



William Shaw, Ph.D, CLIA Laboratory Director
CLIA ID # 17D0919496

First Name Patient 1	Last Name GPL Test	DOB 1/1/79	Sex Male	Ordering Physician	GPP GPP23846
Sample Condition	Sample Type	Test Order Date 6/9/15	Collection Date N/A	Customer Reference N/A	Posting Date 9/14/15

Key

++ Both copies of the chromosome have the described mutation		Likely benign
+ - One copy of the described mutation exists in this patient		Mutation of unknown significance
- - No mutation exists on either chromosome (see appendix)		Small risk of being pathogenic
		Increased pathogenic risk
		Likely pathogenic

Results

DNA METHYLATION

Mutations: 2

The methylation pathway is the complex process by which carbons are added onto folic acid from various substrates and redistributed onto other compounds in the body, through the use of several enzymes. Methylation is often referred to as one-carbon donation. This pathway is responsible for the formation of methionine (a sulfur based amino acid) and thymidylate monophosphate (dTMP). These compounds play critical roles in nucleotide synthesis, neurotransmitter function, detoxification, and numerous other processes. The recycling of carbons for use in the methylation pathway is critical for cellular function. These enzymes are coded for on the end of the 21st chromosome, making them more susceptible to mutations.



Gene	RS Number	Pathogenicity	Genotype	Phenotype	Disease Associated
CBS	rs2851391		T,C	+ -	Homocysteine levels are altered
VDR	rs1544410		C,T	++	Increased risk of osteoporosis

MENTAL HEALTH

Mutations: 2

The mental health panel identifies mutations to many of the genes that influence mental health. The enzymes in this panel either degrade or modify many different types of neurotransmitters. Mutations to these genes can cause an increased risk of developing depression, schizophrenia, anxiety, and bipolar disorder.



Gene	RS Number	Pathogenicity	Genotype	Phenotype	Disease Associated
------	-----------	---------------	----------	-----------	--------------------

DAOA	rs2391191		G,A	+ -	Mood disorders/schizophrenia
CAMKK	rs1063843		T,C	++	Increased risk of schizophrenia

DRUG METABOLISM

Mutations: 2



The cytochrome P450s are a family of monooxygenase enzymes that are expressed throughout the body, but are most prevalent in the liver. The P450s have two main roles: The detoxification of exogenous chemicals (including medical drugs) and the metabolism of endogenous chemicals including hormones, neurotransmitters, cholesterol, and vitamins. Mutations to some of the P450s could alter the appropriate dosage for many medications and could have long-term health consequences.

Gene	RS Number	Pathogenicity	Genotype	Phenotype	Disease Associated
CYP2C9	rs1799853		C,T	+ -	Decreased drug metabolism
CYP2E1	rs3813867		G,C	+ -	Increased risk of ischemic stroke

AUTISM RISK GENES

Mutations: 2


Autism spectrum disorder (ASD) is a developmental disability that is characterized by unique cognitive and behavioral symptoms that vary in degrees of severity. Recently, there have been multiple studies that compared individuals with ASD to the general public. These studies have identified a series of genetic mutations that are more commonly found in patients with ASD than found in the general population. Further studies are needed to determine the degree to which these mutations are relevant in ASD. If a patient has one of these mutations, it does not mean that he/she will develop ASD, but their risk for developing ASD may be higher than the general public.

Gene	RS Number	Pathogenicity	Genotype	Phenotype	Disease Associated
OXT	rs2740210		C,A	+ -	Increased risk of schizophrenia
OXT	rs2740208		A,C	++	Increased risk of autism

OXALATE METABOLISM

Mutations: 1



To some extent, all individuals will obtain oxalates from three sources: liver cells (endogenously), yeast species (exogenously), and food (also exogenously). The degree to which an individual is predisposed to having adverse metabolic consequences from high levels of oxalate is influenced by the metabolism of oxalate in the body. The deposition of oxalates in critical tissues such as brain and blood vessels, and the oxidative damage caused by oxalate salts can lead to symptoms of pain, nephrolithiasis, and possibly neurological symptoms.

Gene	RS Number	Pathogenicity	Genotype	Phenotype	Disease Associated
HAO1	rs941426		A,G	+ -	Primary hyperoxaluria

GLUTEN SENSITIVITY

Mutations: 2



The Gluten sensitivity panel looks at a large portion of the genes that if mutated, could lead to gluten sensitivity. Mutation in any of these genes may indicate the need to limit or eliminate gluten from the diet. Gluten sensitivity can manifest in a variety of symptoms. Many patients with autism spectrum disorder report neurological benefits upon switching to a gluten-free diet.

Gene	RS Number	Pathogenicity	Genotype	Phenotype	Disease Associated
DPP4	rs1558957		T,C	+ -	Increased risk of cardiovascular disease
DPP4	rs17574		A,G	+ +	Increased risk of cardiovascular disease

CHOLESTEROL METABOLISM

Mutations: 2



Cholesterol is a very important molecule for the body. The concept of good and bad cholesterol depends highly on the circumstances of the individual person. Cholesterol is used to produce crucial hormones, vitamins, secondary messengers, and bile acids. Although very high blood serum cholesterol values are associated with heart disease, low values are associated with increased violent behavior, suicide, depression, anxiety, bipolar disease, Parkinson's disease, and increased mortality from cancer. Low cholesterol values are also associated with manganese deficiency, celiac disease, hyperthyroidism, liver disease, malabsorption, and malnutrition.

Gene	RS Number	Pathogenicity	Genotype	Phenotype	Disease Associated
IDI1	rs7075141		G,A	+ -	Increased risk of alzheimer's disease
FDPS	rs11264359		A,G	+ -	Increased risk of crohn's disease

ACETAMINOPHEN TOXICITY

Mutations: 2



Acetaminophen (Paracetamol, Tylenol) is one of the most commonly used pain relievers. Liver toxicity associated with acetaminophen usage has led to over 33,000 hospitalizations a year. Proper metabolism of acetaminophen is critical to preventing toxicity and the resulting damage. Genetic mutations that cause increased levels of the metabolite NAPQI or that limit the body's ability to detoxify acetaminophen will make a person more likely to experience adverse effects from acetaminophen usage.

Gene	RS Number	Pathogenicity	Genotype	Phenotype	Disease Associated
GSTP1	rs1695		A,G	+ -	Increased asthma risk
GSTP1	rs947895		C,A	+ -	Increased risk of lung cancer

TRANSPORTERS

Mutations: 2

Transport proteins are important for shuttling molecules across cellular membranes. Transporters specific to certain molecules control the influx of compounds into and out of the cell. The process of regulating the needs of the cell is tightly controlled through the use of transporters. Mutations to transporter genes impede the ability of cells to uptake vital nutrients and export toxic substances. There are numerous diseases that are associated with faulty transport including Alzheimer's disease, coronary artery disease, aggressive behavior, and decreased drug efficacy.

Gene	RS Number	Pathogenicity	Genotype	Phenotype	Disease Associated
APOE	rs449647		A,T	+ -	Increased risk of alzheimer's disease
APOE	rs405509		T,G	+ -	Increased risk of alzheimer's disease/atherosclerosis

Interpretation

DNA Methylation

Gene: CBS, Pathway: DNA Methylation

Cystathione beta-synthase (CBS) is a pyridoxal-5'-phosphate (vitamin B6) dependent enzyme that converts L-serine and L-homocysteine into L-cystathionine. L-cystathionine is later converted into the amino acid cysteine. Mutations to the CBS gene are the most common cause of hereditary hyperhomocysteinemia (PMID:15581573).

The adverse effects of homocysteine accumulation in the body are related to the substitution of homocysteine for methionine in protein synthesis. The resulting complications include an increase in immune response, increase in cell death, and protein damage (PMID 14999406). The degree of homocysteinemia is relative to the mutation. Hyperhomocysteinemia has been linked to multiple mutations to the CBS gene. The most common of these is the Ile278Thr and the Gly307Ser, which cause homocysteine to build up in the blood. Complications of hyperhomocysteinemia include mental retardation, seizures, and vascular disease. One of the most common causes of death for patients with homocystinuria is heart attack (PMID:14722927).

Spina bifida is a neural tube defect. Spina bifida occurs during neural tube development. During development the top of the tube becomes the brain, while the remainder becomes the spinal cord. This development is usually complete by day 28 of pregnancy, however errors in this process can cause neural tube defects such as spina bifida (PMID:19683694).

Many individuals with mutations to the CBS gene are responsive to high dose vitamin B6 and folate therapy. The degree of responsiveness is related to the type of mutation. Since this gene is responsible for the synthesis of cysteine, this amino acid becomes essential for patients deficient in this enzyme. Reduction of methionine and administration of betaine is also of benefit to reduce homocysteine load (PMID:9587030).

References

PMIDs: [15581573](#), [14999406](#), [14722927](#), [19683694](#), [9587030](#)

Gene: VDR, Pathway: DNA Methylation

Vitamin D receptor (VDR) is a nuclear hormone receptor for vitamin D3. Vitamin D3 interacts with this receptor to influence multiple biological activities by regulating gene transcription. Vitamin D can be obtained from dietary sources or can be endogenously synthesized from 7-dehydrocholesterol by a nonenzymatic ultraviolet B mediated reaction from sun exposure. Vitamin D requires two hydroxylations by vitamin D 25-hydroxylase in the liver. Later vitamin D is hydroxylated (activated) in the kidney to form calcitriol (vitamin D3). This is the form of vitamin D that binds to VDR. VDR activation is important in the regulation of blood pressure, cardiac function, and insulin regulation (PMIDs: 20693391 and 23855914).

Vitamin D3 is associated with maintenance of calcium distribution. More recently, it has been implicated in inflammatory processes, vascular integrity, and collagen formation (PMID: 23814529). Mutations in VDR have been linked to metabolic syndrome (PMID: 23855914). Individuals with VDR mutations have greater propensity for insulin insensitivity, higher triglycerides, and lower HDL levels (PMID: 23855914). Vitamin D receptor mutations can also lead to vitamin-D-dependent rickets type 2 (VDDR2). This disorder is characterized by poor bone formation, which leads to bones that are prone to fracture, bowed legs, hypocalcemia, hyperparathyroidism, osteomalacia, and alopecia (PMID: 9275211).

Restoration of mineral balance through supplementation with additional calcium and phosphate is helpful in ameliorating some of the symptoms associated with this mutation (PMIDs: 9751523, 11705326, and 8530616).

References

PMIDs: [20693391](#), [23855914](#), [9275211](#), [9751523](#), [11705326](#), [8530616](#)

Mental Health**Gene: DAOA, Pathway: Mental Health**

The D-amino acid oxidase activator (DAOA) protein (also known as G72) is a 153 amino acid protein that localizes in the brain, spinal cord, and testis. This protein is located in the endoplasmic reticulum and the mitochondria cellular compartments. DAOA is a modulator of D-amino acid oxidase (DAO) activity. If DAO is hyperactivated it can result in a decrease in the D-serine level and hypofunction of the NMDA receptor. Overexpression of DAOA has been found in schizophrenics and those suffering from bipolar disorder. Mutations to DAOA have been linked to a higher incidence of schizophrenia and bipolar disorder.

References

PMIDs: [24362575](#), [12364586](#), [17684499](#), [15271585](#)

Gene: CAMKK, Pathway: Mental Health

Calcium/calmodulin-dependent serine protein kinase (CaMKK) is a calcium-dependent kinase which phosphorylates the proteins CaMK1 and CaMK4. CaMKK is present mostly in the brain and it influences signaling cascades that affect memory, learning, and synapse formation. CaMKK2 regulates the production of neuropeptide Y. CaMKK2 inhibition in mice causes a reduction in appetite and weight loss.

Mutations to CAMKK2 have been shown to increase the risk of developing schizophrenia (PMID: 23958956).

References

PMIDs: [9662074](#), [23958956](#)

Drug Metabolism

Gene: CYP2C9, Pathway: Drug Metabolism

Cyp2C9 is a member of the cytochrome P450 family of proteins. Cyp2C9 is present in microsomes in the liver. Cyp2c9 is known to metabolize many xenobiotics (medications). Some well known xenobiotics that Cyp2c9 metabolizes are phenytoin, tolbutamide, ibuprofen, and warfarin.

Cyp2c9 is highly polymorphic with many different versions in the human populations. This variability can make dosing of drugs metabolized by Cyp2c9 difficult. Many adverse drug reactions are the results of variability caused by Cyp2c9 activity-caused mutations. Two drugs that often cause toxicity because of reduced metabolic capacity are warfarin and phenytoin.

References

PMIDs: [15822186](#), [18690857](#)

Gene: CYP2E1, Pathway: Drug Metabolism

Cyp2e1 is a member of the cytochrome P450 family of enzymes. It is located in the microsomes of liver cells. Cyp2e1 is involved in the oxidation of multiple therapeutics, endogenous compounds, and toxic substances. Cyp2e1 metabolizes mostly small polar molecules because of the size of its active site. Cyp2e1 deactivates many therapeutics, either by causing them to change to an inactive form or making them easier for the body to excrete.

Cyp2e1 is important for the metabolism of acetaminophen (paracetamol). Cyp2e1 converts acetaminophen into the toxic molecule NAPQI. NAPQI is then able to bind to many different types of proteins causing them to malfunction or be degraded. This can result in tissue damage as well as liver failure.

References

PMIDs: [14527082](#)

Autism Risk Genes**Gene: OXT, Pathway: Autism Risk Genes**

OXT is the gene for the hormone oxytocin. Oxytocin is produced in the hypothalamus and stored in the posterior pituitary gland. Oxytocin is important for cognitive, emotional, and social functions. Some social functions that oxytocin controls include social recognition, pair bonding, anxiety, and maternal behaviors.

Mutations to the OXT gene have been implicated in autism spectrum disorders (ASD). Patients with ASD often have poor verbal communication and decreased social interaction. Intravenous oxytocin has been reported to improve some autistic symptoms.

References

PMIDs: [18566739](#), [25977088](#), [25662821](#)

Oxalate Metabolism

Gene: HAO1, Pathway: Oxalate Metabolism

Glycolate oxidase is expressed primarily in the liver and is involved with converting glycolic acid to oxalate. Mutations which cause a deficit in this enzyme are actually beneficial to individuals with hyperoxaluria (PMID: 24996905). The drug (L)-oxothiazolidine-4-carboxylate (OTZ), which blocks glycolate oxidase has been used for the treatment of primary hyperoxaluria (PH1) with mixed success (PMIDs: 11851603, 11135080, and 9598565). Primary hyperoxaluria is characterized by an overproduction of oxalate which can lead to kidney obstructions, frequent and severe urinary tract infections, and even kidney failure (PMID: 19758989). Mutations to HAO1 that cause overproduction of the enzyme would lead to a more complicated prognosis.

Individuals with mutations to this enzyme who have elevated oxalate markers on the Organic Acids Test may benefit from certain supplemental and diet changes. NADH is a strong inhibitor of oxalate production because it prevents the formation of glyoxalate and converts it back into the less toxic glycolate (PMID: 9038835). Free radical injury may contribute to oxalate stone formation, and supplementation with the antioxidant vitamin E has been shown to be beneficial in some studies (PMID: 12624656). Vitamin B6 therapy has long been the standard therapy for primary hyperoxaluria as it reduces the synthesis of oxalate from glyoxalate (PMIDs: 3974685 and 15610227). More recently it has been discovered that probiotic bacteria are capable of metabolizing oxalates in the gastrointestinal tract (PMID: 16284877). Supplementation with lactobacilli and bifidobacteria may help reduce the oxalates that are formed by yeast and bacteria and that are released from food. It will not prevent the formation of oxalates in the liver. Low oxalate diets and calcium citrate supplementation have also been incorporated by individuals with hyperoxaluria to help improve outcomes (PMID: 11135080).

References

PMIDs: [24996905](#), [11851603](#), [9598565](#), [19758989](#), [9038835](#), [12624656](#), [15610227](#), [3974685](#), [16284877](#), [11135080](#)

Gluten Sensitivity

Gene: DPP4, Pathway: Gluten Sensitivity

Dipeptidyl peptidase-4 (DPP4) is an antigenic enzyme that is expressed on the surface of most cell types and associated with immune regulation, signal transduction, and apoptosis. DPP4 cleaves X-proline dipeptides from the N-terminus of polypeptides. DPP4 plays a major role in glucose metabolism.

DPP4 degrades the proline-containing epitope of gliadin. DPP4 also enhances the degradation of gluten and casein.

References

PMIDs: [17940427](#), [15138176](#)

Cholesterol Metabolism

Gene: IDI1, Pathway: Cholesterol Metabolism

Isopentenyl-diphosphate delta isomerase (IDI1) is an enzyme that catalyzes the isomerization (atomic rearrangement) of isopentenyl pyrophosphate (IPP) to dimethylallyl pyrophosphate (DMAPP). This is a critical step in the cellular production of ubiquinones (such as CoQ10), sterols, cholesterol, Heme A, and farnesylated proteins (involved in intracellular signaling cascades), among others (PMIDs: 24162026 and 11842430).

Mutations to IDI1 may play a role in amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease (PMID: 20955688). Also mutations may increase the risk of developing Alzheimer's disease (PMID: 16385451).

References

PMIDs: [24162026](#), [11842430](#), [20955688](#), [16385451](#).

Gene: FDPS, Pathway: Cholesterol Metabolism

Farnesyl diphosphate synthase (FDPS) is an enzyme that converts geranyl diphosphate and isopentenyl diphosphate to 2Z,6E-farnesyl diphosphate. The farnesyl diphosphate product is involved with cholesterol synthesis, is a substrate product for farnesylated proteins (involved in intracellular signaling cascades), and as an agonist for hormone receptors (PMID: 11842430, <http://www.ncbi.nlm.nih.gov/gene/2224>).

Mutations in this gene are associated with variations in bone mineral density (PMID: 24534219). Inhibition of this enzyme is the target of drugs designed to prevent bone resorption (<http://www.ncbi.nlm.nih.gov/gene/2224>). Mutations to this gene can lead to abnormalities in cholesterol homeostasis and reduced levels of cholesterol esters in the adrenals (PMID: 8827514).

References

PMIDs: [11842430](#), [8827514](#)

Acetaminophen Toxicity**Gene: GSTP1, Pathway: Acetaminophen Toxicity****Glutathione S-transferase P (GSTP1)**

Glutathione S-transferase is made of three different variants. These variants are composed of glutathione S-transferase theta-1 (GSTT1), glutathione S-transferase Mu-1 (GSTM1), and glutathione S-transferase P (GSTP1).

There are two mutations to GSTP1 that have been shown to have a decreased activity compared to wild type. These mutant proteins are Ile105Val and Ala114Val. The Ile105Val mutant is known to have a much lower activity than the other mutant and wild type proteins. The Ile105Val mutant has a significant decrease in GSH-conjugation of acetaminophen, clozapine, and diclofenac.

GSTT1 and GSTM1 mutations have been associated with an increased risk of prostate cancer.

(PMID: 23888321, 24185126, 24907267)

References

PMIDs:

Transporters

Gene: APOE, Pathway: Transporters

Apolipoprotein E (APOE) combines with lipids in the body to form lipoproteins. Lipoproteins are responsible for packaging cholesterol and other lipids and carrying them through the bloodstream. APOE is a major component of very low-density lipoproteins (VLDLs). VLDLs remove excess cholesterol from the blood and transport it to the liver. There are three different gene versions of APOE called e2, e3, and e4. The most common is e3.

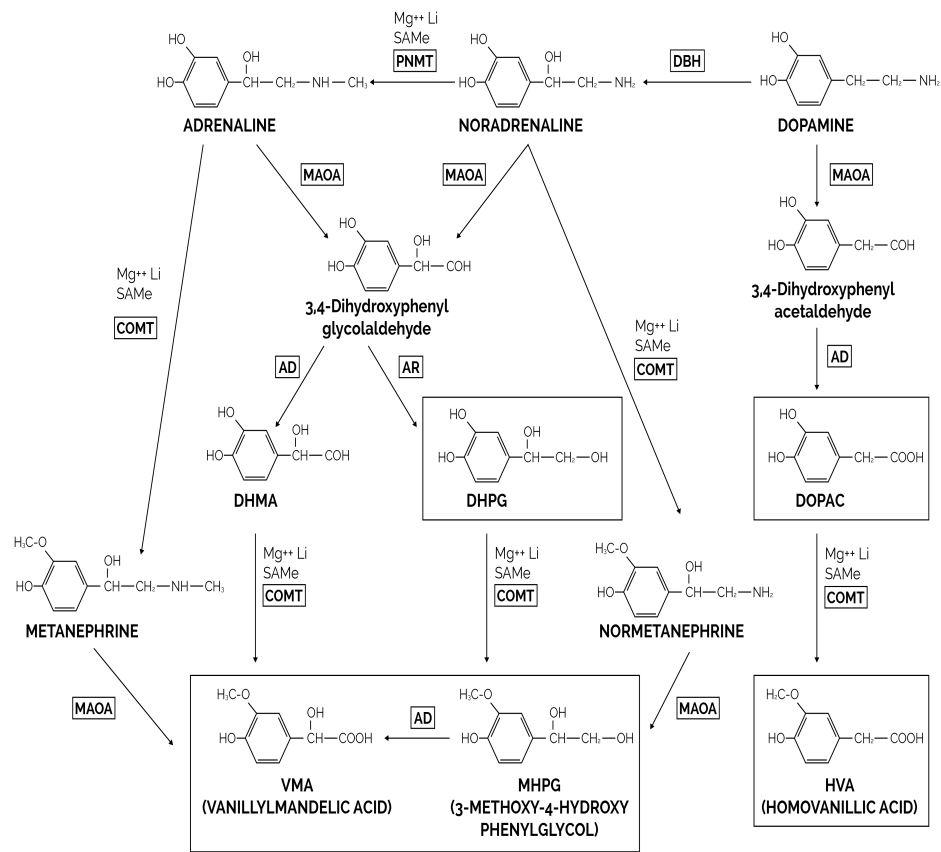
Mutations to APOE can lead to an increased risk of developing Alzheimer's disease. People who inherit one copy of the APOE e4 allele have an increased risk of developing the disease. Besides Alzheimer's disease, patients with mutated version of APOE have an increased chance of developing atherosclerosis, which is an accumulation of fatty deposits and scar-like tissue in the lining of the arteries. APOE mutations have also been linked to an increased risk for age-related macular degeneration. Macular degeneration is a leading cause of vision loss for older people. An increased risk of macular degeneration is associated with having one copy of the APOE e2 allele.

References

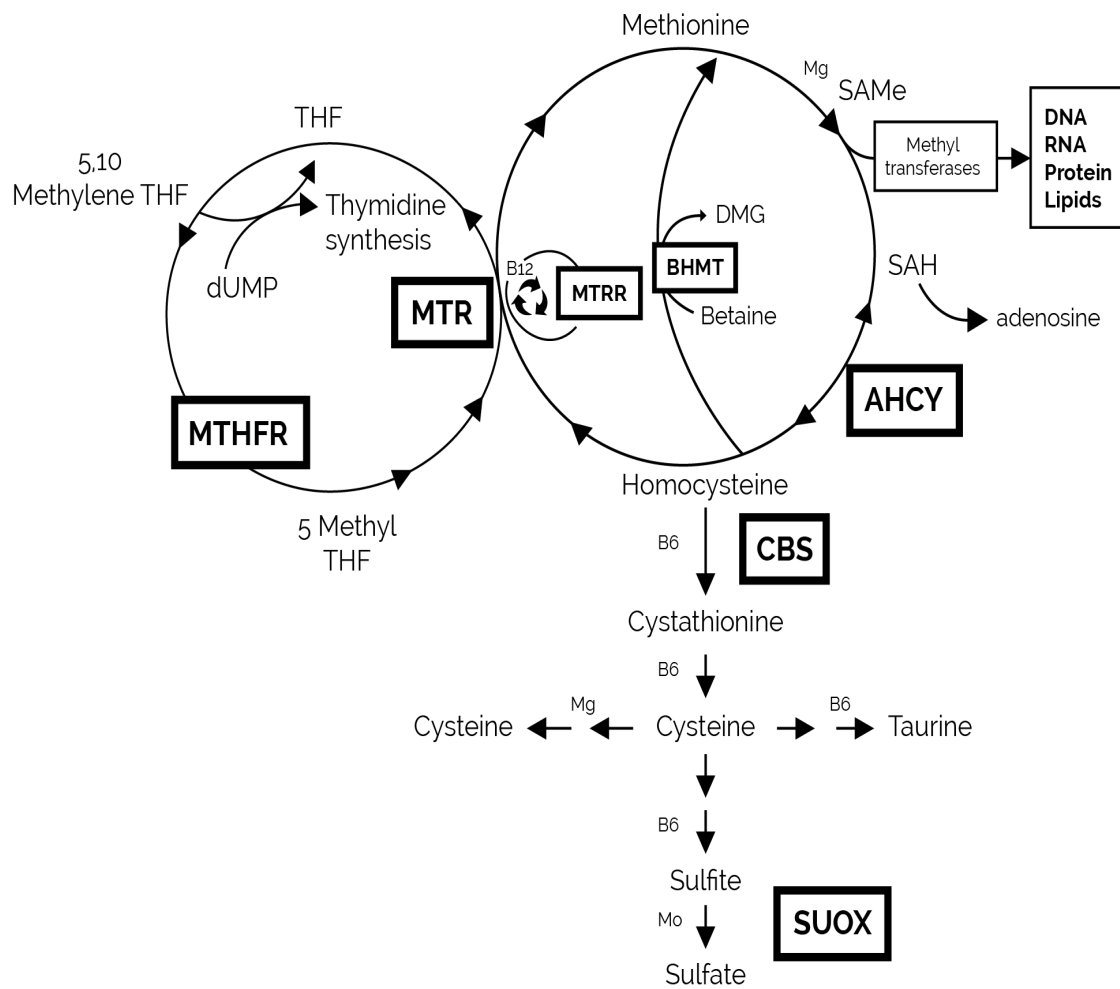
PMIDs: [15181244](#)

Appendix

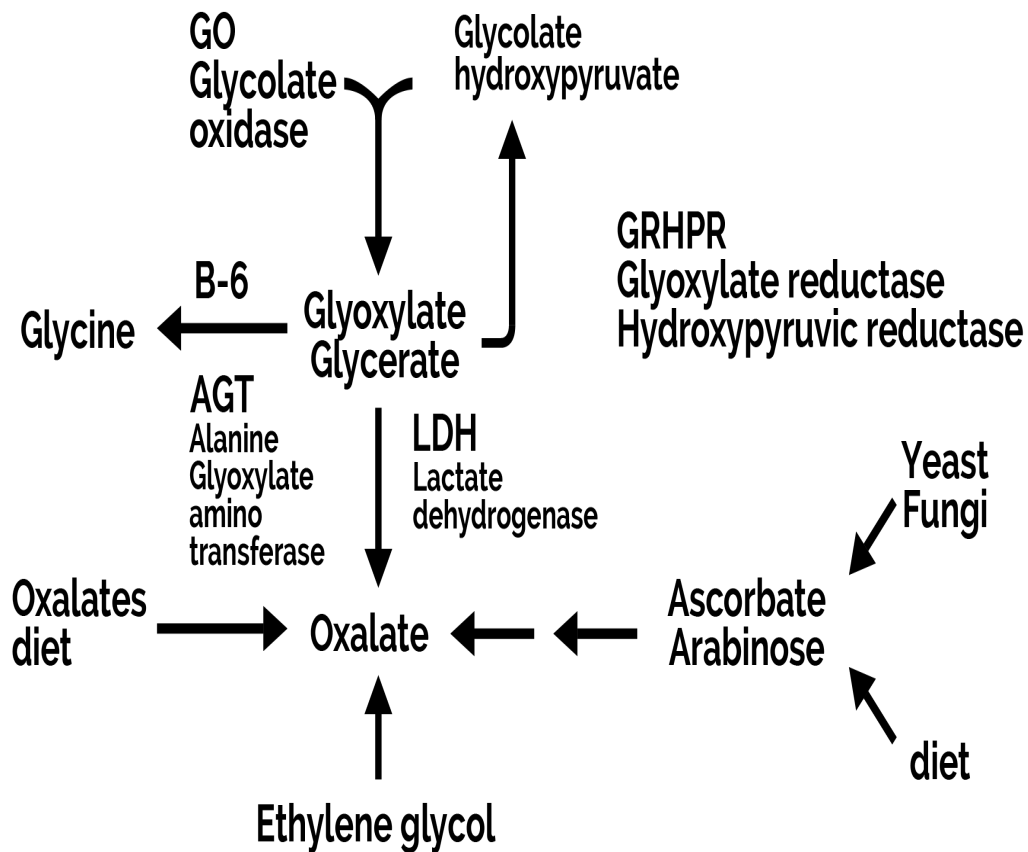
Mental Health Pathway



Methylation Pathway



Oxalate Metabolism



Methods

This panel was designed to sequence over 1,000 Single nucleotide polymorphisms (SNPs) identified through next-generation sequencing. For this test, genomic DNA was extracted from the tissue submitted and captured with an inversion probe method for the SNPs specific to this panel. This captured target was sequenced on Illumina MiSeq sequencing system with 100bp paired-end reads. Sequence results were mapped to UCSC hg19 genomic reference using the Ziphyr® bioinformatics pipeline. Changes from the reference are listed on the front-page table and variants homozygous with the reference are listed in the appendix of the report. Courtagen Life Sciences, 12 Gill St. Ste. 3700, Woburn, MA 01801, performed both the analytic sequencing and bioinformatic filtering of variants; while the interpretation of the results were written, and provided by, Great Plains Laboratory.

Limitations

Single nucleotide polymorphisms (SNPs) are identified with a technical sensitivity of >99% and specificity of >99%. False positives and negatives results may occur due to limitations in technology, bioinformatics, and interpretation. Smaller insertion/deletion events (indels), large rearrangements, including del/dups and CNVs, are generally not detected with this methodology. In addition, this test will not detect other changes in the genes tested outside of the defined SNPs including, single nucleotide variants in the coding region, intronic, variants in the 5' and 3' untranslated regions (UTRs), novel start codons in 5'UTR regions upstream of the reported ATG start site, triplet repeat expansions, methylation or other epigenetic changes, mosaicism, loss of heterozygosity, uniparental disomy, or skewed X inactivation. With sequencing assays of this scope, not all bases can be covered fully. Examples include high GC/AT content and regions with difficult probe design. Bases with a depth of coverage below 10x are not always recovered.