https://doi.org/10.33472/AFJBS.2.1.2020.1-8

ISSN: 2663-2187

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Review Article **Open Access**

Phenylketonuria: Genes in phenylketonuria, diagnosis, and treatments

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Article Info

Volume 2, Issue 1, January 2020
Received : 23 September 2019
Accepted : 02 December 2019
Published : 01 January 2020
doi: 10.33472/AFJBS.2.1.2020.1-8

Abstract

Introduction: Phenylketonuria (PKU) is a rare autosomal-recessive disorder inherited in accordance with the law of segregation. Detection tools for people with PKU can include Sanger Sequencing (SS) and Next Generation Sequencing (NGS). Diet therapy, Large Neutral Amino Acids (LNAA), and Specific Nutrient Combination (SNC) can help alleviate people with PKU. In the future, genetic manipulation techniques can help to eliminate PKU. **Objectives:** In this review, the author describes the progress in a study that focused on detection tools such as SS and NGS, the phenylalanine hydroxylase (PAH) gene and mutations in the *PAH* gene, use of drugs for PKU, and genetic manipulation techniques such as Adeno-associated virus (AAV) vectors and clustered regularly interspaced short palindromic repeats (CRISPR) RNA-guided *Fok*I nuclease system (*Fok*I-dCas9 system). AAV is abbreviation of AAV. CRISPR system is abbreviation of clustered regularly interspaced short palindromic repeats. **Methods:** The author searched the PubMed Database at National Center for Biotechnology Information (NCBI) for articles on PKU disorder. These articles were published between 2014 and 2019. Articles were open access and in English. Searches were also done at Google and ScienceDirect. **Results:** PKU derives from mutations in the *PAH* gene. Features of PKU may include ataxia, intellectual ability, and seizures. MassARRAY method, minisequencing method, SS and NGS can detect PKU on humans. Diet therapy, BH_4, LNAA, SNC, enzyme therapy can help patients with PKU. However, these drugs cannot treat PKU permanently. In the future, genetic manipulation techniques can be used. AAV vectors and *Fok*I-dCas9 system can be useful to eliminate PKU disorder. **Conclusion:** Guthrie method, MassARRAY, minisequencing, SS and NGS are tools for detecting PKU. Treatments with such as diet therapy, LNAA, SNC, and enzyme therapy are useful for PKU disorder. AAV vectors and *Fok*I-dCas9 system are methods that can be useful for eliminating PKU in the future.

Keywords: *phenylalanine hydroxylase (PAH), Phenylalaninase, PKU, PKU1*

1. Introduction

Phenylketonuria (PKU) is a genetic disorder that can affect intellectual disability and other medical problems.[1,2] Other names for this disorder are deficiency disease or, Folling disease, Folling's disease, and phenylalanine hydroxylase (PAH)deficiency disease[1]. Mutations in the *PAH* gene result in PKU that cause reduction of the

[1] Online Menedelian Inheritance in Man, https://omim.org/entry/261600.

[2] Online Mendelian In heritance in Man, https://omim.org/entry/612349.

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activity of PAH.[2] This enzyme catalyzes hydroxylation of phenylalanine to tyroxine[2,3]. This disorder affects about 1 in 2,500 (Wang *et al*., 2019) to 100,000 births (Muntau *et al*., 2019). The carrier frequency is 1 in 50 (Tolve *et al*., 2018). PKU has a high prevalence in Turkey, Ireland, and the United Kingdom. The prevalence PKU is 1 in 2,600 in Turkey, 1 in 4,500 in Ireland, 1 in 10,000 in the United Kingdom, 1 in 11,000 in China, and 1 in 15,000 in the United States. PKU is quite rare in Japan: 1 in 100,000 (Muntau *et al*., 2019) and Thailand 1 in 212,535 births (Chaiyasap *et al*., 2017). This disorder is a rare-autosomal recessive and is inherited in accordance with Mendel's first principle. Both male and female have the same chances of inheriting PKU.

PKU, if undiagnosed, can lead to ataxia (Wang *et al*., 2019) , eczema,[3] (Van Wegberg, 2017) intellectual disability[1] (Van Wegberg, 2017), microcephaly (Van Wegberg, 2017), neurological and psychosocial problems (Wang *et al*., 2019) , and seizures[1] (Wang *et al*., 2019; and Van Wegberg, 2017). Less than 5% of PKU patients have a deficiency in tetrahydrobiopterin (BH_4) synthesis, and essential cofactor for hydroxylation of phenylalanine to tyroxine (Wang *et al*., 2019). Reduction of PAH leads to increase of phenylalanine in blood and brain (Van Wegberg, 2017). Four classification of PKU[4] (Neto *et al*., 2019) severe (higher than 1,200 μM), moderate (between 900 and 1,200 μM), mild (between 600 and 900 μM), and non-PKU mild (lower than 600 μM) (Neto *et al*., 2019).

Screening for PKU in newborns or older is an important method to diagnose this disorder (Muntau *et al*., 2019). Several techniques i.e., Sanger Sequencing (SS) and Next Generation Sequencing (NGS) are available for Screening this disorder. Drugs are available to treat PKU are BH_4, Large Neutral Amino Acids (LNAA) (Daly *et al*., 2019; and Taslimifar *et al*., 2019), Specific Nutrient Combination (SNC) (Bruinenberg *et al*., 2019), and gene therapy (Durrer *et al*., 2017; and Pan *et al*., 2016). Use of these drugs can help patients with PKU to live as normal people. In the future, gene therapy can be a useful tool for eliminating PKU disorder. Gene therapy includes Adeno-associated virus (AAV) vectors and *Fok*I-dCas9 system.

In this review article, the author describes the progress in a study of PKU focused on genetic aspects, diagnosis, treatments including such as SNC and gene therapy. Genetic aspects include the *PAH* gene and mutations in the *PAH* gene, while diagnosis could include SS and NGS. Finally, treatment of PKU is comprised of drugs and gene therapy.

2. Methodology

The author searched the PubMed Database at National Center for Biotechnology Information (NCBI) for articles on PKU. Searches included the *PAH* gene, mutations in the *PAH* gene, diagnosis tools, drugs, and gene therapy for PKU. All articles were open access and in English published between 2014 and 2019. The author references were included in this review. Other relevant publications were also included in the searches.

2.1. Genes in phenylketonuria

The *PAH* gene supplies directives for making PAH.[1,2] Mutations can arise in gene and cause damage of protein (Nelwan, 2018; and Nelwan, 2017a, b and c) of PKU patients. These mutations cause permanent changes in DNA in the *PAH* gene. Mutations in the *PAH* gene result in four classes for PKU: severe, moderate, mild, and non-PKU mild hyperphenylalaninemia (Table 1) (Van Wegberg, 2017).

Table 1: Classifications in PKU

Classifications	Phenylalanine levels	Dietary phenylalanine	References
Severe	≥1,200 μM	┐ <20 mg/kg/day to	Neto *et al*., 2009
	(≥20 mg/dl)	250-350/kg/day	Regier and Greene, 2000
Moderate	900-1,200 μM	20-25 mg/kg/day to	Neto *et al*., 2009
	(15-20 mg/dl)	350-400 mg/kg/day	Regier and Greene, 2000
Mild	600-900 μM	25-50 mg/kg/day to	Neto *et al*., 2009
	(10-15 mg/dl)	400-600 mg/kg/day	Regier and Greene, 2000
Non-PKU mild	>600 μM	Normal diet	Neto *et al*., 2009
HPA	(5-10 mg/dl)		Regier and Greene, 2000

Note: HPA = hyperphenylalaninemia; and PKU = phenylketonuria.

[3] Online Menedelian Inheritance in Man, https://omim.org/entry/261600
[4] Online Mendelian In heritance in Man, https://omim.org/entry/612349.

2.2. The PAH gene

Official name of the *PAH* gene is phenylalanine hydroxylase. Other names include L-Phenylalanine, tetrahydrobiopterin: oxygen oxidoreductase (4-hydroxylating), PH4H_HUMAN, Phenylalaninase, Phenylalanine-4-Hydroxylase, Phenylalanine-4-Monooxygenase, and PKU1.[2] The human *PAH* gene occupies chromosome 12q at position 23.2, 12q23.2.[2,3,4] The *PAH* gene spans 90 kb, contains 13 exons,[4] and includes base pairs 102,836,885 to 102,958,410[2]. The *PAH* gene encodes an aromatic amino acid hydroxylase protein family.[2] (Chaiyasap *et al*., 2017). The encoded PAH enzyme converts phenylalanine to tyrosine[2].

2.3. Mutations in the PAH gene

Neto *et al*. (2019) found that mutations in the *PAH* gene can include deletion with frame shift, in-frame deletion, large deletion, missesnse, nonsense, and splicing sites[5] (Table 2) (Wang *et al*., 2017). There have been identified PAH 1101 variants in PAHvdb Database;[6] about 50% are missense mutations.[7] Gu *et al*. (2014) suggested that mutations in the *PAH* gene consist of 13,5% deletions, 1.8% insertions, 5% nonsense, 60.5% missense, and 11% splice site, (Pecimonova et al., 2019). The p.Arg408Trp is the most common mutation in many populations,[2] Gundorova *et al*. (2019) suggested that the pathogenic variant p.Arg408Trp is widespread in Europe. The p.Arg408Trp is not frequently found in Asia. 80 to 85% of the p.Arg408Trp mutation in the world is in the Baltic countries: Estonia, Latvia, and Lithuania. 50% is in the East European countries: Czech Republic, Hungary, Russia, and Slovakia. In Balkans, the frequency was 30% to 40%. In Western and Middle Europe, the pathogenic variant p.Arg408Trp mutation is 5% to 25%. Other pathogenic variants may include p.Arg261Gln, p.Tyr414Cys, and p.Ser349Pro (Gundorova *et al*., 2019).

2.4. Diagnosis tools for phenylketonuria

Newborn screening for PKU diagnosis depends on diagnosis tools such as Guthrie method, fluorometric method SS and NGS. SS is the method of choice to sequence short pieces of DNA[8] and causative mutations. NGS is a tool for diagnosing newborns with inborn errors of metabolisms (IEMs) (Wang *et al*., 2019) and to sequence whole exome and genome levels.[8] In addition to SS and NGS, tandem mass spectrometry (MS/MS) is a useful tool for detecting IEMs such as phenylalanine and tyrosine (Gu *et al*., 2014). Single-gene testing or

Table 2: Mutations in the *PAH* gene

Mutations	Total	Percentage	References
Frameshift	21	1.73985087	ClinVar[7]
Missense	337	27.92046396	ClinVar[7]
Nonsense	31	2.568351284	ClinVar[7]
Splice site	28	2.31980116	ClinVar[7]
Deletion	109	9.030654515	ClinVar[7]
Duplication	23	1.905550953	ClinVar[7]
Indel	5	0.414250207	ClinVar[7]
Insertion	25	2.071251036	ClinVar[7]
Single nucleotide	628	52.02982601	ClinVar[7]
Total	**1,207**	**100**	

[5] NCBI Gene, https://www.ncbi.nlm.nih.gov/gene/5053.

[6] PAHvdb, http://www.biopku.org.

[7] NCBI ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/?-term=PAH[gene].

[8] Genetics Home Reference, https://ghr.nlm.nih.gov.

multiple gene panel can also be a useful tool for diagnosing PKU disorder (Regier and Greene, 2000) . New methods such as MassARRAY and minisequencing have also been developed.

The newborns' blood samples were collected by heel prick on filter paper that was subsequently tested using Guthrie method and / or the fluorometric method. The positive cases underwent plasma amino acid analysis using high performance liquid chromatography to determine phenylalanine levels. Determination of urinary pterin levels can be used to screen BH_4 deficiency (Chaiyasap *et al*., 2017).

Chaiyasap *et al*. (2017) used NGS method for diagnosing PKU and BH_4-deficiency. The authors took three milliliters of peripheral blood from each patient and extracted genomic DNA was by using Gentra Puregene Blood kit (Qiagen, Hilden, Germany). The extraction process was according to the manufacture's protocol. Genomic DNA was for exome sequencing using service from Macrogen, Inc (Seoul, South Korea). The preparations of samples were according to Agilent SureSelect Target Enrichment kit (Agilent Technologies, Santa Clara, CA, USA) preparation guide. Sequencing for libraries was with HiSeq2000 or HiSeq2500 sequencer. Alignment for the result sequences were according to the human genome sequence using Burrows-Wheeler Alignment. The authors used Picard software for making and removing duplicate sequences. For data quality assessment, genotyping, and variant calling, the authors used Genome Analysis Toolkit (GATK3.v4). For variant annotation, SnpEff_v.4.1 was used. With this method, found mutations in the *PAH* gene (Chaiyasap *et al*., 2017). It shows that NGS is an appropriate molecular genetics technique for diagnosing mutations in the *PAH* gene.

The Life Technologies can help for the sample preparation and SS. The BigDye Terminator v1.1 Cycle Sequencing kit (Gu *et al*., 2014) and by an ABI PRISM 3130XL (Thermo Fisher Scientific) (Tolve *et al*., 2018) can perform SS. An ABI 3730 can run sequencing after ethanol purification.[8] A total of 42 exons of PKU, BH_4-deficiency associated genes are needed to be analyzed.[7] MS / MS testing of blood spots can be performed using the NeoBase™ Non-derivatized MS / MS kit (PerkinElmer, MA, USA) (Wang *et al*., 2019). The NGS procedures can also use Nextera Rapid Capture Enrichment Reference Guide. Runs can use Miseq DX platform and data analysis can use BaseSpace Variant Interpreter (Illumina, San Diego, CA, USA) (Tolve *et al*., 2018). NGS is useful for the clinical genetic analysis of IEMs diagnosis (Chaiyasap *et al*., 2017).

In newborn screening, blood taken from heel stick was spotted on Whatman 903 filter paper and dried for screening need. The time of sampling was between the 3rd and 10th day of life. Dried blood spots were pre-processed following the instruction of NeoBase™ none-derivatized MS / MS kit (PerkinElmer, MA, USA), and then they were analyzed by using TQD MS / MS system (Waters, MA, USA) and NeoBase™ non-derivatized system. The analyses can include phenylalanine, tyrosine, and acylcarnitines. The normal ranges and cut-off values of analytes in the manual of the manual of NeoBase™ non-derivatized MS / MS kit (Cat. 3040-001Z, PerkinElmer, MA, USA) and from the worldwide collaborative project can be applied. Suspected positive cases were recalled for the repeated test by MS / MS (Guo *et al*., 2018).

Sarker *et al.* (2019) validated the method of liquid chromatography tandem mass spectrometry (LC-MS / MS) for diagnosis of IEMs with the target for three different age groups. The authors used a second-tier test, gas chromatography-mass spectrometry, to confirm patients with PKU. There were seven patients older than one year in test of LC-MS / MS. Gas chromatography-mass spectrometry confirmed three patients with phenyketonuria. About 43% of IEM came from consanguineous family across the globe. Sarker *et al*. (2019) collected blood specimens by heel prick method. The authors spotted about 75 μL of blood on Whatman™ Generic Multipart filter paper (GE Healthcare Westborough, MA, USA) to prepare a dried blood spot card for LC-MS / MS analysis. The authors collected about 80% specimens between 24 hours and 72 hours after birth and collected about 20% specimens between day 4 and day 7 after birth. The collections of blood specimens for older children were after 4-hour fasting with standard venipuncture method. Then prepared a dried blood spot card by spotting about 75 μL blood on Whatman™ 903 filter paper (Sarker *et al*., 2019). In addition, 5 mL fasting urine specimens also collected for urinary metabolic screening tests. Tests include ferric chloride test, 2,4-Dinitrophenylhydrazine test, Cyanide nitroprusside test, and test for urine reducing sugar and ketone bodies. Drying of the dried blood specimen cards were as long as four hours at room temperature and stored at –70º C in plastic ziplock bags with desiccants until analysis was done. After metabolic screening tests, about 2 mL urine specimens were stored at –70º C for second-tier tests such as metabolic profiling using gas chromatography-mass spectrometry (Sumaily and Mujamammi, 2017).

Tolve *et al.* (2018) suggested a procedure for diagnosing PKU: minisequencing. The authors have optimized the detection of the 24 probes in four multiplex PCR followed by four single-nucleotide extension reactions

and four electropherograms. The method showed 100% sensitivity and 100% specificity. Tolve *et al*. (2018) successfully tested the method on dried blood spots. This genotyping assay needs 48 hours to do the test and is able to characterize both alleles in more than 50% of the PKU subjects (Tolve *et al*., 2018). This method is a useful tool for detecting PKU disorder.

SS and MassARRAY are techniques for detecting genetic variants in IEMs. SS, which is applied for the known gene locus in genetic analysis, is a golden standard to determine the DNA sequence. Both NGS and MassARRAY, which can be used for genetic analysis of spectrum of gene, are high throughput sequencing methods. Moreover, MassARRAY has been used more widely and longer to detect known mutations. In addition, it is reliable and the cost is less (Guo *et al*., 2018).

Regier and Greena (2000) suggested single-gene testing or multiplegene panel for diagnosing PKU disorder. For single-gene testing, sequence analysis of *PAH* is performed first followed by gene-targeted deletion/duplication analysis if only one or no pathogenic variant is found. In addition, a multiplegene panel that includes *PAH* and other genes of interest may also be considered (Regier and Greene, 2000).

The establishment of the diagnosis of PAH deficiency in a proband is based on two criteria. First, it is a plasma phenylalanine concentration persistently above 120 µmol/L (2mg/dL) and altered ratio of phenylalanine to tyrosine in the untreated state with normal BH_4 cofactor metabolism. Second, it is based on the finding of biallelic pathogenic variants in the *PAH* gene by molecular genetics testing (Regier and Greene, 2000). The follow-up testing commences for the second time positive cases, including biochemical tests or genetic analysis. The recall and follow-up protocol in the guidelines "Follow-Up Testing for Metabolic Disease Using Tandem Mass Spectrometry" may be used. Specialists based on the clinical symptom, screening test, and biochemical and genetic analysis make definitive diagnosis. The parents of all cases with definitive diagnosis are informed, and they are referred to specialists for the treatment (Guo *et al*., 2018).

2.5. Treatments

Treatment of PKU may be performed with such as dietary therapy, sapropterin, LNAA, and gene therapy (Table 3). Dietary restriction of phenylalanine is predominantly treatment for PKU. Sapropterin has been successful treatment in BH_4 responsive patients. However, sapropterin can only be beneficial about 30% of all PKU patients. LNAA has been explored and still needs long-term studies to understand the full effect of this treatment. Gene therapy may be a useful tool for treating PKU in the future. Research has been conducted to get the right tool for treating PKU patients with genetic manipulation techniques.

Dietary therapy is associated with a risk for nutritional deficiencies. For example, growth retardation and early-onset osteoporosis can be caused by deficiencies in specific substances, such as minerals or vitamins, in a phenylalanine-restricted diet (Guo *et al*., 2018). A diet therapy limits food with high phenylalanine; dairy products, eggs, meat, and fish.[7] However, huge social and economic burdens are imposed by the diet therapy. In addition, compared with age-matched individual without PKU, patients with PKU who consume a phenylalanine-restricted diet may not achieve their full neurodevelopment potential. Accordingly, various alternative therapeutic strategies have been proposed for PKU treatment (Guo *et al*., 2018). It could experience deficits in cognitive functioning, for instance in processing speed, attention, working memory, and social-cognitive functioning (Bruinenberg *et al*., 2019). Dietary therapy is the basis of PKU management. It consist of three parts: natural protein restriction, Phe-free–L-amino acid supplements, and low protein food. Treatment should be commenced before the age of 10 days. Many countries will require change in timing of national NBS, logistical and diagnostic procedures (Van Wegberg, 2017). However, treatment with dietary therapy is ineffective in patients with PKU exhibiting BH_4 deficiencies (Guo *et al*., 2018).

BH_4 can help stabilize misfolded mutant enzymes and prevent their proteolysis, and increase enzyme activity was demonstrated by changing the dose and formulation of BH_4 (Guo *et al*., 2018). BH_4 is also involved in catecholamine, serotonin, and nitric oxide biosynthesis (Sarker *et al*., 2019). BH_4 is a cofactor in neurotransmitter synthesis that include dopamine, epinephrine, norepinephrine, and serotonin.[7] Patients with high residual activity of the PAH enzyme have a greater probability of BH_4 response, but a minority of patients with classical PKU also may benefit from BH_4 therapy. Efficacy and safety of BH_4 therapy has been proved in children with <4 years old (Van Wegberg, 2017). Synthetic BH_4 is commercially available. In 2007, the US approved sapropterin dihydrochloride as an adjuvant therapy for PKU. Furthermore, when administering at doses of 5-25 mg/kg/day for up to 22 weeks, BH_4 caused a decrease in the plasma phenylalanine concentration in approximately 32- 50% of treated participants, as illustrated by analyzing

the effects of cofactor treatment in clinical trials (Guo *et al*., 2018). it is important to give an early and precise diagnosis for PKU patients to provide a proper treatment to the patients (Chaiyasap *et al*., 2017) .

LNAA may decrease plasma phenylalanine concentration in affected adolescents and adults. However, women of childbearing age should not take LNAA(Sarker *et al*., 2019). LNAA can be useful to adult patients with PKU that cannot not tolerable with a phenylalanine-restricted diet. Four L-type amino acid transporters are present in the transport system through which certain molecules, such as LNAAs, are transported to the brain. These L transporters transport LNAAs, including the branched-chain amino acids valine, leucine, and isoleucine; the aromatic amino acids tyrocine, triptopane, and phenylalanine. These also include some other amino acids, such as threonine, methionine, and histidine. Thus, to cross the blood-brain barrier, these molecules compete with each other to bind the transporters based on their plasma concentrations. High LNAA supplementation thus inhibits plasma phenylalanine transport and reduces brain phenylalanine concentrations. Additional supplementation of LNAA was of limited value, but it may be of benefit in those unable to adhere to their phenylalanine free L-amino acid supplements. Although some centers routinely administer phenylalanine, free LNAA supplements to older patients who are unable to adhere to dietary treatment. These supplements remained untested in children under the age of 11 years, and not reports in use in pregnant women. In addition, further research is required to ascertain the ideal dosages and amount of each specific L-amino acid within the LNAA supplement (Guo *et al*., 2018).

Bruinenberg *et al*. (2019) suggested that a SNC was postulated to improve brain function in PKU. The authors examined at three-point time; 3, 6, and 9 months for memory and motor function. In the Novel Object Recognition (NOR) test, Bruinenberg *et al*. (2019) found that PKU mice, despite being subjected to high phenylalanine conditions, could master the task on all three-time points when supplemented with SNC. Under low phenylalanine conditions, PKU mice on control diet could master the NOR at all three time points, while PKU mice on the SNC supplemented diet could master the task at time points 6 and 9 months. SNC supplementation did not consistently influence the performance in the open field (OF), spatial object recognition (SOR) test or balance beam (BB) in PKU mice. The low phenylalanine diet was able to normalize concentrations of norepinephrine and serotonin. However, these neurotransmitters were not influenced by SNC supplementation. This study demonstrates that both a long-lasting low phenylalanine diet, the diet enriched with SNC, as well as the combined diet was able to ameliorate some, but not all of these PKU-induced abnormalities. These diets seem to improve some, but not other domains that are impaired in the BTBR PKU mouse model. The authors demonstrate that a long-term intervention study in BTBR PKU mice improves NOR, while a long-term intervention with a low phenylalanine diet nearly normalizes serotonin and norepinephrine levels. Bruinenberg *et al*. (2019) suggest that future research should be aimed at developing an optimal nutritional intervention to target brain function in PKU patients (Bruinenberg *et al*., 2019).

Enzyme therapy for enzyme substitution for PKU patients has been available. Replacement with PAH fusion proteins is effective in mouse studies. However, PAL-replacement therapy appears to be a more promising approach for treating PKU. In addition, this therapy also converts excess phenylalanine into readily excreted and less toxic products. However, mouse models of PKU showed that injection administering or oral version of the PAL enzyme has some limitations. For example, injection of PAL activates immune responses, whereas oral administration of PAL is associated with enzyme degradation. It reduces the effectiveness of the therapy. Notably, conjugation of PAL with polyethylene glycol (PEG) decreased PAL-induced immune responses. Clinical trials on PKU patients are promising. Phase I and II trials using injectable recombinant PAL conjugated with PEG have shown a reduction in the phenylalanine levels (Guo *et al*., 2018).

2.6. Genetic manipulation

Genetic manipulation techniques are promising techniques for treating PKU disorder. Multiple methods are available. These techniques can include such as adenoviral vectors, AAV, and clustered regularly interspaced palindromic repeats (CRISPR / Cas9) system (Nelwan, 2019).

Restriction of dietary phenylalanine is for preventing neuronal damage in PKU patients. deprived compliance by PKU patients and the heavy economic burden confront the dietary therapy. Although dietary therapy recovers cognitive performance, other neuropsychological parameters such as attention, and executive functions are impaired compared to healthy controls. Diet therapy, BH_4, LNAA, SNC, and enzyme therapy may be beneficial for PKU patients. However, the ideal method would be to edit variants in the *PAH* gene. Gene therapy may help for editing the mutated gene (Pan *et al*., 2016).

PKU gene therapy in animal models has been developed for more than two decades (Pan *et al*., 2016). Adeno virus, AAV, and lentivirus are viral vectors that have been developed for treating PKU disorder (Guo *et al*., 2018). However, the temporary recovery a very low gene transfer rate limited this approach. Adenovirus, AAV, and lentivirus still need modification to be used for gene therapy in PKU patients.

Pan *et al.* (2016) used a modified CRISPR system which employs the fusion of inactive Cas9 (dCas9) and the *FokI* endonuclease (*FokI*-dCas9) to correct p.Arg408Trp in the *PAH* gene. This technique can correct allele frequency 21.4%. This correction could rescue residual PAH activity and restore normal function. Co-expression of a single guide RNA plasmid, and the presence of a single stranded oligodeoxynucleotide in *PAH*-_c.1222>TCOS-7 cells –an *in vitrro* model for PKU—corrected the *PAH* variant and restored PAH activity (Nelwan, 2019). It seems that the *FokI*-dCas9 system is a promising tool for treating PKU disorder.

3. Conclusion

PKU has an autosomal recessive inheritance pattern in accordance with the law of segregation. Mutations in the *PAH* gene result in PKU disorder: severe, moderate, mild, and non-PKU mild HPA. To control PKU, the diagnosis has a significant role. Diagnosis techniques for PKU include SS, NGS, MS/MS. Dietary and enzyme therapies can help people with PKU. Drugs for PKU patient include BH_4, LNAA, and SNC. Genetic manipulation techniques are potential and useful tools to control PKU in the future. Adenovirus vectors, AAV vectors, and *Fok*I-dCas9 system may be useful for treating PKU disorder.

Acknowledgment

Exclusively M. Nelwan performed research and manuscript development.

Conflicts of interest

The author has no conflicts of interests regarding the content of this article.

References

Bruinenberg, V.M., van Vliet, D., van der Goot, E., Counotte, D.S., Kuhn, M., van Spronsen, F.J. and van der Zee, E.A. (2019). Long-term dietary intervention with low Phe and / or a specific nutrient combination improve certain aspects of brain functioning in phenylketonuria (PKU). *PloS ONE*. 14(3). e0213391. doi:10.1371 / journal.pone.0213391

Chaiyasap, P., Ittiwut, C., Srichompthong, C., Sangsin, A., Suphapeetiporn, K. and Shotelersuk, V. (2017). Massive parallel sequencing as a new diagnostic approach for phenylketonuria and tetrahydrobiopterin-deficiency in Thailand. *BMC Medical Genetics*. 18(1), 102. doi:10.1186 / s12881-017-0464-x

Daly, A., Evans, S., Chahal, S., Santra, S., Pinto, A., Jackson R. *et al*. (2019). Glycomaropeptide: long-term use and impact on blood phenylalanine, growth and nutritional status in children with PKU. *Orphanet Journal of Rare Diseases*. 14(1), 44. doi:10.1186 / s13023-019-1011-y

Durrer, K.E., Allen, M.S. and von Herbing, L.H. (2017). Genetically engineered probiotic for the for the treatment of phenylketonuria (PKU); assessment of novel treatment *in vitro* in the *PAH*^{enu2} mouse model of PKU. *PloS ONE*. 12(5). e0176286. doi:10.1371 / journal.pone.0176286

Genetics Home Reference. https: / / ghr.nlm.nih.gov

Gu, Y., Lu, K., Yang, G., Cen, Z., Yu, L., Lin L. *et al*. (2014). Mutation spectrum of six genes in Chinese phenylketonuria patients obtained through next-generation sequencing. *PloS ONE*. April 4; 9(4). e94100. doi:101371 / journal.pone.0094100.

Gundorova, P., Stepanova, A.A., Kuznetsova, I.A., Kutsev, S.I. and Polyakov, A.V. (2019). Genotypes of 2579 patients with phenyketonuria reveal a high rate of BH4 non-responders in Russia. *PloS ONE*. January; 14(1). 0211048. doi:10.1371 / journal.pone.0211048

Guo, K., Zhou, X., Chen, X., Wu, Y., Liu, C. and Kong, Q. (2018). Expanded newborn screening for inborn errors of metabolism and genetic characteristics in a Chinese population. *Front. Genet*. 9, 122. doi:10.3389 / fgene.2018.00122

Muntau, A. C., du Moulin, M. and Feillet, F. (2018). Diagnostic and therapeutic recommendations for the treatment of hyperphenylalaninemia in patients 0-4 years of age**.** *Orphanet Journal of Rare Diseases*. 13(1), 173. doi:10.1186 / s13023-018-0911-6

Nelwan, M.L. (2017). Friedreich ataxia: treatment with genetic approach. *Journal of Advances in Biology & Biotechnology*. 14(4),1-12. doi:10.9734/JABB/2017/36113

Nelwan, M.L. (2017). Hemophilia A and induced pluripotent stem cells. *Journal of Advances in Biology & Biotechnology*. 14(3), 1-11. doi:10.9734/JABB/2017/35111

Nelwan, M.L. (2017). Treat occulocutaneous albinism with gene therapy. *Journal of Advances in Biology & Biotechnology*. 16(3), 1-12. doi:10.9734/JABB/2017/38504

Nelwan, M.L. (2018). Control of *MAOA-L* allele expression through genetic manipulation techniques using CRISPR/Cas9 system. *Asian Journal of Biotechnology and Genetic Engineering*. 1(2), 1-10. doi:10.9734/AJBGE/2018/44335

Nelwan, M.L. (2019). Schistosomiasis: Life cycle, diagnosis, and control. *Current Therapeutic Research*. 91, 5-9. doi:10.1016/j.curtheres.2019.06.001

Neto, E.V., Laranjeira, F., Quelhas, D., Ribeiro, I., Seabra, A., Mineiro, M. *et al*. (2019). Genotype-phenotype correlations and BH_4 estimated responsiveness in patients with phenylketonurian from Rio de Janeiro, Southeast Brazil. *Molecular Genetic*. 7(5), e610. doi:10.1002/mgg3.610

Pan, Y., Shen, N., Jung-Klawitter, S., Betzen, C., Hoffman, B.F., Hoheisel, J.D. and Blau, N. (2016). CRISPR RNA-guided *Fok*I nucleases repair a *PAH* variant in a phenylketonuria model. *Scientific Reports*. 6, 35794. doi:10.1038/srep35794

Pecimonova, M., Kluckova, D., Csicsay, F., Reblova, K., Krahuluc, J. and Procházkova, D. (2019). Structural and functional impact of seven missense variants of phenylalanine hydroxylase. *Genes*. 10(6), 459. doi:10.3390/genes10060459

Regier, D.S. and Greene, C.L. (2000). Phenylalanine hydroxylase deficiency. January 10. [Updated 2017 Jan 5]. In Adam M P, Ardinger H H, Pagon R A, *et al*. (Eds.). GeneReviews® [Internet], Seattle (WA): University of Washington, Seattle; 1993-2019.

Sarker, S.K., Islam, M.T., Biswas, A., Bhuyan, G.S., Sultana, R., Sultana, N., *et al*. (2019). Age-specific cut-off values of amino acids and acylcarnitines for diagnosis of inborn errors of metabolism using liquid chromatography tandem mass spectrometry. *Biomed Research International*. 3460902. doi:10.1155/2019/3460902

Sumaily, K.M. and Mujamammi, A.H. (2017). Phenylketonuria: A new look at an old topic, advances in laboratory diagnosis, and therapeutic strategies. *International Journal of Health Sciences*. 11(5), 63.

Taslimifar, M., Buoso, S., Verrey, F. and Kurtcuoglu, V. (2019). Propagation of plasma l-phenylalanine concentration fluctuations to the neurovascular unit in phenylketonuria: an in silico study. *Front Physiol*. 10, 360. doi:10.3389/fphys.2019.00360

Tolve, M., Artiola, C., Pasquali, A., Giovanniello, T. D., Amici, S. and Angeloni, A. (2018). Molecular analysis of PKU-associated PAH mutations: a fast and simple genotyping test. *Methods and Protocols*. 1(3), 30. doi:10.3390/mps1030030

Van Wegberg, A. M. J., MacDonald, A., Ahring, K., Bélanger-Quintana, A., Blau, N. and Bosch, A.M. (2017). The complete European guidelines on phenylketonuria: diagnosis and treatment. *Orphanet Journal of Rare Diseases*. 12(1), 162. doi:10.1186/s13023-017-0685-2

Wang, W., Yang, J., Xue, J., Mu, W., Zhang, X., Wu, W., *et al*. (2019). A comprehensive multiplex PCR based exome-sequencing assay for rapid bloodspot confirmation of inborn errors of metabolism. *BMC Medical Genetics*. 20(1), 3. doi:10.1186/s12881-018-0731-5

Wang, Z.W., Jiang, S.W. and Zhou, B.C. (2017). *PAH* mutation spectrum and correlation with PKU manifestation in north Jiangsu province population. *Kaohsiung Journal of Medical Sciences*. 34, 89-94. doi:10.1016/j.kjms.2017.09.006

Cite this article as: Martin L. Nelwan (2020). Phenylketonuria: Genes in phenylketonuria, diagnosis, and treatments. *African Journal of Biological Sciences* 2 (1), 1-8.