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Phenylketonuria (PKU): Causes, classifications, clinical symptoms, diagnosis and treatment

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Abstract. The autosomal recessive condition phenylketonuria (PKU) is brought on by mutations in the gene that codes for phenylalanine hydroxylase (PAH). Phenylalanine (Phe) builds up in the blood as a result of PAH mutations. Untreated PKU can result in several dangers, including brain damage. Due to factors including enhanced genetic testing and newborn screening, the number of PKU patients is predicted to rise. Therefore, it is necessary to popularize the knowledge of PKU in the society. Additionally, minimizing the occurrence of PKU symptoms is greatly aided by early identification and treatment of PKU. In this context, this review highly summarizes all aspects of knowledge about KPU, focusing on the genetic cause, classification, clinic symptoms, diagnosis, and treatment. Newborn screening and related diagnostic technologies, including the Guthrie test, the McCaman and Robins fluorescence assay, and Tandem mass spectrometry, are introduced in this paper. The dietary measures, PKU Formula, and two effective medications, Kuvan and Palynzio, are involved as well. In addition, state-of-the-art genetic techniques for diagnosis and treatment are also included. With an earlier and more accurate diagnosis, governments around the world should strengthen awareness of PKU and lead to the development of effective diagnostics and therapeutics. Finally, the purpose of this paper is to let more people know about PKU, strive for early detection and treatment, and do everything possible to avoid the occurrence of phenylketonuria symptoms.

Keywords: phenylketonuria (PKU), Phenylalanine (Phe), phenylalanine hydroxylase (PAH), comprehensive introduction, genetic.

1. Introduction

The inborn metabolic mistake PKU, caused by mutations in the gene encoding PAH, results in an accumulation of the amino acid Phe in the body. Phe is one of the essential amino acids in the human body. It is ingested through food and digested in the intestine. In liver cells, most of Phe is converted to a kind of hydrophilic amino acid named tyrosine under the action of PAH. Tyrosine has the ability to synthesize important hormones such as thyroxine, adrenaline, and melanin. Another part of Phe is involved in protein synthesis. A small amount of Phe is converted to phenylpyruvate by the action of liver transaminase. When the gene encoding PAH is mutated, it will cause an activity defect in the related enzyme in the human body, and it cannot convert Phe into tyrosine. For that reason, it accumulates in the blood, cerebrospinal fluid, and tissues. At the same time, due to the obstruction of Phe metabolism into the amino acid pathway, bypass metabolism will be enhanced, and a large

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number of harmful metabolites such as phenylpyruvate generated by Phe metabolism through the liver will lead to brain damage and other hazards. Typical clinical symptoms of phenylketonuria include mental retardation, light pigmentation of skin and hair, and a rat urine smell in urine and sweat. worldwide There is a prevalence of about 1 in 2, 4000 live births worldwide for PKU, and a total of approximately 0.45 million PKUs exist in the world [1].

Since it was discovered in 1934, PKU has been extensively and deeply studied by doctors and scientists around the world. A great deal of literature about PKU can be found. However, most of them are dedicated to one particular aspect, such as PKU diagnosis, PKU treatment, or newborn screening (NBS) for PKU. In order to make people have a preliminary understanding of PKU, a simple but inclusive introduction to this disease is more useful.

This paper gives a comprehensive introduction to PKU, including its genetic cause, classification, diagnosis, and treatment. The most advanced genetic techniques for detection and treatment are also involved. The widespread awareness of PKU is important because it is one of the few genetic disorders that is currently known to be treatable, preventable, and detectable early.

2. Genetic cause

23 pairs of chromosomes make up the human body, 22 of which are autosomes and one of which is a pair of sex chromosomes. The long arms of chromosome 12 (12q22–12q24.1) contain the PAH gene. PKU is caused by a PAH gene mutation and is a recessive autosomal genetic disease that is inherited within families. A kid will only get the condition if all of the PAH genes from both parents are mutated, since each chromosome is passed down to a child in two copies, one from the mother and one from the father. A child has 100% PKU if both parents have it and has a 50% chance if one parent has it and the other is a recessive gene carrier. In the third case, a child has a 25% chance of developing the condition if both parents are carriers (as shown in Figure 1).

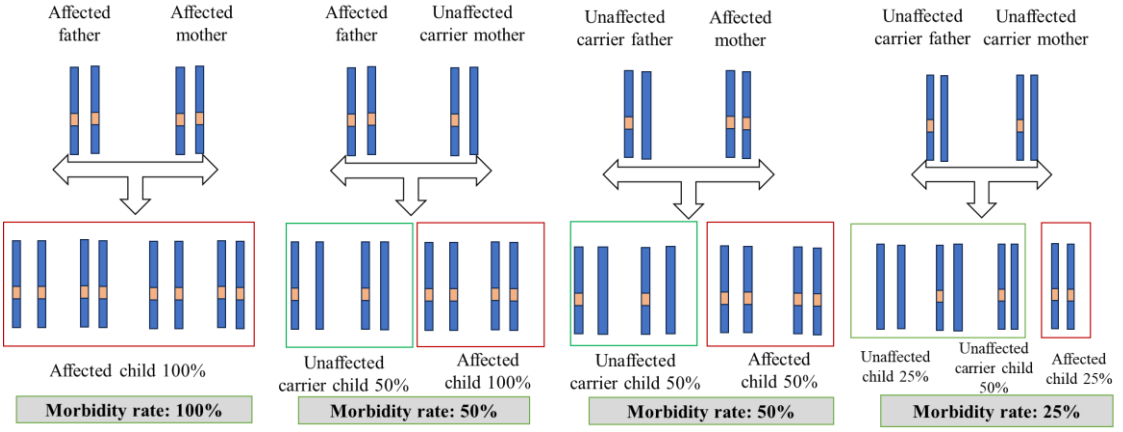

Figure 1. Inheritance of PKU (icture credit: Original).

3. Classification

The normal range for Phe in the blood is under 120 micromoles per liter (μmol/L). Based on the blood Phe concentration, there are generally three types of PKU: mild hyperphenylalaninemia PKU (MHP), mild PKU (mPKU), and classical PKU (cPKU). A Phe concentration between 120 and 600 μmol/L is considered MHP, which is the lowest level above normal. A Phe concentration of 600 to 1200 μmol/L is defined as mild PKU (mPKU). And a Phe level greater than 1200 μmol/L is cPKU, which is the most severe type. From the global data, the phenotypic distribution of PKU was about 16% MHP, 22% mPKU and 62% cPKU. However, the distribution and severity of various phenotypes of PKU vary greatly according to different countries and regions.

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4. Clinical symptoms

If detected early and treated, people with PKU have almost no symptoms. However, if not detected and treated in time, PKU can damage the brain and nerves, leading to cognitive impairment. Eczema, a lighter color of skin and hair compared to other family members, a small head, and a rat odor in the breath, skin, or urine are the common symptoms for untreated PKU patients. Severe symptoms include behavioral problems such as irritability, hyperactivity, poor self-esteem, developmental delay, and intellectual disabilities. PKU patients with MHP have a lower risk of developing intellectual disabilities in the absence of treatment [2]. However, infants with classical PKU will develop a permanent intellectual disability in a few months.

5. Diagnosis

Early diagnosis of PKU is of great significance. At present, newborn screening is basically carried out in most areas of the world for the diagnosis of PKU. Diagnostic techniques are also constantly evolving, increasing diagnostic sensitivity, specificity, and positive prediction rates.

5.1. Newborn screening

Newborn blood screening analyzing Phe concentrations is the main means of detecting PKU, which is currently adopted by many countries. It can almost diagnose all cases of PAH deficiency. Normally, newborn screening is done by having a few drops of blood taken from his or her heel after 24 hours but before 72 hours of birth. If the blood Phe level is higher than 242 μmol/L (4mg/dL), the baby should be considered to have PKU. 1% of tested infants have positive results during the first screening, but only 10% of those with positive results have PKU. That means the false-positive rate is as high as 90 percent. If the initial test shows a positive result, the infant's blood sample should be sent to a referral metabolic center to perform a confirmatory quantitative test. False-negatives are rarely seen.

5.2. Diagnostic technology

Over the past few decades, testing technology has evolved to make newborn screening for PKU more and more accurate. The Guthrie test is also known as the Guthrie bacterial inhibition assay (BIA), which has been used in initial newborn screening tests for PKU for more than half a century in many countries. BIA uses bacteria, which is Bacillus subtilis, to measure the blood Phe concentrations, and if the blood Phe concentration is greater than the cut value (4mg/dL or 242 μmol/L), further examination and diagnosis should be made. BIA has a very high sensitivity of over 99% for mild and classical PKU in infants since it is more sensitive to detect blood Phe levels between 180 and 240 μmol/L (3–4 mg/dL). The identification of MHP, however, is unclear and probably not comprehensive. According to research over 3 years during the 1990s, the specificity of BIA was 99.9% and the positive predictive value was 12.8% for infants tested after 24 hours. BIA is inexpensive, easy to develop, and widely used. The disadvantage factor for BIA is that the bacterial growth is easily impacted by many things; antibiotics present in the blood, for instance, can lead to an inaccurate test result [3].

The McCaman and Robins fluorescence assay is a routine PKU screening method based on the theory that fluorescence will be released from Phe after incubation with fluorophores. The sensitivity of the fluorometry assay is 100%, and the specificity is 51%. This screening method may be more positive than BIA, especially if the patient's Phe level is close to the threshold. In other word, it is more likely to detect PKU infections near the threshold. Although this test has a high degree of precision, its accuracy is still below ideal for fluorescence in related compounds is nonselective [4].

Tandem mass spectrometry (MS/MS) is the most current cutting-edge technology, using two or more mass spectrometers connected to each other to improve the ability of analysis. This method can be used as a screening method for newborns younger than 24 hours. MS/MS have the advantages of high sensitivity, high specificity, and high detection efficiency, with sensitivity and specificity of 100% and 98%, respectively [5]. MS/MS is widely used for newborn screening for PKU now.

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5.3. Carrier screening

Carrier screening, also called a genetic test, is used to detect PKU carriers by looking for variants in the PAH genes. This genetic screening is one of the methods used in recent years to diagnose PKU. The PAH Gene Locus-Specific Database has over 1000 PAH variations, over 600 of which are disease-causing mutations linked to varying levels of PAH deficiency, from mild hyperphenylalaninemia to typical PKU [6]. Both parents need to decide how medical professionals will obtain DNA from the fetus if they are thinking about prenatal genetic testing. The two most frequently used procedures are chorionic villus sampling (CVS) and amniocentesis, which involve taking a sample of amniotic fluid from the mother's uterus for testing. It is strongly advised to conduct a carrier screening if one of the parents carries the PKU gene. However, parents should consider this carefully and go to a genetic consultant since both methods have risks.

6. Treatment

Treatment for PKU patients may last a lifetime, and they should try to keep blood Phe levels within 120–360 μmo/L. Early diagnosis and treatment of PKU are essential since they can lessen the risk of serious health issues like intellectual impairment. Eating a low-protein diet is the predominant treatment for PKU. Over the last two decades, medications have been developed and used to treat PKU in combination with a low-protein diet. Clinical trials for PKU gene therapy are currently under development.

6.1. Dietary measures and formular

Dietary measures aim to keep the blood Phe lever in the normal range and attain normal growth and nutritional status for PKU patients at the same time. A low-protein diet is the main therapy for PKU patients. Reduce about 75% or more of your protein intake to maintain your blood Phe level. Patients should eliminate high-protein foods like eggs, milk, cheese, nuts, and fish, and control their intake of bread, rice, pasta, and some vegetables. Based on their individual tolerance, each PKU patient should be given a daily dose of Phe. This makes it possible for people to consume protein at a constant rate. Phe intake can be calculated based on daily protein consumption, where 1 g of protein will provide roughly 50 mg of Phe. Individualized PKU diets should be followed under the guidance of a metabolic dietitian or medical professional. The amount of adjustment required may differ from person to person and depend on factors such as catabolism, growth, and pregnancy. It's possible that some patients can achieve the goal range by strictly adhering to their current diet. Some patients might have to drastically cut back on their phenylalanine intake. Therefore, it is necessary to regularly test the blood Phe concentrations and adjust the diet plan under the guidance of a doctor according to the test results [7].

PKU sufferers require a special dietary supplement called PKU Formula to obtain the necessary nutrients because a restricted diet might result in malnutrition. The PKU formula has been specially developed for patients with PKU and is an amino acid-based powder that can provide the daily nutrition they need. The recommended daily dose of formula is divided across meals and snacks, not consumed all at once, under the direction of a healthcare professional or nutritionist. A Phe-free infant formula will take the place of the standard formula for infants. A dietitian needs to calculate carefully how much breast milk or regular formula is added to a PKU formula that does not contain Phe.

Although the main PKU symptoms can be prevented by strict adherence to a low-protein diet, PKU patients nevertheless have a higher incidence of attention deficits and certain learning deficits than the general population. Only a tiny percentage of treated people can maintain optimal blood Phe concentrations with food therapy alone, as it becomes increasingly difficult to maintain blood Phe levels over adolescence and adulthood.

6.2. Medication

It is quite challenging to maintain a low-Phe diet over the long run. Even if the patient does, Phe can still accumulate in the patient's blood over time, increasing the level of Phe in the blood. In this case, on the basis of a low-protein diet, it must be combined with medication.

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Kuvan (sapropterin dihydrochloride) was the first approved prescription medication for PKU in 2007, developed by the international biotechnology company BioMarin. It is an oral medication, available as a tablet or powder, used to reduce Phe levels in adult PKU patients and children's PKU patients older than one month. The active component in Kuvan is a pharmaceutical form of BH4 that supplies more BH4 and activates the PAH enzyme to lower Phe in PKU patients. It functions similarly to BH4 in the human body. Kuvan can be used to reduce blood Phe levels along with a low-protein diet in many PKU patients of all ages and in all symptom ranges. Clinical research has demonstrated that Kuvan can considerably lower blood Phe concentrations. However, there was no discernible difference in the lowering of Phe levels in PKU individuals with low blood Phe concentrations [8, 9]. Headaches, runny noses, throat pain, diarrhea, vomiting, coughing, and nasal congestion were the most frequently reported side effects. Recommend treating PKU patients with Kuvan and a low-protein diet if their blood Phe lever is greater than 360 mol/L [10]. However, only 20–50% of patients benefit from Kuvan, and adults with blood Phe levels greater than 600 μmol/L should consider other medications [11].

An unusual enzyme therapy called Palynzio (pegvaliase-pqpz) received its initial FDA approval on May 24, 2018. It is a PEGylated PAL enzyme that can reduce blood Phe levels by converting Phe to ammonia and trans-cinnamic acid. Conversion products that have been created by Palynzio are processed in the liver and then eliminated in the urine. Palynzio is used to treat adult patients with uncontrolled PKU with Phe concentrations over 600 μmo/L with ongoing management. Palynziq did not function in all people, but 95 out of 118 patients who took part in the pivotal trial were able to reduce their Phe levels by at least 20% by the end of the treatment period of 24 months [12]. Although this course of treatment successfully lowers blood Phe, it still requires daily injections and is not a cure.

6.3. Gene therapy

In order to cure genetic problems at their root, therapeutic genes are delivered to cells or tissues using modified viruses or other methods, such as gene therapy. The main platform for delivering changed genetic material into cells and tissues during gene therapy for treating numerous human diseases is the adeno-associated virus (AAV) vector [13, 14]. Genetic therapies for PKU seek to restore liver PAH expression by using nucleic acids as medicines.

Homology Medicines, a genetic medicine company, is creating HMI-102 as an experimental gene therapy to treat adult PKU. The liver-tropic AAVHSC15 vector is used to encode the PKU patient's mutated PAH gene. This gene treatment is in phase II trials.

The BioMarin company developed an AAV5 gene therapy named BMN 307 to treat adults with PKU. AAV5 is a gene therapy vector that is recombinant adeno-associated virus serotype 5-based and carries the liver-specific promoter-controlled human coagulation factor VIII (hFVIII) gene. This gene therapy has been held by the FDA during the phase 1/2 study since 2022 because the FDA required further studies of this therapy [15].

American Gene Technologies (AGT) is researching a new gene therapy based on lentivirus vectors, which can alter liver cells and replace the defective gene with the correct copy. This lentiviral-based gene therapy can trigger the activity of PAH enzymes and rebuild the body's ability to metabolize phenylalanine. AGT's gene therapies for PKU are in the preclinical stages of development [16].

There is no published data for all three clinical trials by now.

7. Conclusion

PKU is a hereditary condition that is passed down from parents and can result in a buildup of the amino acid Phe in the body, which will damage a human's nervous system. Early diagnosis is crucial for the treatment of PKU. A newborn screen is the most effective method for the early diagnosis of PKU. By now, newborn screening rates in developed countries are higher than 99%, but worldwide, only 30% of newborns are screened. Therefore, increasing newborn screening rates in developing countries as soon as possible is the most effective way to achieve early detection of PKU, especially in countries with low newborn screening coverage, such as Latin America and Africa. Treatment for PKU

lasts a lifetime, so clinicians should work closely with patients to guide their diet and medications. Pharmaceutical companies and researchers should speed up the research on PKU gene therapy and strive to realize a cure for PKU in the near future.

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