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Mutation spectrum of phenylketonuria in Syrian population: Genotype–phenotype correlation

Hossam Murad [a,*], Amir Dabboul [a], Faten Moassas [a], Diana Alasmar [b], Walid Al-achkar [a]

[a] Molecular Biology and Biotechnology Department, Human Genetics Division, Atomic Energy Commission, Damascus, Syria
[b] Chlidien's Hospital of Damascus, Syria

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ABSTRACT

Characterization of the molecular basis of phenylketonuria (PKU) in Syria has been accomplished through the analysis of 78 unrelated chromosomes from 39 Syrian patients with PKU. Phenylalanine hydroxylase (*PAH*) gene mutations have been analyzed by using molecular detection methods based on the restriction fragment length polymorphism (RFLP), artificial constructed restriction sites (ACRS) PCR and direct DNA sequencing. 56.4% of the patients had cPKU. A mutation detection rate of 79.49% was achieved and sixteen different mutations were found: missense 56.25%, splice site 37.5%, and frameshift 6.25%. The predominant mutation in this population sample was p.R261Q G>A, p.F55>Lfs and p.R243Q G>A. No mutation in six PKU patients was observed. In 57.9% of patient genotypes, the metabolic phenotype could be predicted. The identification of the mutations in the *PAH* gene and the genotype–phenotype correlation should facilitate the evaluation of metabolic phenotypes, diagnosis, implementation of optimal dietary therapy, and determination of prognosis in the patients and genetic counseling for the patient's relatives.

1. Introduction

Phenylketonuria (PKU) is an autosomal recessive disorder with an incidence of 1/10,000 in Caucasians and a variable frequency in other populations (Eisensmith et al., 1995; Mitchell and Scriver, 1993).

Phenylketonuria is classified by the severity of hyperphenylalaninemia for the patients. The normal range of blood phenylalanine (Phe) concentrations is 50–110 μmol/L. Individuals with blood Phe concentrations of 120–600 μmol/L before starting treatment are classified as having mild hyperphenylalaninemia (MHP); those with concentrations of 600–1200 μmol/L are classified as mild phenylketonuria (miPKU) [sometimes a moderate classification (mPKU) is included for concentrations of 900–1200 μmol/L] and concentrations above 1200 μmol/L denote classic phenylketonuria (cPKU) (Blau et al., 2010).

In most industrialized countries, PKU is diagnosed in newborn screening programs (Guthrie and Susi, 1963). Patients with high serum levels of Phe benefit from a phenylalanine-restricted diet, which prevents the neurotoxic effects of Phe and its metabolites. Phenylketonuria (PKU, MIM# 261600) is caused by a high variety of mutations in the gene for phenylalanine hydroxylase (PAH) enzyme (E.C. 1.14.16.1). The *PAH* gene (GenBank: AF404777) spans about 90 kbp on chromosome 12q22–q24.1 and contains 13 exons (Scriver, 2007). More than 500 different mutations have been identified and listed in the *PAH* mutation database (PAHdb; http://www.mcgill.ca/pahdb). The wide variability in the common mutations between ethnic groups and geographical areas makes PAH deficiency a genetic disease with great allelic heterogeneity. To prevent mental retardation due to the neurotoxic effects of high levels of Phe and pathological metabolites, patients with PKU must be treated early in their life with a low-L-phe diet depending on the severity of their clinical phenotype (Eisensmith et al., 1995; Mitchell and Scriver, 1993). Thus, definition of PKU-causing *PAH* mutation profile in a given population seems worthwhile in order to anticipate dietary requirements through mutation analysis (Guttler and Guldberg, 2000). Given the wide heterogeneity of *PAH* mutations, it is crucial to know the mutation epidemiology of both PKU and hyperphenylalaninemia (HPA) in individual ethnic groups (Zschocke, 2003). Besides these mutations, there are some polymorphisms in the *PAH* gene that can be used in carrier detection (Weiss, 1996). In addition, when the mutation epidemiology is known, interlaboratory quality control strategies can be planned and industrial kits and control samples with known mutations can be produced (Taruscio et al., 2004).

This paper presents data about mutation detection at the *PAH* locus in Syrian PKU patients, and we examined genotype–phenotype correlation for 21 genotype in 39 PKU patients.

Abbreviations: AV, arbitrary value; IVS, intervening sequences; MHP, mild hyperphenylalaninemia; PAH, phenylalanine hydroxylase; Phe, phenylalanine; PKU, phenylketonuria; PRA, predicted residual activity; HPA, hyperphenylalaninemia.

* Corresponding author at: Molecular Biology and Biotechnology Department, Human Genetics Division, Atomic Energy Commission of Syria, P.O. Box 6091, Damascus, Syria. Tel.: +963 11 2132580; fax: +963 11 6112289.
E-mail address: ascientific@aec.org.sy (H. Murad).

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2. Materials and methods

2.1. Patients

A total of 39 unrelated patients with PAH deficiency from different regions in Syria, corresponding to 78 independent alleles, were enrolled in this study. Biochemical testing of all patients included plasma amino acid level determination using ion exchange chromatography (Table 1).

2.2. DNA isolation, amplification, RFLP and sequencing

Blood samples (5 mL) were collected from each patient by venipuncture in EDTA, after obtaining informed consent from their parents.

The degree of consanguinity in our group was 38.46% for first cousins. In families with more than one person with PKU, only one of them was included. DNA was extracted using a QIAamp DNA Blood Mini Kit (Qiagen®) according to the manufacturer's instructions.

At the first step, exons 7, 9, 10 and 11 were amplified. PCR amplification of fragments containing sites of mutations was carried out using primers derived from published data (Zschocke et al., 1995). Amplified fragments were examined for the presence of 6 mutations by restriction digestion, the enzyme used is given in parentheses (Table 2): p.E280K (*MspI-ACRS*), IVS10-11G>A (*DdeI*), IVS11+1G>C (*DdeI*), p.P281L (*MspI-ACRS*), p.R261Q (*HinfI*), and p.S310F (*Mn1I*). If the mutation is not encompassed by an existing sequence for the restriction enzyme, an artificial restriction site was introduced by PCR (ACRS). PCRs were carried out on the Gene Amp PCR system 9700 (Applied Biosystems, CA), with a cycling protocol which consisted of initial denaturation at 95 °C for 3 min, followed by 40 cycles, denaturation at 94 °C for 45 s, annealing at 50–65 °C for 45 s, and extension at 72 °C for 45 s. The PCR product was electrophoresed on 2% agarose gel. Digestion of the PCR product was performed with an appropriate restriction endonuclease for O/N, and restriction fragments were analyzed on either 3% agarose gel or 8% polyacrylamide gel depending on the size of the DNA fragments after digestion. The DNA on the agarose gels or on polyacrylamide was visualized by staining with ethidium bromide. Finally, the other unknown genotypes were determined by sequencing of all coding regions and their flanking intron regions of the *PAH* gene by BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems) in ABI prism 310 genetic analyzer.

2.3. Genotype–phenotype correlation

In the analysis, the frameshift and splice-site mutations are counted as null mutations. For the genotype–phenotype analysis, mutations were classified according to the predicted residual enzymatic activity (PRA) in vitro based on the in vitro expression studies according to PAH database (http://www.pahdb.mcgill.ca), and the predicted phenotype was correlated to the observed phenotype for each mutation (Bercovich et al., 2008; Daniele et al., 2009; Mallolas et al., 1999;

Table 1
Clinical and genetic data of Syrian PKU patients.

Patient no. (age)	1st degree consanguinity	Diagnosis	Mutation allele 1/allele 2	Ex/I	Mutation type	Genotype	Pretreatment Phe level (μmol/L) (phenotype)
P1 (10 years)	No	Lsh, MR, P	p.[R270K];[?]	Ex.7	Missense	Heterozygote	1531 (cPKU)
P2 (23 months)	No	My, P	p.[S310F];[S310F]	Ex.9	Missense	Homozygote	1333 (cPKU)
P3 (9 years)	No	A, Ep, P	[IVS4+5G>T];[IVS9+5G>A]	I.4/I.9	Splice/splice	Compound heterozygote	1551 (cPKU)
P4 (6 years)	Yes	Lsh, P	p.[R243Q];[R243Q]	Ex.7	Missense	Homozygote	1309 (cPKU)
P5 (6.5 years)	Yes	M	[IVS11+1G>A];[IVS11+1G>A]	I.11	Splice	Homozygote	1387 (cPKU)
P6 (6 years)	No	MR	p.[P281L];[P281L]	Ex.7	Missense	Homozygote	2184 (cPKU)
P7 (2.7 years)	No	F, Ep, My, A	p.[E280K];[E280K]	Ex.7	Missense	Homozygote	835 (miPKU)
P8 (3.5 years)	Yes	Ep, My, M, P	p.[E280K];[E280K]	Ex.7	Missense	Homozygote	1310 (cPKU)
P9 (2.5 years)	No	P	[IVS4+5G>T];p.[R270K]	I.4/Ex.7	Splice/missense	Compound heterozygote	1575 (cPKU)
P10 (7 years)	Yes	M, Mild MR, P	[IVS9+5G>A];[IVS9+5G>A]	I.9	Splice	Homozygote	497 (MHP)
P11 (2.5 years)	No	P	p.[R243Q];[R243Q]	Ex.7	Missense	Homozygote	1030 (mPKU)
P12 (25 months)	No	M, P	[IVS10-11G>A];[IVS10-11G>A]	I.10	Splice	Homozygote	1270 (cPKU)
P13 (6 years)	No	MR	p.[D151E];[IVS10-11G>A]	Ex.5/I.10	Missense/splice	Compound heterozygote	1630 (cPKU)
P14 (7 years)	Yes	No data	[IVS7+1G>A];[IVS7+1G>A]	I.7	Splice	Homozygote	2249 (cPKU)
P15 (9 months)	Yes	No data	p.[F55>Lfs];[F55>Lfs]	Ex.2	Frameshift	Homozygote	1677 (cPKU)
P16 (No data)	Yes	No data	[?];[?]	–	–	–	745 (miPKU)
P17 (22 Months)	Yes	A, P	p.[P281L];[P281L]	Ex.7	Missense	Homozygote	1086 (mPKU)
P18 (No data)	No	No data	[?];[?]	–	–	–	1350 (cPKU)
P19 (No data)	Yes	A, P	[?];[?]	–	–	–	843 (miPKU)
P20 (10 years)	No	MR, A, Ep, P	p.[F55>Lfs];[F55>Lfs]	Ex.2	Frameshift	Homozygote	2844 (cPKU)
P21 (8.5 years)	No	Lsh, MR	p.[R270K];[?]	Ex.7	Missense	Heterozygote	1170 (mPKU)
P22 (5 years)	No	MR	p.[R261Q];[?]	Ex.7	Missense	Heterozygote	766 (miPKU)
P23 (10 years)	Yes	A, MR, Lsh, Ep, S, P	[IVS2+5G>C];[IVS2+5G>C]	I.2	Splice	Homozygote	1679 (cPKU)
P24 (7 years)	Yes	A, MR, Lsh	p.[F55>Lfs];[F55>Lfs]	Ex.2	Frameshift	Homozygote	1733 (cPKU)
P25 (7 years)	Yes	A, Lsh	[IVS2+5G>C];[IVS2+5G>C]	I.2	Splice	Homozygote	1643 (cPKU)
P26 (7.5 years)	Yes	No data	p.[S310F];[S310F]	Ex.9	Missense	Homozygote	1267 (cPKU)
P27 (9.5 years)	No	MR, A, P	[IVS4+5G>T];[IVS4+5G>T]	I.4	Splice	Homozygote	1180 (mPKU)
P28 (3.5 years)	No	No data	p.[P281L];[?]	Ex.7	Missense	Heterozygote	1150 (mPKU)
P29 (4 years)	No	MR	[IVS10-11G>T];[IVS10-11G>T]	I.10	Splice	Homozygote	1124 (mPKU)
P30 (2 years)	No	Lsh, P, M	[?];[?]	–	–	–	1275 (cPKU)
P31 (6 years)	No	No data	[?];[?]	–	–	–	455 (MHP)
P32 (4 years)	No	No data	p.[R261Q];[R261Q]	Ex.7	Missense	Homozygote	1221 (cPKU)
P33 (no data)	No	No data	p.[R261Q];[R261Q]	Ex.7	Missense	Homozygote	1407 (cPKU)
P34 (2.5 years)	No	No data	p.[Y387H];[Y387H]	Ex.11	Missense	Homozygote	1752 (cPKU)
P35 (no data)	No	No data	[?];[?]	–	–	–	1026 (mPKU)
P36 (no data)	No	No data	p.[R261Q];[R261Q]	Ex.7	Missense	Homozygote	1045 (mPKU)
P37 (no data)	No	No data	p.[R243Q];[R243Q]	Ex.7	Missense	Homozygote	1093 (mPKU)
P38 (5.5 years)	Yes	No data	p.[R270K];[R270K]	Ex.7	Missense	Homozygote	1188 (mPKU)
P39 (8 years)	Yes	Ep	p.[R408W];[R408W]	Ex.12	Missense	Homozygote	1134 (mPKU)

P: paraplegia; MR: mental retardation; S: short stature; ep: epilepsy; Lsh: light skin and hair; My: myopia; M: microcephaly; A: aphasia; F: fever; ?: unknown mutation; C: classical; Mo: moderate; Mi: mild; MHP: mild hyperphenylalaninemia.