A Nutritional Genomics Approach to Epigenetic Influences on Chronic Disease

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» "Nutritional genomics refers to the application of "omics" technologies, together with systems biology and bioinformatics tools, to understand how nutrients interact with the flow of genetic information to impact various health outcomes" [1]. Nutritional genomics is an umbrella term that encompasses two distinct but related fields: nutrigenomics and nutrigenetics. Nutrigenomics may be defined as the measurable effect of nutrients on the genome, proteome, microbiome and metabolome. Thus, the use of laboratory measures such as organic acids, amino acids, homocysteine, etc. may serve as an indicator of whether a gene is functioning or impaired due to a SNP, and to what degree. This clinical data may then be used to develop a personalized nutrition plan to influence the biochemical pathways in which the SNP interacts. Nutrigenetics is similar as it also explores the measurable interactions of nutrients on the genome, however, nutrigenetics is focused on the measurable interactions between diet and *disease risk* and which dietary interventions influence intervention outcomes. This may help predict how a patient may respond to a dietary intervention in terms of controlling or exacerbating disease risk. It is therefore instrumental in the design of personalized nutrition plans to ameliorate symptoms or in the prevention of the development of various disease types.

Nutrigenetics is focused on genetic variation and disease prediction or the way that diet influences the risk of developing a disease. Nutrigenetics is most often practiced by geneticists and genetic counselors who are skilled in computational statistics. Remember, this is *disease prediction*. Nutrigenomics is focused on how nutrients affect the genome and the biochemistry we can measure related to those lifestyle choices. Measurement in this case is typically through metabolomics and microbiome analyses. Due to the reliance on metabolomics for validation, advanced nutrition professionals are the primary nutrigenomics practitioners. These are two distinct fields under one large umbrella. Epigenetics intermingles easily through both fields, making the scope a bit more challenging to decipher.

In these next sections, we will discover the salient genetics associated with several conditions. Some conditions and interventions will be strictly nutrigenetic in nature while others are more nutrigenomic. There will be some overlap as that is the nature of the field. We will dive deeply into the nutritional genomics and epigenetics of cardiometabolic disease; neurodegenerative diseases like Alzheimer's disease and Parkinson's disease; common psychiatric conditions such as depression, anxiety, schizophrenia and bipolar disorder; understand the genomics of irritable bowel disease, mitochondrial insufficiency, cancer and nutrient-related autoimmune diseases followed by an introduction to pharmacogenomics. We will elucidate the effect that stress has on methylation and the epigenome and discuss how common toxic exposures can influence methylation. Remember, hypermethylation silences gene expression while hypomethylation activates it, thus in essence, hypomethylating means to turn on the gene, while hypermethylation will typically turn it off. The goal is to have proper methylation, not over or undermethylation. This chapter is an overview of the topics and is not an exhaustive review.

17.1 What Is Epigenetics?

The term epigenetics was originally coined by Waddington in 1942. It was derived from the Greek word "epigenesis," which described the influence of genetic processes on development [2, 3]. Modern day epigenetics is the study of gene expression through histone or methyl modulation along with RNA silencing rather than the alteration of the genetic code itself. Epigenes are additional instruction layered on top of our inherited genetic code (A, T, C, G, U); this additional instruction is known as the epigenome. If we think of the genetic code as the manuscript, the epigenetic information could be viewed as the highlighted or tagged sections. These "tags" or markers indicate to the instructional processors that something is either important or that it can be ignored.This is the science that turns "on" or "off" the genes, which can result in both positive and negative outcomes.

There are a multitude of "tags" that guide the genomic processors. Some are methyl groups, others are histone modifiers, and some even modify RNA, one of the processors! These tags work together to either increase or restrict access to the genome, therefore controlling the expression of the proteins found within the genome. The really interesting part about these tags is that they are not corrected like our genetic code. These tags or markers can change based on our lifestyle, experiences, diet, exercise habits, and exposure to chemicals. This fluidity allows the epigenome to "learn" and adapt to the current environmental circumstances. These epigenetic markers can even survive what is called global DNA demethylation, or the wiping clean of epigenetic markers from the gametes when a zygote is formed. Interestingly, there is a process called imprinting whereby one of the parent's genes is "dominant" and silences the other allele. These imprinted genes keep their epigenetic tags, even through global demethylation. These are the tags that survive from generation to generation and influence genetic expression. As the new embryo forms, other epigenetic tags physically record each cell's experiences on the genome. In this recording or writing stage, the epigenome ultimately stabilizes gene expression allowing for proper embryonic development [4].

Maternal diet, smoking status, mental state, and social environment can do one of two things. This environmental information can hypomethylate CpG islands, which in essence turns on the gene, or it can result in RNA silencing, thus turning off the gene [4]. This means that the trauma that your great grandmother experienced at the turn of the twentieth century could potentially increase your risk of developing a mental illness, such as depression. Fathers also have epigenetic transfer as well, thus, it also means that your

"health-nut" father could also confer more favorable cancer and cardiovascular epigenetics [5].

There are three major systems that control epigenetics including DNA methylation, histone modification, and non-coding RNA (ncRNA)-associated gene silencing [4]. There are also less well-studied processes such as acetylation and ubiquitination. In addition to these processes, the "systems" are also modifiable. For example, histone proteins are responsible for packaging DNA via chromatin complexes into dense chromosomes. Histone structures may be modified by acetylation of lysine residues, methylation of lysine and arginine residues, phosphorylation of serine and threonine residues, ubiquitination of lysine residues on histone tails, sumoylation, and ADP ribosylation. Histone acetyltransferases (HATs) add acetyl groups to lysine residues on histone tails, whereas histone deacetylases (HDACs) can remove the acetyl groups [4, 6, 7].

DNA methylation typically occurs at CpG islands, which are most commonly found in gene promoter regions [6]. It is at this CpG island that DNA methyltransferases (DNMTs) coordinate the addition of a methyl group to the 5-carbon position of the cytosine residues where it regulates transcription. DNMT1 exclusively maintains normal methylation by exact duplication of DNA between cell generations. DNMT2, on the other hand, is responsible for methylation within embryonic stem cells. Methylation has also recently been discovered to occur in non-CpG cytosines within undifferentiated stem cells [4, 7–9]. Methylation is important as not all genes are expressed at all times. *In fact, most are repressed.* Methylation is an epigenetic means of keeping genes suppressed until they are needed. Remember, methylation, especially hypermethylation, silences gene expression. Pause and consider for a moment the use of methylated vitamins and what implications this may have on DNA expression. Further, methylation is important for determining chromosomal replication timing. During hypomethylation there is late-replication, which may be demethylated slowly during cell division. Hypermethylation, on the other hand, leads to much earlier replication during S phase. Those hypermethylated genes are typically repackaged with acetylated histones. Hypomethylation will cause the building of nucleosomes that contain deacetylated histones [9]. In this way, methylation may control the "turning off" of genes.

Lastly, small non-coding RNA molecules (MicroRNAs) are a range of molecules that also help to control the expression and function of genes. They are non-coding RNAs that regulate gene expression post-transcriptionally or after the protein has been processed by the ribosome. Typically, they repress protein production by altering the capabilities of messenger RNA and by a process called translational silencing [10]. In this way, they are able to repress gene function and expression. MicroRNAs are accountable for targeting approximately 30% of genes and can influence tumor suppression, apoptosis, cellular proliferation, and cell movement [6].

Agouti mice illustrate how nutrition may modify phenotypic expression, epigenetic expression and disease risk outcome. Maternal dietary differences in methyl (CH_3) intake dictates the presence of a yellow hair color either as a band within primarily brown hair in wild-type mice, or as fully yellow hair in those with mothers who had decreased methylation. This is related to the agouti viable yellow (Avy) gene. This phenotype is accompanied by obesity and is a result of paracrine signaling issues resulting in hyperphagia in mice pups. The consequence of this epigene is that the mice expressing the aberrant agouti gene are more likely to develop diabetes and cancer later in life. The difference between pups expressing the wild-type genetics versus the polymorphism is the overmethylation status allowing for suppression of the *agouti* gene. To further influence the expression of this gene, supplementation with choline, vitamin B12, and folate both prior to and during pregnancy repress *agouti* expression [6, 11, 12] (Fig. 17.1).

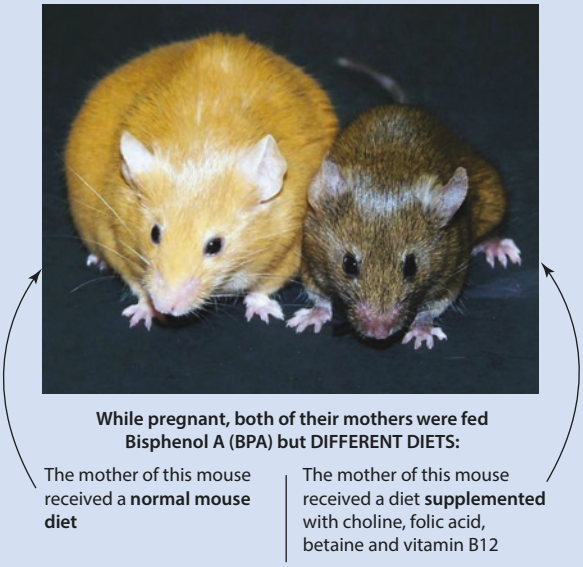

Fig 17.1 These two mice are genetically identical and of the same age. The larger yellow obese Agouti mouse on the left received a diet without methyl nutrients. The smaller brown healthy Agouti mouse on the right received a diet with methyl nutrients (choline, folic acid, betaine and B12). Both mice were fed BPA in each diet. In the laboratory, BPA appears to reduce methylation of the agouti gene. With the mother fed the regular (BPA-modified) diet, the pups were more likely to be yellow and obese and more prone to develop cancer and diabetes. When the mothers were fed the BPA-modified diet plus methyl nutrients, the pups were more likely to be born healthy and brown, and they were of ideal weight. The methyl nutrient supplementation counteracted the negative effects of the exposure. (Adapted from Jirtle R, Dolinoy D. Agouti Mice. Retrieved from: ► https://commons.wikimedia.org/wiki/File:Agouti_Mice.jpg. With permission from Creative Commons License 3.0: ► https://creativecommons.org/licenses/by/3.0/deed.en.)

Another classic example of the influence of nutrition on epigenetic expression is the *Dutch Hunger Winter Study*. This study looked at a cohort of people conceived during a famine that occurred over 6 months during the last winter of World War II (1944–1945). During this time, the caloric intake decreased from approximately 1400 kilocalories in October

1944 to less than 1000 kilocalories towards the end of November 1944. Caloric intake further declined at the peak of the famine to 400–800 kilocalories from December 1944 to April 1945. Despite the plummeting amounts of kilocalories during this time, the proportion of fats, carbohydrates, and proteins remained the same [13–15]. The study found a positive correlation between famine, calorie restriction and obesity in the offspring of women pregnant during this time. This association was further correlated to obesity-related diseases in adulthood such as atherosclerosis, hyperlipidemia, coronary artery diseases, and increased risk of cardiovascular mortality [13–15]. As a segue into obesity and obesity-related disorders, we will explore specific epigenetic influences and single nucleotide polymorphisms (SNPs) associated with cardiometabolic disease.

17.1.1 The Epigenetics of Cardiometabolic Disease

Cardiometabolic disease encompasses a cluster of disease characteristics including atherosclerosis, dyslipidemia, diabetes mellitus type 2 (DM2), hypertension (HTN), increased waist circumference and obesity [16]. All of the aforementioned conditions have genetic etiologies that can increase the risk or prevalence of the disease in affected individuals.

To begin, let us look closely at genetics that increase the risk for cardiovascular disease (CVD). Apolipoprotein E (APOε) is a cholesterol carrier that assists in lipid transport [17]. It does so by merging with endogenous lipids to form lipoproteins that are responsible for restructuring other lipids and cholesterol. These lipoproteins also circulate these lipids in the bloodstream. The three isoforms of APOε are APOε2, APOε3, and APOε4. The APOε3 isoform is considered to be the neutral genotype and confers no additional CVD risk. However, the ε2/ε2 variation is associated with familial hypercholesterolemia, or genetically related high cholesterol [18]. Dysbetalipoproteinemia is a rare familial dyslipidemia characterized by approximately equally elevated serum cholesterol and triglyceride levels due to accumulated remnant lipoproteins in apolipoprotein ε2/ε2 homozygotes [18]. This genotype is very rare with the frequency in European ethnicity at 0.3%, African American ethnicity at 0.4%, and Asian ethnicity at 0.2% as compared to the ε3/ε3 genotype with 57.4%, 41.7% and 74.6%, respectively [19, 20]. This type of predictability is nutrigenetics and is not likely to be a candidate for epigenetic alteration. However, Yang, et al. (2007) found that for those with a single variant of APOε2, there was a 2.2-fold increased risk of myocardial infarction if there was a concurrent high saturated fat intake. They also found that for those that carry a single variation of the APOε4 gene, there was a 1.6-fold increased risk of myocardial infarction when paired with a high saturated fat diet. Compared to non-carriers, the APOε2 carriers who consumed a high saturated fat diet resulted in consistently elevated LDL cholesterol levels by (+ 17%) and carriers of the APOε4 variant had an increase of (+ 14%) [21]. This dietary intervention potentially activated these genes and increased the risk of CVD.

It has been suggested that those with the ε2/ε2 variation restrict their saturated fat intake to less than 12 g/day to prevent dysbetalipoproteinemia and myocardial infarctions [21]. However, there are further complications with suggested saturated fat intake recommendations. The apolipoprotein A2 (APOA2) gene determines the amount of saturated fat required to prevent dyslipidemia in those with DM2. Those with the CC genotype at rs5082 have an increased risk for dyslipidemia and should decrease saturated fat consumption to less than 10% [22]. The current nutritional United States Department of Agriculture (USDA) guideline already recommends the consumption of less than 10% of calories per day from saturated fats (20 grams) [23]. While this may seem auto-confirmatory, recently there is an extreme trend towards ketogenic, or very high fat diets. For those with either the ε2/ε2, ε4/ε4 or APOa2 genotypes, a high fat diet would clearly be contraindicated as it would significantly increase their risk of heart attack and cardiometabolic disease. The frequency of APO ε4/ε4 is much higher than APOε2. For European ethnicity, the frequency is 2.9%, African American ethnicity 3.6%, and Asian ethnicity is 1.0% while carriers of APOε4 are 24%, 34.1% and 15.4%, respectively [19, 20]. The Yang study reviewed carriers, thus the results are associated with having only one deleterious allele.

Hypertension is a hallmark diagnostic of cardiometabolic disease. For patients with hypertension and diabetes, treatment should be initiated when blood pressure is 140/90 mm Hg or higher, regardless of age [24]. Genomically, hypertension is related to the angiotensin-converting enzyme (ACE) and the angiotensinogen gene (AGT). Angiotensinogen, which is formed in the liver and controlled by AGT, is broken down by renin to form angiotensin I. Angiotensin II is formed from angiotensin I by ACE, which is then acted upon by the angiotensin receptor (AT1R) and ultimately converts to aldosterone. Angiotensin II regulates vasodilation and constriction, the sympathetic nervous system, antidiuretic hormone, and hormones in the adrenal glands. Aldosterone is the primary adrenal hormone for this system, as it regulates sodium retention, potassium excretion and ultimately fluid balance [25]. This is the underlying mechanism for hypertension drugs called ACE inhibitors, blocking angiotensin II production and thereby lowering aldosterone production and the above-described regulatory mechanisms. However, when there is a deletion in the ACE gene, there will be an increase in aldosterone and angiotensin II because the regulator has been removed [26]. This can result in anxiety, increased cortisol, memory problems, hypertension, and autoimmunity [27]. This imbalance in aldosterone will also alter the electrolyte balance, specifically, a decrease in sodium excretion, thus sodium retention and/or swelling and an increased excretion of potassium potentially resulting in hypokalemia [25]. Alterations in the AGT gene exacerbate the ACE deletion genotype while also carrying an inherent risk for pre-eclampsia, hypertension and insulin resistance. AGT directly increases angiotensinogen levels, thereby alter-

ing the renin-angiotensin-aldosterone system (RAAS) axis. Caproli, et al. found that almost 50% of all hypertensive cases were what they termed "salt-sensitive." They found that hypertensives carrying both the ACE and AGT polymorphisms responded favorably to a reduced sodium diet (less than 1500 mg per day). Those individuals who did not carry one or both of these polymorphisms are considered non-salt-sensitive and did not have any measurable effect when restricting sodium. This study elucidates that salt restriction is not always beneficial for all hypertensive patients [25].

Diagnostic and Testing Guidelines for Diabetes Mellitus Type 2

The diagnostic criteria for pre-DM2 consist of fasting plasma glucose (F/PG) 100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L) (impaired fasting glycaemia) OR 2-hour PG in the 75-g oral glucose tolerance test (OGTT) 140 mg/dL (7.8 mmol/L) to 199 mg/dL (11.0 mmol/L) (impaired glucose tolerance) OR hemoglobin A1C (A1C) 5.7–6.4%, while the diagnostics for DM2 are A1C ≥6.5% OR FPG ≥126 mg/dL (7.0 mmol/L).

Fasting is defined as no caloric intake for at least 8 hour.*OR 2-hour PG ≥200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by the World Health Organization (WHO), using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water OR in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L) [28].

Diabetes or dysregulated blood glucose is the other pillar of cardiometabolic disease. Type 2 diabetes (DM2) is a disease of insulin resistance, most often caused by central obesity and lack of exercise. This is considered a disease of lifestyle, thus epigenetically influenced. The idea that there may be an underlying genetic component to DM2 is appealing in many ways. Currently, the research is only suggestive of correlations rather than causations. One such example is the Transcription Factor 7 Like 2 (TCF7L2) locus. Those with the TT genotype at rs12255372 have a 67% increased risk of developing DM2. There is also preliminary evidence that this SNP could possibly increase the risk of breast cancer and aggressive prostate cancer [29]. Those with the genotype should include low glycemic index/glycemic load foods, reduce sugar consumption and limit processed grains to mediate this risk [29]. Interestingly, the global risk was not mediated in all study subjects, suggesting that the epigenome and environmental conditions were influencing the disease risk outcomes. This suggests an association between family history, genetics and disease outcomes.

Further, when considering diabetes risk associated with dyslipidemia, it is important to consider the gene Paraoxonase 1 (PON1). PON1 has both esterase and lactonase activity. The esterase enzymes assist in the catabolism of pesticides and certain pharmaceutical drugs while also protecting high and low-density lipoproteins from oxidation. Alterations in this gene could result in abnormally high levels of lipid peroxides, inflammation and compromised detoxification. The lactonase activity is specific to the catabolism of homocysteine thiolactones. When there is an increase in homocysteine, the result is an elevation of oxidizing thiolactones. When there are alterations in this gene, there is the potential for elevated levels of homocysteine and HDL-specific protein damage [30]. A specific SNP in PON1, rs662 (A) confers a higher risk of coronary heart disease and diabetes because it encodes for lower amounts of PON1 activity, thereby increasing oxidative stress and CVD-related disorders [31]. Specifically, when there are errors in PON1, HDL is oxidized, conferring a higher risk of cardiovascular disease. It has been shown that both Vitamin E supplementation and a diet higher in monounsaturated fats can help decrease both oxidized HDL and the activity of altered PON1. It should be noted that a diet high in saturated fat alone can inhibit the activity of PON1 even in the absence of genetic alteration, thus creating a functional enzyme deficiency. In this way, a diet high in saturated fats can increase the risk of both cardiometabolic disease and markers such as homocysteine [32, 33].

When presented with cardiometabolic disease, one of the most researched and used supplements is omega-3-fatty acid, often as fish oil. Typically, when we think of endothelial nitric oxide synthase (eNOS/NOS3), we are associating it with vasodilation, vascular smooth muscle relaxation via cGMP-mediated signal transduction pathway, vascular endothelial growth factor (VEGF)-induced angiogenesis in coronary vessels, and its ability to promote blood clotting via platelet activation. One intriguing hypothesis is that perhaps the mediating effects in CVD from NOS modulation depend on omega 3-fatty acid status. There is a SNP in NOS3 that determines whether increasing omega 3-fatty acids will decrease triglyceride levels in those with dyslipidemia. In rs1799983, it was found that subjects with the risk allele T (TT or GT) who had low levels of omega 3-fatty acids had 25% increases in serum triglyceride levels when compared to those with the GG genotype. This study suggested that those carrying the T allele increase their omega-3-fatty acid intake by 1.24 grams/day. Once subjects repleted with omega 3-fatty acids, their triglyceride levels normalized [34]. This may be the mechanism behind the plethora of research that indicates that a Mediterranean diet [35] or a diet high in fish like the Okinawan diet [36] confer such benefits in regards to CVD and cardiometabolic disease. It also may suggest a potential genetic component to omega-3 therapy in CVD and hypertriglyceridemia and should be a consideration in prescription/supplemental omega-3 therapy. It is also important to consider the corollary for this SNP, meaning those who do not have the risk allele and who may not be fish oil responders.

This brings us to the final pillar of cardiometabolic disease – obesity. It would be helpful for individuals and practitioners if there simply were a "fat" gene to determine whether dieting would be effective. Unfortunately, we have yet to find the genetic holy grail of obesity, but we have made some progress. Enter the fat mass and obesity gene (FTO). There has been quite an evolution of this gene and our understanding of its implications. To start, it was once a five-membered haplotype, meaning that there were five causally associated SNPs within the FTO locus that "predicted" the impact of

FTO [37]. Later, this group was revised to a three-membered haplotype, and then finally in 2015, a so-called causal variant was discovered [38]. SNP rs1421085 (C, C) confers a 1.7× increased obesity risk while the heterozygous form confers 1.3× increased obesity risk [39]. These are pretty low odds risks, and the magnitude does not exceed 4, meaning that this gene may play a minor role in obesity-related risks. This SNP disrupts the pathway for adipocyte thermogenesis involving ARID5B, IRX3, and IRX5, all regulatory genes for adipogenesis. Evidence suggests that because IRX3 binds so strongly to FTO, that other obesity-linked SNPs may be associated with IRX3 but not necessarily with FTO expression [40]. As you can see, we are merely scratching the surface. Unfortunately, we are unlikely to find a single answer to our complex obesity epidemic; instead, understanding and adjusting the epigenetics of obesity and cardiometabolic disease are far more promising. This epigenetic adjustment may include changes to diet and lifestyle, as well as optimizing methylation status.

Two conditions associated with cardiometabolic disease include polycystic ovarian syndrome (PCOS) and hemochromatosis (HFE). PCOS presents with infertility, hirsutism, polycystic ovaries, and insulin resistance, three out of five of these having considerable overlap with cardiometabolic syndrome [41]. The genomics of PCOS are heterogenous and have minimal GWAS (genome-wide associations). The SNPs that are associated with PCOS have been found only in non-obese PCOS patients, which is not the typical presentation of the disease [42]. There is, however, a sulfation gene, sulfotransferase 2A1 (SULT2A1), which may have a role in adrenal androgen excess in women with PCOS. Sulfotransferases are important for the metabolism of drugs and endogenous compounds. They convert them into more hydrophilic, water-soluble sulfate conjugates that then may be safely excreted. SULT2A1 specifically catalyzes the sulfation of steroids like DHEA and bile acids from the liver and adrenal glands. Alterations in these genes may increase the risk of PCOS by increasing endogenous levels of DHEA and/or be associated with hirsutism and insulin resistance [43].

Next is a clearly nutrigenetic condition, hemochromatosis (HFE). HFE C282Y accounts for 85% of all hemochromatosis cases. Another minor variant, HFE H63D, is responsible for the remaining 15% of cases, often with a milder presentation [44]. Hemochromatosis is an iron-overload syndrome that results in cirrhosis of the liver, diabetes, hypermelanotic pigmentation of the skin, heart disease, liver cancer, depression, and fatigue [45]. This condition can be exacerbated by hepatitis infections and it is associated with hypogonadism in males. When these genes are present, it is best to avoid excessive dosages of vitamin C as to not increase the absorption of non-heme iron. When presented with cases of liver disease, diabetes and cardiovascular disease, when the genomics does not necessarily make sense, it is important to rule out genetically inherited conditions such as HFE.

17.1.2 The Epigenetics of Psychiatric and Neurodegenerative Diseases

The gold standard of diagnostics for psychiatric diseases is the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition, DSM-V. Dementia is characterized in the DSM-V; however, Alzheimer's disease (ALZ) is not specifically categorized [46]. This is a conundrum as more than half of dementia cases have ALZ as an etiology [47]. DSM-V characterizes dementia as having "multiple cognitive deficits, which include memory impairment and at least one of the following: aphasia, apraxia, agnosia or disturbance in executive functioning. Social or occupational function is also impaired. A diagnosis of dementia should not be made during the course of a delirium (a dementia and a delirium may both be diagnosed if the dementia is present at times when the delirium is not present) [46]." Interestingly, the same apolipoprotein (APOƐ) that increases the risk for cardiovascular disease (CVD) is also associated with ALZ. This raises the epigenetic question of true disease etiology: Is ALZ a disease of environmental exposures and lifestyle choices rather than Mendelian genetics? Many would argue yes, and some have even gone so far as to rename ALZ type 3 diabetes [48]. When we consider inheritable diseases, we should also remember that families often experience identical environmental influences. Therefore, it is challenging to decipher true genetic etiology and risk when the epigenome greatly confounds such concepts.

Let us consider the nutrigenetics, or Mendelian genetics, that are associated with ALZ. As with cardiovascular disease (CVD), the ε3 variant in APOε is considered to be neutral with an odds ratio of 1 [49]. The ε2/ε2 genotype is actually protective of ALZ with approximately a 0.5 (0.22–1.1) odds ratio [49]. While the frequency of this ε2/ε2 genotype is quite rare and protective for ALZ, it also increases the risk for beta dyslipidemia. This is a case where the phenotype would present with CVD and not ALZ. However, the more common genotype, ε4, connects the two conditions clearly. Carrying one variant of ε4 is associated with approximately 3.4 (3–3.8) times increased odds of developing ALZ. Carrying two copies of the ε4 variant exponentially increases the risk resulting in an approximately 12.9 (10.2–16.2) times increased odds risk of developing ALZ in populations of European ancestry [49]. Thus, for those that carry even one of the ε4 variants, there is an increased risk for both CVD and ALZ. Further associating CVD with ALZ, Corneveaux et al. also found a consistent relationship with ALZ disease risk predictability to polymorphisms in angiotensin-converting enzyme (ACE), one of the hypertension-related SNPs [49]. There are many other genetic factors that play into the overall risk of developing ALZ that should also be considered outside of APOe, ACE, and CVD genetics. Studies have identified certain GWAS SNPs that are associated with both early and late onset ALZ, as well as many other regulating factors such as amyloid precursor protein (APP), Presenilin 1(PSEN1) and Presenilin 2 (PSEN2) [50]. The jury is still out on the etiology of ALZ

and CVD; however, dietary and lifestyle choices clearly play an epigenetic role in the development of both diseases [49]

The DSM-V diagnostic criteria for generalized anxiety disorder (GAD) include the following: "The presence of excessive anxiety and worry about a variety of topics, events, or activities; the worry is experienced as very challenging to control; the anxiety and worry are associated with at least three of the following physical or cognitive symptoms (in children, only one symptom is necessary for a diagnosis of GAD): edginess or restlessness, tiring easily; more fatigued than usual; impaired concentration or feeling as though the mind goes blank; irritability (which may or may not be observable to others); increased muscle aches or soreness; difficulty sleeping (due to trouble falling asleep or staying asleep, restlessness at night, or unsatisfying sleep) [46]."

Generalized anxiety disorder (GAD) affects approximately 22% of the population, and more often in females than males [51]. With GAD affecting approximately one quarter of the population, there are epigenetic lifestyle factors and genomic alterations that need to be addressed.

First, our modern lifestyle prizes working excessive hours, typically away from home, while also trying to manage the basics of life, family, and household. Many work so many hours that they compromise their sleeping habits (impaired cortisol regulation), not to mention their diet (obesity, nutrient deficiency) and activities outdoors (vitamin D deficiency). Each of these factors are individually enough to alter the epigenetic activation of disease. In ▶ Chap. 18, the complex system of methylation is detailed. In brief, methylation is the process of moving methyl groups from one bio-molecule to the next, ultimately functioning in the regulatory capacity for everything from DNA synthesis, expression and modulation to other complex systems such as detoxification. Many of our modern diseases can be connected to alterations in this complex system due to the implications of regulating gene expression [52]. GAD, panic disorder and milder presentations of anxiety are not exceptions.

There is an enzyme called phenylethanolamine N-methyltransferase (PNMT) (notice the word, methyltransferase; it transfers a methyl group from one molecule to another). In this case, PNMT specifically converts norepinephrine to epinephrine [53]. In more common terms, these bio-molecules are called noradrenaline and adrenaline. Chronic activation of this enzyme increases adrenaline and results in anxiety, insomnia, and ultimately adrenal fatigue [53]. The cofactor for this particular enzyme is the universal methyl donor, s-adenosyl-methyltransferase (SAM) [54]. When SAM releases its methyl group, it is first converted to s-adenosyl-homocysteine and ultimately into homocysteine, an inflammatory amino acid metabolite associated with neuroinflammation and CVD. Therefore, chronic stress can result in anxiety, elevated levels of homocysteine, CVD and a depletion of beneficial methyl donors, thereby increasing overall disease risk. This enzyme provides the biological basis for the connection between chronic stress and disease. Mind-body techniques such as yoga, tai-chi, meditation, gentle exercise, sound therapy and prayer are helpful strategies to reduce daily stress [55].

Delving a bit deeper into the genetics and epigenetics of anxiety, there are two major enzymes (genes) that can control either catecholamine regulation (dopamine, epinephrine and norepinephrine) or other excitatory neurotransmitters like glutamate. For more detailed information on catecholamine synthesis, please refer to ▶ Chap. 18. The regulatory enzyme for the catecholamines is called catechol-o-methyltransferase (COMT) (another methyl-transferase). COMT transfers methyl groups from SAM to the catecholamines dopamine, epinephrine and norepinephrine while assisting in their degradation (◘ Fig. 17.1) [56]. COMT also transfers other methyl groups, like those found in foods like green tea, citrus (quercetin), and potatoes, along with the hormone catechol estrogen [57]. The issue with this being a methyltransferase is that it is dependent on the flow of methyl groups generated in one-carbon metabolism, and recycled or processed in methylation pathways. Having both too little SAM and too much s-adenosyl homocysteine (SAH) can create inhibition or downregulation of enzymatic activity resulting in an increase of catechols. This can be independent of genetic alteration creating a functional inhibition of this enzyme [58]. This enzyme has broader applications and connects neurotransmitter regulation, estrogen metabolism and diet. Like most nutrigenomic enzymes, this one is dependent on a nutrient cofactor, or "catalyst" that is required for the enzyme to function properly (note: this is not a true catalyst in chemical terms as the catalyst is not always consumed, rather in these cases, we are describing a circumstance where the substrate is being converted to a product and this reaction requires a cofactor). The cofactor for COMT is the incredibly important mineral, magnesium [59]. In addition to serving as a cofactor for COMT, magnesium is also a cofactor for 300 other enzymatic processes in the body [60, 61]. The phenotype for this type of anxiety typically excludes panic attacks, but there is a ruminating presentation of anxiety and an increased risk for insomnia [62] and palpitations [63]. Anecdotally, those with COMT inhibition (functional or SNPs) typically have "type A" personalities due to increased dopamine and epinephrine levels, are often successful and may or may not have disordered sleep patterns.

Magnesium deficiency can be the result of many things, including downregulation of the COMT enzymes. If you consider basic sciences and relate that all enzymes have a substrate, a catalyst and a product, when there is an upregulation of this pathway, there will be a depletion of the substrate and catalyst with an increase in the product. If there is a downregulation, there is either a catalyst deficiency (nutrient deficiency) or a SNP. In the case of upregulation, COMT depletes methyl donors by moving them too swiftly away from the catecholamines, thus ultimately decreasing these levels while simultaneously decreasing its catalyst cofactor, magnesium. This may present with fatigue and potentially dopamine depression. To validate this on an organic acids test, the markers vanilmandelate (VMA) and homovanillate (HVA) would be decreased showing excessive breakdown. In

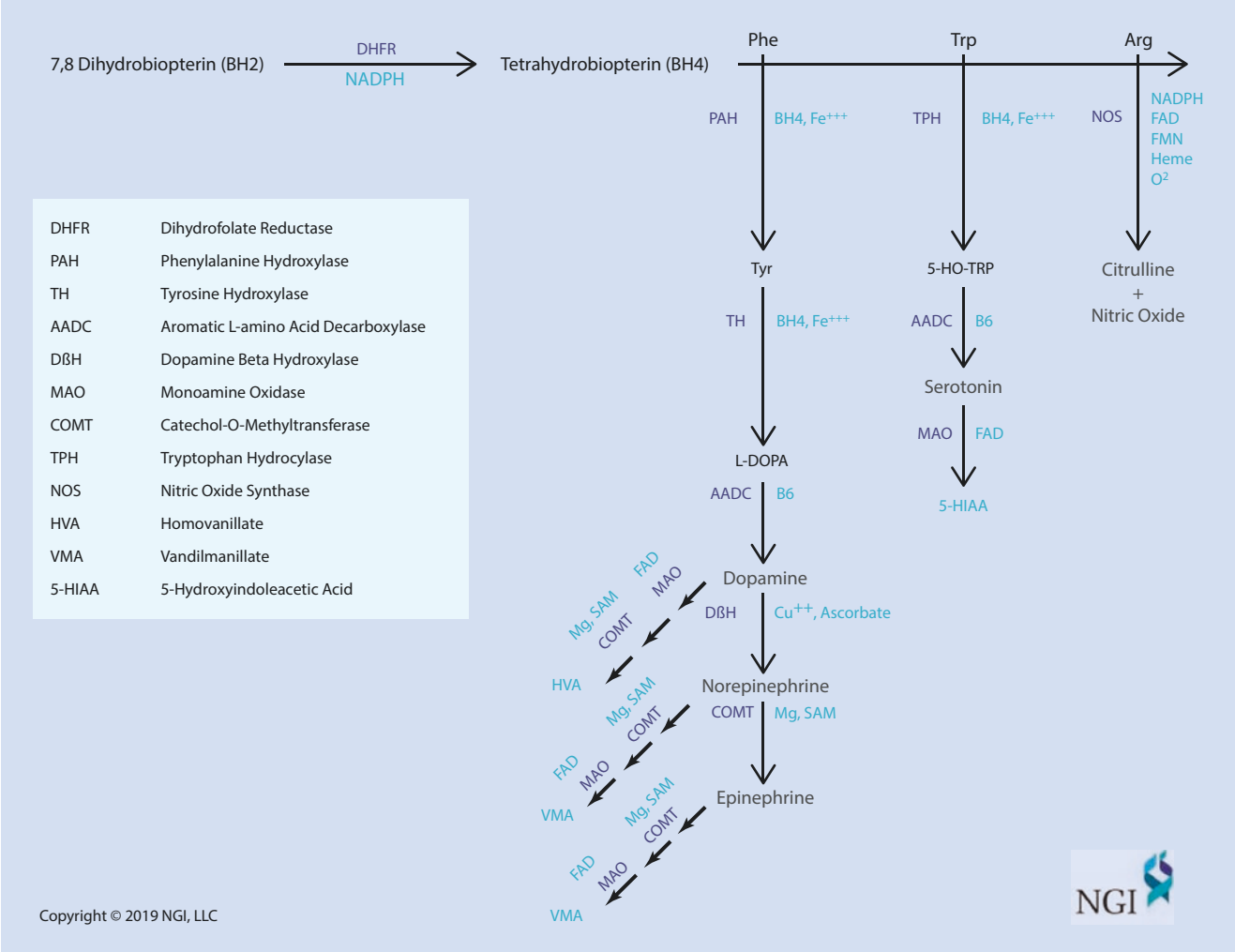

Fig. 17.2 BH4 and neurotransmitter synthesis. (Courtesy of Nutritional Genomics Institute, LLC)

downregulation, there is a "block" in the pathway, resulting in the inhibition of catecholamine breakdown, thereby resulting in increased levels of these neurotransmitters and the presentation of anxiety. The HVA/VMA pattern will be reversed in this case. If the presentation is consistent and there is a known alteration in COMT, magnesium threonate may ameliorate symptoms and anxiety. This particular form of magnesium has the potential to cross the blood-brain barrier and is considered to be a superior form for neurological conditions [64]. Interestingly, clinically, this pathway strives for homeostasis. Many patients will experience a refractory response or increased anxiety or insomnia when given magnesium. In these cases, it is important to investigate the other enzyme that also degrades these neurotransmitters, monoamine oxidase.

According to the DSM-V, panic disorder includes the following: "panic attacks must be associated with longer than 1 month of subsequent persistent worry about: (1) having another attack or consequences of the attack, or (2) significant maladaptive behavioral changes related to the attack. To make the diagnosis of panic disorder, panic attacks cannot directly or physiologically result from substance use (intoxication or withdrawal), medical conditions, or another psychiatric disorder. Other symptoms or signs may include headache, cold hands, diarrhea, insomnia, fatigue, intrusive thoughts, and ruminations." [46] (Fig. 17.2).

There is another presentation of anxiety as alluded to above. While this phenotype of anxiety can also include symptoms such as insomnia, it *includes* panic attacks associated with panic disorder. Panic attacks are associated with an enzyme that converts the excitatory neurotransmitter glutamate into the inhibitory neurotransmitter, γ-Aminobutyric acid (GABA). Not to be confused with generalized anxiety disorder, however, the name of this enzyme is glutamic acid decarboxylase 1 or GAD1. When there are alterations in these enzymes, there is a "block" in the conversion, resulting in an increase in glutamate and a decrease in GABA. This block is typically related to the limitation of the cofactor, vitamin B6 in the active form of pyridoxal-5-phosphate (P5P). To "validate" the decreased activity of this SNP, there is an organic acids marker called xanthurenate. When elevated, this is confirmation of a P5P deficiency [65].

Glutamate is found in a variety of foods, especially processed foods. Its most notorious conformation is monoso-

dium glutamate or MSG. MSG can increase glutamate levels in the brain and increase both neuronal and gastrointestinal inflammation via nuclear factor kappa-beta (NF-κB) [66]. Glutamate is also a modulator of the kynurenine pathway, which is limited by tryptophan and modulated by the nutrients pyridoxal-5-phosphate (vitamin B6), niacin (vitamin B3), iron, and magnesium. This association is consistent with the presentation of anxiety with concurrent depression as tryptophan is the precursor to serotonin. Alterations in this pathway may result in an increase in quinolinate, a neuroinflammatory metabolite responsible for modulating N-methyl-D-aspartate (NMDA) receptors in the brain. Excessive stimulation of the NMDA receptors results in neuronal inflammation and is associated with conditions like autism spectrum disorder and ALZ [67]. These GAD1 isoforms provide potential etiologies for panic disorders being genetically inherited [68]. For those having alterations in GAD1, supplementation with P5P, magnesium, niacin, and potentially iron (if deficient) can help to modulate the panic attack phenotype. If there is concurrent depression, consider also supplementing with tryptophan or 5-hydroxytryptophan.

The GAD enzyme has another interesting association. In cases where there is a homozygous alteration in the GAD enzyme, there is an increased risk for glucose dysregulation. This particular alteration results in a peculiar presentation, whereas there are moderately elevated levels of plasma blood glucose with low levels of fasting insulin. This presentation typically has the phenotype of reactive hypoglycemia, thin habitus and an erroneous diagnosis of DM2 [69]. The connection between these two clinical presentations lies in the physiology of GAD being expressed in both the brain and the beta islet cells of the pancreas [70]. To correct the discrepancy, moderate to high dosages of P5P provide an intervention to stabilize the glucose dysregulation. Interestingly, ACE inhibitors also modulate this particular mechanism, contributing to the complexity of a clinical diagnosis [71]. An ACE deletion and homozygous GAD isoform would create the phenotype of cardiometabolic disease. Clinically, this would be difficult to distinguish, perhaps other than the thin habitus. The lesson is to not make assumptions about the etiology of disease based solely on clinical presentation. The reality is often complex.Depression (major depressive disorder, MMD) is defined by the DSM-V as: "depressed mood or a loss of interest or pleasure in daily activities for more than 2 weeks; mood represents a change from the person's baseline; impaired function: social, occupational, educational; specific symptoms, at least five of these nine, present nearly every day:

1. Depressed mood or irritable most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful)
2. Decreased interest or pleasure in most activities, most of each day
3. Significant weight change (5%) or change in appetite
4. Change in sleep: Insomnia or hypersomnia
5. Change in activity: Psychomotor agitation or retardation
6. Fatigue or loss of energy
7. Guilt/worthlessness: Feelings of worthlessness or excessive or inappropriate guilt
8. Concentration: Diminished ability to think or concentrate, or more indecisiveness
9. Suicidality: Thoughts of death or suicide, or has suicide plan

DSM-V proposed (not yet adopted) anxiety symptoms that may indicate depression: irrational worry, preoccupation with unpleasant worries, trouble relaxing, feeling tense, fear that something awful might happen" [46].

Lastly, we will discuss depression. Please refer to Fig. 17.2 for additional information regarding the synthesis and catabolism of dopamine and serotonin along with specific variant information. Clinically, there are two types of depression [71]. The first and most commonly known is related to serotonin deficiency. This mechanism has spawned an entire class of pharmaceutical drugs called selective serotonin reuptake inhibitors (SSRIs). While success is found in a portion of MDD patients with SSRIs, there is a considerable portion of non-responders [72]. Clinically, serotonin deficiency-related depression encompasses feelings of worthlessness, a tendency to withdraw from social activities, lack of motivation and excessive sleeping. To validate serotonin depression, there is an organic acids marker called 5-hydroxyindoleacetic acid (5-HIAA), a serotonin catabolism metabolite. This marker will be low if there is a restriction in serotonin metabolism with concurrent suppression of the amino acid tryptophan [67]. There may also be variations in the enzymes that create serotonin, such as tryptophan hydroxylase. The cofactors for this enzyme are (tetrahydrobiopterin) BH4, P5P, riboflavin, and copper. Addressing any abnormalities found in these nutrients could potentially relieve the strain on the enzyme responsible for forming serotonin [67]. In cases such as these, consider supplementing with tryptophan, 5-hydroxytryptophan or St. John's wort. It should also be noted that in addition to the expected downregulation phenotype, there are also upregulations in the genes that regulate this pathway. Monoamine oxidase A (MAO-A) specifically regulates serotonin catabolism and is the enzyme that stimulated the class of medications known as MAOIs or monoamine oxidase inhibitors. In these cases, you may find that 5-HIAA is actually elevated along with the validation marker for riboflavin, glutaric acid [73].

The second, less frequently recognized form of depression involves dopamine deficiency. Clinically, these patients present with a decreased interest or pleasure in most activities. Often in these cases, there is an upregulation in dopamine beta hydroxylase (DβH), which converts dopamine to norepinephrine. DβH requires the cofactors of vitamin C and copper, elucidating the characteristic low copper phenotype in dopamine depression [74]. A blockage in this enzyme is associated with schizophrenia treatment outcomes as it can theoretically increase dopamine levels [75]. Dopamine can also be restricted by one of four dopamine receptors. There

may be functional blocks in these receptors due to autoantibodies or polymorphism. Acute neuropsychiatric conditions like pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) and pediatric acute-onset neuropsychiatric syndrome (PANS) are also associated with autoantibodies to dopamine receptors. In the majority of these cases, supplementing with mucuna pruriens, also known as the velvet bean, supplies a direct precursor to dopamine, L-DOPA and resolves the symptoms of dopamine depression [76].

Sometimes, there will be abnormalities in both dopamine and serotonin. If there is evidence to support this conclusion, then there may be an enzyme deficiency in aromatic L-amino acid decarboxylase (AADC). This enzyme is P5P-dependent and is responsible for both the conversion of L-DOPA to dopamine and 5-hydroxytryptophan to tryptophan. Supplemental P5P is again the correct intervention for depression. It is also possible that there are genetic abnormalities or functional blocks in both the serotonin and dopamine pathways, thus this consideration should not be disregarded. For overall support, supplementation with P5P, magnesium and riboflavin are complementary for the resolution of anxiety and depression.

17.2 The Epigenetics of Irritable Bowel Disease and Dysbiosis

For every client that has a neurological condition, there are five more that have a variation of dysbiosis, leaky gut or irritable bowel disease (IBD). In this section, we will briefly discuss dysbiosis, environmental factors such as dietary influences and probiotic use associated with dysbiosis, and the genetics of celiac disease (CD). This is not an exhaustive review and is designed as an introduction. It is important to remember that humans have a symbiotic relationship with our microflora. While it is popularly believed that our microbes outnumber us in a ratio of 10:1, these numbers have recently been recalculated to show that while there are still more microbial species than there are human, the ratio is closer to 1:1 [77]. Regardless of these ratios, the concept that we equally share the control panel with the microbiome has considerable implications on our health, disease, and longevity.

Celiac disease affects only a small percentage of the population. It is estimated that between Europeans and Americans, there is an approximate 0.5–1.26% frequency of CD [78]. CD presents with damaged epithelial cells of the intestine, malnutrition, diarrhea, anemia, osteoporosis, dermatitis herpetiformis, dental enamel hypoplasia, an increased risk of cancer and neurological symptoms such as headache, brain fog, and paresthesia [78, 79]. The etiology of this disease is an autoimmune reaction to the gluten protein found in cereal grains such as wheat, barley and rye [79]. The current guidelines for the diagnosis of CD includes: Signs and symptoms suggestive of CD, anti-transglutaminase type 2 antibody (anti-TG2) with levels typically more than 10 times the upper limit of normal and a positive confirmation test of anti-endomysium-IgA antibodies (EMA) [79]. As we begin to explore the genetic susceptibility of CD, we will encounter a condition that has recently been coined to explain the sudden rise in gluten sensitivity in the absence of CD. This new condition has been named non-celiac gluten sensitivity (NCGS), and it is associated with heterozygous alterations in the human leukocyte antigen (HLA) genes that determine CD. HLAs are a highly polymorphic gene complex that encodes for the major histocompatibility complex (MHC) proteins. These MHC proteins are primarily responsible for immune regulation and are the genes that require matching for organ transplants [80]. Alterations in these genes increase the risk of several autoimmune diseases.

About 90–95% of CD patients carry a certain genotype called HLA-DQ 2.5. When there is a homozygous mutation for this genotype, there is a 50-fold increased risk of developing CD [81]. The other remaining 5–10% of CD patients have a milder alteration in HLA-DQ 8. The presence of a homozygous alteration in this genoset results in a 17-fold increased risk of CD development [81]. In some cases, especially in NCGS, there are heterozygous alterations in these SNPs or other related SNPs that result in a genotype called HLA-DQ 2.2. Please refer to the Epigenetic SNPs chart associated with this chapter for specific details, genes and SNPs regarding HLA-DQ 2.5, HLA-DQ 8, and HLA-DQ 2.2 (see Table 17.1).

Interestingly, 40% of the general population also has alterations in these DQ genes, yet they do not develop CD. NCGS can also be present in the absence of any known genomic alteration, which complicates the suspected etiologies [81]. Dr. Stephanie Seneff proposes that this dramatic rise in NCGS and CD is associated with the relatively recent advances in biotechnology. Glyphosate is commonly known as Roundup ® and is trademarked by Bayer AG [45]. This chemical is not only a ubiquitous herbicide, but the majority of our mega crops have been genetically altered to be "Roundup-ready" or have the ability for the plant organism to survive glyphosate application, while killing the other "weeds." These genetically modified organisms (GMOs) can potentially compromise the cytochrome p450 system, result in NCGS, irritable bowel syndrome and unfavorable alterations in the microbiome as well as negatively stimulate the immune system [82]. Unfortunately, GMO wheat, corn, and soy comprise a large portion of the American diet as recommended by the USDA [83]. Here we see can clearly see the advancements in crop productivity directly impacting the epigenome and risk for CD and NCGS. These environmental conditions (i.e., how much of these foods we consume) ultimately determine if these genes are to become epigenetically activated. Remember, the presence of the genetic alteration increases the risk of the disease, but it not solely diagnostic.

This balance of the microbiota is far more important than we imagined in the age of antibiotics. Further, there is a great deal of variability in the microbiomes of individuals. We have learned that these nuances relate to health status, age, diet, microbial interactions, and even host genotype.

Table 17.1 Epigenetic SNPs hart

SNPS	Co factors	Organic acids/clinical associations	Amino acids/ other tests/ interventions	rs Numbers
Epigenetics of cardiometabolic/ neurodegenerative diseases				 ► www.snppros.com
APOε 2 *Apolipoprotein E 2* ε2/ε2 is protective of Alzheimer's disease but associated with familial hypercholesterolemia Dysbetalipoproteinemia is a rare familial dyslipidemia characterized by approximately equally elevated serum cholesterol and triglyceride levels due to accumulated remnant lipoproteins in apolipoprotein ε2/ε2 homozygotes	N/A	Dysbeta-lipoproteinemia ↑Cholesterol, ↑triglycerides	Lipid panel, CMP; those with this genotype should limit saturated fat intake to 12 g/day; advanced lipid panel/MPO	rs7412 (T) rs429358 (T)
APOε 3 *Apolipoprotein ε 3*	N/A	Considered to be the neutral genotype for both CVD and ALZ.	Lipid panel, CMP	rs7412 (C) rs429358 (T)
APOε 4 *Apolipoprotein ε 4* Carrying a single copy of the ε4 variant is associated with about 1.5–2 times increased odds of developing Alzheimer's disease (ALZ) Two copies of the ε4 variant is associated with approximately nine times increased odds risk of Alzheimer's disease populations of European ancestry	N/A	↑Cholesterol; a high saturated fat intake was associated with a 2.2-fold increased risk of MI among carriers of APO ε∗2 (OR = 3.17; 95% CI, 1.58–6.36) and with a 1.6-fold increase among carriers of the –491T and APOε ∗4 variants together (OR = 2.59; 95% CI, 1.38–4.87). Consistently, a high fat diet elicited a greater response in LDL cholesterol among carriers of APOε ∗2 (+17%) and APOε ∗4 (+14%) compared to noncarriers (+6%)	Lipid panel, CMP, investigate other ALZ-related genetics	rs7412 (C) rs429358 (C)
APOA2 *Apolipoprotein A2* In diabetics, this gene determines the amount of saturated fat required to prevent dyslipidemia	N/A	↑Cholesterol, ↑triglycerides Those with the CC genotype have an increased risk for dyslipidemia and should decrease saturated fat consumption to less than 10%	Lipid panel, CMP, advanced lipid panel/MPO	rs5082 (C)

(continued)

Table 17.1 (continued)

SNPS	Co factors	Organic acids/clinical associations	Amino acids/ other tests/ interventions	rs Numbers
ACE del *Angiotensin converting enzyme* Deletion results in upregulation of activity Results in higher rate of conversion of angiotensin 1 to angiotensin 2 ↑aldosterone	N/A	↑Aldosterone can result in anxiety increased cortisol, learning, memory problems, hypertension and autoimmunity Electrolyte balance issues, specifically, ↓sodium excretion (so sodium retention/ swelling) and ↑excretion of potassium excretion (low potassium); cardiovascular complications	Lipid panel, CMP, monitor blood pressure; modify stress	rs4343(A) is a proxy for ACE Del16; GA, AA genotypes should restrict sodium intake to less than 1500 mg/day
AGT *Angiotensinogen gene* Specifically increases angiotensinogen levels, exacerbating the ACE deletion; requires renin to degrade; works in the adrenals Associated with insulin resistance, especially when combined with ACE Associated with increased hypertension and its associated diseases like pre-eclampsia and heart disease	N/A	↑Insulin resistance, ↑hypertension, ↑angiotensinogen Electrolyte balance issues, specifically, ↓sodium excretion (so sodium retention/ swelling) and ↑excretion of potassium excretion (low potassium); cardiovascular complications	Lipid panel, CMP, monitor blood pressure; modify stress	rs699, M235T (C) When combined with ACEdel16=salt sensitive hypertension— restrict sodium intake to less than 1500 mg/ day
FTO *Fat mass and obesity* Causal variant that disrupts a pathway for adipocyte thermogenesis involving ARID5B, IRX3, and IRX5; mechanistic basis for the genetic association between FTO and obesity	Molecular oxygen, alpha-keto-glutarate and iron	↑Weight gain, ↑insulin resistance, ↑risk for DM2	Lipid panel, CMP, fasting Insulin	rs1421085(C)
TCF7L2 *Transcription factor 7 Like 2* Increased risk of diabetes mellites 2	N/A	TTs have a 67% increased risk of developing DM2. They should include low GI/ GL foods, reduce sugar consumption and limit grains to reduce this risk	Lipid panel, CMP, HBA1C, fasting insulin	rs12255372(T); slightly increases (~1.5×) risk for type-2 diabetes and possibly breast cancer and aggressive prostate cancer
NOS1 *Nitric oxide synthase 1* Catalyzes the conversion of arginine to nitric oxide Also creates the byproduct citrulline	NADPH and NADP+	↑Orotate, ↑citrate, ↑isocitrate, ↑cis-aconitate	Potential abnormalities in arginine, citrulline, nitric oxide Lipid panel to screen for hypertension	rs7298903(C) rs3782206(T) rs2293054 [Val1353Val]

Table 17.1 (continued)

SNPS	Co factors	Organic acids/clinical associations	Amino acids/ other tests/ interventions	rs Numbers
NOS2 *Nitric oxide synthase 2* Inducible nitric oxide synthase (iNOS) is considered a reactive free radical involved in neurotransmission along with antimicrobial and antitumoral activities Found in the immune and cardiovascular systems and is involved in immune defense against pathogens May be induced by lipopolysaccharides and cytokines Commonly found in inflammatory disorders	Manganese	↑Orotate, ↑citrate, ↑isocitrate, ↑cis-aconitate	Potential abnormalities in arginine, citrulline, nitric oxide	rs2297518(A/T) rs2248814(G/C) rs2274894(T)
SOD2 *Superoxide dismutase 2* Superoxide dismutase is an antioxidant enzyme required to catalyze the dismutation of two superoxide radicals into hydrogen peroxide and oxygen Protects tissues from oxidative stress	Manganese	Increased risk of ischemic heart disease in 9188 participants from the Copenhagen City Heart Study Later found to reduce the risk of chronic obstructive pulmonary disease and acute exacerbations of chronic obstructive pulmonary disease in three independent, large population studies.	RBC minerals, lipid panel; Do not practice high intensity exercise without guidance	rs1799895(G) R213G
PON1 *Paraoxonase* Esterase enzymes that break down pesticides and pharmaceutical drugs Involved in protecting both HDL and LDL from oxidation. Its lactonase activity is involved in the breakdown of homocysteine thiolactones	Displaces calcium	Those with alterations have an increased risk of pesticide poisoning, diabetes, atherosclerosis and heart disease High fat diet alone inhibits enzyme	Lipid panel, CMP, fasting insulin, Homocysteine; Increase MUFA and Vitamin E	rs662 (A) confers a higher risk of coronary heart disease
HFE-C282Y Accounts for 85% of all hemochromatosis cases Hemochromatosis results in cirrhosis of the liver, diabetes, hypermelanotic pigmentation of the skin, heart disease, liver cancer, depression and fatigue Made worse with hepatitis infections Associated with hypogonadism in males	Avoid excessive Vitamin C	↑Ferritin levels, sometimes accompanied with ↑transferrin saturation and ↑hepatic iron concentration (HIC)	Hepatic iron concentration in hemochromatosis- affected patients ranges from 5000 to 30,000 µg/g (normal values, 100–2200 µg/g) TIBC ranges from 200 to 300 µg/dL in hemochromatosis-affected patients (normal range, 250–400 µg/dL)	rs1800562 C282Y (AA)

(continued)

Table 17.1 (continued)

SNPS	Co factors	Organic acids/clinical associations	Amino acids/ other tests/ interventions	rs Numbers
HFE-H63D Accounts for 15% of all hemochromatosis cases. Hemochromatosis results in cirrhosis of the liver, diabetes, hypermelanotic pigmentation of the skin, heart disease, liver cancer, depression and fatigue Made worse with hepatitis infections Associated with hypogonadism in males Risk allele C can result in mild presentation, but was also found to be associated with iron deficiency in women	Avoid excessive Vitamin C	↑Ferritin levels, sometimes accompanied with ↑transferrin saturation and ↑hepatic iron concentration (HIC)	Hepatic iron concentration in hemochromatosis-affected patients ranges from 5000 to 30,000 µg/g (normal values, 100–2200 µg/g) TIBC ranges from 200 to 300 µg/dL in hemochromatosis-affected patients (normal range, 250–400 µg/dL)	rs1799945 – H63D (GG/CC) Risk allele C
SULT2A1 *Sulfotransferase 2A1* Sulfotransferases are important for the metabolism of drugs and endogenous compounds and convert them into more hydrophilic water-soluble sulfate conjugates that then may be excreted. SULT2A1 catalyzes the sulfation of steroids and bile acids in the liver and adrenal glands	N/A	May have a role in adrenal androgen excess in women with polycystic ovary syndrome (PCOS)	CMP, 24-hour urinary hormones test, FHS, LH, SHBG	rs11083907(A) rs11569679(T) rs2547231(C) rs296366 (T) rs4149449(T) rs4149452(T)
Epigenetics of psychiatric diseases				
GAD1 *Glutamic acid decarboxylase 1* Converts the excitatory neurotransmitter glutamate into the inhibitory neurotransmitter GABA GAD67 isoform encoded by the GAD1 gene is the rate-limiting enzyme in GABA synthesis from glutamate in the brain	P5P	↑Xanthurenate, mutations may result in anxiety/panic disorders, increased blood glucose levels and hemoglobin A1C, with decreased insulin levels	Fasting insulin, CMP, HBA1C	GAD1: rs2241165(C) rs2058725(C) rs3791850(A)
COMT *Catechol-O-Methyltransferase* Transfers methyl group from SAM to the catecholamines. Breaks down dopamine, epinephrine and norepinephrine. Transfers methyl group from SAM to catechols from foods, (green tea, potatoes), antioxidants (quercetin), and hormone catechols (estrogen)	Magnesium SAM	↓VMA, ↓HVA (downregulation; most common; suggests methyl trapping) ↑VMA, ↑HVA (Upregulation –suggests methyl dumping)	Consider RBC Magnesium	rs4680(A) rs4633(T)
PNMT *Phenylethanolamine N-methyltransferase* Converts norepinephrine to epinephrine	SAM/methyl donor	↑VMA, ↓SAM; Stress, insomnia, anxiety, methyl donor depletion The connection between stress and disease	Check COMT Status	rs149585781(A) rs3764351 rs773317399(A)

Table 17.1 (continued)

SNPS	Co factors	Organic acids/clinical associations	Amino acids/ other tests/ interventions	rs Numbers
DR1 *Dopamine Receptor 1* Most abundant dopamine receptor in the central nervous system Regulates neuronal growth and development Mediates some behavioral responses Modulates dopamine receptor D2-mediated events Reward and pleasure centers	Copper is the cofactor for DBH	↓HVA; Associated with bipolar disorders attention deficit hyperactivity disorder and alcoholism	RBC copper, ceruloplasmin, Iron panel with ferritin (iron and copper have the same trans-porter)	rs4867798(C) rs251937 (C/G) haplotype is: rs265981(C) rs4532(A) rs686(T)
DR2 *Dopamine receptor 2* DR2 is associated with adult walking behavior, associative learning, auditory behavior, behavioral responses to cocaine and ethanol, feeding and grooming behavior and myoclonic dystonia	Copper is the cofactor for DBH	↓HVA; Dopamine is required for fine motor control, cognition, mood and neurotransmitter balance. (High levels = schizophre nia or paranoia; low levels = Parkinson's)	RBC copper, ceruloplasmin, Iron panel with ferritin (iron and copper have the same trans-porter)	rs6277(C) rs1799732(–/–)
DR3 *Dopamine receptor 3* Localized to the limbic areas of the brain that are associated with cognitive, emotional and endocrine functions May be associated with susceptibil-ity to hereditary essential tremor 1	Copper is the cofactor for DBH	↓HVA	RBC copper, ceruloplasmin, Iron panel with ferritin (iron and copper have the same trans-porter)	rs167771(G)
DR4 *Dopamine receptor 4* Responsible for neuronal signaling in the mesolimbic system of the brain Regulates emotion and complex behavior	Copper is the cofactor for DBH	↓HVA; Linked to many neurological and psychiatric conditions such as schizophrenia, bipolar disorder, addictive behaviors and eating disorders (i.e., anorexia nervosa) Target for drugs that treat schizophrenia and Parkinson's disease Involved in behavioral fear response; response to cocaine and ethanol; olfactory learning; response to amphet-amines, histamine and steroid hormones; involved in short term memory and social behavior	RBC copper, ceruloplasmin, Iron panel with ferritin (iron and copper have the same trans-porter)	rs916457(T) rs752306(T) rs3758653(C) rs1800443(G) rs4331145(A)
Nutrient–specific disease associa-tions				
VDR - TAQ *Vitamin D receptor* Its affinity for calcitriol is roughly 1000× greater than its affinity for calcidiol.	Vitamin D	VDR-TAQ SNPs are often linked to better tolerance of methyl donors (conflicting evidence)	Calcidiol	rs731236(A)

(continued)

Table 17.1 (continued)

SNPS	Co factors	Organic acids/clinical associations	Amino acids/ other tests/ interventions	rs Numbers
VDR-Bsm *Vitamin D receptor* Its affinity for calcitriol is roughly 1000× greater than its affinity for calcidiol	Vitamin D	Rapidly converts calcidiol to calcitriol Clinical picture includes consistently low calcidiol levels with elevated calcitriol levels Adverse effects are common when calcidiol is supplemented in higher levels (50,000 IU)	Calcidiol, calcitriol	rs1544410(A) Risk allele A: women have an increased risk of low bone mineral density. Conversely, A 26 study meta-analysis estimated a decreased risk of osteoporosis associated with the G;G genotype
VDR – GWAS *Vitamin D receptor – Genome-wide association*	Vitamin D	Homozygotes were associated with an ~2.5× higher risk of prostate cancer compared to homozygote carriers of the more common (C) allele in the 630 Caucasian patients studied	Calcidiol, calcitriol	rs2107301(T)
TCN1 *Transcobalamin I* Secreted by the salivary glands and is a B12 binding protein. TCN1 helps B12 survive the HCL in the stomach and the complex travels to the intestine	Vitamin B12; hydroxocobalamin bypasses this transporter	↑MMA; Transports up to 80% of vitamin B12. Thought to function as a circulating storage form and may prevent bacterial use of the vitamin	Serum B12; check FIGLU and serum folate to rule out folate trapping	rs526934(G)
TCN2 *Transcobalamin 2* Once inside the enterocytes of the ileum, B12 breaks apart from TCN1 to bind to TCN2, which then carries B12 to the liver	Vitamin B12; hydroxocobalamin bypasses this transporter	↑MMA; major part of the secondary granules found in neutrophils and facilitates the transport of cobalamin to the liver; 20–30% of cobalamin is transported on the TCN2	Serum B12; check FIGLU and serum folate to rule out folate trapping	rs1801198(G)
GIF *Gastric intrinsic factor* Is produced by the parietal cells and is also a B12 binding protein	Vitamin B12	↑MMA, pernicious anemia, familial pernicious anemia, acute lymphoblastic leukemia, high-altitude polycythemia In gastritis (non-*H. pylori* related), those with the FUT2 secretor variant had decreased amounts of GIF secretion. This was true even in heterozygous FUT2 mutations	Intrinsic factor, MMA, serum B12; check FIGLU and serum folate to rule out folate trapping Consider GI/stool testing	rs558660(G)
GSTT1Del *Glutathione S-transferase theta 1* Conjugates reduced glutathione Many exogenous and endogenous hydrophobic electrophiles	1200 mg Vitamin C/ day	Potential ↑ in pyroglutamate, alpha-hydroxybutyrate and alpha-ketobutyrate	Potential abnormalities in glutamate, cysteine, glycine	rs79605217 (G/C) rs796052136(–/–)

Table 17.1 (continued)

SNPS	Co factors	Organic acids/clinical associations	Amino acids/ other tests/ interventions	rs Numbers
BCMO1 *Beta-carotene 15, 15´-monooxygenase 1* Converts beta-carotene into the active form of vitamin A, retinol (beta-carotene ->trans-retinol) **Nomenclature: renamed BCO1 in early 2018**	Iron	May increase plasma beta-carotene and decrease plasma retinol May cause between a 32% and 59% decrease in conversion of beta-carotene to trans-retinol	Signs and symptoms of retinol deficiency; check VDR and vitamin D status Plasma vitamin A	rs12934922(T) rs7501331(T) rs11645428 (A) rs6420424(A) rs6564851(G) confers higher beta carotene levels
Dysbiosis/GI disease SNPs				
FUT2 *Fucosyltransferase 2* Determines secretor status Secretor status allows for expression of the ABH and Lewis histo-blood group antigens in various secretions including the intestinal mucus	N/A	↓*Bifidobacteria*, ↑D-Lactate; ↑MMA, ↓serum B12, ↓microbiome diversity FUT2 non-secretors are at an increased risk of celiac disease FUT2 and MUC2 (mucin 2) polymorphism increases risk for colitis in those with Crohn's disease	Non-secretors are resistant to norovirus Serum B12 Consider stool testing	rs601338 (G>A) causes non-secretor status. Further linkage has been found with rs516246(T)
Celiac HLAs Those with these DQ genotypes have an increased risk for developing celiac disease	N/A	↑D-Lactate, ↑arabinitol, ↑benzoate, ↑phenylacetate, ↑p-hydroxybenzoate, ↑p-hydroxy-phenylacetate, ↑indican	Signs and symptoms suggestive of CD Anti-transglutaminase type 2 antibody (anti-TG2) levels more than 10 times the upper limit of normal Positive confirmation tests of anti-endomysium-IgA antibodies (EMA)	DQ 2.5 = rs2187668 (T) DQ7 = rs4639334 (A) DQ8 = rs7454108 (G) DQ 2.2 requires a test of three genes, two for inclusion, rs2395182 (T) and rs7775228 (C). DQ4 excludes a DQ2.2 as evidenced by rs4713586(C) The rs2040410(A) allele is associated with DRB1∗0301, and the rs7454108(C) allele is associated with DQB1∗0302 (and thus DQ8)
HLA-DQ2.5 90–95% of all celiac patients have this genotype	N/A	↑D-Lactate, ↑arabinitol, ↑benzoate, ↑phenylacetate, ↑p-hydroxy-	Signs and symptoms suggestive of CD	rs2187668 (T) (DQB1∗0201) is linked to DQA1∗0501; creating the
		benzoate, ↑p-hydroxy-phenyl-acetate, ↑indican	Anti-transglutaminase type 2 antibody (anti-TG2) levels more than 10 times the upper limit of normal Positive confirmation tests of anti-endomysium-IgA antibodies (EMA)	DQ2.5 haplotype

(continued)

Table 17.1 (continued)

SNPS	Co factors	Organic acids/clinical associations	Amino acids/ other tests/ interventions	rs Numbers
HLA-DQ8 Half of the remaining 5–10% of celiac patients have DQ8	N/A	↑D-Lactate, ↑arabinitol, ↑benzoate, ↑phenylacetate, ↑p-hydroxybenzoate, ↑p-hydroxyphenylacetate, ↑indican	Signs and symptoms suggestive of CD Anti-transglutaminase type 2 antibody (anti-TG2) levels more than 10 times the upper limit of normal Positive confirmation tests of anti-endomysium-IgA antibodies (EMA)	rs7454108 (G) (DQB1∗0302)
HLA-DQ 2.2; GS221 Increased risk gluten intolerance and for autoimmune disorders such as celiac disease		↑D-Lactate, ↑arabinitol, ↑benzoate, ↑phenylacetate, ↑p-hydroxybenzoate, ↑p-hydroxyphenylacetate, ↑indican	Signs and symptoms suggestive of CD Anti-transglutaminase type 2 antibody (anti-TG2) levels more than 10 times the upper limit of normal Positive confirmation tests of anti-endomysium-IgA antibodies (EMA)	rs2395182(T), rs7775228(C), not rs4713586(C)
APB1 *AP2-like ethylene-responsive transcription factor* Responsible for extracellular histamine degradation (encodes the enzyme Diamine Oxidase)	Copper	↓ DAO, ↑Ulcerative colitis symptoms	RBS copper, cerruloplasmin	rs1049793(G/T)
Mitochondrial Disease				
NDUFs3 NADH: ubiquinone oxidoreductase, iron-sulfur *protein fraction 3; Complex 1* Cleavage of NDUFS3 is the first step in GZMA-induced (AKA blood coagulation factor IX/Christmas factor) cell death Leigh syndrome	N/A May be limited by NAD, electrons and ubiquinone Thiamine is the cofactor for several mitochondrial enzymes and stimulates energy conversion	Fatigue and exercise intolerance Homozygous mutations should respond well to treatment with riboflavin, thiamin, carnitine and higher doses of coQ10	Check CoQ10 status Deficiency is marked by elevated hydroxymethyglutarate. Check fatty acid oxidation—may result in increases in adipate, suberate and ethylmalonate Elevated anion gap can indicate a need for thiamine	rs4147730(A)

Table 17.1 (continued)

SNPS	Co factors	Organic acids/clinical associations	Amino acids/ other tests/ interventions	rs Numbers
NDUFs7 *NADH: ubiquinone oxidoreductase, iron-sulfur protein fraction 7; Complex 1* Highest in heart and skeletal muscle Leigh Syndrome causes poor feeding, episodes of apnea and cyanosis, acute gastroenteritis, moderate hypertrophic obstructive cardiomyopathy, extensive white matter hypodensity, mild ventricular enlargement, and hypodense symmetric lesions in putamen and mesencephalon	N/A May be limited by NAD, electrons and ubiquinone Thiamine is the cofactor for several mitochondrial enzymes and stimulates energy conversion	Fatigue and exercise intolerance Homozygous mutations should respond well to treatment with riboflavin, thiamin, carnitine and higher doses of coQ10	Check CoQ10 status Deficiency is marked by elevated hydroxy-methyglutarate Check fatty acid oxidation, may result in increases in adipate, suberate and ethylmalonate Elevated anion gap can indicate a need for thiamine	rs1142530 (T) rs2332496(A)
NDUFs8 *NADH: ubiquinone oxidoreductase, iron-sulfur protein fraction 8; Complex 1* Required in the electron transfer process	N/A May be limited by NAD, electrons and ubiquinone Thiamine is the cofactor for several mitochondrial enzymes and stimulates energy conversion	Fatigue and exercise intolerance Homozygous mutations should respond well to treatment with riboflavin, thiamin, carnitine and higher doses of coQ10	Check CoQ10 status Deficiency is marked by elevated hydroxy-methyglutarate Check fatty acid oxidation, may result in increases in adipate, suberate and ethylmalonate Elevated anion gap can indicate a need for thiamine	rs1051806(T) rs2075626 (C)
COX5c *Cytochrome oxidase C subunit 5c* Cytochrome C (Complex 4) genes are the last step in the mitochondrial respiratory chain. This last step transfers all of that energy to oxygen, and having multiple SNPs in these genes creates free radicals	May be limited by ubiquinone Responds to riboflavin	COX5A is associated with sideroblastic anemia and cardio-encephalo-myopathy Glutaric Acid	CBC, Echocardiogram, EKG, 8-HO-2DG, Lipid peroxides	rs8042694(G)
COX6c *Cytochrome oxidase C subunit 6c* C Cytochrome C (Complex 4) genes are the last step in the mitochondrial respiratory chain. This last step transfers all of that energy to oxygen, and having multiple SNPs in these genes creates free radicals	May be limited by ubiquinone Responds to riboflavin	COX6C is associated with an increased risk for prostate cancer and kidney disease Glutaric Acid	CMP, 8-HO-2DG, Lipid peroxides	rs4626565(C)

(continued)

Table 17.1 (continued)

SNPS	Co factors	Organic acids/clinical associations	Amino acids/ other tests/ interventions	rs Numbers
ATP5c1 *ATP Synthase, H+ Transporting, Mitochondrial F1 Complex, Gamma Polypeptide* Encodes a subunit of mitochondrial ATP synthase Mainly expressed in heart Located in the mitochondrial matrix, complex V of oxidative phosphorylation	Hydrogen, phosphate group	Extreme fatigue, Fibromyalgia syndrome, decreased viability, decreased caspase 3/7 activity	Check all other mitochondrial subunits to ensure proper nutrient repletion	rs1244414(T)
ATP5g3 *ATP synthase, H+ transporting, mitochondrial Fo complex subunit C3 (Subunit 9)* Encodes a subunit of mitochondrial ATP synthase Mainly expressed in heart, located in the mitochondrial matrix, complex V of oxidative phosphorylation	Hydrogen, phosphate group	Extreme fatigue, fibromyalgia syndrome, decreased viability	Check all other mitochondrial subunits to ensure proper nutrient repletion	rs36089250(C)
Cancer–related SNPS				
TNFa *Tumor necrosis factor alpha* Pro-inflammatory cytokine that is associated with both anti and pro-cancer effects	N/A	AGONIST: Cannabidiol, echinacea, larch arabinogalactan ANTAGONIST: Astragalus, andrographis, resveratrol, alpha lipoic acid, Vitamin C, co-enzyme Q10, *Lactobacillus rhamnosus*, curcumin	Consider regular/ increased cancer screenings Consider stool testing	rs1800629(A) rs361525(A)
PTEN *Phosphatase and tensin homolog* Tumor suppressor gene that regulates apoptosis Diseases associated with PTEN: Bannayan-Riley-Ruvalcaba syndrome, Cowden disease, Cowden syndrome-like phenotype, Cowden syndrome, Endometrial carcinoma, Lhermitte-Duclos disease, Macrocephaly/autism syndrome, Malignant melanoma, Oligodendroglioma, PTEN hamartoma tumor syndrome with granular cell tumor, Prostate cancer, Proteus syndrome, Squamous cell carcinoma, head and neck, Vater association with hydrocephalus; In cancer, PTEN is frequently mutated or lost in human tumors	N/A	AGONIST: Astragalus, butyrate, honokiol, retinoic acid, Vitamin D; PTEN is increased by TNF-α (pro-inflammatory cytokine). ANTAGONIST: PTEN inhibition decreases nitric oxide (NO) production; high fat diet; resveratrol	Consider regular/ increased cancer screenings	rs701848(C) rs121909229 (A/C) rs121909233(A)

Table 17.1 (continued)

SNPS	Co factors	Organic acids/clinical associations	Amino acids/ other tests/ interventions	rs Numbers
NAT1/2 *N-acetyltransferase 1 protein* Involved in phase II xenobiotic metabolism and helps with the biotransformation of aromatic and heterocyclic amines Detoxifies hydrazine and acrylamine drugs	Acetylators like darkly colored berries and grapes (purple and red colored fruits and vegetables)	rs4986782 increases the risk for smoking-induced lung cancer, head and neck cancers Slow phenotype associated with increased risk of several cancers Fast phenotype associated with bladder cancer Remove charred meats and other xenobiotics	Check glutathione status and conjugation Check CYP enzymes to ensure proper detoxification	NAT2∗4 is wild type and considered to be a "rapid" metabolizer (rs1801279, rs1041983 and rs1801280) **Slow phenotype:** 1. rs1801289(C) + rs1799929(T) 2. rs1801280(C) + rs1799929(T) + rs1208(G) 3. rs1801280(C) + rs1208(G) 4. rs1801280(C) 5. rs1801280(C) + rs1799930(A) 6. rs1041983(T) + rs1801280(C) + rs1799929(T) + rs1208(G) 7. rs1041983(T) + rs1801280(C) + 1700020(T) + rs1208(G) 8. rs1041983(T) + rs1801280(C) + rs1799930(A) 9. rs1041983(T) + rs1799930(A) + rs1208(G) 10. rs1799929(T) + rs1799930(A)
Pharmacogenetics				
CYP1A1 Substrates: estrone, brassica vegetables containing diindolylmethane (DIM) or Indole-3-carbinole (I3C), polycyclic aromatic hydrocarbons, caffeine, aflatoxin B1, and acetaminophen	Heme	Fast metabolizer phenotype; associated with increased risk for breast cancer	24-hour urinary hormones testing	CYP1A1∗2C rs1048943(C) CYP1A1∗4 rs1799814(T)
CYP1A2 95% caffeine metabolism Other substrates: theophylline, phenacetin, acetaminophen, estrogen	Heme	GA: 26% increased risk for MI with two or more cups of coffee AA: 53% increased risk of MI with two or more cups of coffee Limit caffeine consumption to less than 200 mg/day High caffeine intake may result in increased estrogen levels and therefore hormone-related cancers (breast, ovarian, uterine, prostate, and testicular). See pharmacogenetics resources	Caffeine suppression test; 24-hour urinary hormones testing	rs2472300(A) rs762551 Risk Allele A increases activity (fast metabolizer) Risk Allele C decreases activity (slow metabolizer)

(continued)

Table 17.1 (continued)

SNPS	Co factors	Organic acids/clinical associations	Amino acids/ other tests/ interventions	rs Numbers
CYP1B1 Substrates: hydroxylation of estrogens, oxidizes 17-beta-estradio to the carcinogenic 4-hydroxy derivative, tamoxifen, polycyclincar-omatic hydrocarbon, aflatoxins, theophylline, phenacetin, and warfarin	Heme	COMT SNPs (downregu-lation) may compound SNPs in CYP1B1 Increases risk for breast, ovarian and prostate cancers	RBC magnesium for COMT status and 24-hour urinary hormones testing	CYP1B1 L432V (rs1056836 (G) Is protective against prostate cancer) CYP1B1 N453S rs1800440(C) is associated with decreased levels of urinary 2-OHE and 16-alpha in premenopausal women and is associated with breast and endometrial cancers CYP1B1 R48G rs10012(C/G)
CYP2E1 Substrates: Acetaminophen, caffeine, alcohol, chlorzoxazone, enflurane, aniline, benzene, chlorzoxazon, dacarbazine, eszopiclone, halothane, isoflurane, isoniazid, methoxyflu-rane, paracetamol, sevoflurane, theophylline, trimethadione, zopiclone	Heme	Chronic detoxication challenges See pharmacogenetics resources	Check glutathi-one synthesis	CYP2W1∗1B C9896G (rs2070676): GG is induced by alcohol, coffee and smoking; CC is wild type with no up or downregulation CYP2E1∗4 A4768G (rs6413419 (A)) Works with other CYP genes to detoxify carcinogens.
CYP2D6 Substrates: antidepressants, antipsychotics, analgesics, antitus-sives, beta-blockers, antiarrythmics, and antiemetics	Heme	Very common in most populations; mixed regulation often results in adverse drug reactions See pharmacogenetics resources	N/A	CYP2D6 T100C (rs1065852): T risk allele is a non-functioning or partially functioning variant causing decreased activity CYP2D6 C2850T (rs16947) AA increased risk for upregulation CYP2D6 S486 S485T (rs1135840): G risk allele causes upregulation
CYP2C9 Substrates: warfarin, NSAIDs, amitriptyline, apixaban, azilsartan, clopidogrel, Benadryl, gliben-calamide sulfonylurea glimepiride	Heme	SNPs indicate down-regulation See pharmacogenetics resources	N/A	CYP2C9∗2 C430T (rs1799853(T)) (~20% decrease) CYP2C9∗3 A1075C (rs1057910(C)) (~40% decrease)
CYP2A6 Substrates: nicotine, aflatoxin B1, cotinine, coumarin, dexmedtomi-dine, docetaxel, efavirenz, irinotecan, letrozole, methoxsalen, oxaliplatin, pilocarpine, tegafur, valproic acid, warfarin, methyl tert-buty (gasoline additive), halothane, methoxyflurane	Heme	Inhibited by grapefruit juice SNPs indicate down-regulation Chronic detoxification challenge phenotype See pharmacogenetics resources	Check VKORC1 status for warfarin sensitivity status	CYP2A6∗2 A1799T rs1801272(T) CYP2A6∗3 rs1057910(C)

Table 17.1 (continued)

SNPS	Co factors	Organic acids/clinical associations	Amino acids/ other tests/ interventions	rs Numbers
CYP2B6 Substrates: nicotine, alfentanil, bupropion, cyclophosphamide, ifosfamide, methadone, nevirapine, propfol, sertraline, sorafenib, tamoxifen, vaprioc acid, methoxetamine	Heme	SNPs indicate down-regulation Chronic detoxification challenge phenotype See pharmacogenetics resources	N/A	A136G rs3530384 C1132T rs34097093 C26470T rs8192719) G23280A rs2279344 G29435A rs7260329 I328T rs28399499 L262A rs2279343 Q172H rs3745274 R22C rs8192709 T1421C rs1042389 T20715C rs36079186 T23499C rs2279345
CYP2C19 Substrates: antidepressants, antiepileptics, proton pump inhibitors, clopidogrel, proguanil, propranolol, gliclazide, carisoprodol, chloramphenicol, c yclophosphamide, indomethacin, nelfinavir, nilutamide, progesterone, teniposide, warfarin	Heme	Mixed regulation may result in adverse drug reactions See pharmacogenetics resources	Urinary Hormones testing Check depression etiology (dopamine vs serotonin)	CYP2C19∗17 806C>T rs12248560 (T) Ultra rapid metabolizer CYP2C19∗2 rs4244285 (A) represents decreased activity or downregulation
CYP3A4 Substrates: Lidocaine, erythromycin, cyclosporine, ketoconazole, testosterone, estradiol, cortisone, alfentanil, alfuzosin, almotriptan, alprazolam, amiodarone, amitriptyline, amlodipine, anastrozole, aprepitant, aripiprazole, astemizole, astazanavir, atorvastatin, bepridil, bexarotene, etc.	Heme	Check CoQ10 markers such as hydroxymethylglutarate Grapefruit causes decreased clearance of a substrate and higher plasma levels See pharmacogenetics resources	Urinary hormones Test; adrenal stress index test	CYP3A4∗1B 392G>A (rs2740574): GG involved in oxidative deactivation of testosterone GG/AG alleles confer 10X increased risk of aggressive prostate cancer in African American men (>54%) CYP3A4∗3 M455T rs4986910(C) involved in estrogen metabolism Significantly decreased risk of breast cancer with the minor allele T
CYP3A5 Substrates: Olanzapine, tacrolimus, nifedipine, cyclosporine, testosterone, progesterone, androstenedione	Heme	N/A See pharmacogenetics resources	Urinary hormones test	CYP3A5∗2 rs28365083(T) is a non-functioning allele CYP3A5∗3 rs776746(G) is associated with down regulation

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Fucosyltransferase 2 (FUT2) mutations, in particular, appear to be the cause of many abnormalities in the microbiome. FUT2 encodes the fucosyltransferase 2 enzyme, which determines secretor status. Secretor status allows for expression of the ABH and Lewis histo-blood group antigens in various secretions including the intestinal mucus. The presence of a particular SNP in FUT2, rs601338 (G>A) results in the non-secretor status. There is also another potential candidate SNP, rs516246 for which there has been considerable linkage to phenotypic expression. It appears that 20% of individuals who are of European descent have this non-secretor FUT2 polymorphism [84].

A 2011 study concluded that those with FUT2 polymorphisms (non-secretors) had only half of the *bifidobacterial* diversity and richness found in secretors. In particular, absence of bacterial denaturing gradient gel electrophoresis (DGGE) genotypes of species such as *Bifidobacterium adolescentis, Bifidobacterium catenulatum/pseudocatenulatum,* and *Bifidobacterium bifidum* were noted. In addition to the noted absence of beneficial bacteria, they also found that there are increased levels of potentially harmful bacteria such as *Blautia* et rel., *Dorea formicigenerans* et rel., *Ruminococcus gnavus* et rel., and *Clostridium sphenoides* et rel [84, 85]. Further, FUT2 non-secretor status also places individuals at higher risk for various diseases including Crohn's disease, ulcerative colitis, type 1 diabetes, vaginal candidiasis and urinary tract infections [84, 85]. Additionally, non-secretors have decreased carbohydrate availability in the intestine, which can cause increased risk of *Salmonella* and *C. difficile* following antibiotic treatment [84]. While more recent studies have called this relationship into question, supplementation with beneficial *bifidobacteria* can still be helpful in those with FUT2 polymorphisms.

A study that investigated Crohn's disease risk in those with FUT2 polymorphism found that mucin 2 (MUC2) might also play a role in this risk [86]. MUC2 is secreted in mucin in the colon and helps provide a barrier that excludes bacteria from the mucosal cell surface. This mechanism ultimately decreases the risk for colitis [86]. Aberrant glycosylation of MUC2 core proteins have been shown to cause spontaneous colitis in mice; thus, it appears that FUT2 negatively impacts microbial adhesion and/or the use of glycans that may lead to dysbiosis.

This association with dysbiosis was further related to the revelation that non-secretors were deficient in several pathways responsible for amino acid metabolism, but interestingly had higher metabolism rates for carbohydrates and lipids, cofactors, and vitamins and glycan biosynthesis [86]. These metabolic abnormalities can alter the integrity of the mucosal epithelium, which then alters the microbial composition. Interestingly, the decrease in the production of amino acids is often reported in those with inflammatory bowel disease (IBD). Alternatively, the metagenome shows an alternate pattern. It showed that while those with IBD had decreased amino acid biosynthesis and carbohydrate metabolism, these conditions resulted in an increased nutrient uptake. This could potentially suggest that the microbiota are compensating for the decreased availability of carbon sources. Further, a condition that results in decreased amino acids may cause a stress response in the individual and therefore the onset of autophagy of intestinal epithelial cells that may ultimately cause IBD [86]. It is important to note that these perturbations can result in sub-clinical intestinal inflammation even in apparently healthy individuals with FUT2 polymorphisms. Supplementation with zonulin tightening nutrients like zinc carnosine can be helpful in those with FUT2 associated intestinal permeability [87].

Lastly, to round out the genomic potential for IBD, specifically Crohn's disease, there is a gene called amiloride binding protein 1 (ABP1/AOC1). This gene is involved in histamine metabolism and poses an interesting intersection between diet and disease. A specific copper dependent SNP in this gene, rs1049793, is associated with an exacerbation in Crohn's disease symptoms. ABP1 is specifically responsible for the regulation of polarized epithelial cells, a mechanism that is dysregulated in IBD [88]. This effect can be compounded if there are also alterations in FUT2 and/or MUC2. It is important to assess copper and cobalamin status in these cases.

There are two mechanisms for histamine degradation, one working intracellularly and the other working via extracellular histamine degradation. Intracellular histamine metabolism is carried out by histamine methyltransferase (HNMT) while extracellular degradation occurs via another enzyme called diamine oxidase (DAO). ABP1 is one gene that encodes DAO, thus regulating extracellular histamine degradation [89]. Histamine is released in the body upon mast cell degranulation and there are four regulatory histamine receptors (H1R-H4R). H1 receptors are found throughout the body in smooth muscle, vascular endothelial cells, the heart and central nervous system. H2 receptors trigger the gastric secretion of histamine to regulate hydrochloric acid production. Less is known about H3 and H4 receptors; however, H1–H3 receptors are primarily found in the brain and H4 receptors are found in the periphery [90]. Histamine is responsible for a staggering number of biological effects, which vary based on receptor, cell location and target cell. Examples include gastric acid secretion, neurotransmitter release, smooth muscle constriction, vasodilation, tachycardia, arrhythmia, stimulation of nociceptive nerve fibers, and an increase in estrogen and endothelial permeability, which results in dysbiosis and an increase in mucus production [89].

The signs and symptoms associated with Crohn's disease include increases in *Bacteroides,* decreases in *Firmicutes* and anti-inflammatory *F. prausnitzii,* chronic diarrhea, increased smooth muscle contractions, excessive bowel mucus production, bleeding from the rectum, weight loss, and fever [91]. As evidenced, there is the potential for considerable overlap between histamine intolerance and Crohn's disease. Crohn's disease presentation and the histamine intolerance associated with the consumption of certain trigger foods appear to have a linear relationship, whereas alterations in ABP1 decrease DAO, which ultimately results in histamine intolerance and intestinal permeability [92]. There are several foods

(i.e., epigenetic activators) that are thought to be high in histamine that may need to be avoided. Examples include fermented foods, alcohol, pickled foods, mature cheeses, smoked meats, shellfish, beans, nuts, and wheat. Some foods are also considered to be histamine liberators. These include most citrus fruits, strawberries, cocoa and chocolate, nuts, papaya, beans, tomatoes, wheat germ, and additives (benzoate, sulfites, nitrites, and glutamate). There are also foods that block diamine oxidase (DAO) which can be problematic, as a block in extracellular histamine degradation would ultimately result in excessive circulating histamine. Examples include alcohol, black tea, energy drinks, green tea, and mate tea. Most fresh meats, fruits, vegetables, eggs, grains, cooking oils, herbs, and non-citrus juices are low in histamine and are non-degranulating. Other known dietary triggers for histamine intolerance may include sulfur, gluten, oxalates, salicylates, and lectin, all of which may play a role in IBD [93].

The relationships between diet, genomics, microbiota, and disease are being discovered exponentially. Once we have mastered the other "omics," there is the potential for truly precise medicine, disease modulation and nutritional intervention.

17.2.1 The Epigenetics of Nutrient-Associated Diseases

To continue with the relationship between nutrients and diseases, we will discuss the relationship between vitamin B12, vitamin D, and autoimmune disease. These two vitamins are two of the most vital nutrients for not only proper one-carbon metabolism and methylation, but also for nutrient absorption and hormone regulation. The first of these is a vitamin that, if measured accurately, would be found to be deficient in such a large subset of the population that the governments would consider fortification. Enter the cobalamins, commonly known as vitamin B12.

There are technically four forms of cobalamin [94]. The most commonly supplemented is called cyanocobalamin. If we break apart the word into fundamental blocks, we have a cobalamin structure, which resembles hemoglobin, and this four-membered ring has a special center group, which in this case would be cyanide. This is the synthetic version of vitamin B12. That means that it is man-made, and is the cheapest supplement option. Biochemically, cyanocobalamin is the most stable form of B12. This molecular stability is the result of having the non-reactive cyanide molecule housed in the center [95]. While the amount of cyanide found in typical B12 supplementation is not inherently dangerous, it does require additional energy, such as ATP, for the body to safely excrete the cyanide from the body. This means that there is a potential net loss of ATP, or cellular energy, when this form of the vitamin is taken internally [96]. There are three better options: adenosylcobalamin, hydroxocobalamin and methylcobalamin.

To start, we need to understand vitamin B12 metabolism and deficiency. It can take a very long time to develop a vitamin B12 deficiency. This is not something that occurs overnight as vitamin B12 is stored long term in the liver, typically, anywhere from 3 to 5 years. Specifically, approximately 2–4 mg of vitamin B12 is stored in the body, of which 50% is found in the liver [95]. Of those 2–4 mg, adenosylcobalamin represents 70% of the vitamin B12 stored in the body, which is also mostly found in the liver. Humans cannot utilize a nutrient if it is only in the storage form, held captive by the liver. Thus, the non-storage form, methylcobalamin, is the main form of vitamin B12 found in the blood. Please take notice of the specific words here, this one is methylcobalamin; therefore, it has a methyl group that can be donated, located in the center of the cobalamin ring. This is important when we consider the vital role that vitamin B12 plays as the connection between one carbon metabolism and methylation. Methionine synthase and methionine synthase reductase (MTR and MTRR) recycle homocysteine back to methionine to produce SAM. This enzyme is the connection between the one-carbon cycle and methylation. MTR and MTRR must be able to convert adenosylcobalamin into methylcobalamin, gaining the methyl group from methylenetetrahydrofolate reductase (MTHFR). Lastly, there is the hydroxyl form of cobalamin, and as the name implies, it carries a hydroxyl group in the center of the cobalamin ring. A hydroxyl group is one molecule of hydrogen and one molecule of oxygen. If you remember from basic chemistry, water is made of two molecules of hydrogen bonded to one molecule of oxygen. Water is in the most stable electrochemical form; thus, a hydroxyl group will be searching for another hydrogen to stabilize its structure. This particular biochemistry allows this form of B12 to bypass several of the transport mechanisms for vitamin B12 [97]. Hydroxocobalamin and methylcobalamin are also stored, but to a lesser extent, in the muscles, bone, kidney, heart, brain, and spleen. When analyzing whole blood, methylcobalamin comprises 60–80% of vitamin B12 found in the blood and adenosylcobalamin comprises up to 20% of total plasma cobalamin [95]. It is important to remember this when measuring serum cobalamin as it is primarily a proxy for methylation status and not a true assay for cobalamin status or cellular levels.

Now that we understand the basics of cobalamin, we need to discuss how these molecules are transported and utilized in vivo. There are two major transporters for cobalamin. The first is transcobalamin I (TCN1 or haptocorrin), and it is a binding protein secreted by the salivary glands and assists in the complexes' survival from the acidity in the stomach. Once bonded with vitamin B12, and it has successfully survived digestion from hydrochloric acid (HCL), the complex travels to the intestine. Meanwhile, gastric intrinsic factor (GIF), a vitamin B12 binding protein which requires HCL for production, is produced by the parietal cells. After the TCN1/B12 complex has arrived in the less acidic environment of the intestine, the TCN1/B12 complex can then bind to the intrinsic factor formed from GIF and be absorbed in the ileum. Once inside the enterocytes of the ileum, vitamin B12 breaks apart from TCN1 to bind to TCN2, which then carries vitamin B12 to the liver [95].

TCN1 transports up to 80% of vitamin B12 and is thought to function as a circulating storage form. The remaining 20–30% of cobalamin is transported on TCN2. When cobalamin is bound to TCN1, it may prevent bacterial use of the vitamin, which has implications on and from the microbiome. In addition to being a B12 transporter, TCN2 is a major part of the secondary granules found in neutrophils connecting B12 status to the immune system [95]. If there are polymorphisms in either of these transporters, it may cause an increase in serum vitamin B12 (often even without supplementation) with an increase in the organic acid methylmalonic acid (MMA). This means that there will be high serum levels and low tissue concentration. Sometimes, if only TCN1 is present, serum levels may also be low. The use of hydroxocobalamin can bypass this transport issue and assist with repletion [95].

So, we have learned that TCN2 is involved in the function of neutrophils, but are there other, more overt autoimmune diseases associated with defects in any of these genes? The most notorious disease is pernicious anemia, which is the result of intrinsic factor deficiency or malfunction. Alterations in GIF are also associated with an increased prevalence of familial pernicious anemia, acute lymphoblastic leukemia, and high-altitude polycythemia. GIF deficiency is also responsible for many cases of non-*H. pylori* related gastritis [98]. This non-*H. pylori*-related gastritis is compounded by the presence of even a heterozygous FUT2 non-secretor variant, as FUT2 decreases GIF secretion. This circumstance would result in cobalamin deficiency [99]. On the contrary, the FUT2 secretor status is associated with elevated levels of plasma vitamin B12. This is because the non-secretor variants do not produce H-type antigens, the antigen which is present in blood group O. There is some association with decreased plasma vitamin B12 levels on non-type O blood [99].

Next, we will briefly discuss vitamin D, its receptors (VDR) and consequences associated with defects in these receptors. There are many additional resources on the associations between vitamin D and autoimmune disease beyond those discussed in this chapter. First, we will review vitamin D nomenclature. Calcidiol (25(OH)D) is the form typically measured in serum. This is a combination of both vitamin D2 and D3 levels from endogenous and exogenous sources. Next is calcitriol, or 1–25-OH(D), the active form of vitamin D. Calcidiol is converted into calcitriol and this mechanism is tightly controlled in the absence of VDR polymorphisms.

VDRs have many regulatory roles. To start, VDRs control genes and have hormone-binding and DNA-binding domains. Specifically looking at DNA, VDRs form a complex with the retinoid-X receptor (vitamin A receptor), and that heterodimer is what binds to DNA, either turning it on or turning it off (vitamin A and vitamin D are required cofactors) [100]. In most cases, VDRs activate transcription and gene expression, but VDRs have also been known to suppress transcription. The affinity VDRs have for calcitriol, the active form of Vitamin D, is roughly 1000×x greater than its affinity for calcidiol [100]. Thus, VDRs are specifically implicated in epigenetic control. Pollutants like smoke and lack of sunshine all contribute to the function of VDR and disease [100]. VDRs also control calcium homeostasis. Certain VDRs can theoretically elevate calcitriol while lowering calcidiol. Calcitriol increases the level of calcium in serum by increasing the uptake of calcium from the intestines, increasing the release of calcium from bones [100]. VDRs are also involved in tissue modulation. They can regulate apoptosis, cellular proliferation and differentiation via matrix metalloproteinases and plasminogen activators. They also modulate the immune system and have the potential to modulate B cells, T cells, dendritic cells, monocytes, and natural killer cells. Low levels of vitamin D, calcitriol specifically, are associated with autoimmune disease [100].

Here are four VDR SNPs that may be encountered. The first represents a causal link to cancer development. It was found that rs2107301 (T;T) homozygotes were associated with an ~2.5× higher risk of prostate cancer compared to homozygote carriers of the more common (C) allele in the 630 Caucasian patients studied [101]. Vitamin D status as an epigenetic influencer can ultimately increase one's risk for prostate cancer, elucidating the importance of vitamin D repletion. Interestingly, vitamin D status is also a regulator for melanoma, suggesting that those that spend more time indoors are ultimately at an increased risk for both forms of cancer [102].

There are two other *commonly reviewed* VDR SNPs; however, the evidence for and against them is inconclusive. These two SNPs are called VDR fok and VDR taq. The first has circumstantial evidence to suggest that polymorphisms can result in blood sugar regulatory issues. VDR-taq SNPs are also circumstantially linked to better tolerance of methyl donors and may cause a decrease in calcidiol levels [103, 104]. Unfortunately, there is insufficient evidence to support nutrigenomic interventions based on these SNPs.

Lastly is VDR-bsm. This VDR provides instructions for making nuclear vitamin D receptors and is involved in the binding of calcitriol and calcitriol receptor activity. When the risk allele A is found in rs1544410, women have an increased risk of low bone mineral density. Conversely, a 26 study meta-analysis estimated a decreased risk of osteoporosis associated with the G;G genotype [103, 104]. Clinically, this VDR often causes rapid conversion of vitamin D2/3 to the active form calcitriol. This over-conversion results in elevated calcitriol/1,25-dihydroxy vitamin D with normal to low 25(OH)D levels. This phenotype erroneously presents with an apparent vitamin D deficiency, because calcidiol is low. However, the gene activating, hormone modulating, immune system influencer calcitriol is too high. In these cases, the patient is often prescribed more supplemental vitamin D, which can result in adverse reactions like nausea, dizziness, syncope, tachycardia, and autoimmunity [105]. Calcitriol initiates the transcription of genes of your immune system (modulates B cells, T cells, dendritic cells, monocytes, and natural killer cells). If there is not enough calcitriol, these genes are not activated. However, if there is too much calcitriol from VDR bsm, these immune cells receptors are blocked. As you can see, at either end of the spectrum there

is a connection between inflammation, autoimmunity and VDR bsm SNPs [106]. An interesting intersection to methylation is that excess calcitriol causes more copies of the cystathionine beta synthase (CBS) gene to be transcribed, thereby increasing hydrogen sulfide and may exacerbate transsulfuration/detoxification issues. Please see ▶ Chap. 18 for more information on CBS [107].

17.3 Epigenetics of Cancer

Cancer is a multifactorial disease state that, again, could have an entire textbook devoted to epigenetic regulation. In this section, we will bridge mitochondrial insufficiency and disease to key cancer pathway genomics. We will begin with an overview of glutathione synthesis. This begins in transsulfuration with an enzyme called CBS. This enzyme is responsible for the catabolism of a regulatory molecule homocysteine into our endogenous antioxidant, glutathione. When there is proper function of CBS, the results are taurine, sulfate and glutathione; all anti-inflammatory biomolecules, if within normal limits. In a normally functioning CBS, hydrogen sulfide enhances the production of reactive oxygen species (ROS) while simultaneously increasing glutathione, the cells' self-protection mechanism against ROS. This results in a net zero of ROS. Hydrogen sulfide in physiological dosages is beneficial to mitochondrial function as it protects it from cytotoxicity. In the case of ischemia, hydrogen sulfide actually blocks cytochrome oxidase, resulting in a decrease in mitochondrial damage. It also upregulates an important mitochondrial detoxification enzyme called superoxide dismutase (SOD) while concurrently decreasing ROS. Hydrogen sulfide is also a regulator of apoptosis, a precursor to sulfation and acts as a neuroprotectant by increasing glutathione and moving another important enzyme, cystathionine gamma lyase (CGL – also vitamin B6 dependent) to the mitochondria which results in an increase of cellular ATP [108].

However, there are variants for this CBS enzyme (Fig. 17.3) that result in up or down regulation of this process. While somewhat controversial, clinically, CBS upregulation can result in sulfur sensitivity, meaning there is an increased sensitivity to sulfur-containing foods and drugs. In upregulation (CBS C699T), we end up with excess sulfate, glutamate, ammonia, and cortisol with decreased levels of glutathione, all of which are inflammatory. This results in a net increase of ROS and the excess hydrogen sulfide interferes with proper mitochondrial function [108]. In downregulation (CBS A360A), there is a functional block that results in elevated levels of homocysteine, a higher incidence of CVD, and decreased glutathione due to limitation [109]. Glutathione is also processed through the gamma-glutamyl cycle, which is a transport system for the amino acids that form glutathione, cysteine, glutamate, and glycine. If there is a decrease in glutathione in the absence of a CBS upregulation, investigating SNPs within this cycle could provide an etiology to the deficiency [110].

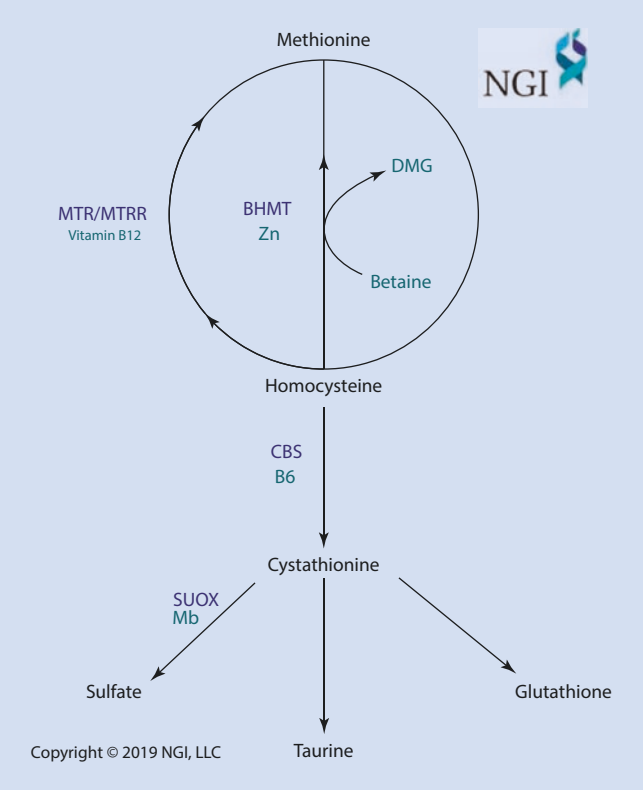

Fig. 17.3 Transsulfuration and CBS upregulation. There are three regulatory pathways of homocysteine recycling to consider: MTR/MTRR, BHMT, and CBS; CBS is the only disposal route. CBS upregulation will result in decreased levels of homocysteine, whereas CBS downregulation will result in elevated levels of homocysteine. (Courtesy of Nutritional Genomics Institute, LLC)

Now that we know how glutathione is produced and have an introduction to a few of the regulatory mechanisms, what does glutathione have to do with cancer? There are two glutathione-S-transferase genes that are directly implicated in cancer prevalence. These enzymes are responsible for binding the powerhouse antioxidant, glutathione, in its special reduced form, to toxicants, which assists in the cytochrome P450 system's removal of them. These genes, rather the absence of them, have a defined role in human carcinogenesis.

Glutathione S-transferase theta 1 deletion (GSTT1) conjugates reduced glutathione along with many exogenous and reactive oxygen species (hydrophobic electrophiles). It is absent in 38% of the general population [111]. This enzyme is concentrated in the liver, heart, brain, skin, and blood and the deletion results in a higher prevalence of cancer development in these tissues [111]. Glutathione S-Transferase Mu 1 (GSTM1) also conjugates reduced glutathione and functions in the detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress [112]. Null mutations or deletions in this gene are associated with an increase in various cancers and compromised detoxification [112]. Both enzymes are associated with vitamin C deficiency [113]. Not having the coding genes for either of the enzymes places one

at considerable risk for the development of several types of cancer, depending on the environmental toxin exposure. In addition to vitamin C deficiency status, both enzymes can be modulated by selenium and vitamin E [114].

In addition to these glutathione- and transsulfuration-related genes, there are also acetylation-related genomics. The N-acetyltransferase 1 and 2 (NAT1 and NAT2) proteins are involved in phase II xenobiotic metabolism and help with the biotransformation of aromatic and heterocyclic amines. It also detoxifies hydrazine and acrylamine drugs [115]. This is done via N-acetylation, which results in the detoxification of monocyclic aromatic amines. This detoxification process is ultimately responsible for the formation of DNA adducts, or a segment of DNA bound to a cancer-causing chemical that precipitates the onset of cancer development.

NAT2 is specifically responsible for the detoxification of smoke, caffeine, drugs, exhaust fumes and many other environmental toxins (heterocyclic aromatic amines). Drugs reported to be metabolized by NAT2 include isoniazid, sulfadimidine, hydralazine, dapsone, procaine amide, sulfapyridine, nitrazepam and some sulfa drugs [116]. These polymorphic conditions can result in fast and slow acetylators (N-acetyl transferase) phenotypes. The slow acetylators have a higher incidence of breast, lung, colon, head and neck, and bladder cancer, with the latter seeming to be the predominant cancer for this group [117]. The fast acetylator variants predominantly result in colon cancer [117]. The key to assisting these enzymes is to reduce the toxin exposure to reduce the stress on these enzymes. Avoidance of smoke, pesticides, insect sprays, charred meats, red meat, metal toxicity, chemicals, and solvents and the addition of acetylators like darkly colored fruits and vegetables high in anthocyanins balanced with adequate fiber will help mediate the cancer predisposition.

Next, let us look into regulatory pathways for cancer. The first is a tumor suppressor gene that regulates apoptosis, phosphatase and tensin homolog (PTEN). There are several diseases and cancers associated with a loss of PTEN function: Bannayan-Riley-Ruvalcaba syndrome, Cowden disease, Cowden syndrome-like phenotype, Cowden syndrome, endometrial carcinoma, Lhermitte-Duclos disease, macrocephaly/autism syndrome, malignant melanoma, oligodendroglioma, PTEN hamartoma tumor syndrome with granular cell tumor, prostate cancer, proteus syndrome, squamous cell carcinoma, head and neck and vertebral (V) abnormalities, anal (A) atresia, tracheoesophageal (T) fistula, esophageal (E) atresia, renal (R) abnormalities (VATER) associated with hydrocephalus [118]. There are several herbs and vitamins that can either upregulate or downregulate this enzyme. Substances that would upregulate this enzyme, or agonists, include astragalus, butyrate, honokiol, retinoic acid, and vitamin D [119, 120]. PTEN is also increased by TNF-α (pro-inflammatory cytokine), which we will discuss next. Substances that block PTEN, or antagonists, include a high fat diet and resveratrol [119, 120]. PTEN inhibition also decreases nitric oxide (NO) production, which could potentially result in hypertension and neurotransmitter imbalances.

Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine that is associated with both anti- and pro-cancer effects. This means that in some cases, such as melanoma, we would not want to upregulate this enzyme [121]. However, the majority of the current research does suggest that for most cancers, it would be wise to upregulate this protein, as it is pro-inflammatory to the cancer itself. Like PTEN, there are several factors that can increase or decrease transcription. Agonists include cannabidiol, echinacea and larch arabinogalactan while antagonists include astragalus, andrographis, resveratrol, alpha lipoic acid, vitamin C, ubiquinol, curcumin, and *Lactobacillus rhamnosus* [122–127]. As evidenced, there are many common interventions that are actually epigenetic modulators that can ultimately increase or decrease one's overall risk of cancer development.

17.3.1 Epigenetics of Mitochondrial Insufficiency

Mitochondrial function was alluded to in regard to cystathionine gamma-lyase. Without a full review of mitochondrial function, we will focus on several key regulatory enzymes. As a refresher, mitochondria are the cells' powerhouse for ATP production. There are five major complexes, and the majority of these mitochondrial genes (mtDNA) are maternally inherited. Mitochondria convert energy into ATP through the process of oxidative phosphorylation via the electron transport chain, with the final conversion being carried out by an enzyme called ATP synthase. This process is dependent on hydrogen, ubiquinol, riboflavin, niacin, carnitine, thiamine, manganese, antioxidation, and succinate [128]. Any deficiency in these biomolecules can result in a functional mitochondrial deficiency.

The first enzyme we will discuss is part of complex 1. There are eight sulfur subunits in NADH ubiquinone oxidoreductase, iron-sulfur protein fraction; however, only three have actionable interventions (NADH-ubiquinone oxidoreductase 76 kDa subunits 3/7/8—NDUFS3/7/8). Children that have severe mutations in these genes have Leigh's syndrome, which is fatal in infancy. Non-ClinVar homozygous alterations in these subunits typically respond well to treatment with riboflavin, thiamin, niacin, carnitine, and higher doses of coenzyme Q10 (coQ10) [67]. Ultimately polymorphisms increase the production of free radicals (ROS) and addressing the subunit ahead in the chain (NDUF) will lessen stress on these enzymes. Broad-spectrum antioxidants are helpful in these cases, antioxidant vitamins such as C and E, and the mineral selenium help to quench the excess ROS. Minor alterations in these enzymes can result in fatigue and exercise intolerance and typically respond well to treatment with riboflavin, carnitine and higher doses of coQ10 [128–130].

SOD 2A16V is the antioxidant enzyme responsible for mitochondrial detoxification. It codes for the superoxide dismutase 2 enzyme and its role is to bind to the oxidant super-

oxide and convert it to less toxic by-products to be processed and removed. It has the cofactor of manganese and not having this functioning enzyme increases the risk of certain cancers and idiopathic cardiomyopathy. Unfortunately, intense exercise compromises this enzyme and, in terms of intense exercise, is related to a "dose-dependent" activity rate based on the number of substitutions [131]. Thus, those with alterations in this mitochondrial enzyme should take caution with high-intensity exercise and supplement with manganese to help assist with mitochondrial function.

The COX or cytochrome C oxidase genes code for the carrier protein between complex 3 and 4. This last transfer allows for all of the built-up energy in the form of hydrogen to bind with oxygen to make water. This mechanism is the cellular requirement for breathing. Like complex I insufficiency, having multiple SNPs in these genes creates free radicals. Specifically, alterations in COX6C are associated with prostate cancer and kidney disease and COX5A has associations with sideroblastic anemia and cardioencephalomyopathy. Both of these enzymes are concentrated in heart and skeletal muscle, elucidating the associated disease pathologies [132].

The last step is the conversion of this collected energy into ATP. ATP synthase is required for the final conversion of adenosine diphosphate (ADP) into the usable form of energy, ATP. There are SNPs in the ATP5C1 gene that code ATP synthase. Alterations in this gene result in a decreased output of ATP and increased ROS. Remember, ATP is our cellular currency, and a decrease in production will ultimately result in increased ROS. Alterations in ATP synthase have also been associated with accelerated aging, ALZ and an increased prevalence for cancer [133]. Overall, we want to upregulate mitochondrial function, starting with complex one and moving along through the complexes while assuring that there is adequate antioxidation. It is important to recognize that mitochondria are sequential chains. Therefore, nutrient cofactors should be added in order and not all at one time for optimal expression.

17.4 Introduction to Pharmacogenetics

Pharmacogenomics is one of the more defined areas of personalized medicine. It describes how genes may affect a person's response to a medication, combining pharmacology and genomics to tailor medical treatment on an individual basis. This field assists physicians on the selection of pharmaceuticals, length of treatment, and dosage. Studies in pharmacogenomics work to associate SNP biomarkers with pharmaceutical treatment outcomes. Challenges occur in that related pharmaceuticals such as statins have different degrees of heritability, meaning that each must be investigated separately [134]. The modern pharmaceutical industry will often develop drugs in a genetically guided manner. This has allowed for adoption of genetic testing to be used clinically prior to prescribing a medication [135].

Phase 1 detoxification is comprised of cytochrome P450, a superfamily of enzymes with many sub-divided families that are used in detoxification. All of the cytochrome enzymes are named using "CYP" for cytochrome P450 and are followed by an Arabic numeral (i.e., CYP1, CYP2, CYP3, etc.). These families are then further subdivided into subfamilies with the addition of a capital letter (i.e., CYP1A, CY1B, CYP1C, etc.). Individual members of each subfamily are then numbered in the order they were identified (i.e., CYP1A1, CYP1A2, CYP1A3, etc.). Phase 1 detoxification converts lipid-soluble molecules entering the liver into more water-soluble intermediary metabolites. These metabolites are often more toxic, not less. A range of substances including drugs, dietary components such as charcoal-broiled meats, steroid hormones, the vitamins niacin and riboflavin, as well as xenobiotics such as dioxin, exhaust and paint fumes, organophosphorus pesticides, and fragrances may induce P450 enzymes [136].

Phase 2 detoxification includes acetylation, glucuronidation, glutathione conjugation, peptide conjugation, methylation, and sulfation. Glutathione is our primary endogenous antioxidant that is essential for proper phase 2 detoxification. Glutathione is a tripeptide formed from the amino acids glutamate, cysteine, and glycine. It accounts for approximately half of our cysteine requirements. In phase 2 detoxification, glutathione is used to conjugate and excrete toxins and drugs, making them more water-soluble. Genetic SNPs in glutathione synthase (GSS) may impair glutathione production within the gamma-glutamyl cycle [137]. This enzyme aids in the production of glutathione and requires magnesium as its cofactor. When GSS levels diminish, it is associated with hemolytic anemia [138]. Further, mutations may lead to elevation of the urinary organic acid pyroglutamate [67]. Methylation connects to phase 2 detoxification via the adjoining pathway transsulfuration. Here we see the enzyme cystathionine beta-synthase (CBS) converts homocysteine to cystathionine, which ultimately leads to the production of taurine, sulfate, and glutathione (Fig. 17.2) [139].

Acetylation is associated with the biotransformation of aromatic and heterocyclic amines. The enzymes N-acetyltransferase 1 and 2 (NAT1 and NAT2) determine whether an individual is considered to be a slow, intermediate, or rapid metabolizer. It requires two slow metabolizer alleles to result in the slow metabolizer phenotype [116, 117]. This means that the rapid metabolizer allele is dominant, and the slow metabolizer allele is recessive. Sulfation includes 12 phase 2 enzymes used in the biotransformation of drugs, hormones, and xenobiotics, as well as bioactivation of carcinogens. Sulfotransferases are important for the metabolism of drugs and endogenous compounds and convert them into more hydrophilic water-soluble sulfate conjugates that then may be excreted. Sulfotransferase 2A1 (SULT2A1) catalyzes sulfation of steroids and bile acids in the liver and adrenals [43].

Glucuronidation converts fat-soluble compounds to water-soluble compounds for excretion and conjugates drugs such as salicylates, morphine, acetaminophen, and benzodi-

azepines; xenobiotics such as phenols, polycyclic aromatic hydrocarbons, nitrosamines, aflatoxin, and heterocyclic amines; and dietary and endogenous substances such as bilirubin, melatonin, bile acid, steroid hormones, and fat-soluble vitamins [67].

Currently, pharmacogenomics is only used for a handful of current diseases and conditions such as depression, mood disorders, heart disease, cancer, asthma, and HIV/AIDS. For example, the breast cancer medication herceptin or trastuzumab, only works for women who have an overproduction of the herceptin 2 (HER2) protein [135]. Mercaptopurine is a medication used for acute lymphoblastic leukemia. It can only be used for those who do not have a genetic variant in NUDT15 c.515C>T, as it may interfere with the clearance of the drug. Improper dosing can result in severe side effects and increased risk for infection. Dosage may be adjusted to an individual's genetics. In the case of children with acute lymphoblastic leukemia, it may increase the risk of 6-mercaptopurine induced myelosuppression and may also cause liver function abnormalities [15, 135]. Lastly, the antiviral drug abacavir may only be used in HIV-infected patients without polymorphism to HLA-B∗57:01, as this SNP can be associated with severe cutaneous adverse drug reactions (SCARs) which may be potentially life threatening [140].

Warfarin genetics are one of the few cases where there is more than one genetic variant related to understanding genetic risk. Both CYP2C9 and vitamin K epoxide reductase (VKORC1) variants influence metabolism, as well as risk for side effects such as hemorrhage [141]. It is important to understand that there are several variants within these classes of gene as well that help to predict the decrease in response. For example, CYP2C9∗3 carriers have an average of 40% reduction in warfarin metabolism and CYP2C9∗2 carriers have an average of 20% reduction in warfarin metabolism [142]. SNPs in CYP2D6, CYP1A2 and CYP3A4 can be particularly relevant in pharmacogenomics as these enzymes are important for the metabolism of nearly three quarters of medications [134, 143].

The quest to find ways of perfecting cholesterol levels has led to medications that target cholesteryl ester transfer protein (CETP) and proprotein convertase subtilisin/kexin type 9 (PCSK9). CETP is associated with high levels of high-density lipoprotein (HDL) and PCSK9 is associated with low levels of low-density lipoprotein (LDL). For this reason, medications have been created as inhibitors of CETP and PCSK9 in order to raise HDL and lower LDL [135]. To round out pharmacogenomics and cardiovascular disease, there is the antiplatelet drug clopidogrel. For patients who have suffered from acute coronary syndrome, stroke or percutaneous coronary intervention, clopidogrel is used as a blood thinner. CYP2C19 status relates to variability in drug metabolism as the CYP2C19∗2 (rs4244285, c.681G>A) variant causes loss of function causing a decrease in the ability to metabolize clopidogrel and other medication inducers of CYP2C19. However, it is important to not view this enzyme–drug interaction in singularity. This one variant alone does not completely predict the drugs' efficacy. Other CYP2C19 variants may have differing effects, either inducing or inhibiting, while other genes such as PON1 and ATP binding cassette subfamily B member 1 (ABCB1) also influence the metabolism of this drug [143].

17.4.1 Final Thoughts

Epigenetics and the technology associated with the "omics" revolution have and will continue to shape the way medicine and precision nutrition is practiced. As with the FTO SNP revolution, this field requires constant review of (and contribution to) the literature and an ever-expansive open mind. It is imperative that practitioners view the complex interactions between diet, lifestyle, genomics, and medications to accurately practice precision medicine and nutritional genomics. In essence, one may not "treat" a SNP or combination of SNPs. Rather, practitioners of the future will be required to not only understand genetics, but also epigenetics, genomics and the complexity of influencers that determine gene expression.

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