Identification of the Most Common Mutations in the Phenylalanine Hydroxylase (*PAH*) Gene in Jordanian Patients.

R. Fathallah,[1] H. Daggag,[1] M. Al-Shboul,[2] R. Abu-Khashabeh,[1] M. Al-Masri,[1] and S. Al-Hait,[3] M. El-Khateeb,[1]

[1]National Center for Diabetes, Endocrinology and Genetics (*Amman*), [2]Institute of Medical Biology: Human Embryology (*Singapore)*, [3] Jordan PKU Clinic (*Amman)*

rajaafathalla@gmail.com

ABSTRACT:

Phenylketonuria (PKU) is an autosomal recessive metabolic disorder caused by mutations in the phenylalanine hydroxylase (*PAH*) gene. PKU is included in the National Jordanian Screening Program, with its incidence recorded at 1 in 4000. In spite of existence of such program, the spectrum of *PAH* gene mutations in Jordanian patients has not been examined yet. The current study was initiated by the National Centre for Diabetes, Endocrinology and Genetics (NCDEG) in collaboration with the Jordanian Ministry of Health, with the aim of establishing DNA diagnostic facilities, identifying the mutations and implementing prenatal and Pre-implantation Genetic Diagnosis (PGD) testing.

INTRODUCTION:

PKU is one of the most common inborn metabolic disorders, with an average incidence of 1 in 10000 in Caucasians and a variable frequency in other populations (6). It is an autosomal recessive disorder, caused by a genetic error in the hepatic enzyme *PAH* gene, located on chromosome 12q22-q24.2, comprising 13 exons (5, 7).

Up to date, five hundred and sixty four mutations have been identified in the *PAH* gene with the majority of the mutations clustering in exon 6 and 7 (**Fig. 1**) (www.pahdb.mcgill.cal). According to the National Jordanian Screening Program *(NJSP)*, the average incidence of PKU in the Jordanian population is 1/4000. In association with the newly established newborn screening program, it is necessary to establish common mutational screening for the affected individuals and families.

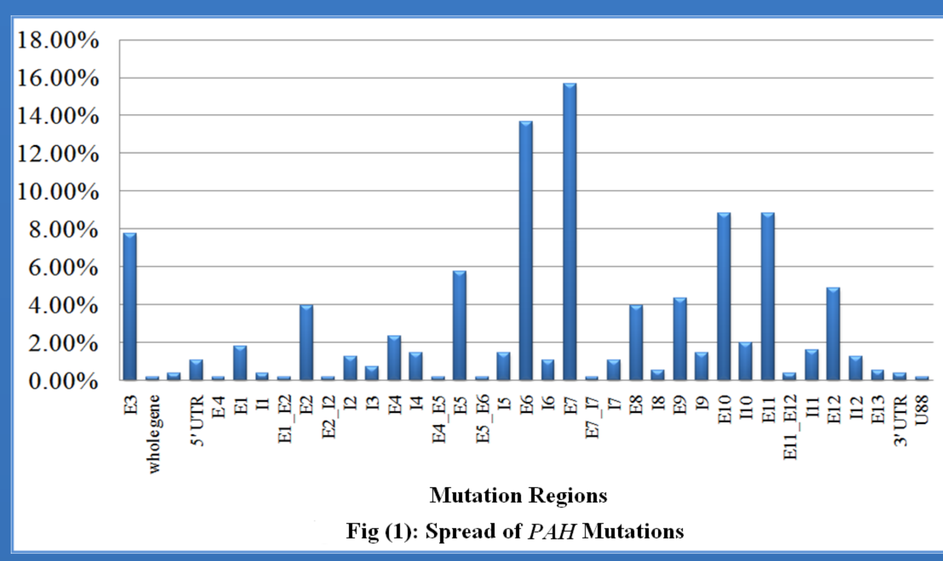

Fig (1): Spread of *PAH* Mutations

PATIENTS AND METHODS:

In the current study, forty seven unrelated PKU patients were recruited from the Jordanian Ministry of Health. Patients were clinically diagnosed with PKU based on plasma phenylalanine level, in addition to other clinical findings. Methods used to study those patients were as follows: gDNA was extracted from whole blood using the Phenol/ Chloroform method. Samples were screened for five mutations, I224T, Y206D, Y198_E205>Cfs, L197_Y204>XfsX1 and R243Q **(Fig. 2)**, using the amplification refractory mutation system- PCR (ARMS- PCR) and restriction enzyme- PCR (RE- PCR). Five samples were sent overseas for the sequencing of the *PAH* gene. Furthermore, sequencing analysis of exon 2 in the *PAH* gene has been done in our laboratory using Beckman Coulter CEQ™ 8000 Genetic Analyzer System.

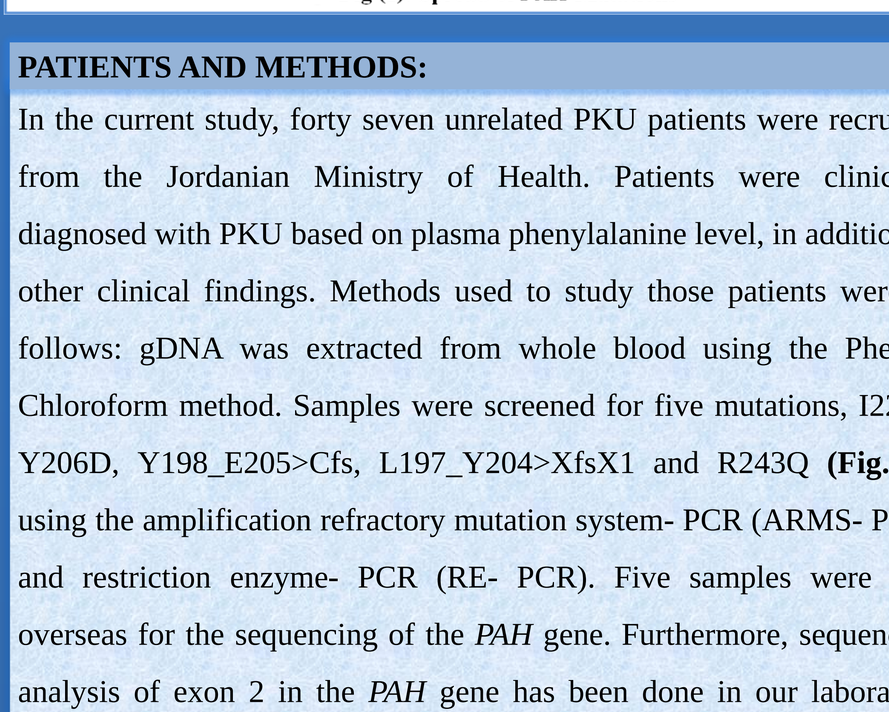

Fig (2): The Most Common Mutations within Caucasian and Mediterranean Populations

RESULTS:

None of the forty seven PKU patients carried any of the mutations screened in this study **(Fig. 3)**. Sequencing of the *PAH* gene in the five PKU index patients revealed that two were homozygous for the E56E; IVS2+1G>A mutation in exon 2- intron 2 boundary (E2- I2), which was previously reported in the Palestinian population (3). One patient was heterozygous for the L48S mutation in exon 2, which was detected in the Turkish population (4). And the remaining two mutations identified in our samples, the IVS10-11G>A located in intron 10 and the F39del in exon 2 respectively, have been reported in the European populations (1, 2). In order to confirm the obtained results, we sequenced exon 2 of the *PAH* gene in three of the patients, with the mutation also identified through our methodology **(Fig. 4)**.

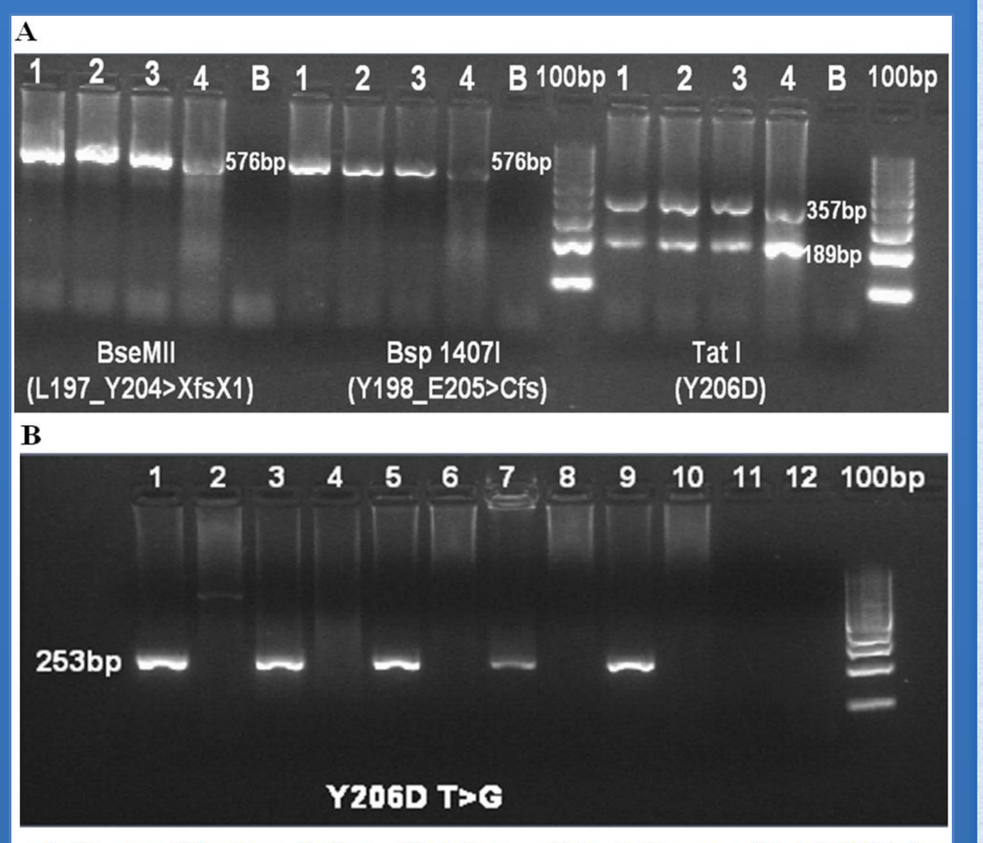

Fig (3): Amplification of Three Mutations within *PAH* gene. (A) RE-PCR for Y206D, L197_Y204>XfsX1, Y198_E205>Cufs. (B) ARMS-PCR for Y206D.

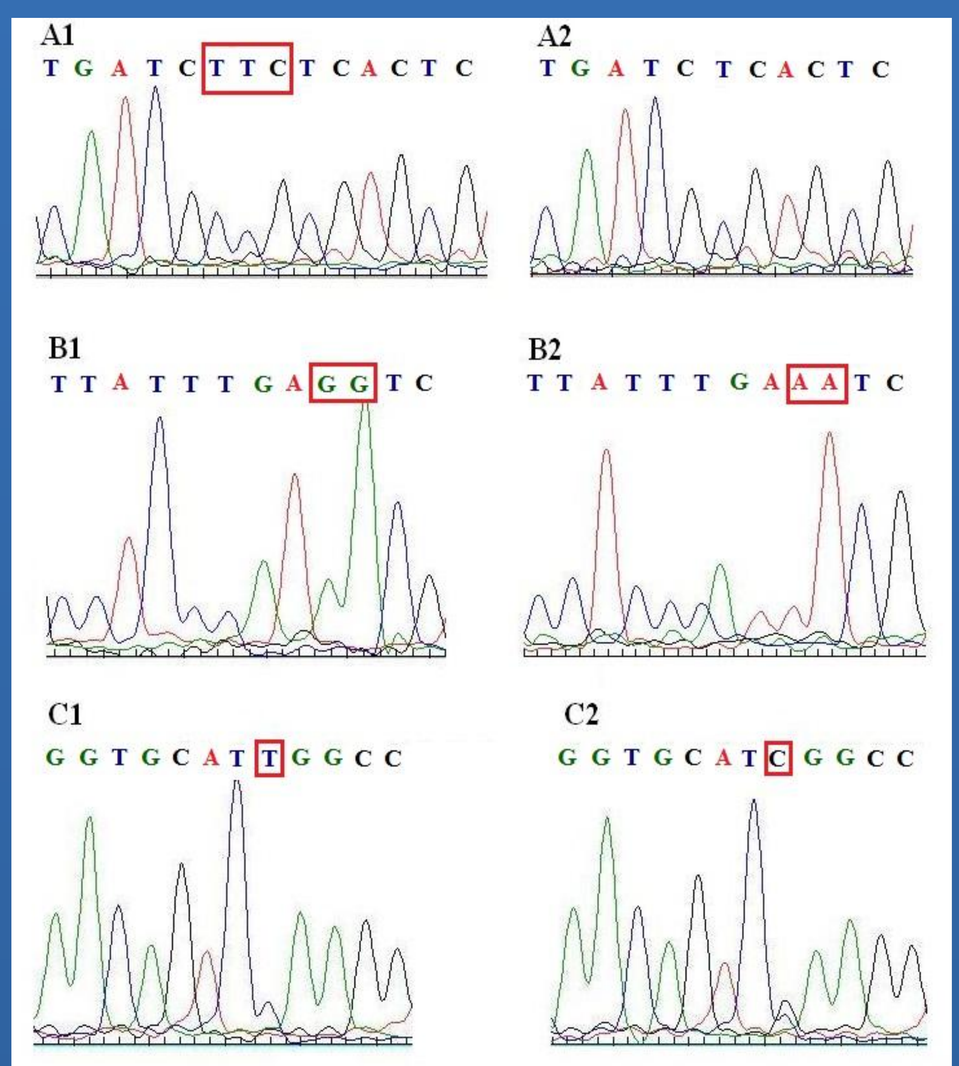

Fig (4): Sequencing of Exon 2 of the *PAH* gene. (A1, B1, C1) Negative Control for Exon 2. (A1) F39del (3- nucleotide deletion), (B2) E56E;IVS2+1G>A (c.168G>A; c.168+1G>A) Mutation, (C2) L48S (c.143T>C).

DISCUSSION AND CONCLUSION:

The molecular basis of the most common mutations within the Arab Jordanian population has not been extensively analyzed. Nevertheless, several studies have reported a *PAH* gene mutation pattern within the Mediterranean population **(Fig. 2)**.

So far, the five mutations examined in our lab (common within Mediterranean populations), were not present within the forty seven samples screened. Sequencing of the remaining patients presenting with PKU might reveal novel mutations that may be present only in the Jordanian population. Preliminary data indicates that common mutations within the Jordanian population might be clustered within exon 2 and not within exons 6 and 7 as predicated. Furthermore, the current study of PKU may reveal a presence of novel, not yet identified, mutations in the Jordanian population. In the future, we hope that through mutual collaboration between the medical community, Jordan Charity Association for *Phenylkeronuria (JCA PKU)* and molecular genetics laboratory, we will be able to identify PAH mutations present in Jordan, and provide better services related to diagnosis and prenatal testing of PKU.

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REFERENCES:

1. DWORNICZAK B, AULEHLA-SCHOLZ C, KALAYDJIEVA L, BARTHOLOME K, GRUDDA K, HORST J (1991) Aberrant splicing of phenylalanine hydroxylase mRNA: The major cause for phenylketonuria in parts of Southern Europe. *Genomics* 11:242-246.
2. GULDBERG P, HENRIKSEN KF, GUTTLER F (1993) Molecular analysis of phenylketonuria in Denmark: 99% of the mutations detected by denaturing gradient gel electrophoresis. *Genomics* 17:141-146.
3. KLEIMAN S, BERNSTEIN J, SCHWARTZ G, EISENSMITH RC, WOO SLC, SHILOH Y (1992) A defective splice site at the phenylalanine hydroxylase gene in phenylketonuria and benign hyperphenylalaninemia among Palestinian Arabs. *Hum Mut* 1:340-343.
4. KONECKI DS, SCHLOTTER M, TREFZ FK, LICHTER-KONECKI U (1991) The identification of two missence mutations at the PAH gene locus in a Turkish patient with phenylketonuria. *Hum Genet* 87:389-393.
5. Liem H, Susan B, Lynne P, and Charles R. S (1996) PAH Mutation Analysis Consortium Database: A Database for Disease-Producing and Other Allelic Variation at the Human *PAH* Locus. *Nucl. Acids Res* 24(1): 127-131.
6. Yang Y, Drummond-Borg M, Garcia-Heras J (2001) Molecular analysis of phenylketonuria (PKU) in newborns from Texas. *Hum Mutat* (6):523.
7. Zschocke J (2003) Phenylketonuria mutations in Europe. *Hum Mutat* 4:345-56.