An Overview of Phenylketonuria:

Genetics, Symptoms, & Treatment

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In 1934, Asbjorn Folling, a doctor practicing in Oslo, Norway, made a landmark discovery among patients with certain physical dysfunctions (Centerwall and Centerwall 2000). During a chemical analysis , Folling identified phenylpyruvic acid in urine samples from two young siblings suffering from severe mental retardation and behavioral abnormalities (Folling, 2000). Because phenylpyruvic acid is absent in urine of healthy individuals, Folling examined patients in medical facilities to verify these unique findings (Folling 2000). Folling's research demonstrated that phenylpyruvic acid is a byproduct associated with a distinct set of physical abnormalities, and eventually this research was published in scientific journals (Williams et al. 2008). Folling called the disorder " imbecillitas phenylpyruvica," now referred to as "Phenylketonuria" or "PKU" (Williams et al. 2008). To understand PKU, we will briefly explore its genetic basis, common symptoms, and the process of treatment.

PKU is an inherited metabolic disorder that affects about 1 in 10,000 births worldwide (Jarnes Utz et al. 2012) and about 1 in 16,000 in North America (Harding 2008). The disorder results in hyperphenylalaninemia (HPA), a condition in which the amino acid phenylalanine (Phe) escalates to abnormally high levels in the body (Harms & Olgemoller 2011). Patients who may accumulate blood Phe levels greater than 1200 μmol/L are usually considered to have "classical PKU" while those who may accumulate levels greater than 400 and less than 1200 μmol/L are usually considered to have "mild" to "moderate PKU" or "variant HPA" (Vernon et al. 2010). Phe levels below 400 μmol/L are generally considered clinically benign and levels between 120 and 360 μmol/L are common treatment targets (Vernon et al. 2010). HPA is commonly attributed to over five hundred mutations of the gene responsible for production of a metabolic protein produced by hepatocytes in the liver (Cunningham et al. 2012)

Phenylalanine hydroxylase (PAH) is the liver enzyme commonly associated with PKU metabolic dysfunction (Gassio et al. 2010). The dysfunction is characterized by PAH deficiency (Harding 2008), however, impaired biosynthesis of the human cofactor tetrahydrobiopterin (BH4) may also produce HPA (Elsas et al. 2011). The catabolic reaction of PAH with BH4 converts Phe to tyrosine (Tyr) by supplying a hydroxyl group to its side chain (Gassio et al. 2010). PAH deficiency is attributed to a mutation of a single

gene governing the production of PAH (Harding 2008). The gene is found on the long arm of chromosome twelve (12q23.2) and blueprints the polypeptide sequence necessary for PAH synthesis (Williams et al. 2008). The majority of dysfunctional PAH alleles impede proper protein folding (Schoemans et al. 2010). The mutant alleles are recessive, however, heterozygous genotypes often express incompletely dominant phenotypes (Brooker et al. 2011). These intermediary phenotypes often result in relatively unproblematic cases of HPA (Brooker et al. 2011).

Untreated PKU results in irreversible neurological and cognitive dysfunction among several other complications (Williams et al. 2008). Hence, early detection of the disorder is essential to preventing and treating its detrimental effects (Burke et al. 2011). Newborn screening programs became widely standardized in the 1960s mainly as a result of social and political movements advocating for standardized detection and treatment of PKU (Burke et al. 2011). Today, nearly all developed countries implement programs designed to detect diseases such as PKU (Pitt 2010). Screening methods for the disease involve analysis of Phe levels in dried blood spots (Pitt 2010). Among early methods used to detect the amino acid was a bacterial inhibition assay involving the growth of strains of *Bacillus subtilis*, which require external sources of Phe for growth (Pitt 2010). The size of propagated colonies would be used as a parameter on which to assess blood Phe levels (Pitt 2010). However, modern methods of screening, including tandem mass spectrometry (TMS), are replacing older methods like the bacterial inhibition assay (Pitt 2010). TMS has the advantage of being less prone to producing false positives and is capable of screening for other disorders simultaneously (Blau et al. 2011).

Untreated PKU causes a wide range of biological dysfunctions including hypopigmentation, growth inhibition, microcephaly, seizures, pregnancy complications, and severe cognitive and motor dysfunction (Rebuffat et al. 2010; Harding 2008). The dysfunctions have been partially linked to hypomyelination of neurons in the central nervous system (Martynyuk et al. 2010). The myelin deficiency is attributed to an impairment of synthesis, although other studies have demonstrated normal synthesis with degeneration (Martynyuk et al. 2010). Ironically, excessive Phe and some of its derivatives,

including phenylacetate and phenylpyruvate, do not appear to directly account for this abnormality according to one study (Schoemans et al. 2010).

PKU may additionally cause emotional disorders including depression (Sharman et al. 2012). These issues have been linked to synthesis disruption and deregulation of the neurotransmitters serotonin and dopamine according to one study (Sharman et al. 2012). Sharman et al. (2012) suggested a connection between prevalence of depressive symptoms with a decrease in executive function. The same study indicates that poor dietary management may be a significant correlative factor in the onset of symptoms (Sharman et al. 2012). Symptoms appear to be significantly associated with high ratios of Phe to Tyr or low Tyr (Sharman et al. 2012). One study looked into the possible benefits of providing high-Tyr supplementation to PKU patients but recorded no benefits (Pietz et al. 1995).

For decades, the primary treatment for patients afflicted with PKU has been a low Phe diet (Belanger-Quintana et al. 2012). After implementing newborn screening and diet management protocols in the 1960s, the medical community remained unsure of when or if there may be an appropriate time to relax or cease low-Phe diet management (van Calcar & Ney 2012). Randomized trials were performed to test whether or not PKU symptoms would reappear in individuals who ceased diet management (van Calcar & Ney 2012). These trials suggested that diet management should not be discontinued even after childhood neurological development (van Calcar & Ney 2012). Modern PKU healthcare operates under the assumption that the special diet is lifelong (van Calcar & Ney 2012). The dietary intake normally consists of providing synthetic or low-protein foods and Phe-free protein substitutes (Belanger-Quintana et al. 2012). Patients and parents often spend significant amounts of time managing Phe-restricted diets (Belanger-Quintana et al. 2012). Properly managed special diets are very successful at treating PKU and while they do not completely eliminate the neurological consequences of PKU, the diets are capable of maintaining patient mental and executive function within the normal range (MacLeod & Ney 2010). Unfortunately, as much as 75% of adult patients discontinue the special diet because they are often considered unpalatable and expensive (Vernon et al. 2010).

Because of the drawbacks associated with the PKU special diet, alternative PKU treatment options are being explored (Belanger-Quintana et al. 2011). In 2007, sapropterin dihydrochloride (SDHC) was approved for commercial distribution in the United States to treat patients with PKU and HPA (Belanger-Quintana et al. 2011). SDHC is a synthetic variant of the natural human cofactor BH4 (Giovannini et al. 2012) and is currently available as a pharmaceutical under the trademark "Kuvan" (Levy et al. 2007). A significant proportion of PKU patients have been responsive to the drug but its effectiveness is variable (Belanger-Quintana et al. 2011). Clinical definitions of a responsive patient vary (Elsas et al. 2011) but most definitions use significant reductions in plasma Phe as the fundamental parameter (Singh & Quirk 2011). Thirty percent decreases or more have been considered clinically significant in many studies (Burton et al. 2010; Elsas et al. 2011; Harding 2010).

Phenylalanine ammonia lyase (PAL) has also proven useful in reducing plasma Phe levels (Giovannini et al. 2012). Early clinical preparations of PAL have encountered a variety of issues, including protease vulnerability but modern PAL applications are becoming more practical (Wang et al. 2008). PAL is a stable chemical produced by plants and fungi and is capable of deaminating Phe to trans-cinnamic acid and trace amounts of ammonia (Hyun et al. 2011). Cinnamic acid is a harmless metabolite that can be quickly converted to hippuric acid and expelled in the urine (Belanger-Quintana et al. 2011). One of the novel attributes of PAL deamination is that it does not require a cofactor nor does it yield a Tyr product (Hyun et al. 2011).

Among the most modern treatment options currently being developed for PKU are gene therapies. Rebuffat et al. (2010) demonstrated successful viral-mediated gene transfer in PKU mice via intramuscular injection. The gene transfer resulted in successful PAH gene expression (Rebuffat et al. 2010) The study demonstrated dramatic long-term increases in PAH activity in male mice with somewhat less significant results in females (Rebuffat et al. 2010). Another similar study demonstrated the efficacy of viral-mediated gene transfer in a mouse model and showed hepatic PAH activity restored to 65-70% in females and 90% in males (Jung et al. 2008).

PKU is a potentially devastating neurodegenerative disease. The PKU special diet is unappealing, inconvenient, and expensive. Treatment strategies are not perfect. However, it is also a great example of how effective management can dramatically alter the course of a disease. Excitingly effective therapies such as SDHC, PAL, and gene transfer may offer patient outcomes that were previously unobtainable. Given how far our understanding of the disorder has come since its discovery, it is not hard to imagine how much farther it will probably come in the near future. Medical science is providing us with the tools necessary to reduce the burden of afflicted individuals and in time, perhaps that burden will become insignificant.

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