Should genetic analysis in newborn screening and a heterozygote test for hyperphenylalaninaemia be recommended? An Italian study

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Abstract

***Objective*—To determine whether the introduction of genetic analysis for phenylalanine hydroxylase (PAH) deficiency into regional screening programmes can be supported by the benefit-cost ratio.**

***Method*—Tests for the genetic PAH locus were carried out in 151 patients with hyperphenylalaninaemia originally from all of the Italian regions. PAH mutations were identified by extraction of genomic DNA from leucocytes (whole blood in EDTA), PAH exon amplification was determined by polymerase chain reaction, restriction enzyme analysis was carried out for some recognised mutations, and DNA sequence analysis for the other mutations.**

***Results*—It was found that the eight most common mutations in the population accounted for 49% of the mutant alleles, which is well below the required standard for effective population screening (90%).**

***Conclusions*—Genetic screening for PAH deficiency in Italy does not increase the sensitivity of the methodology and the benefit-cost ratio, and thus provides no advantage, particularly as the correlation between genotype and the metabolic phenotype needed to optimise dietary intervention is still being studied.**

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Hyperphenylalaninaemia is caused by deficiency of the phenylalanine hydroxylase (PAH; EC 1.14.16.1) enzyme activity.[1]

The PAH gene, located on chromosome 12, consists of 13 exons.[2] According to the PAH mutation analysis consortium, more than 300 different mutations have been reported to date.[3] Each mutation may influence the enzyme activity in a different way, so that various combinations of mutations result in a spectrum of metabolic phenotypes, ranging from severe, moderate, and mild phenylketonuria requiring strict dietary management, to mild hyperphenylalaninaemia, in which the dietary restriction of phenylalanine is not necessary.

At present, newborn screening of hyperphenylalaninaemia is carried out by an immunoenzymatic assay on Guthrie card (normal values of blood phenylalanine $\leqslant$180 μmol/l). This simple, cheap test (costing around £2) does not distinguish between patients with phenylketonuria and mild hyperphenylalaninaemia.

When hyperphenylalaninaemia gene carriers are screened, which is usually required by parents and relatives of a patient with hyperphenylalaninaemia, the current biochemical methods based on the phenylalanine loading test (costing around £30) may misclassify heterozygosity and normality in 10–20% of cases.[4]

Genetic analysis—that is, identification of all the PAH mutations characterised so far, would provide a sensitivity of at least 98% (because some mutations are located in unscreened region of the PAH gene, such as introns or promoter regions) and a specificity of 100% for the test.[5] The cost of such analysis has been estimated as around £420 for both an affected patient and an identified carrier.

Advantages of the genetic test would be, firstly, a 98% sensitivity in detection of PAH carriers compared with a sensitivity of 80–90% for the biochemical test and, secondly, early discrimination between patients with phenylketonuria and mild hyperphenylalaninaemia. The latter might lead to a definition of the individual biochemical pattern before treatment once the relation between genotype and metabolic phenotype is shown.

This study aimed at identifying the most common mutations in the Italian regions and evaluating whether the introduction of genetic analysis for PAH deficiency into regional screening programmes could be supported by the benefit-cost ratio.

Patients and methods

We tested for the genetic PAH locus in 151 patients with hyperphenylalaninaemia (containing 15 sib pairs), corresponding to 272 unrelated alleles. The median age of the patients, who came from all the Italian regions, was 9.2 years (range 0.1–20.4); male/female ratio 1.25.

Isolation of genomic DNA and amplification of the 13 exons was carried out by standard protocols.[6] Denaturing gradient gel electrophoresis analysis of the polymerase chain reaction (PCR)-amplified exons and flanking intronic sequences of the PAH gene was carried out as described by Guldberg *et al*.[7] Samples with a pattern of bands differing from those of normal controls were further processed by sequence analysis. DNA sequencing was carried out directly on PCR-amplified products with the non-guanine-cytidine-clamped primers of each exon, using the

Table 1 Mutations of the phenylalanine hydroxylase gene and their prevalence in a sample of Italian patients

No	Mutation	Exon	No of alleles	%
1	R261Q	7	26	9.5
2	IVS10nt546	10	25	9.2
3	L48S	2	18	6.6
5	Y414C	12	17	6.2
6	R158Q	5	16	5.9
4	P281L	7	15	5.5
7	R408W	12	8	2.9
8	A300S	8	8	2.9
9	R252W	7	4	1.8
10	E390G	11	4	1.5
11	Y204X	6	3	1.1
12	G352fsdelG	10	2	0.7
13	A395P	11	2	0.7
14	del F39	2	2	0.7
15	A395G	11	2	0.7
16	R68S	3	2	0.7
17	V230I	6	2	0.7
18	R297H	8	2	0.7
19	T287N	7	2	0.7
20	A259V	7	1	0.4
21	R408Q	12	1	0.4
22	V388M	11	1	0.4
23	R252Q	7	1	0.4
24	del l94	3	1	0.4
25	A309V	11	1	0.4
26	L15/S16fsdelCT	1	1	0.4
27	F55fs	2	1	0.4
28	I65T	3	1	0.4
29	E178G	6	1	0.4
30	C334S	10	1	0.4
31	D415N	12	1	0.4
32	V386C	11	1	0.4
33	T380M	11	1	0.4
34	IVS8nt1 g→a	9	1	0.4
35	E6nt96 A→g	6	1	0.4
36	R261X	7	1	0.4
37	D84Y	3	1	0.4
38	T92I	3	1	0.4
39	E360X	11	1	0.4
40	L367V	11	1	0.4
41	Y417D	12	1	0.4
42	IVS12nt1g→a	12	1	0.4
43	P366H	11	1	0.4
44	R176X	6	1	0.4
45	V245E	7	1	0.4
46	G257S	7	1	0.4
47	G247V	7	1	0.4
48	G257D	7	1	0.4
49	G257C	7	1	0.4

Applied Biosystem Model 310 DNA Sequencing System and the Rhodamine Terminator Ready Reaction (Perkin Elmer, Italy).

Restriction enzyme analysis was used to identify some recognised mutations (DdeI for IVS10nt546, HinfI for R261Q, MluNI for L48S, MspI for R158Q and P281L, HhaI for A300S).[8]

The costs estimated throughout this paper include both material and labour.

Results

Mutations were characterised in 227 of 272 (83%) unrelated alleles. Mutations for two alleles were found in 102 patients, 11 sib pairs (182 unrelated alleles) and a mutation for one allele only was identified in 49 patients, four sib pairs (45 unrelated alleles). Among the 49 different PAH mutations, we also identified three new mutations at the PAH locus—two missense (L367V and Y417D) and one non-sense (E380X).[9] The prevalence of each identified mutation was evaluated within the panel of the 272 unrelated alleles (table 1).

The eight most common mutations in our population (R261Q, IVS10nt546, L48S, P281L, Y414C, R158Q, R408W, A300S) accounted for 49% of the mutant alleles—a value that is well below the required standard for effective population screening (90%).

Fifty seven patients, including five sib pairs, had two alleles mutant for one of the above eight mutations. When the five sibling pairs were excluded the sensitivity was 53% (only 52 affected out of a total of 97 subjects), with a cost of around £180 for each patient.

Conclusions

Our results confirm the high heterogeneity of PAH gene mutations in Italy in comparison with other countries. For example, in Denmark four mutations account for 70% of the total, and in Poland and Ireland a single mutation accounts for around 40–50% of PAH mutant alleles.[10–12]

Accordingly, genetic screening for PAH deficiency in Italy does not increase the sensitivity of the methodology and the benefit-cost ratio, and thus cannot be proposed for use in that country. Indeed, even if the sensitivity were higher, the cost would still be prohibitive as the correlation between genotype and metabolic phenotype (and from this the possibility of optimising dietary intervention) is still unclear. In conclusion, genetic analysis in hyperphenylalaninaemia might provide additional information on the metabolic phenotype in those patients already identified as hyperphenylalaninaemic once a correlation between genotype and metabolic phenotype has been shown.[13] However, further investigation of this correlation is required.

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