaluating putative spina bifida susceptibility loci.

Aberrant DNA methylation is a common feature of human neoplasia. Paz et al. (2002) studied interindividual inherited susceptibility to the epigenetic processes of CpG island hypermethylation and global genomic hypomethylation, which are observed simultaneously in cancer cells. They genotyped 233 patients with colorectal, breast, or lung tumors for 4 germline variants in 3 key genes involved in the metabolism of the methyl group. A positive association of aberrant methylation was found with homozygosity for the MTR 2756G allele as well as with the 677T allele of the MTHFR gene.

NOS3: NITRIC OXIDE SYNTHASE 3 .0001 CORONARY SPASMS, SUSCEPTIBILITY TO [NOS3, GLU-298ASP]

Angiotensin I-converting enzyme (EC 3.4.15.1), or kininase II, is a dipeptidyl carboxypeptidase that plays an important role in blood pressure regulation and electrolyte balance by hydrolyzing angiotensin I into angiotensin II, a potent vasopressor, and aldosterone-stimulating peptide. The enzyme is

also able to inactivate bradykinin, a potent vasodilator.

Yoshimura et al. (1998) found a missense glu298-to-asp variant (E298D) in exon 7 of the NOS3 gene. In a study of 113 patients with coronary spasm, in whom the diagnosis of coronary spasm was made by intracoronary injection of acetylcholine, and 100 control subjects, they found a significant difference in the distribution of the variant; 21.2% of the coronary spasm group and 9.0% of the control group ($p = 0.014$ for dominant effect) showed the variant. Hingorani et al. (1999) investigated the relationship between the glu298-to-asp variant in atherosclerotic coronary artery disease, using 2 independent case-controlled studies. In the first study, cases consisted of 298 unrelated patients with positive coronary angiograms and controls were 138 unrelated healthy individuals ascertained through a population health screen. In the second study, the cases were 249 patients with recent MI and a further 183 unrelated controls. There was an excess of homozygotes for the asp298 variant among patients with angiographic coronary artery disease, and among patients with recent MI when compared with their respective controls (35.9% vs 10.2% in the first study, and 18.1% vs 8.7% in the second study). In comparison to glu298 homozygotes, homozygosity for asp298 was associated with an odds ratio of 4.2 (95% confidence interval, 2.3 to 7.9) for angiographic coronary artery disease and 2.5 (95% confidence interval, 1.3 to 4.2) for MI.

Dahiyat et al. (1999) found a significant association of late-onset Alzheimer disease (AD; 104300) and homozygosity for the glu allele in a study of 122 early-onset and 317 late-onset Alzheimer cases compared to 392 controls.

This was independent of apoE status. The authors remarked on the interaction of beta-amyloid with endothelial cells. In contrast, Higuchi et al. (2000) studied 411 Japanese patients with sporadic AD and 2 groups of controls: 350 Japanese controls and 52 Caucasian controls. They found no difference in the glu298-to-asp polymorphism between AD patients and controls, even when stratifying for age of onset and presence of the APOE E4 allele. However, they observed that the glu allele frequency was significantly higher in Japanese controls than in Caucasian controls, suggesting that the association reported by Dahiyat et al. (1999) may be a function of race.

In a study of 35 patients with histories of placental abruption and 170 control subjects, Yoshimura et al. (2001) found that the frequency of glu298-to-asp homozygotes and heterozygotes was higher in the placental abruption group than in the control group (40% vs 14%; p less than 0.001).

Kobashi et al. (2001) found in a study in Japan that the frequency of

heterozygotes and homozygotes for asp298 in the NOS3 gene was significantly higher in patients with hypertension in pregnancy (0.23) (see 189800) than in controls (0.12) (p less than 0.01). Multivariate analysis showed that a family history of hypertension, the TT genotype of the angiotensinogen gene (AGT; 106150.0001), the GA+AA NOS3 genotype, and a prepregnancy body mass index of more than 24 were independent potent risk factors, after adjustment for maternal age and parity. The odds ratios of these factors were 2.7, 2.3, 2.2, and 2.1, respectively. The results suggested that the asp298 of NOS3 is a potent, independent risk factor for hypertension in pregnancy.

Jachymova et al. (2001) found a significantly higher frequency of T alleles (related to the E298D polymorphism) in hypertensives (see 145500) as compared to normotensives. Significant association was found in patients showing resistance to conventional antihypertensive therapy.

SHMT; SERINE HYDROXYMETHYLTRANSFERASE, CYTOSOLIC; SHMT1

Serine hydroxymethyltransferase (SHMT), a pyridoxal phosphate-containing enzyme, catalyzes the reversible conversion of serine and tetrahydrofolate to glycine and 5,10-methylene tetrahydrofolate. Some eukaryotic cells, including human cells, contain both cytosolic and mitochondrial forms of SHMT. Mammalian cells that lack mitochondrial SHMT activity are auxotrophic for glycine (SHMT2; 138450). It has been suggested that glycine synthesis from serine occurs in the mitochondria, whereas cytosolic SHMT may catalyze the conversion of glycine to serine, although direct evidence for this proposal is lacking. Garrow et al. (1993) cloned human cDNAs for cytosolic and mitochondrial SHMT by functional complementation of an *Escherichia coli* glyA mutant with a human cDNA library. The cDNA for the cytosolic enzyme encoded a 483-residue protein of M(r) 53,020. The deduced protein sequence shared 63% identity with that of the SHMT2 protein. By isotopic *in situ* hybridization, Garrow et al. (1993) assigned the cytosolic and mitochondrial SHMT genes to 17p11.2 and 12q13, respectively. The high degree of nucleotide sequence identity between the 2 isozymes as well as the presence of keratin genes in both chromosomal regions was consistent with these regions of chromosomes 12 and 17 having arisen by a duplication event.

Folate-dependent one-carbon metabolism is critical for the synthesis of numerous cellular constituents required for cell growth, and SHMT is central to this process. Elsea et al. (1995) found that the SHMT1 gene maps to the critical interval for Smith-Magenis syndrome (SMS; 182290)

on 17p11.2. They found that the gene spans approximately 40 kb. It was found to be deleted in all 26 SMS patients examined by PCR, fluorescence in situ hybridization, and/or Southern analysis. Furthermore, haploinsufficiency was indicated by the fact that SHMT enzyme activity in patient lymphoblasts was approximately 50% that of unaffected parent lymphoblasts. They suggested that haploinsufficiency may play a role in the SMS phenotype and that this finding may point to possible therapeutic interventions.

Byrne et al. (1996) described an SHMT pseudogene with 90% identity to SHMT cDNA. By fluorescence in situ hybridization, they mapped the pseudogene to 1p33-p32.3.

SUOX; SULFITE OXIDASE

Sulfite oxidase (EC 1.8.3.1) is the terminal enzyme in the oxidative degradation pathway of sulfur-containing amino acids. Kisker et al. (1997) characterized 4 missense mutations in the SUOX gene in cell lines from patients with isolated sulfite oxidase deficiency (606887.0001-606887.0004).

The molybdenum-containing enzyme sulfite oxidase catalyzes the conversion of sulfite to sulfate, the terminal step in the oxidative degradation of cysteine and methionine. Deficiency of this enzyme in humans usually leads to major neurological abnormalities. Four variants associated with sulfite oxidase deficiency have been identified: two mutations are near the sulfate binding site, while the other mutations occur within the domain mediating dimerization. PMID: 9428520

Neonatal sulphite oxidase deficiency is characterised by severe neurologic dysfunction, brain atrophy, dislocation of the lens and increased urinary excretion of sulphite, thiosulphate, taurine and S-sulphocysteine, and by a low plasma cystine. Barbot, C. et al. present clinical, neuroradiological and biochemical data of a patient with late onset symptoms comparing this presentation with the neonatal form and stressing the difficulties of the diagnosis of this disorder. PMID: 8719749

VDR; VITAMIN D RECEPTOR

The vitamin D₃ receptors are intracellular polypeptides of 50 to 60 kD that specifically bind 1,25(OH)₂D₃ and interact with target-cell nuclei to produce a variety of biologic effects. Baker et al. (1988) described the cloning and characterization of cDNAs encoding the human vitamin D receptor. Analysis of the deduced amino acid sequence showed that the VDR protein belongs to the superfamily of trans-acting transcriptional regulatory factors, including the steroid and thyroid hormone receptors. VDR is more closely related to the thyroid hormone receptors than to the others. Baker et al. (1988)

Ferrari et al. (1999) investigated the relationship between 3-prime and 5-prime VDR gene polymorphisms (as determined by the restriction enzymes BsmI and FokI) and bone mineral mass and calcium and inorganic phosphate (Pi) metabolism in a homogeneous cohort of young, healthy men. Among BB homozygotes, BMD (z-scores) was significantly lower only in subjects also carrying the f allele at the VDR 5-prime polymorphic site (FokI). Serum PTH levels were significantly higher in the BB genotype at baseline and remained so under either a low or a high calcium-phosphorus diet. Moreover, on the low calcium-phosphorus diet, BB subjects had significantly decreased tubular Pi reabsorptive capacity and plasma Pi levels. The authors emphasized the importance of identifying multiple single-base mutation polymorphisms within candidate genes of bone mineral mass and suggested a role for environmental/dietary factor interactions with VDR gene polymorphisms in peak bone mineral mass in men.

Makishima et al. (2002) demonstrated that the vitamin D receptor also functions as a receptor for the secondary bile acid lithocholic acid, which is hepatotoxic and a potential enteric carcinogen. The vitamin D receptor is an order of magnitude more sensitive to lithocholic acid and its metabolites than are other nuclear receptors. Activation of the vitamin D receptor by lithocholic acid or vitamin D induced expression in vivo of CYP3A (124010), a cytochrome P450 enzyme that detoxifies lithocholic acid in the liver and intestine. Makishima et al. (2002) concluded that their studies offer a mechanism that may explain the proposed protective effects of vitamin D and its receptor against colon cancer.

Previous studies have suggested that lead exposure may be associated with increased risk of amyotrophic lateral sclerosis (ALS). Polymorphisms in the genes for α -aminolevulinic acid dehydratase (ALAD) and the vitamin D receptor (VDR) may affect susceptibility to lead exposure. We used data from a case-control study conducted in New England from 1993 to 1996 to evaluate the relationship of ALS to polymorphisms in ALAD and VDR and the effect of these polymorphisms on the association of ALS with lead exposure. PMID: 12896855

Operating through the vitamin D receptor (VDR), vitamin D inhibits prostate cancer growth and increases insulin-like growth factor binding protein (IGFBP) expression, suggesting that the vitamin D and insulin-like growth factor (IGF) regulatory systems may operate together. We found that among men with the ff FokI genotype, those in the highest tertile of plasma IGFBP-3 had a decreased risk versus those in the lowest tertile (odds ratio, 0.14; 95% confidence interval, 0.04-0.56; P(trend) < 0.01), whereas among men with the FF and Ff genotypes, IGFBP-3 was not associated with risk.

Our findings in a low-risk population suggest that the IGF and vitamin D regulatory systems may interact to affect prostate cancer risk. Larger studies are needed to confirm these findings and clarify the underlying mechanisms. PMID: 11389055

Puchacz et al. examined expression of the 1,25-dihydroxyvitamin D3 [1,25-(OH)₂ D₃] receptors in chromaffin cells of the adrenal medulla and the effects of 1,25(OH)₂ D₃ on expression of the tyrosine hydroxylase (TH) gene. By increasing expression of the rate limiting enzyme in the catecholamine biosynthetic pathway, 1,25-(OH)₂ D₃ may participate in the regulation of catecholamine production in adrenal chromaffin cells. This regulation provides mechanisms through which 1,25(OH)₂ D₃ may control response and adaptation to stress. PMID: 9011759

Yu et al looked at fibroblast growth factor-23 (FGF23) is a circulating factor that plays critical roles in phosphate and vitamin D metabolism. The goal of our studies was to dissect the pathways directing the vitamin D-phosphate-FGF23 homeostatic axis. In summary, Fgf23 is suppressed in diet-induced hypophosphatemia and in hypophosphatemia associated with secondary hyperparathyroidism. Thus, our results demonstrate that Fgf23 is independently regulated by phosphate and by vitamin D. PMID: 15869926

Terms and Conditions for the Advanced Nutrigenomic System

The Company's services and the Advanced Nutrigenomic System are provided to you subject to the following terms and conditions:

1. Minimum Age for Screening Consent: When the person whose sample is being tested is under the age of 18 (a minor), the minor's parent or guardian must sign the Terms and Conditions signature page and on the Informed Consent page authorizing the screening.
2. Consent to Nutrigenomic Screening: You must read and sign the enclosed Informed Consent form. Nutrigenomic screening will not be performed without receiving your signature on both the Informed Consent and the Terms and Conditions signature page. If the Company receives a sample without both the signed Informed Consent and the signed Terms

and Conditions, Company will make a reasonable effort to notify the sender of the missing documents. If the missing documents are not supplied within five business days, Company will dispose of the sample.

3. Purpose of Advanced Nutrigenomic Screening: The enclosed nutrigenomic panel(s) from Company identify(ies) the presence or absence of genetic variants in a specified number of genes. Those variants are known to be associated with the efficiency with which your body metabolizes certain foods and nutrients. Knowing whether or not you have any of these genetic variants may help you to make better diet and lifestyle choices.

The Advanced Nutrigenomic Screening does not test for and is not intended to test for disease, predisposition to disease, or medical conditions.

4. Limitation of Nutrigenomic Screening:

a. The information supplied to you is not diagnostic and neither reveals the presence of disease nor recommends a treatment for disease. Individuals with specific concerns about their medical status or genetic status should consult a licensed physician.

b. You understand and agree that the Screening Results Report the Company provides only includes results solely for the single nucleotides screened in the context of the particular nutrigenomic screening profile you select. The Company cannot and will not provide any additional information about your genetic profile.

c. Health-related information provided through this service is not a substitute for medical advice. It is important that you not make medical decisions based upon the information. You should consult a licensed and qualified physician for diagnosis and treatment of disease. The receipt of any questions or feedback you submit to the Company does not create a professional relationship and does not create any confidentiality privileges beyond those described in our privacy policy.

d. The information provided in Advanced Nutrigenomic Screening is not a comprehensive assessment of your nutritional needs. It is a screening tool for identifying genetic variations that may affect certain aspects of your nutrition. Any decisions you make based on the information provided by

this screen are your sole responsibility. Your Screening Results Report is not a complete screening of your genetic profile. You should not alter your diet or medical care based on those results alone. For a complete assessment of your nutritional needs and the adequacies of your daily diet please consult a qualified physician or nutritional counselor.

5. Limitations on Genetic Variation Results:

a. Nutrigenomics is an emerging field of nutrition science. Research in nutrigenomics is ongoing and is likely to produce new information far into the future. The screening results the Company provides in the Advanced Nutrigenomic System are based on knowledge regarding genetics and nutrition publicly available and determined to be relevant and trustworthy at the time of your screening. Once your screening results are sent to you, the Company will not supplement those results with any additional test information or analysis.

b. Nutrigenomics is an evolving discipline. Your use of these services and the Advanced Nutrigenomic System in your personal health and nutrition decision-making is at your sole risk. Your Screening Results Report constitutes the extent of the information the Company will provide to you. Once your report is produced, the Company will not provide further information, feedback or updates as a part of your nutrigenomic screening.

6. Privacy Policy:

a. The Company regards your personal information, including your test results, as your personal and confidential information. It will do its utmost to keep your information confidential. The testing of your sample is done on a strictly confidential and de-identified basis. De-identified means that all personal identifying information has been removed from your sample and a unique identifier number assigned to it when it is screened for genetic variations.

b. Protecting your privacy is at the core of the Company's screening service. The handling of your genetic information is done without any personal information attached. No personally identifiable information will be disclosed to anyone outside those personnel necessary to assemble your Screening Results Report.

- c. The Company will not disclose your personal information to any third party unless you request it in writing or the Company is required to do so by law.
- d. Upon your request the Company will provide you with a copy of all your personal information then possessed by the Company. The Company will charge an administrative fee if the request requires the Company to de-identify your Screening Results Report. You may request that your personal information be modified, corrected, updated or removed from our database with the exception that the Company may retain your de-identified screening results. In response to your request, the Company will send your personal information only to your address noted in the Company's files. If you wish to validate, change or delete any of the personal information held about you or if you have any questions about this privacy statement please contact our distributor Holistic Health International, LLC
info@holistichealth.com
279 Walkers Mills Road, Bethel, ME 04217 Tel# 207-824-8501

7. Use of Personal Data Collected:

By submitting your sample for nutrigenomic screening with the Company, you agree that we may retain and store your de-identified screening results in a report generated from your sample. The Company may maintain and analyze a database of sample results, including yours. The Company may analyze that de-identified results database for commercial, academic, educational, or other purposes, but any analysis will not disclose personally identifiable information.

8. Warranties and Representations:

- a. There is no warranty or representation, either express or implied, that this service will satisfy your expectations and that the results obtained from your use of the Company's Advanced Screening System will be accurate or reliable in your case.
- b. No advice or information, whether oral or written, obtained through or from the Company's Advanced Screening System shall create any warranty or other obligation unless otherwise expressly stated herein.

c. **The Company makes no representations and extends no warranties of any kind, either express or implied, including but not limited to the provision of services meeting a standard of reasonable care and skill.** The Advanced Nutrigenomic System is provided “as is.” The Company disclaims any and all such representations and warranties to the maximum extent permitted by law. Without limiting the generality of the foregoing, The Company disclaims to the maximum extent permitted by law any and all:

- (i) Warranties of Merchantability or Fitness for a particular purpose;
- (ii) Warranties against infringement of any third party's intellectual property or proprietary rights;
- (iii) Warranties relating to delays, interruptions, errors, or omissions in the service, or a part thereof,
- (iv) Warranties relating to the transmission or deliver of the service,
- (v) Warranties relating to the accuracy, reliability, correctness or completeness of data; and
- (vi) Warranties otherwise relating to performance, nonperformance, or other acts or omissions by the Company or any third party

d. To the fullest extent permitted by applicable laws, you agree to hold the Company harmless and waive any claim for liability including, but not limited to, any claims, loss, demands or damages of any kind whatsoever concerning the services the Company provides and any information, content, advertisements or products provided or distributed in connection with the Company's services. The foregoing will apply whether such claims, loss, demands, or damages arise in tort, contract, negligence, under statute or otherwise.

9. Refunds:

a. The sample kits are returnable to the Company whether opened or unopened. Upon receipt of the kit with a written statement that you are declining the screening, the Company will refund your payment less \$25.

b. The Company will not issue any refunds if you request that your sample's screening be discontinued or stopped after it is submitted for screening and processing at the Company's laboratory.

10. Right to Alter Screening Service Procedure and Scope:

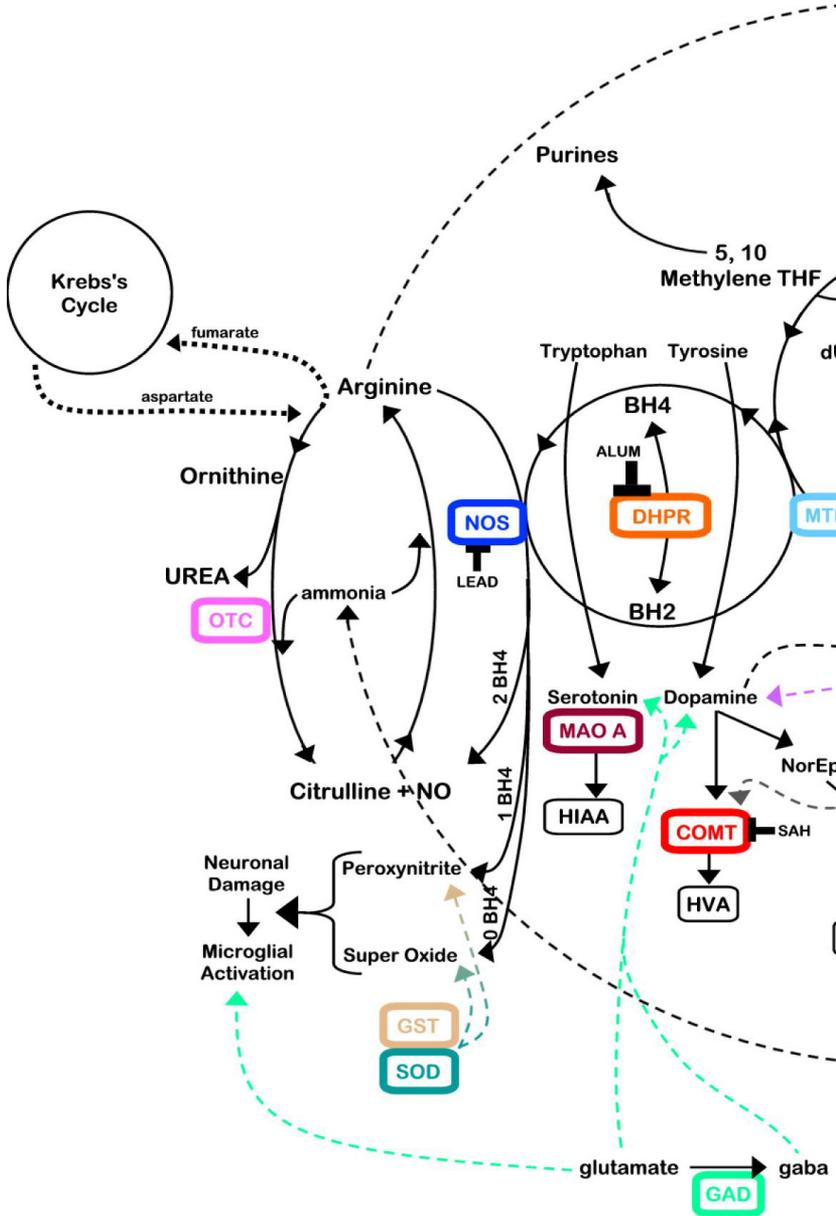
You understand that the Company reserves the right to alter the content and scope of the Advanced Nutrigenomic System in any way, at any time, for any reason, without prior notification to you. At times we may utilize uncharacterized SNPs to further research and knowledge.

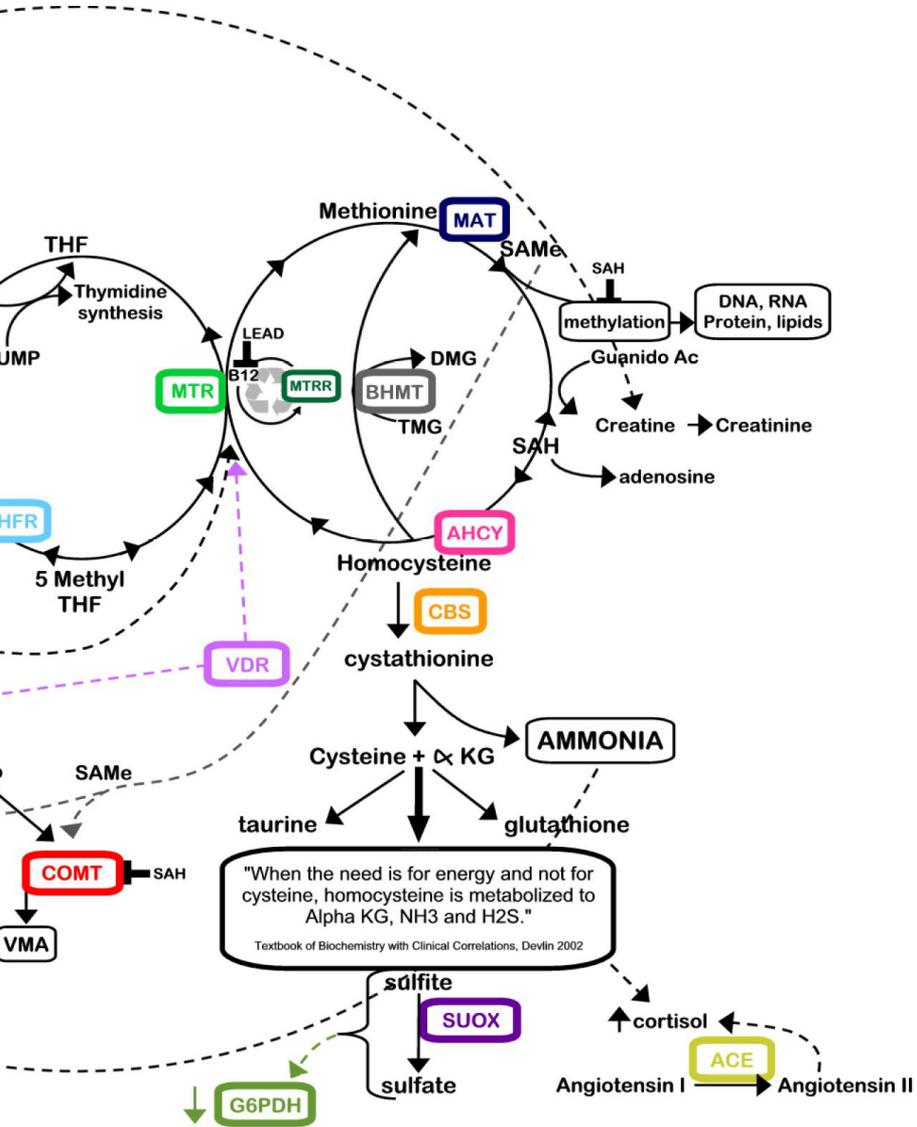
11. General:

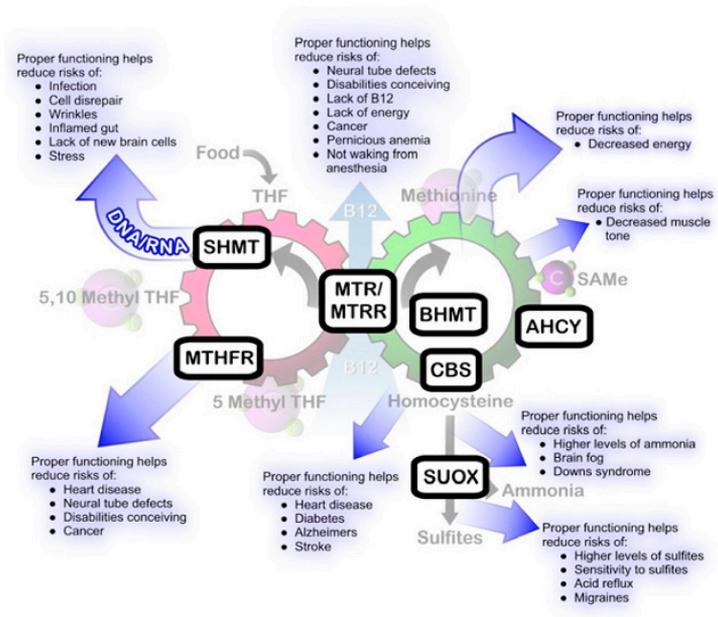
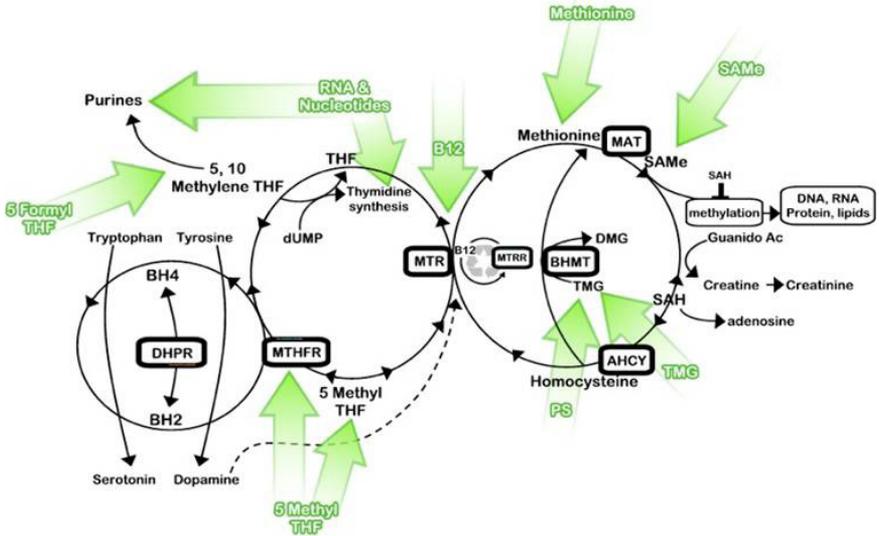
These Terms and Conditions (including the documents referred to herein) govern your use of the Company's services, superseding any prior Terms and Conditions in any form. Any failure to exercise or enforce any right or provision of the Terms and Conditions by the Company shall not constitute a waiver of such right or provision.

12. Governing Law and Jurisdiction:

These Terms and Conditions and the agreement between us will be governed by the law of the State of Virginia, and you agree that any disputes relating to the Advanced Nutrigenomic Test or these Terms and Conditions or the agreement between us will be covered by the exclusive jurisdiction of the courts of the State of Virginia.







Holistic Health International, LLC
 279 Walkers Mills Road, Bethel, ME 04217.
 info@holistichealth.com