Review article

DNA methylation in phenylketonuria: a narrative review

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Abstract: Phenylketonuria (PKU) occurs due to a mutation in the gene encoding phenylalanine hydroxylase, which results in inability to convert phenylalanine into tyrosine. DNA methylation is an important epigenetic modification of the genome. Many human diseases have been detected to be related to aberrant DNA methylation. Investigating the leukocytes of PKU patients exposed to phenylalanine has shown a wide range of methylation, which indicates DNA methylation changes as a biochemical marker. In this article, we reviewed evidence of DNA methylation in pathophysiology of PKU.

Keyword: DNA methylation; Phenylketonuria; phenylalanine

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1. Introduction

Phenylketonuria (PKU), known as the most common inborn error of amino acid metabolism, is due to a mutation in the gene encoding phenylalanine hydroxylase, which results in inability to convert phenylalanine into tyrosine, and consequently a high level of phenylalanine in the blood (1, 2). This disease is a recessive autosomal genetic disorder caused by a deficiency in phenylalanine hydroxylase that leads to intellectual disability if not treated.

Maternal PKU (MPKU) often affects the fetus and causes congenital heart defects and microcephaly. Management of MPKU has primarily concentrated on controlling the high level of phenylalanine in the blood (3). Recently, stem cell therapy (4) such as other disease(5-15) and antioxidant therapy (16-21) present for PKU management. The deficiency of phenylalanine hydroxylase activity in the liver is due to a mutation in chromosome 12, which causes metabolic disorders. The locus of the human chromosome 12q23.2 contains a gene that codes for the phenylalanine hydroxylase enzyme, which has hundreds of alleles, often homozygous phenotypes (22). Investigation has shown that diet alone is not enough to cure this disease (23).

2. Epidemiology

PKU is transmitted as an autosomal recessive trait and has a collective prevalence of about one per 10,000 people, therefore, 2 percent of people carry PKU gene (24, 25). The incidence of PKU was reported to be 1 per 1500 birth in the USA (26). It varies around the world, but its average frequency is 1 in 10000 (27).

3. Etiology

This congenital disorder is caused by a deficiency in phenylalanine hydroxylase (PAH) (25), the enzyme which converts phenylalanine to tyrosine, consequently, the conversion of phenylalanine to tyrosine is interrupted (2, 25). PAH is a liver enzyme that increases the hydroxylation rate of 1-phenylalanine to 1-tyrosine by using tetrahydrobiopterin (BH4) cofactor (26). The polyhydroxyalkanoate (PHA) gene is about 90 kb and contains 13 exons encoding 451 amino acids for synthe-

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sis of the protein (28). PKU gene is located on chromosome 12q23.1, with more than 500 mutations reported in PAH gene, most of which are point mutations (29, 30). Loci that cover phenylalanine hydroxylase are 1.5 mbp. Exons in the PAH gene make approximately 2.88% of the genomic sequence lying between the start codon and 3' poly A. The longest exon and intron are exon 13 (892 bp) and intron 2 (17.87 bp), respectively. The shortest ones are exon 9 (57 bp) and intron 10 (556 bp). The PHA gene contains 40.7% GC in its sequence (31). The most common mutation with a relative frequency of 42% is the substitution of arginine with tryptophan (27). PKU occurs only due to defective activity of the PAH enzyme that is expressed only in the liver. The normal phenylalanine concentration is usually between 50 and 120 µmol/L (32).

In case of PKU, genotype is the more important factor compared to phenylalanine intake. However, people may carry this genotype, but the disease will only be developed by phenylalanine intake and the severity of disease can be improved by diet control (33). Genetics and epigenetics are likely to play a significant role in onset of diseases (34). Aberrant methylation in CpG island of the promoter results in the silencing of argininosuccinate synthetase (ASS) and consequently, arginine biosynthesis disorders. The changes in expression of ASS enzyme affect vascular contraction and metabolic functions (35). ASS is the rate-limiting enzyme in arginine synthesis pathway. The cell is able to convert citrulline to arginine via this metabolic pathway.

Investigating the leukocytes of PKU patients exposed to phenylalanine has shown a wide range of methylation, which indicates DNA methylation changes as a biochemical marker. Studies have shown that a gene that plays a role in development of the nervous system has a particular methylation pattern that affects the expression of downstream genes. GTGTG demethylation and GTGC / TG, PAH partial methylation have been reported in healthy people, indicating that they are not pathogenic alleles. The analysis of GPX3 promoter DNA methylation (GPX3) indicated increased production of primary radicals (36). Phosphodiester bond guanine positions overlap with CCAAT box/metal. The response element (CGATTGGCTG) of the active GPX3 promoter is analyzed by the oxidative stimulus. The CCAAT-box / metal interference is an interesting response element. Because this response element can not only be activated by reactive oxygen species (ROS) induced phenylalanine, it can also be activated by metal ions resulting from ROS hemostatic disorder by creating an imbalance (37, 38). Studies on GTGTC methylation and partial methylation of GTGC/TG PHA have shown that these alleles are not pathogenic (38).

It has been shown that ASS methylation can be detected before the obvious PKU symptoms (35). The relative frequency of mutations in PKU can be estimated as 0.01 in the population, but among these mutations, C1222 C>T allele (P.R408W) has the highest rate. Studies have shown that deamination-methylation of 5-methylcytosine (5mC) has an important role in CpG mutations in the human genome (39).

Table 1: Studies for methylation process in PKU

Author, year, country	Number of control and treated patients	Treatment protocols	Main findings
Murphy, et al., 2006, Canada	3 PKU patients / 12 normal controls	Antonarakis and the Nomenclature Working Group 1998, Invitrogen; Carlsbad, CA Wizard DNA Clean-up System, Promega; Madison, WI	Methylation-mediated deamination of c.1222mC can be a source of recurring c.1222C>T, p.R408W alleles on different background haplotypes.
Dobrowolski, et al., 2014. United States	19 fetuses of PHE unrestricted mice	Harlan Laboratories	In utero exposure to phenylalanine toxicity is associated with aberrant DNA methylation in the brain.
Dobrowolski, et al., 2015, United States	2 classical PKU patients / 5 control brain tissues	A protocol was obtained through the University of Pittsburgh	Abnormal DNA methylation is presented in PKU patients and is influenced by phenylalanine exposure.

PKU: Phenylketonuria

4. DNA methylation and PKU

Regarding methylation process in PKU, there are limited studies (Table 1). Murphy et al., have stated that concentrated deaminization on methylation of 5mC is implicated in CpG mutation. In fact, methylated cytosine in mCpG nucleotide field may induce by itself and initiate C>T replacement. It has been shown that PHA allele is probably a repeated mutation C.1222 C>T, containing a methylated cytosine. There is the similar pattern of methylation in CpG dinucleotide. In homozygous patient samples of p.R408W mutation, T has been recorded in c.1222 nucleotide (A in complementary DNA). In general, based on these evidence, it has been hypothesized that methylation-mediated deamination of c.1222 mc is the main mechanism involved in the repeat of c.1222 C>T, P.r408w alleles in different background haplotypes. The highest observed amounts of c.1222 C>T,P.R408W is the main reason for outbreak of PKU disease in the Caucasian population (40).

Dobrowlski et al., stated that phenylalanine is known as a toxic component in PKU patients, but the exact mechanism of its toxicity is unclear. They concluded that the pattern of DNA methylation may be a critical biomarker relating to historic phenylalanine exposure. These data may improve quality of therapy (41).

Findings of Scriver et al., showed that altered DNA methylation in brain due to phenylalanine toxicity is fetal. In the mentioned research, PKU mice were used as a model. The diet with limited phenylalanine led to the levels of PHE≤ 150µm in blood, while in unlimited condition, the concentration of phenylalanine was 100 µm. Assessment of methylation process in promoters of 17 samples revealed that in contrast to MPKU, expression of these genes was reduced in PKU (31).

This opinion that "phenylalanine toxicity may modify DNA methylation in brain tissue" is a key hypothesis for clarifying pathophysiological mechanisms. Various kinds of individual cell types have different sensitivities to phenylalanine poisoning, resulting in different responses in methylome and consequently, changes in gene expression occur. The exact alterations in gene expression are not clear (42). It is obvious that more research is needed to clarify the exact implicated mechanisms.

5. Conclusion

Data on the role of DNA methylation in pathology of phenylketonuria is very limited. Studies have suggested that DNA methylation may play a role in the mechanism of phenylalanine toxicity in PKU. Accordingly, some studies have suggested DNA methylation as a possible biomarker relating to historic phenylalanine exposure.

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9. Author contribution

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10. Reference

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