Available online at www.sciencedirect.com
ScienceDirect
Clinical Biochemistry 43 (2010) 76–81
CLINICAL BIOCHEMISTRY

Mutation analysis of phenylketonuria patients from Morocco: High prevalence of mutation G352fsdelG and detection of a novel mutation p.K85X

Saloua Dahri [a], Lourdes R. Desviat [b], Belén Pérez [b], Fátima Leal [b], Magdalena Ugarte [b,*], Layachi Chabraoui [a,*]

[a] *Service de Biochimie, Centre d'Etudes des Maladies Héréditaires du Métabolisme, Hôpital d'Enfants de Rabat et Faculté de Médecine et de Pharmacie, Université Mohammed V Souissi, Rabat, Moroccco*

[b] *Centro de Diagnóstico de Enfermedades Moleculares, Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Departamento de Biología Molecular, Universidad Autónoma de Madrid, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER) Madrid, Spain*

Received 7 April 2009; received in revised form 15 September 2009; accepted 16 September 2009
Available online 26 September 2009

Abstract

Objective: The knowledge of the molecular basis of the Phenylketonuria (PKU, MIM# 261600) in different countries provides relevant information for undertaking specific and rational mutation detection strategies in each population and for the implementation of adequate dietary and cofactor treatment. There are no data available in Moroccan population.

Design and methods: In this work we describe the genetic analysis by mutation scanning using denaturing gradient gel electrophoresis (DGGE) and subsequent direct sequencing of 20 different PKU families from Morocco. We have also included the study of 7 Moroccan PKU patients living in Spain detected by the Spanish newborn screening program.

Results: The mutational spectrum in the first sample included eight different changes, one of them, p.K85X, was novel. The most common mutation was the frame shift change p.G352fsdelG identified in 62.5% of the mutant chromosomes studied. Other changes (p.R176X, IVS10nt-11 g>a, p.W120X, p.A165T, p.R243X and p.R243Q) were identified, respectively, in 2 or 3 mutant alleles. All detected mutations were severe according to the classical phenotype of the patients. In the 7 patients living in Spain we have detected 4 severe mutations (p.G352fs, p.R176X, Y198fs and Exon3del) and also milder changes such as p.A403V, p.S196T, p.D145V and p.R408Q detected in 3 mild hyperphenylalaninemia (MHP) patients and a novel p.L258P found in a mild PKU patient.

Conclusion: The results provide important information on the distribution of PKU mutations in this Mediterranean area gaining insight into the genetic epidemiology of the disease.

Keywords: PKU; Moroccan; PAH gene; G352fsdelG mutation; p.K85X mutation

Introduction

Phenylketonuria (PKU, MIM# 261600) is the most common inborn error of amino acid metabolism. It is caused by an autosomal recessive deficiency of the hepatic enzyme phenylalanine hydroxylase (PAH, EC 1.14.16.1). Failure to convert phenylalanine to tyrosine leads to an increase of phenylalanine in body fluids and severe mental retardation unless phenylalanine intake is restricted. Currently, different alternatives to dietary therapy are available at least for a subset of PKU patients, namely tetrahydrobiopterin supplementation [1], and

Abbreviations: PAH, phenylalanine hydroxylase; PKU, phenylketonuria; MHP, mild hyperphenylalaninemia; DGGE, denaturing gradient gel electrophoresis; MLPA, multiplex ligation probe amplification.

* Corresponding authors. L. Chabraoui is to be contacted at Department of Biochemistry and Molecular Biology, Faculty of Medicine and Pharmacy of Rabat, BP 8048 Rabat Nations Unies, Rabat Morocco. Fax: +212 537670224. M. Ugarte, Centro de Biología Molecular "Severo Ochoa" CSIC-UAM, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain. Fax: +34 917347797.

E-mail addresses: mugarte@cbm.uam.es (M. Ugarte), lchabraoui@yahoo.fr (L. Chabraoui).

0009-9120/$ - see front matter © 2009 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.clinbiochem.2009.09.018

enzyme replacement therapy using formulations of PEG-modified phenylalanine ammonia lyase [2].

To date almost 500 different disease-causing mutations in the PAH gene have been identified and reported to the PAH mutation consortium database (http://www.pahdb.mcgill.ca). The molecular bases of PKU have been studied in different populations and the mutation profile spreads throughout the entire PAH structural domains and shows enormous diversity. Some of these mutations cause classical PKU; others cause milder forms such as mild hyperphenylalaninemia (MHP) also known as non-PKU hyperphenylalaninemia, while still others are silent polymorphisms present at elevated frequencies. These PAH mutations generate numerous possible genotypic combinations and contribute to the clinical heterogeneity. Thus, predicting enzymatic activity based on the PAH genotype is possible but sometimes difficult, as the relationship between the clinical-biochemical phenotype and the genotype is not always consistent. Indeed, the existence of discordant phenotypes among siblings who share the same genotype at the PAH locus implies the existence of other genetic and environmental factors that influence clinical phenotype and PKU, as other monogenic diseases, has been described to show features of a complex trait [3,4].

At the functional level mutations may be classified as "null" mutations (some splicing changes, nonsense and frameshift mutations as well as several missense changes) when they cause severe structural alterations or destroy the catalytic domain and result in the absence of PAH residual activity. In contrast, other, mostly missense and some splicing mutations interfere primarily with protein folding, regulation, or parameters of enzyme activity and leave variable residual activity [5].

Comprehensive mutation data has become available for European Mediterranean countries as well as for Iran, Turkey or Egypt [6-8] but not for other populations in North Africa. In this study, we report for the first time the PKU mutational spectrum in 25 Moroccan patients and also in seven patients of Moroccan descent living in Spain and detected by the Spanish newborn metabolic disease screening program. The identification of mutations present at the PAH locus in this population offers the possibility of proper diagnosis of probands, facilitates the identification of asymptomatic carriers and provides genetic counseling for affected families.

Materials and methods

Patients

Twenty-five PKU patients, from 20 unrelated families, collected over 4-year period (2003–2006) were included in the study. They came from hospitals located in different regions of the country because our service is the only Moroccan center specialized in the study of inherited disorders of metabolism. Patients were diagnosed after presenting clinical symptoms using plasma and urinary amino acids analysis by Thin Layer Chromatography [9]. Positive cases were confirmed by fluorometric assay determining serum phenylalanine levels [10].

Patients were classified as having PKU when their serum phenylalanine levels exceeded 600 μmol/L before treatment and when other causes of hyperphenylalaninemia had been ruled out. We have also taken into account the results of the genetic analysis of seven patients from Morocco living in Spain and exhibiting from classical to the mildest form of the disease.

Genetic analysis

The study has included PKU patients as well as their available family members including parents, brothers and sisters according to the consent of the parents. Genomic DNA was extracted from leukocytes isolated from EDTA anti-coagulated blood following standard procedures and screened for mutations by PCR amplification of the 13 exonic genomic fragments of the PAH gene, followed by DGGE [11] and sequencing [12].

All fragments displaying an aberrant migrating band pattern in the DGGE gel were subjected to direct sequencing with BigDye Terminator v.3.1 mix and subsequent analysis by capillary electrophoresis on an ABI Prism® 3700 Genetic Analyzer (Applied Biosystems). Mendelian inheritance in parents and carrier analysis was performed by DGGE. Thus, it was possible to confirm homozygosity due to mutation inheritance from both parents and also to define the carriers.

To identify the large genomic deletions the multiplex ligation probe amplification (MLPA) technique was used (SALSA MLPA Kit P055 PAH, MRC Holland: www.mlpa.com) according to the procedure previously described [13].

Results

Phenotypic classification of patients

Because of the lack of a systematic newborn screening for PKU in Morocco, all studied patients except one were late diagnosed (more than 6 months of age) and showed a high blood phenylalanine equal or exceeding 1200 μmol/L. Mean plasma phenylalanine levels at diagnosis was 1685 μmol/L. Only one patient (patient 2 in Table 1) born in a family with children known to be affected of PKU has been diagnosed during the neonatal period (72 h after birth) and showed a phenylalanine blood level of 960 μmol/L.

Correlating with their high phenylalanine levels, the patients showed different clinical symptoms including mental retardation (96%), convulsions (16%), autism (16%), and speech delay (96%). Eighty percent of the patients are born from a consanguineous marriage (Table 1). When diagnosed during the first 4 years, PKU patients were treated with a phenylalanine restricted diet, without supplementation of BH4 because of its unavailability in our country.

Mutational spectrum

Our study included 20 PKU families from different regions of Morocco, implementing the use of broad range DGGE (Fig. 1). Samples displaying a deviant band pattern were directly sequenced to localize the mutation. Using this