Molecular basis of mild hyperphenylalaninaemia in Poland

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Abstract
The major cause of the different forms of hyperphenylalaninaemia (HPA) is mutations in the gene encoding phenylalanine hydroxylase (PAH). The aim of this study was to determine the mutations responsible for mild forms of HPA and to relate different clinical phenotypes of HPA patients to their PAH genotypes. Four “mild” mutations, including the most frequent A403V and R297H mutations, occurred exclusively in mild hyperphenylalaninaemia (MHP). Mutations A104D, R243Q, R241H, and Y414C were detected in patients with mild phenylketonuria (mild PKU) only. These results may be useful in establishing a molecular differential diagnosis for PAH deficiency in Poland.
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Keywords: mild hyperphenylalaninaemia; phenylketonuria; PAH gene

Phenylketonuria (PKU) is caused by a deficiency of hepatic phenylalanine hydroxylase (OMIM 261600). PAH deficiency is phenotypically heterogeneous as shown in neonatal screening. Mild forms of hyperphenylalaninaemia can be divided clinically into (1) mild PKU with blood phenylalanine levels of 600-1200 µmol/l and (2) non-PKU HPA (MHP) with concentrations below 600 µmol/l.¹ In Poland, the frequency of MHP and mild PKU is approximately 30% of total HPA.²

A total of 316 mutations in the PAH gene have been identified so far.³ Different mutations cause a wide spectrum of in vitro enzyme activities which correlate with the different phenotypes observed. According to phenotypic effects, “severe” and “mild” mutations in the PAH gene can be differentiated.⁴⁻⁵ The purpose of this study is to determine the molecular basis of mild forms of hyperphenylalaninaemia in Poland and to correlate the mutations detected with clinical phenotypes.

Genomic DNA was isolated from total blood of 34 MHP and eight mild PKU probands from unrelated Polish families. All patients were detected through Guthrie newborn screening. Quantitative estimations of phenylalanine concentrations were made if mild hyperphenylalaninaemia was found to be persistent. Serum phenylalanine levels were determined fluorimetrically for the first time at an average age of 2-4 months. The patients were classified according to Güttler.¹ All mild PKU patients were on a diet with partially restricted phenyla-

lanine intake. All the MHP patients were without dietary restrictions.

Routine screening for seven common mutations (Y414C, R408W, IVS12nt1g→a (IVS12), IVS10nt-11g→a (IVS10), R261Q, R252W, and R158Q) was performed as described previously.⁶ Then SSCP analysis of PAH exons with flanking sequences and direct sequencing (Sequenase 2.0 and ΔTaq) was performed.

Three novel mutations were identified in the group studied, IVS2nt5g→a (168+5g→a, IVS2), IVS4nt5g→t (441+5g→t, IVS4), and D222G (GAT→GGT). All the patients were compound heterozygotes for “severe” and

Table 1 Genotype-phenotype relations in MHP and mild PKU patients

Genotype	Pretreatment phenylalanine level (µmol/l)	Phenylalanine concentrations Range (µmol/l)
Mild phenylketonuria (patients on diet)		
A104D/D222G*	545	121-605 (borderline)
R241H/R252W	454	242-484
R241H/IVS10	793	242-484
R241H/R408W	484	278-696
R243Q/A104D	854	726-1211
R243Q?	1138	121-726
Y414C/A104D	1453	121-605 (rapid normalisation)
Y414C/R408W	3190	121-1200 (rapid normalisation)
Mild hyperphenylalaninaemia (patients off diet)		
F55L/IVS10	345	303-605
P89/R408W	672	605-726 (borderline)
P211T/R408W	400	484-726
V245A/R252W	442	484-605
V245A/R408W	394	242-394
R297H/IVS2*	363	363-484
R297H/R252W	484	484-726
R297H/R408W	394	242-660
R297H/R408W	387	333-424
R297H/R408W	327	272-430
R297H/R408W	527	242-484
A300S/IVS4*	272	242-484
A300S/R158Q	605	363-605
A300S/R158Q	363	242-896
A300S/R408W	309	363-484
A300S/R408W	327	484-605
I306V/R408W	563	363-605
I306V/R408W	605	363-605
I306V/R408W	642	484-666
T380M/R408W	339	363-484
T380M/R408W	339	242-363
A403V/R158Q	490	242-363
A403V/P281L	339	363-605
A403V/IVS10	303	242-605
A403V/IVS10	448	363-605
A403V/R408W	611	363-484
A403V/R408W	496	363-484
A403V/R408W	260	363-484
A403V/R408W	327	236-448
A403V/R408W	381	363-605
A403V/R408W	490	260-496
A403V/R408W	484	363-605
A403V/R408W	605	363-605
A403V/R408W	300	260-605

Mild and moderate mutations are shown first. Novel mutations are marked with an asterisk.

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“mild” mutations. The genotypic and phenotypic data obtained for the patients are summarised in table 1.

In the MHP group, A403V and R297H were the most frequent “mild” mutations. Mutations A403V, A300S, and R297H were not detected in a group of 140 classical PKU patients (data not shown) or in mild PKU patients. Mutation A403V has been detected previously in southern Europe, and in Spain it is a relatively common mutation (14%).⁷ In the group reported here its frequency is even higher (19%), but the limited number of cases does not allow significant statistical comparison. In both countries it has been detected in combination with “severe” mutations (R408W, R252W).⁸ R297H has been identified in the USA with no indication of its severity.³ In the MHP group, however, it is connected with “severe” mutations, known to cause classical homozygous PKU (R408W, R252W). It is postulated that A403V and R297H could both be unambiguously ascribed to a “mild” group.

A rare mutation, F55L, was detected in a patient with MHP; it occurred with an IVS10nt-11g→a mutation, known to produce a classical homozygous PKU phenotype.³ A patient with P89S/R408W presented a borderline MHP phenotype, with relatively high phenylalanine levels and stable high IQ scores and it is postulated that P89S is a “mild” mutation.

The next four mutations (Y414C, R243Q, R241H, and A104D) occurred exclusively with mild PKU. The SSCP patterns indicate that these mutations are absent in a group of 140 classical PKU patients.

An A104D/D222G patient had a mild PKU phenotype with high phenylalanine tolerance (50 mg/kg/day) and normal IQ scores at the age

of 2 years. Precise assessment of D222G “severity” requires identification of other patients bearing this mutation.

The novel splicing mutations (IVS2 and IVS4) were “severe” ones and when combined with mild mutations (R297H and A300S, respectively) produced a MHP phenotype.

The results presented provide preliminary data on the molecular basis of mild forms of hyperphenylalaninaemia in Poland. Identification of relatively frequent mutations connected with either MHP or mild PKU enables neonatal differential genotyping of HPA and identification of patients not requiring dietary therapy.^{9 10}

- 1 Güttler F. Hyperphenylalaninemia: diagnosis and classification of various types of phenylalanine hydroxylase deficiency in childhood. *Acta Paediatr Scand Suppl* 1980;280:1-80.
- 2 Cabalska B, Sendek E, Nowaczewska I, Zorska K. Mild hyperphenylalaninemia - frequency, physical and mental development (in Polish). *Pediatr Pol* 1994;59:339-44.
- 3 Byck S, Nowacki P, Prevost L, Scriver CR. *PAH Mutation Analysis Consortium Newsletter*. Winter 1997.
- 4 Economou-Petersen E, Henriksen KF, Guldborg P, Güttler F. Molecular basis for nonphenylketonuria hyperphenylalaninemia. *Genomics* 1992;14:1-5.
- 5 Güttler F, Guldborg P, Henriksen I, et al. Molecular basis for the phenotypic diversity of phenylketonuria and related hyperphenylalaninemias. *J Inher Metab Dis* 1993;16:602-4.
- 6 Żekanowski C, Nowacka M, Żygulska M, et al. Frequencies of the most common mutations responsible for phenylketonuria in Poland. *Mol Cell Probes* 1994;8:323-4.
- 7 Desviat LR, Perez B, Ugarte M. Molecular basis of non-PKU hyperphenylalaninemia in Spain: prevalence of A403V, a mutation with high residual activity. *J Inher Metab Dis* 1996;19:227-30.
- 8 Romano V, Guldborg P, Güttler F, et al. PAH deficiency in Italy: correlation of genotype with phenotype in the Sicilian population. *J Inher Metab Dis* 1996;19:15-24.
- 9 Güttler F, Guldborg P. Mutations in the phenylalanine hydroxylase gene: genetic determinants for the phenotypic variability of hyperphenylalaninemia. *Acta Paediatr Scand Suppl* 1994;407:49-56.
- 10 Lichter-Konecki U, Rupp A, Konecki DS, et al. Relation between phenylalanine hydroxylase genotypes and phenotypic parameters of diagnosis and treatment of hyperphenylalaninemic disorders. *J Inher Metab Dis* 1994;17:362-5.