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PHENYLKETONURIA - YESTERDAY, TODAY.....TOMORROW

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ABSTRACT

Phenylketonuria is a genetic disorder wherein there is an inability to convert phenylalanine to tyrosine leading to toxic elevations of this aminoacid in the blood that ultimately causes mental retardation. This disease occurs at a frequency of 1 in 10,000 births. Early detection and appropriate therapy can prevent permanent brain damage. The historical aspects related to disease and the newer options for diagnosis and treatment are herewith presented.

KEYWORDS: **phenylketonuria, hyperphenylalanininemias ,PAH, enzyme replacement, gene therapy.**

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INTRODUCTION

Phenylketonuria (PKU) is an autosomal recessively inherited metabolic disorder characterized by a mutation in the gene for the hepatic enzyme phenylalanine hydroxylase (PAH) that is essential for the conversion of the amino acid Phenylalanine to Tyrosine.The consequences of the gene mutation in PKU can range from mild deficiency or impaired functioning of the enzyme to a profound deficiency or complete absence of the enzyme. This typically results in elevations of blood phenylalanine greater than 20 mg/dL or 1200 micromol/L (reference range 0-3mg/dl or 21-136 micromol/L). Partial deficiency of the enzyme results in hyperphenylalaninemia, characterized by lower elevation of blood phenylalanine.[1]When PAH activity is reduced or absent, phenylalanine accumulates and is converted into phenylpyruvate (also known as phenylketone), which is excreted in the urine and can be detected by simple tests. These metabolites of phenylalanine are also excreted in the sweat and is responsible for the characteristic mousy odour of these patients. Untreated PKU can lead to mental retardation, seizures, and other serious medical problems. It is a treatable condition with strict dietary restrictions of phenylalanine. The incidence varies according to ethnic background of the child, with a higher incidence in White and Native American populations and lower incidence in African American, Hispanic, and Asian populations.[2] There is great genetic and clinical variability among persons with PKU, as there is for all genetic diseases. More than 400 different mutations in the phenylalanine hydroxylase gene have now been identified. Certain mutations are associated with PKU while others are associated with hyperphenylalaninemia.[3] In addition to genetic factors, environmental and lifestyle also contribute to the variations. For example, age when treatment is started and degree of diet control can explain differences in outcome between two people with the same genetic mutations. This assumes significance in populations of multi-ethnicity.

Historical aspects of Phenylketonuria

It was in the year 1934 a Norwegian doctor Ashjorn Folling discovered Phenylketonuria by testing the urine of a mentally retarded brother and sister and published the first documented biochemical cause of mental retardation , and it has been a long journey since then. The availability of sophisticated diagnostic facilities for early detection coupled with effective dietary therapy now allows a PKU child to grow almost normally and lead a productive life and today we are looking forward to complete and permanent cure for PKU patients by gene therapy.(Table 1)

Table 1
Milestones in the history of Phenylketonuria

Year /Period	Event	Outcome
1934	**The Discovery** Norwegian doctor Asbjorn Folling discovers PKU by testing urine of a mentally retarded brother and sister and published this first documented biochemical cause of mental retardation	First documented evidence of a biochemical cause for mental retardation
1953	**Dietary Therapy** Dr.Horst Bickel reports treating PKU patients with a special low phe diet	PKU children on the road to normalcy
1963	**Prevention is better than cure** Newborn screening programme is developed by Dr.Robert Guthrie	PKU diagnosis in newborn period combined with dietary therapy proved to be a boon for PKU children.
1983	**PAH gene identified** Gene for PAH identified by Dr.Savio Woo	Paved the way for mutation analysis

1990	**More research... more understanding** Animal models of PKU developed	Opportunity for gene replacement therapy
1994	**Gene therapy -Animal trials** Successful treatment of mouse models of PKU Pah^{enu2} mouse by gene therapy directed at liver(Fang et al)[4]	Results were temporary immune reaction developed.
1998	**The enzyme .. the protein...** Structure of PAH elucidated	Helped in understanding the genotype and phenotype relationships.
2006	**More animal trials** Successful treatment of mouse models of PKU, Pah^{enu2} mouse using Adeno-assisted virus (AAV) serotype 8 by gene therapy. (Ding et al ; Harding et al) [5,6]	Results were temporary as the vector did not get incorporated with the hepatocyte DNA and was lost following normal cell turnover immune reaction developed
2007	**Pharmacological treatment for PKU** FDA approves treatment of PKU with tetrahydrobiopterin(BH_4)in BH_4 responsive cases	Improved quality of life in BH_4 responsive hyperphenylalaninemias
2008	Murine models of successful muscle directed gene therapy using AAV vector (Ding et al 2008)[7]	Disadvantage of viral vectors
Current	**Towards permanent cure..** Work underway to develop novel muscle directed gene transfer systems that avoid the use of recombinant viral vectors which can trigger an immune response	Possibility of a permanent cure for PKU

Diagnosis

1. Simple urine screening test -Ferric chloride test**-** an olive green colour is obtained when ferric chloride reacts with phenylpyruvic acid. This test is not specific for phenylpyruvate and gives a positive reaction with many other metabolites and drugs.
2. The Guthrie bacterial inhibition test - a semiquantitative assay designed to detect elevated blood levels of phenylalanine, using the ability of phenylalanine to facilitate bacterial growth in a culture medium with an inhibitor. This is a more reliable diagnostic test and has been popularly used in new born screening program until the advent of Tandem Mass Spectrometry.[8]
3. Quantitation of Phenylalanine by ELISA - a microplate-based enzymatic assay for the simultaneous determination of phenylalanine and tyrosine in serum described by Flemming Wilbrand (2004), is a reliable and simpler test for routine monitoring of Phe levels and has proven to be comparable to Ion exchange chromatography methods.[9]
4. Paper chromatography, Thin layer chromatography, High performance liquid chromatography and Tandem Mass spectrometry are other methods available for detection /quantification of Phenylalanine.
5. The advent of tandem mass spectrometry has revolutionised the newborn screening programmes for inherited metabolic disorders including PKU, in which about 40 metabolites can be detected from a single spot of blood on a filter paper.
6. Genotyping and mutation analysis - Since the gene for PAH has been identified and also cloned , genetic diagnosis is now possible and several hundred mutations have been detected so far.[3]Genetic diagnosis is important not only for confirmation, carrier status, and genetic counselling, but also for differentiating hyperphenylalanininemias due to defective metabolism of Tetrahydrobiopterin.
7.

Management of PKU

The primary aim of PKU treatment is to achieve and maintain low blood levels of Phenylalanine so that the toxicity to the brain associated with high Phenylalanine levels is eliminated or minimised. The neurological impairment in phenylketonuria can be

prevented if appropriate treatment measures are started quite early before irreversible brain damage can occur.

Traditional low Phenylalanine diets

Special formula feeds are available which has revolutionised the treatment of phenylketonurics. However their cost is prohibitive for many patients and they require financial support to continue life long special feeds.

Glycomacropeptide - this is a Casein derived whey peptide that does not contain aromatic amino acids , but a rich source of branched chain amino acids. Useful as a low Phenylalanine protein supplement .

Novel treatment strategies

i. Pharmacotherapy

Treatment with Tetrahydrobiopterin (BH4/Saptropterin dihydrochloride) in those cases responsive to BH4, activates the enzyme PAH and thereby eliminates the dietary restrictions.

ii. LNAA Supplementation (LNAA)

Supplementation with large neutral amino acids (LNAA) other than phenylalanine has proven to be beneficial. LNAA supplementation may have multiple treatment targets: a specific reduction in brain phenylalanine concentrations, a reduction in blood (and consequently brain) phenylalanine concentrations, an increase in brain neurotransmitter concentrations, and an increase in brain essential amino acid concentrations. LNAA treatment is seen as an alternative to conventional dietary PKU treatment.[10]

iii. Enzyme replacement therapy

Enzyme replacement therapy with Phenylalanine ammonia lyase (PAL) is currently being considered as a possible alternative treatment for PKU. The enzyme PAL catalyses the conversion of Phenylalanine to free ammonia and trans-cinnamic acid which can be easily converted to hippuric acid and excreted in urine.[11]

iv. Gene therapy

Gene transfer studies in murine models have been successful.[12] Experiments on liver directed mouse models using recombinant Adeno -assisted virus (rAAV) did not provide permanent correction of the PAH activity as the vector's genome is not integrated into the host hepatocyte DNA, and during normal cellular turnover the viral vector is eliminated.Skeletal muscle directed gene therapy eliminates the disadvantage of liver cell turnover, and has been successfully tried in mouse models and is expected to provide permanent corrections in PKU patients very soon.[13]

Management of PKU should also include promoting normal physical growth and development, periodic monitoring of blood levels of Phenylalanine. G.M.Enns and co-workers (2004) have reported that in PKU patients treated by strict dietary restrictions alone, the neurocognitive, psychosocial quality of life , growth , nutrition, bone pathology and maternal PKU outcomes are suboptimal among all age groups. They have recommended that with the availability of newer treatment options revised PKU management strategies need to be planned to optimise the treatment outcomes.[14]

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