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SHORT REPORT

Haplotypes and mutations of the PAH locus in Egyptian families with PKU

L Effat[1], A Kuzmin[2], N Kasem[3], N Abdel Meguid[1], S Kotb[3], RC Eisensmith[2], SA Temtamy[1], S Rushdi[3], S Woo[2] and M El-Awady[1]

[1]*Department of Human Genetics, National Research Center, Egypt*
[2]*Baylor College of Medicine, Houston, Texas, USA*
[3]*Department of Human Genetics Medical Research Institute, Alexandria University, Egypt*

A high degree of molecular heterogeneneity at the phenylalanine hydroxylase (PAH) locus was established by examining RFLP haplotypes and PAH mutations in the families of 13 Egyptians with phenylketenouria (PKU). Thirteen different haplotypes were unequivocally determined in these kindreds. Haplotypes 1.8, 3.9, 4.3, 7.8, 22.11, 27.6, and 52.8 were found segregating with normal chromosomes, whilst haplotypes 1.8, 5.9, 23.8, 32.8, the newly assigned 73.9, and two as yet incomplete but novel haplotypes were found segregating with the mutant chromosomes. There was no particular preference for a single haplotype among normal or mutant chromosomes. Nine different mutations were also identified among the 26 alleles. IVS 10nt11g (8/26), IVS 2nt5g-c (4/26), R261Q (3/26), R176X (2/26), Y206D (2/26), S231P (2/26), Y198fs [593-614del22bp]; (2/26), G46fs [136/137delG]; (1/26), and E178G (1/26). Six of these mutations (IVS 2nt5g-c, R176X, Y198fs, R261Q, S231P, and IVS 10nt11g) are common to other Mediterranean populations. Two mutations not previously reported in the Mediterranean basin were also observed (Y206D and G46fs). These intriguing preliminary findings confirm IVS 10nt11g as a major mutation among Mediterranean mutations and demonstrate the need for a more comprehensive study of Arab populations to confirm the uniqueness of the two novel mutations to the Egyptian population.

Keywords: Mutation; PAH locus; PKU diagnosis; Haplotypes; polymorphism population genetics

Introduction

Phenylketonuria (PKU), one of the most common inborn errors of amino acid metabolism, is associated with the presence of a large number of molecular defects in the phenylalanine hydroxylase (PAH) gene.[1] Most of these mutations greatly reduce or abolish the activity of the liver PAH enzyme,[2] which normally converts the essential dietary amino acid L-phenylalanine to L-tyrosine.[3] In individuals affected with PKU, levels of phenylalanine in the blood and other tissues increase significantly due to the PAH deficiency.[4] If these high levels are not detected very early in infancy and significantly reduced by administration of a special diet low in phenylalanine, severe and irreversible mental retardation ensues.[5]

Isolation of the gene and the determination of the sequence of full length PAH cDNA[6] not only permitted the characterisation of restriction fragment length

Correspondence: Laila Effat PhD, Department of Human Genetics, National Research Center, Tahrir Street, Dokki 12622, Cairo. Fax: 202 3559169; E-mail: reffat@tc.cac.edu.eg
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polymorphism (RFLP) haplotypes and their application in prenatal diagnostic procedures,[7] but also facilitated the initial mutational analysis at the PAH locus. Subsequent studies in European, Mediterranean and Asian communities disclosed a variety of PAH mutations.[8,9]

Prenatal diagnosis of PKU is an important adjunct to dietary treatment for the prevention of the ensuing mental retardation, as the limited availability of the low-phenylalanine diet and poor compliance are still major problems in Egypt. The identification of different haplotypes and mutations present at the PAH locus in Egyptians offers the possibility of proper diagnosis of probands, facilitates the identification of carriers and provides genetic counselling regarding the relative risk of the disease in their offspring. In addition prenatal diagnosis of PKU can be performed in families whenever mutational or haplotype analysis is informative.

Materials and Methods

Subjects

Eighteen affected individuals from 13 families presenting with mental retardation were diagnosed at the National Research Center to have hyperphenylalaninaemia. Elevated levels of phenylalanine in serum were diagnosed by Guthrie test, and confirmed by thin layer chromatography and HPLC.

Determination of Normal and Mutant Haplotypes (HTs) at the PAH Locus

Leucocytic DNA was extracted from venous blood withdrawn from affected individuals, parents and normal sibs, using the salting-out technique. Haplotype (HT) analysis was based on the eight polymorphic RFLP/VNTR sites scattered throughout the PAH gene.[10] Five restriction sites (BglII, PvuIIa & b, MspI, and XmnI) were analysed by PCR amplification followed by restriction endonuclease digestion as described by Dworniczak *et al*.[11–14] The VNTR site was amplified according to Golstov *et al*.[15] Both the Eco RI and Eco RV were analysed by Southern blotting as described by Woo.[16] The haplotype assignments were determined from the polymorphic sites according to the tabulation of Eisensmith and Woo.[17] Complete HT analysis was not possible in some families, since both parents were not available. In other families, heterozygosity of all family members for one or two of the sites prevented the assignment of definitive haplotypes.

Mutation Detection

Based on the haplotype data and the mutation/haplotype associations in Southern Europeans, Turks and Palestinian Arabs, seven mutations common to these populations (L48S, E221G, R252W, R261Q, E280K, P281L and IVS 10 nt 11g) were screened by PCR amplification of the exons 2, 6, 7, and 11. This amplification was followed by restriction enzyme digestion as described by Eiken *et al*.[18] Following this initial screening, all exons were examined using a combination of DGGE and direct sequencing as described by Kuzmin *et al*.[19]

Results

Polymorphic Haplotypes

Eight loci spread throughout the PAH structural gene were analysed for polymorphism to identify the different HTs present in the Egyptian PKU kindreds. A high degree of heterogeneity was found among both normal and mutant alleles. Within the fully informative families HTs1.8 (n = 1), 3.9 (n = 1), 4.3 (n = 1), 7.8 (n = 2), 22.11 (n = 1), 27.6 (n = 1), 52.8 (n = 1) were observed on normal chromosomes, whilst haplotypes 1.8 (n = 1), 5.9 (n = 1), 23.8 (n = 1), 32.8 (n = 1) were found on the mutant chromosome. Mutant chromosomes also contained the novel HT 73.9 (n = 2) and two incomplete, but tentatively novel haplotypes. Half the patients studied appeared to be the result of consanguineous marriages, as the probands were homozygous for both haplotype and mutation. In non-consanguineous marriages different HT combinations were always found (eg HT1.8/HT32.8; HT23.8/HT5.9). Complete HT analysis was not possible in some families, since not all parents were available. In other families, heterozygosity of all family members for one or two of the sites prevented the assignment completion of the haplotypes.

Mutations

The mutational screening analysis revealed nine different mutations IVS 10 nt11g (8/26), IVS 2nt5g-c (4/26), R261Q (3/26), R176X (2/26), Y206D (2/26), S231P (2/26), Y198fs [593-614del22bp]; (2/26), G46fs [136/137delG]; (1/26), and E178G (1/26) with a high frequency of the IVS 10 nt11g in particular (Table 1). Six of these mutations, namely IVS 2nt5g-c, R176X,

Table 1 Point mutations from the studied sample

	Mutation	*Frequency*	
1	IVS 10 nt1 1g	8/26	30.8%
2	IVS2 nt5g-c	4/26	15.4%
3	R261Q	3/26	11.5%
4	R176X	2/26	7.7%
5	Y206D	2/26	7.7%
6	S231P	2/26	7.7%
7	Y198fs	2/26	7.7%
8	G46fs	1/26	3.8%
9	E178G	1/26	3.8%
10	not identified	1/26	3.8%
Total		26/26	100%

Table 2 VNTR, haplotype, genotype relationship

Family	*Haplotype*	*VNTR*	*Genotype*
A	HT1*/HT32*	8*/8*	R176X/R261Q
B	HTN#*/HTN#	9*/9*	IVS2nt5/IVS2nt5
C	HT23*/HT5*	8*/9*	G46fs/IVS2nt
D	HT?/HT?	3*/3*	Y206D/Y206D
E	HTN#*/HTN#	9*/9*	E178G/Y198fs
F	HT?/HT?	7*/7*	IVS10nt11g/IVS10nt11g
G	HT?/HT?	9*/9*	IVS2nt5/?
H	HT?/HT?	7*/7*	IVS10nt11g/IVS10nt11g
I	HT?/HT?	9*/8*	Y198fs/R176X
J	HTN#*/HTN#	7*/7*	IVS10nt11g/IVS10nt11g
K	HT?/HT?	7*/7*	IVS10nt11g/IVS10nt11g
M	HT?/HT?	3*/3*	R261Q/R261Q/
N	HT?/HT?	11*/11*	S213P/S213P

Y198fs, R261Q, S231P, and IVS 10nt11g are common Mediterranean mutations. The IVS 2nt5g-c was associated with two different HT backgrounds, HT 5.9 and the new HT 73.9 (Table 2). Two new mutations that have not been reported elsewhere in the Mediterranean basin, G46fs (136/137delG) and Y206D were found among our sample, on one and two alleles, respectively. Further screening in the area will determine the haplotype associations of these novel mutations and resolve whether they are unique to Egypt or common to other Arab populations.

Discussion

The rich variety of polymorphism and mutations at the PAH locus makes it a useful tool in molecular population genetics. And many investigators have delineated PAH haplotypes and established their distribution among normal and mutant chromosomes in different populations (see Konecki[20] for a review). Although most of these studies have shown the PAH locus to be very heterogenous, with more than 46 distinct HTs in Europe alone,[21] only six HTs (1,2,3,4,5 and 7) were identified on 76% of all normal chromosomes,[15] with predominance of HTs 1 and 4. These latter two haplotypes have been detected in all populations studied thus far, with only a few exceptions.[20] In the Mediterranean population most relevant to the present study (Italy, Turkey, Palestenian Arabs), HTs 1, 4 and to a lesser extent 7 were also predominant among normal chromosomes.[22] Based on our preliminary findings, the distribution of normal haplotypes in the Egyptian population is not significantly different from that of other Mediterranean populations, with HTs 1.8, 3.9, 4.3 and 7.8 present on more than half of the normal chromosomes of known haplotypes. The other normal HTs (22.11, 27.6 and 52.8) are rare in both Egyptian and European populations.

Haplotype and mutational studies of mutant PAH chromosomes have also demonstrated a high degree of molecular heterogeneity in Egyptian PKU families. Haplotype studies identified seven different mutant haplotypes and nine different mutations among the 26 mutant chromosomes analysed. In comparison with Dr Hashem's study[23] suggesting the rarity of IVS 10nt11g in Egypt, this study showed a high frequency of this mutation (8/26) in Table 1. The relative predominance of this mutation among Southern European mutations has also been demonstrated in several other Mediterranean populations, including Spain, Italy, Greece, Turkey and Palestinian, Moroccan and other Jewish populations of the region.[22] The heterogeneity at the PAH locus in Egyptian PKU families may easily be explained on the basis of Egypt's unique geographic location and rich historical past. Egypt lies at the crossroads of three continents and since ancient Pharaonic times, has been the interest of many conquerors. The inclusion of Egypt as a part of the Osmani Empire for over 400 years produced many social intermarriages between the two populations, intertwining the genetic backgrounds. Thus, the high frequency of the IVS 10nt11g mutation may reflect these close familial relationships that exist between Egyptians and Turks.

Five of the other mutations detected in this study, IVS 2nt5g-c, R176X, Y198fs, S231P and R261Q, have been observed in certain other Mediterranean populations, although at low frequencies. Of these mutations, IVS 2nt5g-c was the most frequent. At a relative frequency of 4/26, it was the second most prevalent mutation identified in this study and much more frequent in Egypt than in any other population in which it has been detected.[24] This mutation was present on two different HT backgrounds, HT 5.9 and HT 73.9; IVS 2nt5g-c has previously been reported to be associated with HTs 1 and 5.[24] As in other populations, HT 1 in Egypt contained multiple mutations; R261Q and R176X were both present on this haplotype background (Table 2). The association between R261Q and HT 1 was not exclusive, as this mutation was also observed on a single chromosome of HT 32.8. The haplotype association of R176X in the Sicilian population was not reported. Of the remaining mutations previously identified in other populations, E178G and Y198fs were each present on the incomplete, but tentatively novel haplotype also containing the 9-copy VNTR. The haplotype association of S231P could not

be established. In addition to these previously identified mutations, two novel mutations were detected among these Egyptian PKU families. These were G46fs and Y206D, present on one and two chromosomes, respectively. G46fs was associated with HT 23.7. Further screening in the area will resolve whether these mutations are unique or common in the Arab population.

These studies defining the molecular bases of PKU in the Egyptian population are critically important for the management of this disease in this developing country. The information gleaned from these studies can facilitate the choice of priorities for mutational screening, thus saving time, effort and expense. Identification of the mutations responsible for PKU can assist in providing accurate information to the families with regard to prognosis in the absence of treatment and response to treatment if available. These data can also be used to perform carrier detection and offer reproductive counselling to the apparently normal sibs. Finally, prenatal diagnosis can be offered at an early stage, thus avoiding the birth of an affected child facing the prospects of profound mental retardation in a society where low-phenylalanine dietary products are scarce or absent.

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