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CLINICAL STUDY

Screening for six Mediterranean mutations in 90 Egyptian patients with phenylketonuria

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Abstract: *Objectives:* The aim of this study is to assess the prevalence of six common mutations in the Mediterranean basin and Turkey among a large group of Egyptian PKU cases
Background: Phenylketonuria (PKU) is one of the most common inborn errors of amino acid metabolism that is caused by deficiency of hepatic phenylalanine hydroxylase (PAH). This deficiency is attributed to more than 528 mutations in the PAH gene.
Methods: Ninety unrelated patients with PKU (180 alleles) were screened for six mutations (IVS10-11G>A, R261Q, R252W, Y277D, E221G and G272S) using polymerase chain reaction-restriction fragment length polymorphism.
Results: The IVS10-11G>A mutation was found in thirty alleles (17 %), the R261Q in twelve (7 %) and R252W in three (1.6 %), while Y277D, E221G and G272S were not found in this patient group.
Conclusion: Screening for six Mediterranean mutations identified a heterogeneous pattern among Egyptian PKU patients with a high frequency of IVS10-11 G>A (17 %) *(Tab. 2, Ref. 31)*. Full Text (Free, PDF) *www.bmj.sk*.
Key words: phenylketonuria, PAH, mutation pattern, PCR-RFLP analysis.

Abbreviations: PKU – phenylketonuria, PAH – phenylalanine hydroxylase, PCR_RFLP – polymerase chain reaction-restriction fragment length polymorphism, DNA – deoxyribonucleic acid, MPH – mild hyperphenylalanemia.

Phenylketonuria (PKU; OMIM#261600) is one of the most common inborn errors of amino acid metabolism with an average incidence of 1/10000. It is an autosomal recessive disorder caused by a deficiency of phenylalanine hydroxylase (PAH, EC 1, 14, 1, 1). PAH deficiency is attributed to mutations in the PAH gene (1). This deficiency results in mental retardation if not treated with phenylalanine restricted diet very early in life (2).

More than 528 mutations have been described within the PAH gene (3). This large number of mutations reduces the enzymatic activity to different degrees, explaining the broad phenotypic heterogeneity of the disease from classic PKU to mild hyperphenylalanemia (MHP) (4).

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Characterizations of PKU mutation patterns have been performed in many countries around the world. To date, two limited studies have been reported on Egyptian PKU patients (5, 6), identifying only 10 mutations with relatively low frequencies reflecting the heterogeneous background of PKU in Egypt. This study assesses the prevalence of six common mutations in the Mediterranean basin and Turkey among a large group of Egyptian PKU cases.

Patients and methods

Ninety unrelated patients with PKU were recruited from the National Research Centre and Ain Shams University hospitals. Patients were classified into three phenotypes based on pre-treatment plasma phenylalanine level: classic PKU (phe >1200 umol/l), mild PKU (phe 600–1200 umol/l) and MHP (phe<600 umol/l) (7). Genomic DNA was extracted from peripheral blood leukocytes using the salting out procedure (8). One hundred and eighty alleles from the ninety patients were screened for the following mutations (IVS10-11 G>A, E221G, R2525W, R261Q, G272S, Y277D) using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (9) (Tab. 1).

Results

Screening 90 patients for the six mutations allowed the characterization of 25.6 % of the studied alleles. Three mutations (E221G, G272S, and Y277D) were not detected in this study. The data for the other three detected mutations ((IVS10-11 G>A,

Tab. 1. PCR-RFLP analysis for 6 PAH mutations detection.

Mutation	Exon	Enzyme	NA (bp)	MA (bp)
E221G	6	NcoI	326	229/97
R252W	7	AvaI	177/86	263
R261Q	7	HinfI	116/147	263
G272S	7	BamHI	115/148	263
Y277D	7	XmiI	163/100	263
IVS10-11G>A	11	DdeI	301	222/79

NA – normal allele, MA – mutant allele

R261Q, R2525W) is shown in comparison to those in other countries (Tab. 2).

Discussion

The molecular basis of PKU among the populations inhabiting the southern rim of the Mediterranean basin and the rest of the Middle East has been largely unexplored. A few studies have reported on the PAH mutation patterns among North Africans Jews (10), Israelite Jews and Palestinian Arabs (11) and Iranians (12). Screening for six mutations that are relatively common in the geographical area extending from western Mediterranean to Iran has come up with a number of findings. First, the IVS10-11 G>A frequency among Egyptian patients was high (17 %) but not as high as in Turkey and Bulgaria or southern Italy. It is interesting to note the decrease in frequency westwards in Spain and Portugal, southwards in Greece and Sicily, and eastwards in Iran (Tab. 2). Moreover, it is intriguing that the mutation frequency is high in Brazil and Chile.

The east-west gradient in the Mediterranean basin with the highest focus in Turkey, has suggested a spread from Asia Minor during the Neolithic period (13, 14). Moreover, recent migration has brought the mutation to northern European countries like Germany (15).

This predominantly Mediterranean mutation has crossed to the other side of the Atlantic Ocean, where the frequency is high in Mexico (16), Chile (17) and Northern but not Southern Brazil (18). The IVS10-11 G>A high frequency among Egyptians probably reflects the remarkable gene flow from Turkey to the southern Mediterranean and refers to the possibility that it may be also frequent among other North African and Middle-Eastern populations.

However, it remains to be studied when this Turkish or Asian mutation was introduced into the Egyptian gene pool. Extensive polymorphism studies can be of great help in this regard, where PKU has been already taken as a good example for tracing the mutation lineages among various populations (19).

The R261Q mutation has also shown a relatively common occurrence (7 %) in the Egyptian patient sample. The relative prevalence of R261Q among Egyptian cases stands at a middle position among the high teen rates reported in Netherlands (20), Southern Italy (21), Galician Spain (22) and Portugal (23) and the lower prevalence (<7 %) in Serbia and Montengero (24) and most Northeastern and Northwestern Europe (25). The distribution of R261Q defies the suggestion of a particular migration movement throughout history. The R261Q mutation has a moderate allele frequency among several populations unconnected by known movements of the people in the past (25). Moreover, R261Q is linked to various haplotypes and as a CpG mutation; independent recurrence in more than one founder is possible. Further work is required to establish the associated haplotype(s) in the Egyptian cases and to compare them to other populations.

The R252W mutation has been detected with a low frequency (1.6 %) among the Egyptian PKU cases. Its high rate of 15% reported in Piemont area of Northern Italy represents an isolated focus among all European countries that have a less than 5 % rate (25).

On the other hand, R252W has shown a high rate in Iran and a moderate one in some Latin American populations (Tab. 2). The high rate of R252W among Iranians has apparently not been represented in the Egyptian gene pool. Although geographically distinct, the old kingdoms of Egypt and Persia (i.e. Iran) may have witnessed some genetic interchange during the Persian occupation of Egypt from 525 BC till 404 BC.

In conclusion, screening for six Mediterranean mutations identified a heterogeneous pattern among Egyptian PKU patients with a high frequency of IVS10-11 G>A (17 %).

Tab. 2. Comparison of the frequency of IVS10-11 G>A, R261Q and R252W in this study and those in various populations.

Mutation	IVS10-11 G>A	R261Q	R252W
This study	17%	7%	1.6%
Other studies	Turkey (13) (25%) Bulgaria (28) (25%) South Italy (14) (19%) Brazil (18) (17%) Spain (22) (15%) Sicily (39) (15%) Greece (31) (13%) Portugal (23) (11%) Serbia (24) (1.5%)	Switzerland (32%) Netherlands (29) (18%) Cuba (27) (16%) South Italy (14) (14%) Brazil (18) (12%) Portugal (23) (10%) Sicily (30) (9%) Iran (12) (8%) France (29) (8%) Serbia (24) (5.9%)	Italy'Piemont' (26) (15%) Iran (12) (15%) Chile (37) (9%) Brazil (18) (7%) Cuba (22) (5%) Portugal (23) (4%) Central Italy (21) (4%)

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