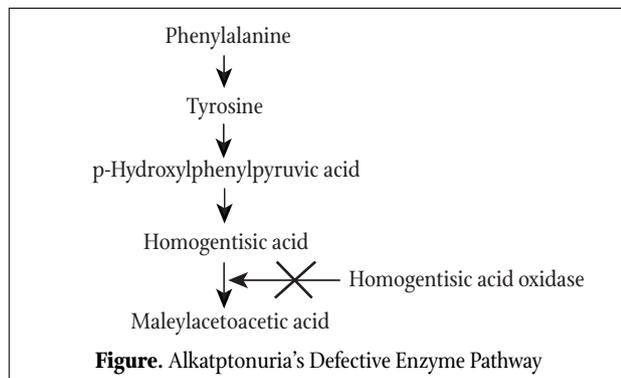


## Biochemical Individuality

John Neustadt, ND, and Steve Pieczenik, MD, PhD

Biochemistry is a complex web of interactions involving an interplay among genetics, diet, and lifestyle. One of the earliest works to discuss biochemical individuality was written in 1902. In that year, Archibald E. Garrod, MD, a clinician in London, England, published a paper in the journal *Lancet* detailing observations on patients with alkaptonuria, an autosomal recessive metabolic disorder that results in a decreased ability to metabolize the amino acids phenylalanine and tyrosine. Phenylalanine, an essential amino acid, is metabolized in vivo to tyrosine, which continues down its pathway eventually to become thyroid hormone, melanin, dopamine, and epinephrine. Alkaptonuria results in a defect in the enzyme homogentisic acid oxidase (HGAO), which catalyzes a single step in the metabolism of phenylalanine and tyrosine—the transformation of homogentisic acid (HA) to maleylacetoacetic acid. HA is cleared by the kidneys and, upon contact with the air, causes urine to turn black. Oxidation of HA also causes an accumulation of dark pigment in cartilage and skin, called ochronosis, and leads to arthritis in adulthood, particularly in the spine and large joints.



Dr Garrod reported, “There are good reasons for thinking that alkaptonuria is not the manifestation of a disease but is rather the nature of an alternative course of metabolism. . . . If it be a correct inference from the available facts that the individuals of a species do not conform to an absolutely rigid standard of metabolism but differ slightly in their chemistry as they do in their structure, it is no more surprising that they should occasionally exhibit conspicuous deviations from the specific type of metabolism than that we should meet with such wide departures from the structural uniformity of the species as the presence of supernumerary digits or transposition of the viscera.”<sup>1</sup> This astute observer was able to extrapolate subtle changes in biochemical function to anatomical variations.

It was not until 1956 that Roger Williams, PhD, a pioneer in nutrition often credited with popularizing the term “biochemical individuality,” wrote the book *Biochemical Individuality: The Basis for the Genetotrophic Concept* (McGraw-Hill, 1998).<sup>2</sup>

Williams believed that people have different requirements for nutrients, and that some may need much greater quantities of nutrients than others for proper functioning of their unique biochemistry. He wrote, “Individuality in nutritional needs is the basis for the genetotrophic approach and for the belief that nutrition applied with due concern for individual genetic variations, which may be large, offers the solution to many baffling health problems.”

Dr Williams based his assertions on anatomical and physiological variations among individuals, and only in the latter years of the last century would researchers such as Bruce Ames, PhD, a biochemist from UC Berkeley, explain the biochemical bases for these assertions. Dr Ames applied the concept of Dr Williams’ work to studying gene–nutrient interactions. Dr Ames’ research showed that variations among genes producing the same enzyme for a biochemical reaction commonly decrease the enzyme’s binding affinity (its ability to use its cofactors).<sup>3,6</sup> The result is an accumulation of reactants and decreased quantities of products, which can be corrected by providing higher amounts of the cofactors.<sup>3,6</sup>

Phenylketonuria (PKU) provides an excellent illustration of this concept. PKU is an autosomal recessive disorder resulting from phenylalanine hydroxylase (PAH) deficiency.<sup>7</sup> PAH catalyzes the reaction of phenylalanine (Phe) to tyrosine (Tyr). Decreased PAH activity results in too much Phe, called hyperphenylalaninemia (high amounts of phenylalanine in the blood), which results in neurotoxicity and consequent mental retardation from the phenylalanine accumulation. More than 500 mutations in gene coding for PAH have been determined, resulting in PAH activity ranging from severe inhibition, when PAH is as low as 5 percent of normal, to mild PKU when PAH is near normal.<sup>8</sup> More than half of children with PKU have one of the milder phenotypes.<sup>9</sup>

Neonates are screened for this disorder at birth, and it has an incidence of 1 in 10,000. Phenylalanine is found in all protein foods and neonates diagnosed with this condition are placed on a phenylalanine-restricted diet and supplemented with tyrosine, vitamins, minerals, and other amino acids. Milk, cheese, egg, meat, and fish are typically omitted or greatly restricted in diets for those with PKU, not because they are disproportionately high in Phe alone, but because they are disproportionately high in protein and, therefore, disproportionately high in most amino acids, including Phe. Vegetables and cereal grains are often emphasized in PKU diets, even though they are protein-containing foods, because they are relatively low in Phe. While effective, maintaining this severely limited diet is quite difficult in school-age children, leads to socially awkward situations for adults, and is complicated in pregnant women.

Since 1999 several clinical trials have reported positive results in decreasing serum Phe in people with PAH deficiencies

by administering pharmacological doses of tetrahydrobiopterin (BH<sub>4</sub>), a PAH cofactor. In 1 small study, a loading dose of 10 mg BH<sub>4</sub> per kg/body weight decreased serum Phe concentrations in 4 of 5 children.<sup>10</sup>

A larger, 12-month study conducted in from December 2000 through December 2002 stratified 38 children into 3 groups: mild hyperphenylalaninemia (pre-treatment plasma phenylalanine less than 600 μmol/L, N = 10, age 15 days to 10 years), mild PKU (pre-treatment plasma phenylalanine 600–1200 μmol/L, N = 21, age 8 days to 17 years), and “classic” or severe PKU (pre-treatment plasma phenylalanine greater than 1200 μmol/L, N = 7, age 1 day to 9 years).<sup>9</sup> Participants consumed a meal containing 100 mg Phe per kg/body weight loading dose, followed 1 hour later with 20 mg BH<sub>4</sub> per kg/body weight. Blood Phe levels were determined at baseline, before BH<sub>4</sub>, and at 4, 8, and 15 hours after BH<sub>4</sub>. A child whose Phe level decreased by more than 30% after BH<sub>4</sub>, compared with levels observed after the Phe loading test, was considered responsive to therapy. All children classified as having mild hyperphenylalaninemia and 17 of the 21 children (87%) with mild PKU responded to BH<sub>4</sub> treatment. Among responders, the decrease in Phe ranged from 37–92%.

These studies indicate that infants and children with this disorder should now also be tested for their responsiveness to BH<sub>4</sub> therapy, as it may allow many of them to consume a less-restrictive diet, and some to even cease the therapeutic diet altogether. Additionally, this research demonstrates that even in “severe” genetic conditions, there may be some enzyme activity that can be stimulated with pharmacologic dosages of nutrients, and that diseases once viewed as incurable may in fact be ameliorated with nutrients.

### Application to Single Nucleotide Polymorphisms

This field of study has been applied to less severe forms of genetic variations known as single nucleotide polymorphisms (SNPs). A SNP is defined as a change in a gene that appears in more than 1% of the population, which suggests a pattern of inheritability rather than chance alone.<sup>11</sup> Additionally, at least one-third of all SNPs alter the binding affinity for an enzyme and its substrate.<sup>3</sup> When this occurs, providing higher dosages of the cofactors necessary for enzyme function can help restore enzyme activity and essentially force the enzyme reaction in the desired direction.<sup>3</sup>

A common example of this less-severe genetic variation is the SNP for the methylene tetrahydrofolate reductase (MTHFR) enzyme. This enzyme is responsible for activating folic acid so that it can function to decrease homocysteine, a protein that, in epidemiological studies, has been shown to increase risk for cardiovascular disease, osteoporosis, cancer (colorectal, lung, cervical), and dementia.<sup>12-18</sup> In vitro evidence suggests that homocysteine may be a causative factor in these diseases through 3 distinct mechanisms: 1) It directly damages DNA by causing fragmentation in a way that mimics radiation exposure. 2) It directly damages the cells lining blood vessels (endothelial cells) and causes a decrease in oxygen and nutrient delivery to tissues including heart, brain, and bone. 3) It decreases nitric oxide

(NO), an important signaling molecule in the body that plays a role in blood vessel dilatation and neurotransmission.<sup>19</sup>

A relatively common genetic alteration in the gene coding for MTHFR is the C677T autosomal recessive mutation. Heterozygotes for this mutation exhibit a 30% reduction and homozygotes a 65% reduction in enzyme activity compared with normozygotes.<sup>11</sup> That is, the enzymes created by the different MTHFR SNPs, such as the C→T polymorphism, require higher amounts of folic acid to function. These polymorphisms occur in up to 50% of Caucasians, 47% of Koreans, 42% of Hispanics, 29% of Native Americans, 12% of African-Americans, and 10% of Asian Indians.<sup>11</sup>

The result is that people with the MTHFR C→T polymorphism have higher concentrations of homocysteine due to a decreased ability to utilize folic acid. The deleterious effects of this genetic polymorphism can be overcome simply by providing higher dosages of folic acid, as has been demonstrated in dose-response studies.<sup>20,21</sup>

In addition to genetics, an enzyme's requirement for nutrients can increase in response to hormones secreted during physical and psychological stress, lack of exercise, poor diet, and free radical damage related to the aging process. As people age, non-genetic factors (eg, diet and lifestyle) play more important roles in the development of diseases than do genetics. In the vast majority of situations, genetics merely predispose someone to a disease, but lifestyle and nutritional status determine whether or not that disease will actually develop. A decreased ability to utilize nutrients can be overcome in many instances by giving higher dosages of the nutrient. This forces biological reactions to go in the desired direction, and helps reverse the deleterious effects of aging and other stressors.

This fundamental concept has revolutionary implications for medicine. Medicine often looks at genetic alterations in their extreme forms, eg, Down syndrome and Marfan syndrome. However, much more common are subtle functional changes in biochemistry caused by genetic individuality, nutritional status, and lifestyle that do not cause readily apparent physical or mental abnormalities. Instead, the changes in biochemical function are more insidious, and the resultant diseases do not even appear until later in life.

### Liver Detoxification Pathways and Breast Cancer

Liver detoxification pathways serve as excellent examples of an organ-specific genetic expression that exert powerful control over the development, maintenance, and progression of diseases. For example, liver detoxification pathways greatly influence the risk for and development of cancers.

Metabolism of endogenous and exogenous compounds occurs in the liver via 2 major complementary pathways called phase I and phase II. These 2 phases work in sequence, with most metabolites of phase I passing through phase II. Phase I enzymes are a super-family of hemoproteins called cytochrome P450s (CYP) enzymes. They oxidize relatively nonpolar molecules, increasing their polarity and allowing them to be excreted in the urine. The main CYP isoforms are 1A2, 1B1, 2D6, 2C9, 2C19, and 3A4.<sup>22-24</sup> Phase II enzymes catalyze conjugation

reactions to compounds, such as glutathione, that facilitate elimination.<sup>11</sup> Phase II enzymes include glutathione *S*-transferases, UDP-glucuronosyl-transferases, N-acetyltransferases, microsomal epoxide hydrolase, and sulfotransferases.<sup>25</sup>

Decreased activities of phase II enzymes are associated with the pathogenesis of diseases. Over-expression of the CYP1B1 isoform has been associated with development of numerous cancers, including prostate, breast, and ovarian cancers, as well as serous and mucinous carcinomas, but its over-expression is not detected in normal tissues.<sup>26-30</sup>

Estrogen is predominantly metabolized in the liver via 2 pathways. One pathway produces 2-hydroxyestrogen (2-OHE). The other first metabolizes estrogen to 4-hydroxyestrogen (4-OHE), then to 16-hydroxyestrogen (16-OHE). The 2-OHE is metabolized via the CYP1A system, while 4-OHE is metabolized via the CYP1B1 pathway.

Exact concentrations of these estrogen metabolites don't appear to be as important as the ratio of their metabolites. A 2-OHE:16-OHE ratio (called a 2:16 OHE) less than 1.8 is associated with increased risk of breast cancer.<sup>31</sup> Increasing this ratio may decrease a woman's risk of developing breast and uterine cancer. In one epidemiological study, a decrease in breast cancer risk of 45% was seen in the highest quintile of 2:16-OHE compared to the first quintile.<sup>31</sup> The 2-OHE pathway is inducible, meaning that its activity can be increased so that 2-OHE production is increased. This can be accomplished by consuming higher amounts of cruciferous vegetables such as broccoli and cauliflower, and also by taking a dietary supplement containing adequate amounts of diindolylmethane (DIM). In a pilot study, administration of 108 mg DIM daily increased the 2:16-OHE ratio in postmenopausal women by 47% (from 1.46 to 2.14), which approached statistical significance ( $P=.059$ ).<sup>32</sup> This study provides another good illustration of how a person's unique biochemistry can be modulated to affect disease risk.

## Conclusion

Examples of rare genetic disorders that produce extremely large requirements for a particular nutrient are numerous; however, other more common alleles and phenotypes merely render people more susceptible to the effects of nutritional deficiencies. The implications of this are wide-ranging. Effects of genetic diseases previously considered untreatable may now be ameliorated by administering higher amounts of the coenzyme(s) required for more efficient metabolic function. Through genetic and functional testing (eg, existence of MTHFR gene or a woman's urinary estrogen ratio, respectively), risk factors may be identified and truly personalized interventions to improve health designed.

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