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INVESTIGATION THE PREVALENCE OF MUTATIONS IVS 10 AND R158Q IN A NUMBER OF IRANIAN PATIENTS WITH PKU

SHIRIN JAHANBAZI, FATEMEHKESHAVARZI*

Department of Biology, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran

(gol.keshavarzi@gmail.com), (09183704918)

ABSTRACT

Phenylketonuria is one of the most common genetic disorders in metabolism of amino acids and important genetic disease. Cause of disease is phenylalanine hydroxylase enzyme deficiency. So far, more than 520 different mutation of this gene have been reported in the world. But mutation (C.472GA) R 158Q is the pathogenic mutation able to create medium and classic form of the disease. The mutation IVS10 has been reported with high frequency in specific populations. By analyzing data by seventeenth edition of software SPSS.Frequency of mutations IVS 10 and R158Q were 10% and 4% respectively. This study aimed to investigate the prevalence of mutations in a number of Iranian patients with PKU.

Keywords: Phenylketonuria, phenylalanine hydroxylase, IVS10 gene, R 158Q gene

INTRODUCTION:

Phenylketonuria (PKU) disease is the most common form of hyper phenylealaninemy and is inherited in recessive autosomal (Mallolas 1999). The disease was discovered by Foling in 1934 and was presented as an inherited metabolicdisorder. Phenylketonuria is the most common congential errors of amino acid metabolism – related disease and its mean prevalence is 1 in 10000, caused by the liver enzyme phenylalanine hydroxylase (pheOH) deficiency. This enzyme is expressed in the liver and causes phenylalanine converts to tyrosine. In the absence of timely diagnosis and treatment, thus increasing the toxicity of phenylalanine in the brain followed by mental retardation and psychological problems. This enzyme id responsible for the convert reaction of phenylalanine to tyrosin in the presence of

its cofactor, tetrahydrobiopterin (BH_4) (Williams 2008). Loss of enzyme PAH activity due to chronic hyper phenylalaninemya (Scriver 1995) Mutation in the gene encoding PAH id the major cause of PKU disease. PAH gene on chromosome 12 approximately 90 klo in length and is located in the band q22 –q24.1.This gene are of 13 exons and 12 introns (Dilella 1986. So far, more than 520 different mutation of this gene have been reported in the world. but mutation (C.472GA) R 158Q is the pathogenic mutation able to create medium and classic form of the disease (Guldberg, 1998). The mutation IVS10 has been reported with high frequency in specific populations. This study aimed to investigate the prevalence of mutations in a number of Iranian patients with PKU.

2. Sample collection

In this study, gene polymorphisms IVS10.R158Q were analyzed in 50 patients with phenylketonuria. The people of this group were selected of the patients referred to Isfahan laboratory noble for diagnosis and control. Patients were selected from among the people of the provinces Chahar Mahal-o-Bakhtyari Isfahan and Yasouj. 2 ml samples of blood were taken from individuals in sterile test tubes containing the anticoagulant EDTA collected and was maintained until needed at 20℃. The classical phenylketonuria patients was found after measuring in serum by UPLC method.PCR-RFLP techniques was used to identify mutation in patients. Then they were in specific restriction digestion .Reaction were used in 25 micro liters containing 50 ng genomic DNA, 2/5 micro liters PCR Buffer 10 x, 0/25mμ of each dNTP ,1/5 mμ $Mgcl_2$, 5-7 pic moles of each primer and 0/5 units of enzyme Taq DNA polymerase (Sinagene – Iran) . And amplified in 32 cycles,a temperature of 94℃ for 1 min, 62℃ for 1 minute and 72℃ for 1 min. initial denaturation for 5 minutes at 94℃ and final long in 10 minutes and 72℃ took place.Products of digestion was carried out on acrylamide gel and then fragments were studied.

3. Data Analysis:

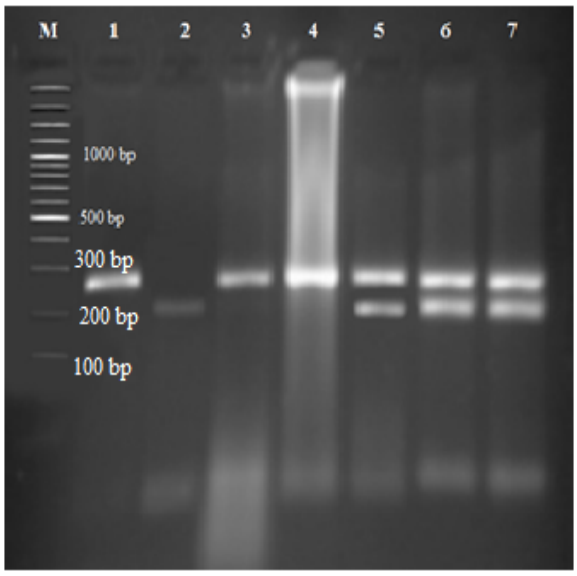

Fig1.enzyme digestion and restriction fragments by enzyme Dde1 on Agarose Gel2% M.Sink, Marker 100bp

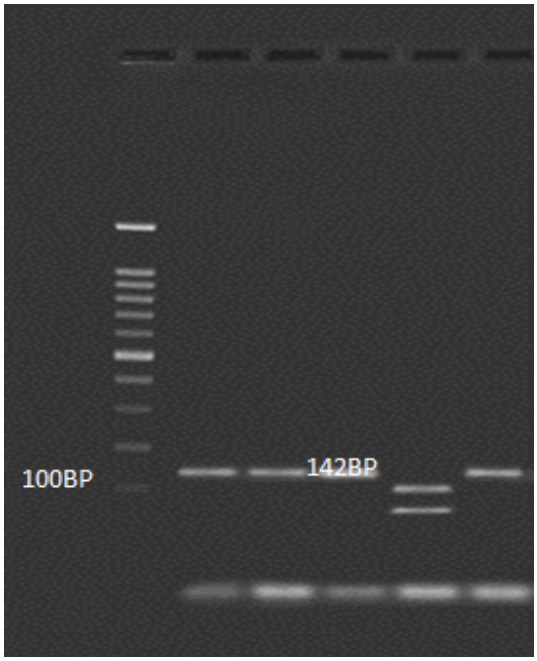

Fig2.enzyme digestion and restriction fragments by enzymeMspIon Agarose Gel2% M.Sink, Marker 100bpFermentas

CONCLUSIONS:

This study aimed to determine the prevalence of mutations R 158Q and IVS10 in a number of Iranian patients with phenylketonuria. A total of 50 patients with PKU were identified and they were satisfied to do this review. At first the cases with BH_4 deficiency were excluded. The three _day regimen of patients were discontinued and the patient was under normal diet. On the third day, 20 ml of urine sample was token to determine the amount of urine pterins (Bio pterin and neo pterin) and

tested. This step was performed to identify patients with BH_4 deficiency. It should be noted that the determination of urine protein was performed by HPLC. Then the people with cofactor BH_4 deficiency were diagnosed and excluded. Then, PKU patients with mutations in PAH gene, 10 ml of peripheral blood were collected and kit QIA amp DNA mini kit (Qiagen ,USA) were used to extract DNA according to manufacturer's instructions . Then PCR reaction was performed in a thermocycler. PCR reaction results were analyzed by statistical software SPSS.By analyzing data by seventeenth edition of software SPSS. Frequency of mutations IVS 10 and R158Q were 10% and 4% respectively. In one study in 1993 on 44 patients in turkey, 28 people had mutation in gene IVS10 and only 2 patients had a mutation in R158Q (Ozgfil 1993). In another study conducted in Turkey in 2010,19.3% of patients had the mutation in IVS10 and 23% patients had a mutation in gene R158Q (William s 2008) Identify common mutations in a given population can greatly help prenatal diagnosis programs in families at risk of child birth with PKU in that population . As well as to identify common mutations can somewhat realize close or not close of different population's history. Data from this study and identification of mutations lead to facilitate analysis of metabolic phenotypes, accurate diagnosis and adopting optimal treatment regimen. You can also use data from this study to detect carriers and pregnancy counseling for siblings of a person with PKU and prenatal diagnosis in early stages of embryonic. Analysis of mutations associated with recessive diseases make possible identifying genetic relationship between different populations and the role of early humans in the new gene pool of new populations.Collect information on common diseases such as PKU using large number of mutations, make it possible drawing a detailed map of the migration and distribution of different ethnic groups in the past. This information can be used to screen mutation through common mutation in populations.

Suggestions

1- Review and analysis by sequencing all exons of the gene for PAH.

2- Design plans to do the same in other parts of the country

3- Review and analysis of mutations reported from other ethnicities site.

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