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Genetic background of clinical homogeneity of phenylketonuria in Poland

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Abstract

In order to elucidate the clinical homogeneity and severity of the hyperphenylalaninaemias in Poland, a total of 71 children with typical phenylketonuria (PKU) originating from western and northern Poland were screened for 13 mutations in the phenylalanine hydroxylase (PAH) gene. Eighty percent of all PKU alleles tested were found to carry an identified mutation. One mutation, namely the R408W mutation, accounted for more than 63% of mutant PAH alleles in Poland, the other 27% being accounted for by six mutations: IVS12nt1 (5%), IVSnt546 (5%), Y414C (4%), R252W (1·5%), R261Q (<1%), and G272ter (<1%). The predominance of the R408W mutation resulted in a high rate of homozygotes (35·2%) and compound heterozygotes for this mutation in children from western and northern Poland. The frequency and deleterious nature of this mutation probably accounts for the clinical homogeneity and severity of the hyperphenylalaninaemias in Poland. In addition, the high rate of the R408W mutation and its association with mutant haplotype 2 at the PAH locus in Poland give additional support to the Balto-Slavic origin of this mutant gene.

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Phenylketonuria (PKU) is caused by deficiency of the hepatic enzyme phenylalanine hydroxylase (PAH: E.C.1.14.16.1) and, if untreated, results in severe mental retardation.[1] More than 50 mutations of the PAH gene have been identified so far, which accounts for the clinical heterogeneity of PAH deficiency shown by newborn screening programmes in a number of European populations.[2] In several eastern European countries including Poland, the clinical severity of the hyperphenylalaninaemias has long been recognised.[3] In order to elucidate the basis of this clinical severity at the molecular level, we screened 71 Polish PKU children for 13 identified mutations of the PAH gene. The present study indicates that the clinical severity and homogeneity of PKU in Poland can be largely ascribed to a small number of deleterious mutations including the R408W mutation whose frequency reaches the highest level reported so far.

Patients and methods

PATIENTS

The 71 hyperphenylalaninaemic children reported here had (1) plasma phenylalanine levels over 1·2 mmol/l on both neonatal screening and re-evaluation at 1 month of age, and (2) dietary tolerance for phenylalanine of approximately 250 mg per day. They therefore met the criteria for typical PKU. The children were born to unrelated healthy parents and originated from either western Poland (36) or northern Poland (35). The haplotypes of 36 patients originating from western Poland have been previously reported.[3]

METHODS

For allele specific oligonucleotide (ASO) screening, amplification primers, ASO probes, and experimental procedures for three reported mutations were as described: (1) R252W,[4] (2) R261Q,[4] and (3) E280K.[5]

For detection of the mutations that are known to suppress restriction sites (G272ter, S273F, and Δ364L), exons 7 and 11 were amplified using intronic primers[5,6] and the amplification products (246 bp and 295 bp respectively) were digested using restriction enzymes *Bam*HI, *Mbo*I (G272ter[7] and S273F[8]), and *Hin*dIII (ΔL364[7]). The expected fragments were 174 bp + 72 bp and 228 bp + 67 bp for mutations at codons 272 to 273 and 364 respectively.

The screening for mutations R158Q[9] and IVS12nt1[10] was performed using the amplification created restriction site (ACRS) technique previously reported.[11] Finally, the screening for mutations R243ter,[12] P281L,[13] IVS10nt546,[6] R408W,[14] and Y414C[15] was performed by the ACRS technique using amplification conditions and oligonucleotide primers shown in table 1. The restriction sites were devised so as to be abolished in case of mutations altering these sites.

Comparison of mutation frequencies between northern and western Poland was made using the χ^2 test and Yates's correction was introduced when necessary.

Results

SCREENING FOR PKU MUTATIONS IN POLAND

Table 2 shows that 80% of all PKU alleles studied (114/142) were found to carry an identified mutation, regardless of the geographical origin of the patients. One mutation (R408W) was found to predominate, accounting for 63% of mutant PAH alleles in Poland (90/142). The other 27% were accounted for by six muta-

Table 1 Amplification conditions and restriction analysis for mutations screened by the ACRS technique. The nucleotides substituted for creation of the restriction sites are shown in bold letters. Mismatches introduced for creation of the restriction site are shown in the last column.

Mutant codon	5′ (up) 3′ (down)	Primers	Anneal temp (°C)	Created restr site	Length of PCR prod (bp)	Length of restr frag (bp)	Created mis-match
R158G	5′	5′-TCCTGTGTACCGTGCAAGCC-3′	55	*Msp*I	142	123+19	C-A
	3′	5′-CCATCCTCAACTGGATGAGG-3′*					
R243ter	5′	5′-CTCCTAGTGCCTCTGACTCA-3′†	57	*Msp*I	102	83+19	C-A
	3′	5′-AAGCAGGCCAGCCACAGGCC-3′					
P281L	5′	5′-CTCCTAGTGCCTCTGACTCA-3′†	57	*Msp*I	217	196+21	C-T
	3′	5′-AGCTGTAGCACAGTACTCCC-3′					
IVS10nt546	5′	5′-AAGGGGCACAAATGGCCTAT-3′	55	*Apa*I	96	80+16	G-G
	3′	5′-ATAAGCAGTACTGTAGGGCC-3′					
R408W	5′	5′-ATGCCACTGAGAACTCTCTT-3′‡	55	*Taq*I	120	99+21	T-G
	3′	5′-GTAGCGAACTGAGAAGGGTC-3′					
Y414C	5′	5′-TCGGCCCTTCTCAGTTCGGT-3′	50	*Rsa*I	147	127+20	G-G
	3′	5′-AGTCTTCGATTACTGAGAAA-3′‡					
IVS12	5′	5′-ATGCCACTGAGAACTCTCTT-3′‡	50	*Rsa*I	214	195+19	G-A
	3′	5′-CGTAAGGTGTAAATTACGTA-3′					

* Ref 9, † ref 16, ‡ ref 14.

Table 2 Distribution of PKU mutations in 142 mutant PAH genes from Poland (n = number of mutant genes tested).

Mutant codon	Exon/ intron	Total (n=142)	Mutant genes: Western Poland (n=72)	Mutant genes: Northern Poland (n=70)	Statistical tests
R408W	12	90	42	48	NS
IVS10nt546	IVS10	7	5	2	NS
IVS12	IVS12	7	2	5	NS
Y414C	12	6	6	0	<0·05
R252W	7	2	0	2	NS
G272ter	7	1	0	1	NS
R261G	7	1	1	0	NS
R158G	5	0	0	0	NS
R243ter	7	0	0	0	NS
S273F	7	0	0	0	NS
E280K	7	0	0	0	NS
P281L	7	0	0	0	NS
ΔL364	11	0	0	0	NS
Total identified		114 (80%)	56 (78%)	58 (83%)	
Total unidentified		28 (20%)	16 (22%)	12 (17%)	

tions, namely: IVS12nt1 (5%), IVS10nt546 (5%), Y414C (4%), R252W (1·5%), R261Q (<1%), and G272ter (<1%). Among the 13 mutations tested, six were not represented in this series (R158Q, R243ter, S273F, E280K, P281L, and ΔL364). No significant discrepancy in the frequency of mutations appeared when the population of mutant alleles was split into two groups according to the geographical origin of the patients, except for the Y414C mutation (table 2).

DISTRIBUTION OF MUTATIONS IN AFFECTED SUBJECTS

Table 3 shows that the 114 mutations identified were distributed among 71 subjects, most of them (45/71) being compound heterozygotes (63·4%). Interestingly, 40/71 children (56·3%) were heterozygous for the R408W mutation, 25/71 (35·2%) were homozygous for this R408W mutation, and 1/71 was homozygous for the IVS12nt1 mutation (total of homozygotes = 36·6%, table 3).

CORRELATION BETWEEN MUTATIONS AND RFLP HAPLOTYPES AT THE PAH LOCUS

Correlation between mutations and RFLP haplotypes was possible in 56 mutant PAH alleles from western Poland. Table 4 shows that each mutant RFLP haplotype was associated with only one mutation and also that each mutation was associated with only one RFLP haplotype. Thus, a strict genotype–haplotype association was observed in this series.

Discussion

The study reported here shows that one single mutation, namely R408W, accounted for more than 60% of PKU alleles in Poland. This mutation is associated with mutant haplotype 2 at the PAH locus in western Poland as originally reported in the Danish population.[14] This association is both inclusive and exclusive, as no other mutation was found associated with haplotype 2 and no other mutant haplotype was found to be associated with the R408W mutation.[3] The high rate of the R408W mutation in Poland accounts for the high proportion of homozygotes (35·2%) and compound heterozygotes (56·3%) for this mutation. These data are consistent with previous reports from geographically related areas, namely southern Poland (57%),[17] Lithuania (69%), and Czechoslovakia (68%).[18] These results, along with those found in eastern Germany, contrast with the lower frequency of the R408W mutation in northern Europe[15] and

Table 3 Distribution of PKU mutations in affected children from Poland.

Mutant codon	No of alleles	No of patients	Homozygotes	Compound heterozygotes
R408W	90	65	25	40
IVS12	7	6	1	5
IVS10nt546	7	7	0	7
Y414C	6	6	0	6
R252W	2	2	0	1
R261Q	1	1	0	1
G272W	1	1	0	2
Total	114		26 (36·6%)	

Table 4 Association between PKU mutations and RFLP haplotypes at the PAH locus in 56 mutant PAH alleles from western Poland.

Mutant codon	No of alleles	Haplotype	No of mutant genotypes
R408W	42	2	1/1
Y414C	6	4	6/6
IVS10nt546	5	6	5/5
IVS12	2	3	2/2
R261Q	1	1	1/1
Total	56		

with its even lower frequency in western Europe (5%).[19] Therefore, the present data support the Balto-Slavic origin of the R408W mutation which probably spread across Europe from the north east to the south west.[18]

It is interesting to note that the two most frequent mutations after R408W are the IVS12nt1 (5%) and IVS10nt546 (5%) mutations, which were originally described in northern[10] and southern[6] Europe. While no significant linkage disequilibrium could be shown, owing to the small number of mutant alleles, it is worth noting that these mutations were associated with haplotypes 3 and 6 respectively, suggesting that the presence of these mutations in Poland could be related to both a founder effect and to genetic drift.

It is also interesting to note that several mutations originally reported in other European countries were extremely rare (R261Q, R252W,[4] G272ter[7]) or even absent in Poland (E280K,[5] S273F,[8] ΔL364,[7] R158Q,[9] R243ter,[12] P281L[13]). Indeed, we found no R158Q mutation in western and northern Poland. This result contrasts with data reporting this mutation as one of the four most frequent mutations in southern Poland and central Europe.[17 20]

As far as genotype-phenotype correlations are concerned, it is important to bear in mind that all mutations detected in this population are known to result in severe forms of the disease. This is not only true for the R408W mutation but for IVS12nt1 and IVS10nt546 mutations as well.[6 10] These features, along with the high rate of homozygosity for these mutations (36·6%) in our series, probably account for the clinical severity of PAH deficiencies in Poland.

In conclusion, the present study provides genetic evidence for a regional specificity in the distribution of mutant PAH genes in western and northern Poland. In addition, it suggests that the high rate of mutant homozygotes for deleterious mutations in this geographical area accounts for the clinical homogeneity of PAH deficiencies in Poland.

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