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Human Phenylalanine Hydroxylase Mutations and Hyperphenylalaninemia Phenotypes: A Metanalysis of Genotype-Phenotype Correlations

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Summary

We analyzed correlations between mutant genotypes at the human phenylalanine hydroxylase locus (gene symbol *PAH*) and the corresponding hyperphenylalaninemia (HPA) phenotypes (notably, phenylketonuria [OMIM 261600]). We used reports, both published and in the *PAH* Mutation Analysis Consortium Database, on 365 patients harboring 73 different *PAH* mutations in 161 different genotypes. HPA phenotypes were classified as phenylketonuria (PKU), variant PKU, and non-PKU HPA. By analysis both of homoallelic mutant genotypes and of "functionally hemizygous" heteroallelic genotypes, we characterized the phenotypic effect of 48 of the 73 different, largely missense mutations. Among those with consistent in vivo expression, 24 caused PKU, 3 caused variant PKU, and 10 caused non-PKU HPA. However, 11 mutations were inconsistent in their effect: 9 appeared in two different phenotype classes, and 2 (I65T and Y414C) appeared in all three classes. Seven mutations were inconsistent in phenotypic effect when in vitro (unit-protein) expression was compared with the corresponding in vivo phenotype (an emergent property). We conclude that the majority of *PAH* mutations confer a consistent phenotype and that this is concordant with their effects, when known, predicted from in vitro expression analysis. However, significant inconsistencies, both between in vitro and in vivo phenotypes and between different individuals with similar *PAH* genotypes, reveal that the HPA-phenotype is more complex than that predicted by Mendelian inheritance of alleles at the *PAH* locus.

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Introduction

Phenylketonuria (PKU [OMIM 261600]) and allied forms of hyperphenylalaninemia (HPA) (Scriver et al. 1995) are among the most widely ascertained "rare" Mendelian traits in humans, because newborn screening for HPA has become a universal public-health practice in many regions of the world. Now that the major gene (phenylalanine hydroxylase; symbol *PAH*) harboring mutations causing HPA has been characterized, mutation analysis at the *PAH* locus is feasible.

Ever since its discovery in 1934, it has been customary to consider PKU as a typical Mendelian trait with autosomal recessive inheritance. However, within the first 2 decades of its discovery, it became apparent that the disorder is also "multifactorial," with inherited (genetic) and acquired (dietary) components, both of which are necessary to establish the variant metabolic phenotype (HPA); accordingly, the latter can be modified by diet (Penrose 1946; Bickel et al. 1954; Armstrong and Tyler 1955; Woolf et al. 1955). Thereafter, it became apparent that HPA also reflects locus heterogeneity (Scriver et al. 1995); although the vast majority of mutations responsible for HPA map to the *PAH* locus, some occur at loci controlling the synthesis and recycling of tetrahydrobiopterin, the essential cofactor for catalytic activity of phenylalanine hydroxylase enzyme. Finally, after the *PAH* gene had been cloned, characterized, and made accessible to mutation analysis (Woo et al. 1983; Kwok et al. 1985; Lidsky et al. 1985; DiLella et al. 1986; Konecki et al. 1992), many recessive mutant alleles were identified at the *PAH* locus—indeed, so many (>325 different disease-associated mutations and many neutral polymorphic alleles) that, to record them, a locus-specific database (Nowacki et al. 1997) has been developed by an international consortium.

The metabolic HPA phenotype appeared, at first, to correlate broadly with genotype; "severe" mutations caused PKU, and "mild" ones caused non-PKU HPA (Okano et al. 1991*a*; Scriver 1991). However, this now appears to be an oversimplification. We have analyzed data on genotype/phenotype correlations, either pub-

Table 1

Classification, by Type, of 73 *PAH* Mutations Carried by 365 HPA Individuals

Mutation Type	Mutations
Missense ($n = 44$)	F39L, L41F, K42I, A47V, L48S, I65T, S67P, S87R, T92I, A104D, R158Q, I164T, G171A, R176L, L194P, P211T, G218V, D222V, V230I, G239S, R241H, P244L, V245A, R252Q, R261Q, Y277D, E280K, I283F, A300S, I306V, A322G, F331L, D338Y, L348V, T380M, Y386C, V388M, E390G, D394A, A395P, A403V, R408Q, Y414C, D415N
Null[a] ($n = 29$)	
Demonstrated[b] ($n = 7$)	M1V, G46S, R252W, A259V, P281L, F299C, S349P, R408W
Protein truncation ($n = 7$)	R111X, W187X, Y204X, R243X, R261X, G272X, S359X
Deletion ($n = 2$)	delF39, delL364
Frameshift ($n = 4$)	F55fsdelT, T186/W187fsdelAT, K363fsdelG, P407fsdelC
Splice defective ($n = 8$)	IVS2nt5, IVS4nt−5, IVS6nt−2, IVS7nt1, IVS9nt−2, IVS10nt−11, IVS11nt−8, IVS12nt1

[a] The term implies a nonfunctional PAH enzyme phenotype.

[b] Null effect demonstrated by in vitro expression analysis. A null phenotype effect was declared when enzyme activity was stated to be below the level of detection in the system (typically, <3% or <1% of normal).

lished or available through the *PAH*-mutation database, in 365 persons harboring 161 different mutant *PAH* genotypes derived from 73 different mutant alleles. Whereas the majority of *PAH* alleles are consistent in their effects on phenotype and are broadly predictive of HPA severity, we show that the observed metabolic phenotype is not always consistent with the predicted effect of genotype at the *PAH* locus. The findings indicate that the HPA phenotype is a complex trait (an emergent property), whereas inheritance of the mutant genotype is Mendelian.

Subjects and Methods

Source of Data

Subjects (cases) were identified both from submissions to the *PAH* Mutation Analysis Consortium Database (http://www.mcgill.ca/pahdb) and from published sources; the latter are listed in the Appendix. Data unpublished but submitted to the *PAH* database and listed there as formal entries with accession numbers ("electronic publications") are identified in the appropriate spreadsheets and can be located in the database.

How Reports Were Selected

Only reports that described the mutant *genotype* (i.e., both mutant *PAH* alleles) were selected, which then were reviewed for information about *phenotypes*. To define the latter, we used (i) plasma phenylalanine levels either in the newborn, prior to the initiation of dietary treatment, or later in life, either under conditions off diet or by using controlled protein loading during the treatment period, and (ii) the dietary phenylalanine tolerance, to achieve and maintain "safe" plasma phenylalanine levels (below *approximately* 500 μM) during either late infancy or the first 5 years of childhood, as reported by the authors. We gave priority to dietary-tolerance data. When information about phenotype was incomplete or ambiguous, we contacted the report authors. Only those patients with corresponding data on genotype and phenotype are included in the present report. In this way, we identified 365 patients.

Criteria for Classification and Phenotype

We divided phenotypes associated with a mutant *PAH* genotype into three broad categories: phenylketonuria (PKU), variant PKU, and non-PKU HPA (Güttler et al. 1987; Scriver et al. 1995). The more stringent the dietary phenylalanine tolerance (i.e., when <500 mg/d), and the higher the plasma phenylalanine value in the untreated state (i.e., when >1,000 μM), the more PKU like is the phenotype, with high risk of severely impaired cognitive development. If the plasma phenylalanine value is consistently above normal (i.e., >120 μM) but <1,000 μM when the patient is on a normal diet, then the phenotype is more like non-PKU HPA and apparently is associated with a much lower risk of impaired cognitive development. The variant PKU category is, by default, the one that unambiguously fits neither PKU nor non-PKU HPA.

Process

Primary data were retrieved from published or in-press reports (see Appendix) and from the database http://www.mcgill.ca/pahdb. Each case was entered on a spreadsheet (Microsoft Excel software), as a separate row, under defined fields describing genotype, plasma

Table 2

Homoallelic Subjects (n = 109) and Corresponding HPA Phenotype, Related to Available Data from In Vitro Expression in Mammalian Cells

Mutation[a]	No. of Patients			PAH Activity in COS Cells in Vitro[b] (% of Normal)
	PKU	Variant PKU	Non-PKU HPA	
M1V	3			<3
IVSnt5	1			
I65T	1[c]	1[c]	1	26
R158Q	2[c]			10
T186/W187fsdelAT	1			
R252W	1			<1
R261Q	3[c]	8[c]		30
R261X	2			
E280K	6			1–3
P281L	5			<1
F299C	1			<3
I306V			1	
IVS9nt−2	1			
S349P	1			<1
IVS10nt−11	8			
K363fsdelG	1			
V388M		4[c]		43
R408Q			4	55
R408W	30			<1
Y414C		4[c]	4	~50
IVS12nt1	15			

[a] Homoallelic state.

[b] From Waters et al. (1998); also see in vitro expression data field at http://www.mcgill.ca/pahdb

[c] In vitro and in vivo phenotypes are inconsistent.

phenylalanine value, dietary tolerance, and other features. The source was given an identifier number (see Appendix), and each subject was given a unique identifier.

Information about genotype and phenotype was then collated, for each person, on a second spreadsheet, in which the uppermost rows describe *PAH* mutations at the 5′ end of the gene, in homoallelic or heteroallelic states, proceeding toward the 3′ end as one reads down the table. Each genotype (row) is keyed ultimately to the individual (by identifiers). The number of individuals in each cell is thus known. The complete data set can be found in the *PAH* mutation database (for a link to the "Supporting Data Repository," where the data are available as a compressed file and a table in HTML, see the homepage http://www.mcgill.ca/pahdb).

Data on In Vitro Expression Analysis

By the time that the present report was written, 35 human *PAH* mutations, putatively impairing enzyme activity in vivo, had been studied by in vitro (unit protein) expression analysis (Waters et al. 1998). These data were compared with corresponding in vivo phenotypes in patients analyzed in the present study.

Use of Null Mutations

We used putative "functionally hemizygous" genotypes (missense/null) (Guldberg et al. 1995; Romano et al. 1996) to evaluate the effect of missense alleles in many patients. The following nulls, grouped as in table 1, were useful for this purpose: M1V, G46S, R252W, A259V, P281L, F299C, S349P, and R408W; R111X, W187X, Y204X, and S359X; delF39; F55fs, and P407fs; IVS4nt−5, IVS6nt−2, IVS7nt1, and IVS11nt−8.

Nomenclature

It has been the convention in the *PAH* Mutation Analysis Consortium to use "trivial names" (Ad Hoc Committee 1996; Beutler et al. 1996). The first letter is the reference amino acid (single-letter code), the number is the *PAH* codon (cDNA [GenBank U49897]), and the second letter indicates the amino acid substituted by a missense mutation. The letter "X" denotes a nonsense (stop) mutation; "del" denotes a deletion; and "fs" denotes a frameshift. Splice mutations are named on the basis of intron and region, with positive numbers indicating a nucleotide change at the 5′ end of the intron and with negative numbers indicating such a change at the 3′ end. Use of systematic names is recommended (see S. Antonarakis [http://ariel.ucs.unimelb.edu.au:80/~cotton/antonara.htm]); they are given in the *PAH* Mutation Analysis Consortium Database.

Results

Seventy-three different *PAH* mutations, all presumed to be phenotype modifying, were inherited in 161 genotype combinations, by 365 patients in our study. The mutations are classified as missense or null (putative or proved) (table 1). The majority (n = 258 [71%]) of subjects were heteroallelic, but, of these, only 181 were "functionally hemizygous."

Homoallelic Mutant PAH *Genotypes*

There were 21 different homoallelic mutant genotypes harbored by 109 individuals (table 2). All other things being equal, the corresponding in vivo phenotypes should reflect an effect of the mutation on *PAH* enzyme activity and thus on the HPA phenotype. We classified 15 *PAH* mutations as "severe" (the homoallelic state confers the PKU phenotype); 2 mutations (I65T and V388M) conferred the variant PKU status; and 2 others (I306V and R408Q) were classified as "mild" (non-PKU HPA). Notably, two different homoallelic mutant genotypes conferred more than one HPA phenotype in different probands; R261Q/R261Q was associated with either PKU or variant PKU, and Y414C/Y414C was associated with either variant PKU or non-PKU HPA.

Table 3

Heteroallelic Subjects (n = 181) with "Functionally Hemizygous" (Missense/Null) Genotypes, and Corresponding HPA Phenotype, Related to Available Data from In Vitro Expression of Missense Alleles in Mammalian Cells

Mutation	No. of Patients			PAH Activity in Vitro (Cell Type)[a] (% of Normal)
	PKU	Variant PKU	Non-PKU HPA	
F39L	2			
G46S	10	1[b]		Not measurable (human kidney)
A47V			1	
L48S	7	4		
I65T	4[b]	3[b]	1	26
S87R			1	
T92I			2	
A104D		5		26 (human kidney)
R158Q	6[b]	3		10
I164T	1			
G171A		1		
R176L			1	
L194P	1			
P211T	1			
D222V	1			
V230I			1	
G239S	1			
V245A			3	
R252Q	1			
R261Q	19[b]	5[b]		30
E280K	1			1–3
I283F	1			
A300S	1		3	
I306V			3	
A322G			3	75
F331L	1			
A359P	1			
T380M			2	
V388M	7[b]			43
E390G		1	1	
A403V		2	1	
R408Q		1[b]		55
Y414C	6[b]	44[b]	6	~50
D415N			9	

[a] From Waters et al. (1998); also see in vitro expression data at http://www.mcgill.ca/pahdb

[b] In vivo and in vitro phenotypes are inconsistent.

Accordingly, the homoallelic genotypes are, in general, predictive, but sometimes they reveal an apparent inconsistency in the effect of *PAH* genotype on the HPA phenotype.

Heteroallelic Mutant PAH *Genotypes*

Thirty-five different missense *PAH* mutations were inherited in combination with a putative null mutation, by 181 "functionally hemizygous" individuals (table 3); 11 alleles consistently conferred the severe PKU phenotype, 10 conferred the non-PKU HPA phenotype, and 3 conferred the variant PKU phenotype. The remaining 11 mutations were inconsistent in effect, because each was associated with more than one HPA phenotype.

Discordance in Classification by In Vitro (Unit Protein) and In Vivo (Metabolic) Phenotype

Predicted and observed phenotypes were not always concordant. The discrepancies were most apparent with the R158Q, R261Q, V388M, and Y414C mutations in the homoallelic state (table 2) and with these and other mutations (G46S and R408Q) in the "functionally hemizygous" state (table 3). These findings show that the in vivo (metabolic) phenotype is not necessarily the equivalent of the in vitro enzymic (unit protein) phenotype.

Interindividual Inconsistency in the In Vivo PAH-*Mutation Effect*

Thirty-seven *PAH* mutations could be classified with reasonable confidence (table 4), but 11 were inconsistent in their effect on phenotype in vivo. Whereas one can imagine how a particular mutation might, by misclassification of phenotype, be associated with two adjacent phenotype classes (e.g., either PKU and variant PKU or variant PKU and non-PKU HPA), it is unlikely that a mutation would appear either in both outlier classes (e.g., A300S in both PKU and non-PKU HPA) or in all three classes (e.g., I65T and Y414C) in a large number of probands (e.g., 55 patients with the Y414C mutation). Although, for classification of allele effect, the use of a functionally hemizygous genotype (missense/null; table 3) is less robust than a homoallelic genotype (table 2), both approaches reveal a similar problem: the HPA phenotype is not always consistent with the corresponding *PAH* genotype.

We identified further ambiguities, particularly in the functionally hemizygous state. Two probands, one with the Y204X/IVS4nt−5 genotype and the other with S349P/IVS10nt−11, both expected to have nonfunctional enzyme phenotypes, had the variant PKU phenotype (data shown in the field [http://www.mcgill.ca/pahdb]) .

Discussion

We analyzed 31 reports documenting 365 persons with persistent HPA due to mutations at the *PAH* locus on chromosome 12q24.1; these persons have 161 different mutant *PAH* genotypes arising from 73 different HPA-producing alleles. We did not include an analysis of the associated (neutral) polymorphic marker haplotypes at the *PAH* locus on which the HPA mutations occur; hence, we have not excluded the possibility that a polymorphic allele might modify expression of the *PAH* gene. In this metanalysis there were several instances of "inconsistent" genotype-phenotype correla-

Table 4

Classification of *PAH* Mutations, According to Their In Vivo HPA Phenotypes

	Mutation Type ($n = 48$)[a]	
In Vivo Phenotype	Missense	Null
Consistent:[b]		
PKU	F39L, I164T, L194P, P211T, D222V, G239S, R252Q, E280K, I283F, F331L, A359P	M1V, IVS2nt5, T186/W187fsdelAT, R252W, R261X, P281L, F299C, IVS9nt−2, S349P, IVS10nt−11, K363fsdelG, R408W, IVS12nt1
Variant PKU	A104D, G171A, D338Y	
Non-PKU HPA	A47V, S87R, T92I, R176L, V230I, V245A, I306V, A322G, T380M, D415N	
Inconsistent:[c]		
PKU or variant PKU	G46S, L48S, R158Q, R261Q, V388M	
Variant PKU or non-PKU HPA	E390G, A403V, R408Q	
PKU or non-PKU HPA	A300S	
All:	I65T, Y414C	

[a] Expressed in either the homoallelic or heteroallelic (missense/null) state.

[b] Unambiguous expression of mutations.

[c] Ambiguous expression of mutations.

tions; therefore, we took special efforts to confirm that the reported assignments of HPA phenotype and *PAH* genotype were correct in these subjects (see How Reports Were Selected section, above).

Our findings reveal several features common to most genetic diseases due to mutation at a major locus (Weiss 1996); for example, the *PAH* locus harbors many alleles, most of which are probably unique by descent and rare in prevalence; most *PAH* alleles are dependent on history (migration and range expansion) for their present geographic distribution; most probands with HPA due to PAH-enzyme deficiency are heteroallelic; and genotype-phenotype relationships are more complex than would be predicted for a Mendelian disorder. Thus, the challenge to understand PKU is no different than that for so many other genetic diseases.

Our report is a first approximation of genotype-phenotype correlations in a large sample of HPA patients. In broad terms, the findings corroborate those made in earlier, pioneering studies (Okano et al. 1991*a*; Svensson et al. 1993; Trefz et al. 1993), in others listed here in the Appendix, and in a forthcoming multicenter report (F. Güttler, personal communication). In brief, there are *PAH* mutations with consistent severe (PKU-like) effects, and there are others that consistently cause only minimal HPA; and, as noted by others (Güttler et al. 1993), there are alleles associated with an intermediate degree of HPA. On the other hand, how mutations actually modify the gene product (the PAH-enzyme subunit) and alter tertiary structure and catalytic activity of the homotetrameric enzyme is still poorly understood. Some *PAH* mutations appear to affect protein stability, and a few will affect substrate or cofactor kinetics (Waters et al. 1998). Meantime, three different approaches are being used to analyze *PAH* genotype-phenotype relationships: (i) in vitro expression analysis of inherited human mutations (Waters et al. 1998), (ii) site-directed mutagenesis and in vitro analysis of the corresponding mutant rat enzyme (Gibbs et al. 1993; Dickson et al. 1994; Kowlessur et al. 1995; Quinsey et al. 1997), and (iii) analysis of phenylalanine metabolism in a person with a classified mutant HPA phenotype (Treacy et al. 1996, 1997). Because our analysis focuses on HPA phenotypes in vivo, it deals with an "emergent property," which is not the equivalent of the unit-protein enzymic phenotype measured by in vitro expression analysis of *PAH* mutations. "Emergence" (or the emergent property) is that peculiarity in which the character of the whole cannot be deduced from even complete knowledge of the components taken separately, in partial combinations, or in hierarchical combinations (Mayr 1982, pp. 63–67).

Whereas it appeared initially that to know the *PAH* genotype would reliably predict the HPA phenotype (Okano et al. 1991*a*; Scriver 1991), it now seems that this will not always be the case, for at least five reasons. First, there is both the evidence of great allelic heterogeneity at the *PAH* locus and a high probability that the proband (in any outbred population) will be heteroallelic—and, therefore, a correspondingly greater potential for variant interactions between mutant *PAH*-enzyme subunits, as well as a corresponding effect on phenylalanine hydroxylation; second, there is evidence for intrafamilial inconsistency in HPA phenotypes, explained in some families by segregation of more than two different *PAH* alleles (Ledley et al. 1986; Guldberg et al. 1995) but in other families attributed to nonallelic factors (Di Silvestre et al. 1991; Tyfield et al. 1995; Treacy et al. 1996); third, phenylalanine outflow from the plasma pool involves two factors, each with high-sensitivity coefficients (Kacser and Burns 1981; Salter et al. 1986)—namely, transport into hepatocytes and phenylalanine hydroxylation—as well as other factors, including protein incorporation, transamination, and regulation (transcriptional and post transcriptional) of *PAH*-enzyme activity, none of which (excepting hydrox-

ylation) is under *PAH*-locus control and each of which is eligible for an effect of allelic variation; fourth, biological individuality clearly does contribute to the moment-to-moment control of plasma phenylalanine value (Scriver and Rosenberg 1973, pp. 290; Scriver et al. 1985); and fifth, cosegregation of a second mutation in *cis* on a mutant *PAH* allele may have an effect on phenotype (Guldberg et al. 1996); the possible effect of a polymorphic allele has already been mentioned .

There are indeed broad correlations between mutant *PAH* genotypes and HPA phenotypes; they will have real practical value and will give guidance for counseling and treatment of HPA persons. However, our particular interest is in the evidence both for inconsistencies among subjects with similar genotypes and for discordance between the in vitro and in vivo effects of some mutant alleles. The latter effects imply that it will always be better to observe and monitor the phenotype in the particular individual than to assume that it can always be predicted with confidence.

The inconsistencies and discordances seen here were of two types: First, the in vivo phenotype did not necessarily fit that predicted on the basis of in vitro expression analysis. The reasons why there may be a discrepancy between the unit-protein phenotype in vitro and that observed in vivo have been discussed more extensively elsewhere (Waters et al. 1998). Here, we briefly note that protein expression of the in vitro systems is both high level and transient. In addition, the in vitro expression of a single mutant allele produces a homoallelic unit-protein phenotype, whereas most HPA subjects are heteroallelic for PAH mutations. Accordingly, the possibility of allelic complementation, which is not analyzed in vitro in the conventional systems, will have to be studied in a "yeast 2–hybrid system," for example. Meanwhile, the R261Q allele, documented here and elsewhere (Apold et al. 1993; Kunert et al. 1993; Burgard et al. 1996), is an interesting example of possible negative complementation in vivo, in a manner not apparent in vitro.

The second form of inconsistency reveals itself in patients who have different phenotypes yet similar genotypes and was seen here with the I65T and Y414C mutations. It is unlikely that misclassification of phenotype or genotype is the explanation for these inconsistencies. They imply that events other than expression of the mutant genotype at the major (i.e., *PAH*) locus itself contribute to the HPA phenotype in the patient. Elsewhere, by means of in vivo isotopic studies, we have shown that phenylalanine hydroxylation per se does not fully account for the in vivo disposal of phenylalanine in some HPA probands (Treacy et al. 1996, 1997). What the events are and how they explain the inconsistencies shown here remain unknown. The present findings simply suggest that the HPA phenotype, largely accounted for by allelic variation at the *PAH* locus, sometimes behaves as a complex trait controlled by events not fully accounted for by those seen at the major locus itself—an idea expressed earlier and by others in the history of PKU research (Langenbeck et al. 1988; Scriver et al. 1995).

We are not the first to notice discordance between the mutant *PAH* genotype and a corresponding phenotype. Untreated PKU patients do not have IQ scores fully concordant with the predicted severity of the *PAH* genotype (Ramus et al. 1993). However, IQ is indeed a complex trait (or emergent property), and close correlation between its metrical value in PKU subjects and the *PAH* genotype would surely be unlikely. Our own analysis of genotype-phenotype correlations shows that the HPA component, which functionally links the *PAH* genotype with IQ, can itself behave as a complex trait.

Appendix

Table A1

Published Sources of Cases

Reference or Source	Patient ID[a]	Article Number[b]
Bénit et al. (1994)	PB94.*xx*	1
Desviat et al. (1995)	LD95.*xx*	2
Dianzani et al. (1995)	ID95.*xx*	3
Dianzani and Knappskog (1995)	ID95.*xx*	4
Dworniczak et al. (1991)	BD91.*xx*	5
Eiken et al. (1996*b*)	HGE95b.*xx*	6
Eiken et al. (1996*a*)	HGE96a.*xx*	7
Guldberg et al. (1994*b*)	PG94b.*xx*	8
Guldberg et al. (1994*a*)	PG94a.*xx*	9
Guldberg et al. (1995)	PG95.*xx*	10
John et al. (1992)	SWMJ92.*xx*	11
Kalaydjieva et al. (1992)	LK92.*xx*	12
Kleiman et al. (1993)	SK93.*xx*	13
L. Kozak (submission to database, 1996)	LK96.*xx*	14
Kunert et al. (1993)	EK93.*xx*	15
Lyonnet et al. (1989)	SL89.*xx*	16
Martinez-Pardo et al. (1994)	MP94.*xx*	17
Okano et al. (1990)	YO90.*xx*	18
Okano et al. (1991*b*)	YO91b.*xx*	19
Pérez et al. (1994)	BP94.*xx*	20
Pérez et al. (1995)	BP95.*xx*	21
Romano et al. (1996)	VR96.*xx*	22
Svensson et al. (1992)	ES92.*xx*	23
Svensson et al. (1993)	ES93.*xx*	24
E. Treacy (personal communication)	ET.ML96.*xx*	25
Tyfield et al. (1995)	LT95.*xx*	26
L. Tyfield (submission to database, 1995)	LT95.*xx*	27
Weinstein et al. (1993)	MW93.*xx*	28
Zschocke et al. (1994)	JZ94.*xx*	29
Zygulska et al. (1991)	MZ91.*xx*	30
Zygulska et al. (1994)	MZ94.*xx*	31

[a] For all 365 cases studied, the code comprises the initials of the first author of the reference, the year of publication, and the case number (here denoted as "*xx*"), the latter of which has been assigned on the basis of the order of appearance in the reference.

[b] As used in the complete table of genotype-phenotype correlations available on homepage http://www.mcgill.ca/pahdb (see directions for "supporting data repository").

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