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A European Multicenter Study of Phenylalanine Hydroxylase Deficiency: Classification of 105 Mutations and a General System for Genotype-Based Prediction of Metabolic Phenotype

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Summary

Phenylketonuria (PKU) and mild hyperphenylalaninemia (MHP) are allelic disorders caused by mutations in the gene encoding phenylalanine hydroxylase (PAH). Previous studies have suggested that the highly variable metabolic phenotypes of PAH deficiency correlate with *PAH* genotypes. We identified both causative mutations in 686 patients from seven European centers. On the basis of the phenotypic characteristics of 297 functionally hemizygous patients, 105 of the mutations were assigned to one of four arbitrary phenotype categories. We proposed and tested a simple model for correlation between genotype and phenotypic outcome. The observed phenotype matched the predicted phenotype in 79% of the cases, and in only 5 of 184 patients was the observed phenotype more than one category away from that expected. Among the seven contributing centers, the proportion of patients for whom the observed phenotype did not match the predicted phenotype was 4%–23% ($P < .0001$), suggesting that differences in methods used for mutation detection or phenotype classification may account for a considerable proportion of genotype-phenotype inconsistencies. Our data indicate that the *PAH*-mutation genotype is the main determinant of metabolic phenotype in most patients with PAH deficiency. In the present study, the classification of 105 *PAH* mutations may allow the prediction of the biochemical phenotype in >10,000 genotypes, which may be useful for the management of hyperphenylalaninemia in newborns.

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Introduction

Deficiency of phenylalanine hydroxylase (PAH [MIM 261600]) impairs hepatic hydroxylation of phenylalanine to tyrosine, the major route of phenylalanine metabolism in humans (Scriver et al. 1995). The disorder is transmitted in an autosomal recessive pattern and is the most common inborn error of amino acid metabolism in the white population, with an average incidence of 1/10,000. The key feature and marker for neonatal detection is elevated serum concentrations of phenylalanine (Scriver et al. 1995).

PAH deficiency is a highly heterogeneous trait showing a broad continuum of phenotypes. Complete or near-complete deficiency of PAH activity is designated "classic PKU" and results in profound and irreversible mental retardation unless the dietary exposure to phenylalanine is drastically reduced. For diagnostic and therapeutic purposes, the continuum of milder forms has been subdivided into arbitrary categories, generally termed "moderate PKU," "mild PKU," and "mild hyperphenylalaninemia" (MHP). As in the case of classic PKU, well-adjusted restriction of phenylalanine intake is required to ensure normal physical, neurological, and cognitive development in patients with the milder forms of PKU, whereas no dietary correction is required to prevent neurological symptoms in individuals with MHP (Güttler 1980; Scriver et al. 1995).

It is now well established that PAH deficiency is caused by mutations in the gene encoding PAH and that the different forms of PAH deficiency are allelic (Ledley et al. 1986; Eisensmith and Woo 1992; Guldberg et al. 1994*b*). As is true of most other inherited disorders, the genetic heterogeneity of PAH deficiency is enormous, with >300 different mutations now identified, worldwide, by the PAH Mutation Analysis Consortium (Nowacki et al. 1998). Some mutations cause complete ab-

olition of PAH function, whereas others are associated with residual in vitro activity in the range of 2%–70% (Okano et al. 1991; John et al. 1992; Svensson et al. 1992; Desviat et al. 1995, 1996; Eiken et al. 1996*a*). This continuous spectrum of mutation-related enzyme activities, together with the plethora of possible mutation combinations (genotypes), may explain the observed interindividual heterogeneity of metabolic phenotypes.

The capability to predict the phenotypic outcome in newborn hyperphenylalaninemics would be highly useful as a tool for refining the diagnosis and implementing an optimal dietary therapy. Several studies have suggested a simple correlation between *PAH* genotype and metabolic phenotype and have reported the phenotypic associations of particular mutation combinations (Okano et al. 1991; Rey et al. 1992; Svensson et al. 1993; Guldberg et al. 1994*a;* Zschocke et al. 1994; Dianzani et al. 1995; Eiken et al. 1996*b;* Eisensmith et al. 1996; Romano et al. 1996; Desviat et al. 1997; Kayaalp et al. 1997). However, three factors have hampered the establishment of a more universal system for predicting the phenotype on the basis of genotype data: (1) the enormous mutational heterogeneity, (2) the different methods and criteria used for diagnosis and classification, and (3) lack of knowledge about how the possible interaction between two different mutant polypeptides in the tetrameric holoenzyme may influence the phenotypic outcome. Herein, we have attempted to overcome some of these limitations by analyzing a large number of patients referred to seven European centers and by aiming at similar criteria and methods for phenotype classification. On the basis of this compilation we have assigned each of 105 *PAH* mutations to one of the four phenotype categories, and we have proposed a general system that allows prediction of metabolic phenotype in the majority of mutation combinations.

Patients and Methods

Patients and Phenotypes

Seven centers in France, Italy, Belgium, Germany, and Denmark participated in this collaborative study. Some centers contributed patient data from several regional treatment centers. The John F. Kennedy Institute in Denmark was assigned as coordinating center and was responsible for compilation of data in a relational database (Microsoft Access software). In all patients, hyperphenylalaninemia had been detected by national mass screening programs, and PAH deficiency had been assessed after exclusion of a defect in tetrahydrobiopterin metabolism (Scriver et al. 1995). Patients were assigned to one of four arbitrary phenotype categories, on the basis of data on either dietary phenylalanine tolerance (in patients with PKU) or pretreatment blood-phenylalanine levels (in individuals with MHP) (Güttler 1980; Güttler and Guldberg 1996). Patients with classic PKU tolerate <20 mg phenylalanine/kg body wt/d (equivalent to 250–350 mg/d), to keep blood phenylalanine values at 300 mmol/liter; patients with moderate PKU tolerate 20–25 mg/kg body wt/d (350–400 mg/d); and patients with mild PKU tolerate 25–50 mg/kg body wt/d (400–600 mg/d). Phenylalanine tolerance was determined in patients >2 years of age; the majority of determinations were made in children 5 years of age. Patients who have phenylalanine levels <600 mmol/liter on a normal diet were classified as having MHP.

Genotype Data and Mutation Nomenclature

For all patients recorded in the database, two presumably causative alterations in the *PAH* gene were identified. Genotyping was performed at the treatment center or at the John F. Kennedy Institute. The approach for genotyping varied among the participating centers and was based on the use of a variety of PCR-based mutation-detection assays, including diagnostic assays (e.g., allele-specific oligonucleotide [ASO] analysis, allele-specific PCR, and restriction-enzyme analysis), mutation-scanning assays (e.g., SSCP, chemical cleavage analysis, heteroduplex analysis, and denaturing gradient–gel electrophoresis [DGGE]), and sequence analysis (for a recent review of mutation-detection methods, see Nollau and Wagener 1997). Most centers used a combination of different techniques.

Mutations were referred to by their "trivial names," as registered in the PAH Mutation Analysis Consortium Database. The corresponding systematic names, as recommended by Antonarakis (1998), are given in the database as well. For some of the patients, associations between genotype and various phenotypic parameters have been reported elsewhere (Rey et al. 1992; Guldberg et al. 1994*a,* 1995; Dianzani et al. 1995; Burgard et al. 1996; Romano et al. 1996). The same data may be found in the PAH Mutation Analysis Consortium Database, and they may have been included in a recent metanalysis (Kayaalp et al. 1997).

Results

Mutation and Phenotype Distributions

A total of 686 patients with PAH deficiency were recorded in the database. The number of patients contributed by each of the seven centers was 46–180. For all patients, one criterion for inclusion was that two presumably causative mutations in the *PAH* gene could be ascertained. The 133 different mutations (listed in the Appendix) include 87 missense mutations, 16 splice-site mutations, 13 nonsense mutations, 10 frameshift dele-

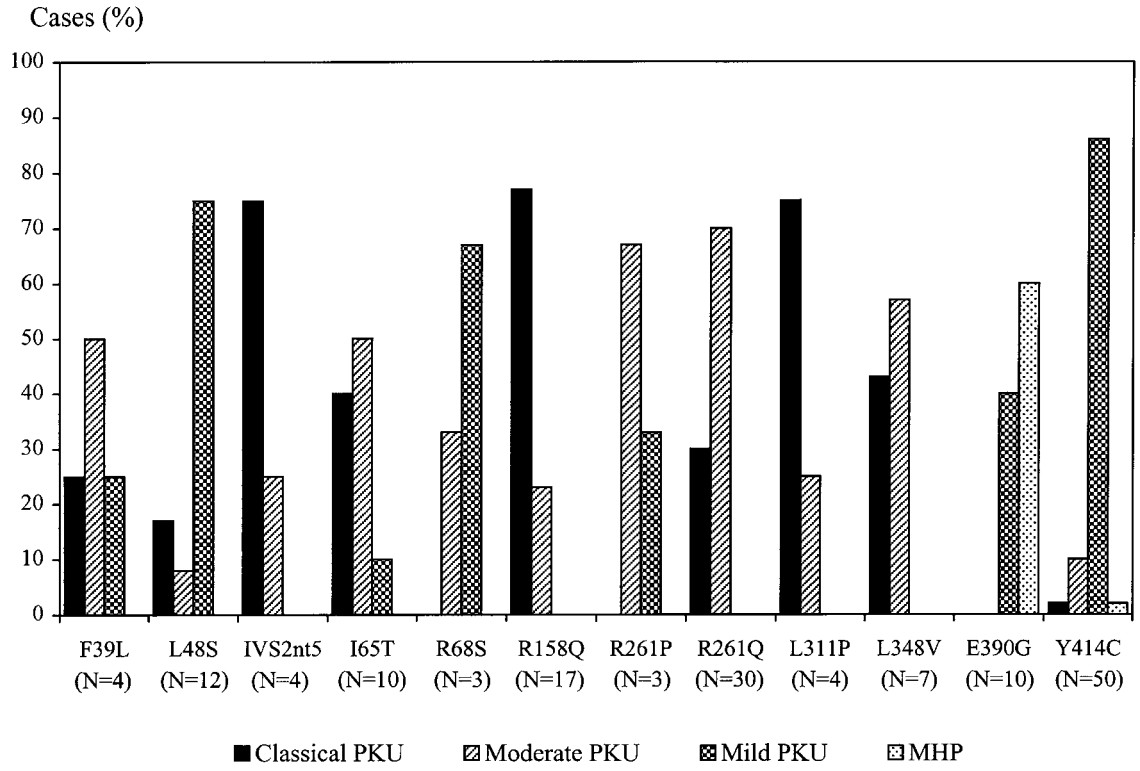

Figure 1 Distribution of phenotypes for the 12 *PAH* mutations that were found in more than one phenotype category, in functionally hemizygous patients.

tions, 3 frameshift insertions, 2 in-frame deletions, and 2 mutations (G10G and Q304Q) that could not be unambiguously classified but that may affect mRNA processing or stability (Richard and Beckmann 1995). On the basis of individual data on phenylalanine tolerance and pretreatment serum phenylalanine levels, the patients were assigned to one of the four arbitrary phenotype categories. A total of 332 patients (48%) had classic PKU; 95 (14%) had moderate PKU; 149 (22%) had mild PKU; and 110 (16%) had MHP.

Classification of Mutations

We have previously suggested that the most reliable and useful approach for the assignment of individual *PAH* mutations to particular phenotype categories would rely on patients in whom the mutant allele acts on its own—that is, in functionally hemizygous patients (Guldberg et al. 1995). Among the 133 different mutations identified in the present study, 37 are known or predicted to completely abolish PAH activity (Appendix). The majority of these mutations are frameshift mutations, splice-site mutations, and base substitutions that introduce a premature stop codon. Base substitutions affecting splice-site consensus regions outside the invariant AG-GT dinucleotides may not completely impair correct mRNA splicing (Krawczak et al. 1992) and, accordingly, were not classified as null mutations. The missense mutations that were considered as null mutations have been shown to result in zero enzyme activity in vitro. The assignment of the 37 mutations as null mutations was in agreement with the general observation, in this and previous studies, that homozygotes and genetic compounds show a classic-PKU phenotype. Among 169 patients with two predefined null mutations, we identified 7 patients who had not been diagnosed as having classic PKU—2 IVS12nt1g→a/IVS7nt1g→a siblings with mild PKU, 1 IVS12nt1g→a homozygote with moderate PKU, and 4 additional patients (genotypes Y356X/P281L, S349P/R252W, and S349P/IVS12nt1g→a, and R408W/IVS12nt1g→a) with moderate PKU. Six of these patients were contributed by one center (see below).

To assess the intrinsic severity of the remaining mutations, we studied the phenotype characteristics in 297 patients who carry a putative null mutation on one of their chromosomes and who therefore can be considered as functionally hemizygous for the mutation on the other chromosome. For 19 mutations, the assignment was unambiguous because the phenotype classification was consistent in two or more functionally hemizygous pa-

Table 1

Proposed Model for Phenotypic Effect of Two Mutant *PAH* Alleles, Expressed as the Sum of Their AVs

	Phenotypic Effect When AV of First Mutation Is			
AV of Second Mutation	1 (Classic PKU)	2 (Moderate PKU)	4 (Mild PKU)	8 (MHP)
1 (Classic PKU)	2 (Classic PKU)	3 (Moderate PKU)	5 (Mild PKU)	9 (MHP)
2 (Moderate PKU)		4 (Moderate PKU/mild PKU)	6 (Mild PKU)	10 (MHP)
4 (Mild PKU)			8 (Mild PKU/MHP)	12 (MHP)
8 (MHP)				16 (MHP)

tients. Thirty-seven mutations were found in only one functionally hemizygous patient each, and their assignment to particular phenotype categories may therefore be somewhat uncertain. For 12 mutations, patients had been recorded in more than one phenotype category (fig. 1). Nine of these mutations (all except R68S, L348V, and E390G) were assigned to two categories by at least one of the seven contributing centers. It is notable that R261Q had been assigned to two phenotype categories (classic PKU and moderate PKU) by five of the centers. In all cases, the mutation was assigned to the category in which it appeared most often. For example, the R158Q mutation was found in 13 functionally hemizygous patients diagnosed as having classic PKU and in 4 patients classified as having moderate PKU and, accordingly, was classified as a classic-PKU mutation.

On the basis of the criteria outlined above, 105 of the mutations were assigned to one of the four phenotype categories (Appendix). Five of the seven mutations that were assigned to the moderate-PKU category were also represented in at least one other phenotype category. Among the MHP mutations, only one (E390G) was occasionally found in patients with mild PKU, suggesting that it confers a phenotype at the border between mild PKU and MHP. On the basis of strict criteria for MHP, E390G would be classified as a mild-PKU mutation (Zschocke et al. 1994).

A Simple Correlation between Genotype and Phenotype

Results from previous studies of PAH deficiency have suggested a simple correlation between genotype and phenotype, implying (1) that the milder of two mutations is "quasi-dominant" and determines the phenotypic outcome and (2) that gene dosage has a significant effect—that is, a patient homozygous for a mutation with residual enzyme activity may exhibit a milder phenotype than is seen in a patient carrying the mutation in the functionally hemizygous constellation. To convert these observations into a more formalized system, we assigned each mutation an arbitrary assigned value (AV): AV = 1, for classic-PKU mutations; AV = 2, for moderate-PKU mutations; AV = 4, for mild-PKU mutations; and AV = 8, for MHP mutations. These values were chosen as the lowest positive whole numbers that allow discrimination between the different mutation combinations. By means of this classification, the phenotype resulting from the combination of two mutant *PAH* alleles may be expressed numerically as the sum of the two mutations' AVs (table 1).

The general validity of this relatively simplistic model was tested in 184 patients who did not carry one of the predefined null mutations—and who therefore were not used for mutation classification—but who were homozygous or compound heterozygous for mutations of assigned severity (Appendix). There were 48 different mutations in these patients. As shown in table 2, the observed phenotype matched the expected phenotype in 145 (78.8%) of the patients. The lowest "concordance rate" (44.8%) was observed for patients with expected moderate-PKU phenotype, whereas the correlation was virtually complete for the two groups expected to show either an MHP phenotype or a phenotype at the border between MHP and mild PKU.

In five patients, the observed phenotype was more than one category away from the expected phenotype. Four of these patients (three R158Q homozygotes and one F299C/R158Q genetic compound with mild PKU) were contributed by one center (see below). The remaining patient had the genotype IVS10nt-11g→a/L48S and was diagnosed as having classic PKU.

Possible Causes of Genotype-Phenotype Inconsistencies

Among 650 patients carrying two mutations that had both been classified with respect to intrinsic severity (Appendix), 88 (13.5%) had been phenotypically classified in disagreement with the genotype-based consensus. To address whether this relatively high number of inconsistencies is due to a true lack of correlation between genotype and phenotype or may relate to intercenter differences in methods and criteria for phenotype classification, the proportion of patients for whom the observed phenotype did not exactly match the expected phenotype was determined, for each of the seven centers. As shown in table 3, the "inconsistency rate" was 4%–23% ($\chi^2 = 28.9$; $P < .0001$). Other observations that may suggest a possible implication of discrepant

Table 2

Observed versus Expected Metabolic Phenotypes in 184 Individuals with PAH Deficiency

AV1 + AV2	Expected Phenotype[a]	No. with Observed Phenotype[b]					Perfect Matches[c] (%)
		PKU					
		Classic	Moderate	Mild	MHP	Total	
2	Classic PKU	42*	11	4	…	57	73.7
3	Moderate PKU	10	13*	6	…	29	44.8
4	Moderate/mild PKU	1	6*	6*	…	13	92.3
5 and 6	Mild PKU	1	3	23*	2	29	79.3
8	Mild PKU/MHP	…	…	10*	10*	20	100
9–16	MHP	…	…	1	35*	36	97.2
Total		54	33	50	47	184	

[a] Determined, for each patient, on the basis of the sum of the AVs of the two *PAH* mutations (according to the entries in the Appendix and table 1).

[b] Groups in which the expected phenotype matches the observed phenotype are marked by asterisks (*).

[c] Between the observed phenotype and the expected phenotype.

procedures for phenotype classification were (1) that 6 of 10 patients for whom the observed phenotype was more than one category away from the expected phenotype were contributed by one center and (2) that one center had classified three patients homozygous for the R158Q mutation as having mild PKU whereas another center had classified four R158Q homozygotes as having classic PKU.

We also addressed whether particular mutations or mutation combinations were prevalent in the 88 patients with discordant phenotypes. This analysis revealed an overrepresentation of three mutations—I65T, R261Q, and R158Q. These mutations were found in 50 (57%) of the discordant patients, whereas they were found in only 14% of the patients for whom there was concordance between expected and observed phenotypes. We did not, however, find any relation between the frequency of these three mutations and the inconsistency rate for the seven contributing centers; for example, the highest proportion of patients carrying I65T, R261Q, or R158Q on at least one chromosome was found in centers "D" (39.5%) and "F" (34.5%), which both showed relatively low inconsistency rates (table 3).

Discussion

We have compiled data on *PAH*-mutation genotypes and corresponding metabolic phenotypes in 686 patients with PAH deficiency who had been referred to seven European centers. This has allowed us to (1) assess the intrinsic severity of 105 *PAH* mutations, (2) test a simple model for correlation between *PAH* genotypes and metabolic phenotypes of PAH deficiency, and (3) address possible explanations for those cases in which the association between genotype and phenotype deviates from that expected.

The main purpose of our study was to determine whether a simple correlation exists between PAH genotypes and metabolic phenotypes, implying (1) that other components of the phenylalanine homeostatic system (reviewed by Scriver et al. 1995) contribute insignificantly to the phenylalanine-hydroxylating capacity in patients with PAH deficiency and (2) that there are generally no relevant positive or negative interactions between two different mutant monomers in the mature PAH tetramer (allelic complementation).

Our data provide both direct and indirect evidence that a simple genotype-phenotype correlation does exist in most patients with PAH deficiency. First, having assigned each of 68 mutations to a particular phenotype category, on the basis of the analysis of functionally hemizygous patients, we observed a perfect match between the expected and observed phenotypes in nearly 80% of the patients who had not been used for the assessment of mutations. It is notable that in only 3% of the patients was the observed phenotype more than one category away from that expected. Second, there was virtually complete association between genotype and phenotype in the group of individuals presenting with MHP in whom phenotype classification was based solely on serum phenylalanine values and was not complicated by a dietary-therapy regimen. Third, the proportion of patients for whom the observed and predicted phenotypes did not coincide differed significantly between the centers, even considering that the centers had contributed unequal numbers of patients for mutation classification. Other things being equal, if all genotype-phenotype inconsistencies were real, they would be expected to be distributed equally among the participating centers.

Taken together, these observations (1) suggest that a significant proportion of patients may have been misclassified and (2) underline previous observations that

Table 3

Patients Carrying Two *PAH* Mutations Who Could Be Classified, According to Data in the Appendix

	No. of Patients (Rate)							
	Center							
	A	B	C	D	E	F	G	Total
Total	174	50	145	41	60	55	125	650
Inconsistent[a]	7 (.04)	8 (.16)	33 (.23)	7 (.17)	4 (.07)	4 (.07)	25 (.25)	88

[a] Observed phenotype did not match expected phenotype (according to data in table 1).

genotypes and phenotypes, although exact in their nature, may be difficult to accurately assess. Some of the observed inconsistencies may be due to the inadequacy of some mutation-detection assays, as has recently been reported in a cystic fibrosis quality-control study (Cuppens and Cassiman 1995). Most centers participating in the present study had used diagnostic assays, such as allele-specific PCR and restriction-enzyme analysis, to rapidly screen for the most common *PAH* mutations. Since two mutations had been identified in all cases, the majority of incorrect typings possibly relating to the use of these methods would be due to false-positives. Alternatively, the use of methods, including sequencing, that examine only parts of the *PAH* gene would leave double mutant alleles undetected (Savov et al. 1995; Guldberg et al. 1996). Nevertheless, we also noticed genotype-phenotype inconsistencies among patients who had been genotyped by whole-gene DGGE scanning and sequence analysis—for example, in the case of a classic-PKU patient with genotype IVS12nt1g→a/Y414C.

Other classification discrepancies may relate to the arbitrary four-level phenotype-classification system used here, which does not fully respect the continuum of PAH phenotypes and which will appear inadequate for classification of phenotypes that occur at the border between two different classes. It is noteworthy in this respect that many of the patients with phenotypes deviating from the majority carried mutations that have been associated with residual in vitro activities in the range of 10%–30% and have been assigned to the moderate-PKU category (AV = 2). The moderate-PKU category, intermediate between classic PKU and mild PKU, has not been used routinely by some of the participating centers and, in general, may be difficult to assess. If moderate PKU were excluded as a separate category and if classic PKU and mild PKU were extended to include the phenylalanine-tolerance levels of moderate PKU, the number of genotype-phenotype inconsistencies observed in this study would be significantly reduced.

Another interesting observation was that one of the centers with a high inconsistency rate generally had diagnosed its patients as having a phenotype more severe than would be expected on the basis of the genotype. A closer look at the therapeutic guidelines at this center revealed that the patients were generally kept at a lower serum phenylalanine level than patients from the other centers, which would cause a relatively lower phenylalanine tolerance and, accordingly, a shift in genotype-phenotype correlations. Other genotype-phenotype discordances may be due either to similar discrepancies from the standard phenylalanine-tolerance classification criteria—including both more or less strict adherence to the target phenylalanine level and age of patients at the time of testing—or to recording of phenylalanine tolerance in periods of growth-rate fluctuation.

Despite the different lines of evidence speaking in favor of a simple correlation between *PAH* genotypes and PAH-deficiency phenotypes, we also identified a number of cases that may not be explained by genotypic or phenotypic misclassifications. One example of such discrepancy is the multiple phenotypic associations of the I65T mutation. In line with previous studies (John et al. 1992; Desviat et al. 1997; Kayaalp et al. 1997), we identified four functionally hemizygous patients with classic PKU, five patients with moderate PKU, and one patient with mild PKU. A potential cause of these inconsistencies may relate to the biological properties and functions of the mutant protein. I65T affects a residue that may reside in the putative regulatory phenylalanine-binding site of PAH (Hufton et al. 1995), implying that the activity of the enzyme may be subject to regulation by substrate concentrations. Accordingly, the phenylalanine tolerance in patients under treatment may depend on the target plasma phenylalanine levels. Several of the authors of the present article have noticed that some patients with mild PKU (such as patients who are Y414C genetic compounds) have a relatively low phenylalanine tolerance when treated, although plasma phenylalanine levels were only a little above the therapeutic threshold when these patients were untreated (J. Zschocke, G.F. Hoffman, P. Burgard, H. Schmidt, unpublished observation). We are presently investigating the possibility that phenylalanine levels may modulate the effect of I65T and other mutations—including R261Q, R158Q, L48S, and Y414C—that are frequently represented in discordant genotype-phenotype associations.

In conclusion, our data lend further support to the long-standing notion that allelic variation at the *PAH*

locus is the major determinant of the metabolic phenotypes of PAH deficiency. They also suggest a simple correlation between *PAH* genotype and metabolic PAH deficiency phenotype, which implies (1) that disease severity in most cases is determined by the least severe of two *PAH* mutations and (2) that two mutations with similar severity may confer a milder phenotype than either of the mutations would do if it acted alone. The general observation that phenotype in patients with PAH deficiency may be predicted solely on the basis of prior knowledge about the individual *PAH* mutations, rather than on the basis of knowledge about the effect of specific mutation combinations, together with the classification of >100 mutations in the present study, may form the basis for assessment of the phenotypic outcome in the majority of possible mutation combinations in Caucasians. In some patients there are inconsistencies in the relationship between genotype and phenotype that require further investigation, and neonatal screening and regular monitoring of blood phenylalanine levels remain the primary strategy for the diagnosis and treatment of PKU. Nevertheless, mutation analysis in the newborn with hyperphenylalaninemia can provide, at a very early stage, additional information on the biochemical phenotype. This is useful for counseling of patients' families and for the clinical management of patients, particularly when, because of borderline phenylalanine levels, it is unclear whether treatment is required.

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Appendix

Assignment of *PAH* Mutations to Metabolic Phenotypes and AVs, Based on Phenotype Characteristics of Functionally Hemizygous Individuals (Number of Patients/Number of Centers)

Classic PKU		Moderate PKU	Mild PKU	MHP	Unclassified
M1V[a]	E280K (7/2)	F39L (2/1)[b]	(F39L) (1/1)	A47V (1/1)	G10G
Q20X[a]	P281L[a]	(L48S) (1/1)	G46S (5/1)	S87R (2/2)	delF39
IVS1nt5g→t (1/1)	IVS7nt1g→a[a]	(IVS2nt5g→c) (1/1)	L48S (9/2)[b]	T92I (1/1)	L41F
(F39L) (1/1)	IVS7nt5g→a (1/1)	I65T (5/2)[b]	T63P/H64N (1/1)	R155H (1/1)	S67P
(L48S) (2/2)	D282N (1/1)	(R68S) (1/1)	(I65T) (1/1)	G171A (1/1)	delI94
F55L (1/1)	H285Y (1/1)	(R158Q) (4/1)	R68S (2/2)[b]	R176L (1/1)	L98S
F55fsdelT[a]	S295X[a]	E6nt-96A→g (1/1)	A104D (9/3)	E178G (1/1)	R158W
IVS2nt5g→c (3/1)[b]	F299C (1/1)	R261P (2/1)[b]	IVS4nt-5c→g (1/1)	V190A (1/1)	N167I
(I65T) (4/3)	IVS8nt1g→a[a]	R261Q (21/7)[b]	I164T (1/1)	V230I (3/1)	C203Y
D84Y (1/1)	IVS8nt-7a→g (1/1)	(L311P) (1/1)	V177A (1/1)	R241C (1/1)	P211T
P89fsinsC[a]	S310fsdel11bp[a]	L348V (4/2)[b]	R241H (1/1)	V245A (7/3)	G218V[c]
I94S (1/1)	L311P (3/2)[b]	V388M (1/1)	A246V (1/1)	A300S (7/4)	V245E
R111X[a]	F327L (1/1)	(Y414C) (5/2)	(R261P) (1/1)	I306V (3/1)	L249F
R158Q (13/5)[b]	F331L (1/1)		Y277D (1/1)	T380M (1/1)	M276V
I174T (2/1)	Q336X[a]		G344S (1/1)	E390G (6/3)[b]	IVS7nt-2a→t[c]
R176X[a]	A342T (1/1)		(E390G) (4/2)	A403V (14/5)	I283F
W187X[a]	A342fsdelG[a]		R408Q (3/2)	R413S (1/1)	S303P
L194P (1/1)	G346fsdelG[a]		Y414C (43/5)[b]	(Y414C) (1/1)	Q304Q
L197fsdel22bp[a]	G346R (2/1)			D415N (5/3)	A309V
Y198fsdel22bp[a]	(L348V) (3/2)				P314H[d]
Y204X[a]	S349P[a]				L333F[c]
Y206X[a]	G352R (2/1)				S350T[c]
E221D222fsdelAG[a]	IVS10nt-11g→a (29/7)				IVS10nt3a→g
S231P[a]	IVS10nt-3c→t (2/1)				P366H
G239S (1/1)	IVS10nt-1g→a[a]				Y386C[c]
R243Q (4/3)	Y356X[a]				D394A
R243X[a]	S359X[a]				A395G
R252G (1/1)	K363fsdelG[a]				IVS12nt2t→c[c]
R252Q (2/2)	R367fsinsC[a]				
R252W[a]	A395P (2/2)				
A259V (1/1)	IVS11nt1g→a[a]				
(R261Q) (9/5)	P407fsdelC[a]				
R261X[a]	R408W[a]				
I269N (1/1)	(Y414C) (1/1)				
G272X[a]	IVS12nt1→a[a]				
K274fsdel11bp[a]	K452fsinsA[a]				

[a] Putative null mutation.

[b] Also represented in other phenotype categories, although at lower frequencies. In these other categories, the mutation is in parenthesis.

[c] Identified in at least one patient with classic PKU and, therefore, may be formally classified as a classic-PKU mutation.

[d] Identified in two functionally hemizygous patients, one of which had classic PKU and the other of which had mild PKU.

Electronic-Database Information

URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim (for PAH)

PAH Mutation Analysis Consortium Database, http://www.mcgill.ca/pahdb (for PAH mutations)

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