Phenylalanine Hydroxylase (PAH) Genotyping in PKU Argentine Patients

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Abstract

Phenylketonuria (PKU, OMIM 261600) is predominantly caused by mutations in the *PAH* gene. One hundred and three Argentine PKU patients were studied by Sanger sequencing; 101 were completely characterized (90.3% were compound heterozygotes). Fifty-four different pathogenic variants were identified. Mutations were distributed all along the PAH gene but concentrated in exon 7 (26%), 12 (12%), 11 (10%), and 6 (10%). 77% were missense, and 77% affected the enzyme catalytic domain, nine mutations accounted for 57% of 179 studied alleles: p.Arg261Gln (Allele frequency(AF):10.6%), c.1066-11G>A (AF:9,5%), p.Arg408Trp (AF:8,3%), p.Tyr414Cys (AF:5,5%), p.Ala403Val, p.Val388Met, and p.Arg158Gln (AF: 5% each), p.Leu48Ser, and p.Ile65Thr (AF:4% each). The predicted phenotype was assigned by Guldberg´s arbitrary value (AV) and compared with the clinical phenotype based in tolerance to Phe intake. 29.1% (n:30) were hyperphenylalaninemias, 18.5% (n:19) mild-PKU, 27.2% (n:28) moderate-PKU and 25.2 % (n:26) classical-PKU. Genotype/phenotype correlation was statistically significant (p<0.001) Overall concordance was 62,5%: 93.3% in hyperphenylalaninemia, 64.7% in mild-PKU and 65.4% in classical patients. The moderate-PKU showed a weak concordance (17%) with milder AV prediction than clinical assessment. 74% of discordant moderate patients harbored p.Arg261Gln, and p.Val388Met. Our cohort is highly heterogeneous, with predominant Mediterranean influence (mainly Spanish), but with differences with other Latin-American countries.

Keywords

Pku, genotype, phenylalanine hydroxylase.

Introduction

Phenylketonuria (PKU, OMIM 261600) is an inborn error of metabolism of phenylalanine (Phe), predominantly caused by mutations in the phenylalanine hydroxylase (*PAH*) gene located on chromosome 12 (12q22-q24.2) [1]

To date, more than 1100 variants have been reported in *PAH,* and new data are continuously added to databases (http://www.biopku.org/home/pah.asp) *PAH* pathogenic variants lead to impaired function of the hepatic enzyme which catalyzes the conversion of the essential amino acid L-phenylalanine (Phe) to L-tyrosine (Tyr) , a precursor of the neurotransmitters dopamine, noradrenaline, and adrenaline.

Although the exact pathogenesis of the disease remains unclear, the defect causes Phenylalanine (Phe) accumulation (hyperphenylalaninemia) and neurotransmitter depletion and, unless treated opportunely, patients suffer from intellectual impairment of different magnitude. The disease is inherited as an autosomal recessive disorder, and its severity is a spectrum that reflects in the phenylalanine blood levels the residual activity of the affected enzyme. The majority of reported patients are

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compound heterozygotes, with high heterogeneity. Genotype varies in different populations, but ancestral origin can be followed deriving from regions of Europe and Asia.[2,3] Also, biochemical and clinical phenotypes are highly variable, suggesting the influence of other modifier genes, possibly those responsible for amino acid brain barrier transporters. Consequently, it is often difficult to ascertain the phenotypic consequences of a pathogenic variant. [4,5]

In the last decades, new treatments became suitable for some PKU patients.[6,7] Some of their therapeutic effects are based on rescuing the residual activity of the affected enzyme.[8,9] In this context, PKU genotyping provides valuable information and available increasing data on databases represents a useful resource for the clinical management of the disease. Thus, the report of PKU cohorts helps to clarify the phenothype-genotype relationships and allows the individualized follow up of affected patients and their selection for new treatment options. [3,10,11]

Newborn screening for PKU was established in Argentina in 1985 and developed in a decentralized model based on phenylalanine determinations in DBS at maternity discharge with confirmation with Phe, tyrosine levels, and Phe/Tyr ratio.

Since then, among the settled programs, the ones of Buenos Aires Province, and FEI (Fundación de Endocrinología Infantil) (a non-profit private institution) found in >6.000.000 births and incidence of PKU and HPA of 1:13000. [12] The detected patients are followed in two specialized centers.

Aim

In this study, we analyzed for the first time the genotype of a large cohort of Argentine PKU patients detected and followed up by both centers to identify the PAH mutation spectrum prevalent in our country and describe the phenotype-genotype associations.

Patients and Methods

Patients were recruited among the 420 patients with hyperphenylalaninemia followed up either in the metabolic unit of the Sor Maria Ludovica Children's hospital (La Plata, Buenos Aires Province, Argentina) or in the PKU clinic of FEI (Fundación de Endocrinología Infantil) (City of Buenos Aires). Their diagnosis was based on the determination of phenylalanine levels >120ug/dl and Phe/Tyr ratio.

Genotype was carried out in 103 patients (52 female, 51 males) from 90 unrelated families. Thirteen of them had more than one affected individual (12 pairs of brothers and a mother and child born from a blood relationship). To assess phenotype, patients were classified according to their Phe tolerance at age 4-7 years, following Guldberg´s phenotype classification as classic PKU, moderate PKU, mild PKU, or Hyperphenylalaninemia.[13]

Genomic DNA was obtained from peripheral blood lymphocytes using Puregene DNA Purification Kit (QIAGEN). Coding exons and exon-intron boundaries of *PAH* (NM_000277.1) were PCR-amplified and analyzed by Sanger sequencing. Mutation nomenclature follows the HGVS guidelines (http://www.HGVS.org/varnomen). Once genotyped, the arbitrary value (AV) by Guldberg´s was assigned to each known mutation obtained from BIOPKU database and phenotype was predicted accordingly. Phenotypes resulting from a combination of the two mutant alleles were expressed as the sum of the two AVs (SUM AVs).[13] The correlation between tolerance and predicted phenotype was calculated with Spearman correlation (significance p:<0.005). Concordance between the predicted and the observed phenotype for patients with complete mutation analysis was also calculated for the whole group and every phenotype category.

Aditionally, the recently communicated genotypic phenotype value (GPVs) for each patient was asigned with the APV scores provided for each mutation in http://www.biopku.org/home/pah.asp following Garbade et al algorithm.[14]

The allelic frequency was calculated in 179 identified affected alleles, calculating only one patient in each pair of siblings that shared two common alleles and three alleles in the discordant couple (sharing only one allele).

The study was approved by the Human Ethics Committee of the Buenos Aires Children's Hospital. Informed written consent was obtained from parents and assent from children older than seven years.

Results

Phenotype

According to Guldberg´s classification 26 (25.2%) patients had classical PKU, 28 (27.2%) moderate PKU, 19 (18.5%) mild PKU and 30 (29.1%) hyperphenylalaninemia (HPA).

Genotype: PAH mutation spectrum

One hundred and one out of 103 patients were characterized entirely, and in 2, only one mutation was found. Fifty four different pathogenic mutations, including three previously unreported variations and one intron duplication spanning an entire exon that needs further characterization, were identified in 179 out of the181 independent alleles. Ninety-one patients (90.3%) were compound heterozygotes.

Mutations were distributed all along the PAH gene being 14% intronic and the others concentrated in exon 7 (26%), exon 12 (12%), exon 11 (10%), exon 6 (10%) and exons 10, 2 and 3 (6% each). Seventy seven percent were missense,12% nonsense, 11% frameshift. 77% were placed in the enzyme catalytic domain, 16% in the regulatory, and 7% in the oligomerization zone. Twelve pairs of siblings that had a similar phenotype shared the same two pathogenic mutations, while the one with discordant phenotype shared only one allele (p.Leu348Val), being the second p.Arg261Gln for one sibling, that was classified as moderate PKU, and p.Val245Ala for his brother that had a persistent HPA.

Their mother was not initially suspected of having PKU, but Phe levels revealed slight hyperphenylalaninemia.

Nine mutations accounted for 57% of alleles screened: p.Arg261Gln (Allele frequency (AF):10.6%), c.1066-11G>A (AF:9.5%), p.Arg408Trp (AF:8.3%), p.Tyr414Cys (AF:5.5%), p.Ala403Val (AF:5%), p.Val388Met (AF:5%), p.Arg158Gln (AF:5%), p.Leu48Ser (AF:4%) and p.Ile65Thr (4%).

Table 1 shows information on genotype, calculated SUM AVs for both alleles,phe tolerance mg/day, phenotype assessed clinically and classified by Guldberg´s, concordance with predicted genotype (Yes/No), GPVs and results of the 48hs BH_4 test when performed, considering as a positive response a decrease ≥30% of the Phe basal levels under a stable Phe consumption.[15]

The p.Arg261Gln was the most frequent mutation in our cohort, affecting 18 (17.4%) of our patients.Two patients were homozygous with classic and moderate phenotypes. The other 16 were heterozygous and showed phenotypes that covered the whole clinical spectrum, depending on the second mutation found. (six HPA, two mild, eight moderates). AVs values ranged from 5 to 12. Response to BH_4 test was positive in the 12 patients tested, including the homozygous.

The c.1066-11G>A variant affected 16 patients (15.5%). Their phenotype was classic in the two homozygous, and nine heterozygous, mild in 4 and HPA in 1. Response to BH4 was positive in 4 out of 6 patient tested.

The p. Arg408Trp was present 16 patients (15.5%). Four were siblings, homozygous, and had the classical phenotype. In the remaining patients, 4 showed classical, 5 moderate and 5 the HPA phenotype, depending on the other mutation found. Response to BH_4 was positive when combined with mutations with higher AVs. Three of these patients were tested, and two responded to BH_4.

The p.Tyr414Cys change was present in 11 patients (10.6%). All were heterozygous with phenotype going from classical to moderate when associated with pArg408Trp or p.Val388Met and developing HPA in the two patients that associated p.Asp415Asn. BH_4 response was present in 4 out of 6 patients tested.

The p.Ala403Val, p.Val388Met, and p.Arg158Gln variants accounted for 10 (9.7%), 9 (8.7%) and 8 (7.7%) patients respectively. While p.Ala403Val was present always in mild forms patients with p.Val388Met, and p.Arg158Gln had more restricted tolerances ranging from classical to mild forms. While our patients with p.Ala403Val did not need to try BH4, responses in those carrying the other two mutations were variable and related to the allelic combination.

Finally the p.Leu48Ser, and p.Ile65Thr changes were present in 8 (7.7%), and 7 (6,8%) patients respectively. While the first showed a milder clinical spectrum, the second included the whole range of clinical features depending on the other present mutation. Accordingly, responses to BH_4 were variable.

Phenotype-genotype correlation

In 96 patients, the predicted phenotype assigned by Guldberg´s activity value for both alleles (SUM AVs) was compared with the observed phenotype. Overall correlation with predicted phenotype was significant (r.0.77; $p<0.001$) with overall concordance between prediction and clinical assessment of 62,5%. This concordance was 93% for HPA, 65% for mild PKU, and 65% for classical PKU patients. On the contrary, a weak concordance (17%) was found in the moderate group where predicted empiric phenotype was milder than the one assessed by

Table 1. Genotype (cDNA and protein) , SUM AVs, Phe tolerance(mg/day) , Phenotype, concordance (C), GPVs and BH4 response in the 103 studied patients

	Allele 1 cDNA	Protein	Allele2 cDNA	Protein	SUM AVs	Phe Tolerance mg/day	Phenotype	(C)	GPVs	BH4 response
1	c.782G>A	p.Arg261Gln	c.782G>A	p.Arg261Gln	8	370	Moderate	no	1.6	yes
2	c.782G>A	p.Arg261Gln	c.782G>A	p.Arg261Gln	8	300	Classical	no	1.6	nt
3	c.782G>A	p.Arg261Gln	c.1208C>T	p.Ala403Val	12	1750	HPA	yes	9.8	nt
4	c.782G>A	p.Arg261Gln	c.1208C>T	p.Ala403Val	12	1600	HPA	yes	9.8	nt
5	c.824C>G	p.Pro275Arg	c.782G>A	p.Arg261Gln	9	900	HPA	yes	5	yes
6	c.824C>G	p.Pro275Arg	c.782G>A	p.Arg261Gln	9	900	HPA	yes	5	yes
7	c.782G>A	p.Arg261Gln	c.204A>T	p.Arg68Ser	8	1000	HPA	yes	5.4	nt
8	c.782G>A	p.Arg261Gln	c.1223G>A	p.Arg408Gln	8	900	HPA	yes	5.2	yes
9	c.782G>A	p.Arg261Gln	c.1222C>T	p.Arg408Trp	5	360	Moderate	no	5.2	no
10	c.782G>A	p.Arg261Gln	c.1042C>G	p.Leu348Val	8	580	Mild	yes	1,5	yes
11	c.782G>A	p.Arg261Gln	c.838G>A	p.Glu280Lys	5	500	Mild	yes	1.6	yes
12	c.782G>A	p.Arg261Gln	c.194T>C	p.Ile65Thr	8	360	Moderate	no	1.6	yes
13	c.782G>A	p.Arg261Gln	c.728G>A	p.Arg243Gln	5	360	Moderate	no	1.6	yes
14	c.782G>A	p.Arg261Gln	c.842+3G>C		5	350	Moderate	no	1.6	yes

Table 1. Cont.

	Allele 1 cDNA	Protein	Allele2 cDNA	Protein	SUM AVs	Phe Tolerance mg/day	Phenotype	(C)	GPVs	BH4 response
15	c.782G>A	p.Arg261Gln	* c.707-2_711del17		?	350	Moderate	?	?	nt
16	c.782G>A	p.Arg261Gln	c.1162G>A	p.Val388Met	8	350	Moderate	no	1.8	nt
17	c.782G>A	p.Arg261Gln	c.117C>G	p.Phe39Leu	8	320	Moderate	no	1.6	yes
18	c.782G>A	p.Arg261Gln	c.842+1G>A		5	310	Moderate	no	1.6	yes
19	c.1066-11G>A		c.1066-11G>A		2	290	Classical	yes	0	nt
20	c.1066-11G>A		c.1066-11G>A		2	290	Classical	yes	0	nt
21	c.1066-11G>A		c.1241A>G	p.Tyr414Cys	5	450	Mild	yes	5.1	yes
22	c.1066-11G>A		c.1241A>G	p.Tyr414Cys	5	450	Mild	yes	5.1	no
23	c.1066-11G>A		c.1045T>C	p.Ser349Pro	2	300	Classical	yes	0	nt
24	c.1066-11G>A		c.1045T>C	p.Ser349Pro	2	300	Classical	yes	0	nt
25	c.473G>A	p.Arg158Gln	c.1066-11G>A		2	300	Classical	yes	0	yes
26	c.1066-11G>A		c.473G>A	p.Arg158Gln	2	220	Classical	yes	0	nt
27	c.1066-11G>A		c.898G>T	p.Ala300Ser	9	900	HPA	yes	9.7	nt
28	c.1066-11G>A		c.165delT	p.Phe55LeufsTer6	2	450	Mild	no	8	nt
29	c.727C>T	p.Arg243Ter	c.1066-11G>A		2	450	Mild	no	0	nt
30	c.1055delG	p.Gly352ValfsTer48	c.1066-11G>A		2	300	Classical	yes	0	nt
31	c.1066-11G>A		c.194T>C	p.Ile65Thr	5	300	Classical	no	1.1	yes
32	c.754C>T	p.Arg252Trp	c.1066-11G>A		2	290	Classical	yes	0	yes
33	c.809G>A	p.Arg270Lys	c.1066-11G>A		2	260	Classical	yes	0	nt
34	c.1066-11G>A		c.592_613del	p.Tyr198SerfsTer136	2	250	Classical	yes	0	no
35	c.1222C>T	p.Arg408Trp	c.1222C>T	p.Arg408Trp	2	300	Classical	yes	0	nt
36	c.1222C>T	p.Arg408Trp	c.1222C>T	p.Arg408Trp	2	300	Classical	yes	0	nt
37	c.1222C>T	p.Arg408Trp	c.1222C>T	p.Arg408Trp	2	300	Classical	yes	0	nt
38	c.1222C>T	p.Arg408Trp	c.1222C>T	p.Arg408Trp	2	300	Classical	yes	0	nt
39	c.1222C>T	p.Arg408Trp	c.1208C>T	p.Ala403Val	9	2500	HPA	yes	9.8	nt
40	c.1222C>T	p.Arg408Trp	c.1208C>T	p.Ala403Val	9	1800	HPA	yes	9.8	nt
41	c.1222C>T	p.Arg408Trp	c.1208C>T	p.Ala403Val	9	1600	HPA	yes	9.8	nt
42	c.1241A>G	p.Tyr414Cys	c.1222C>T	p.Arg408Trp	5	340	Moderate	no	5.1	nt
43	c.1241A>G	p.Tyr414Cys	c.1222C>T	p.Arg408Trp	5	340	Moderate	no	5.1	nt
44	c.1241A>G	p.Tyr414Cys	c.1222C>T	p.Arg408Trp	5	300	Classical	no	5.1	yes
45	c.898G>T	p.Ala300Ser	c.1222C>T	p.Arg408Trp	9	2000	HPA	yes	9.7	nt
46	c.734T>C	p.Val245Ala	c.1222C>T	p.Arg408Trp	9	1900	HPA	yes	10	nt
47	c.1162G>A	p.Val388Met	c.1222C>T	p.Arg408Trp	5	390	Moderate	no	1.8	yes
48	c.441+5G>T		c.1223G>A	p.Arg408Gln	5	380	Moderate	yes	0	yes
49	c.838G>A	p.Glu280Lys	c.1222C>T	p.Arg408Trp	2	340	Moderate	yes	0	nt
50	c.503delA	p.Tyr168SerfsTer27	c.1222C>T	p.Arg408Trp	2	280	Classical	yes	0	nt
51	c.1162G>A	p.Val388Met	c.1241A>G	p.Tyr414Cys	8	400	Moderate	no	5.1	nt
52	c.1162G>A	p.Val388Met	c.1241A>G	p.Tyr414Cys	8	400	Moderate	no	5.1	yes
53	c.1162G>A	p.Val388Met	c.1241A>G	p.Tyr414Cys	8	380	Moderate	no	5.1	yes
54	c.1241A>G	p.Tyr414Cys	c.1243G>A	p.Asp415Asn	12	1500	HPA	yes	10	nt
55	c.1241A>G	p.Tyr414Cys	c.1243G>A	p.Asp415Asn	12	1500	HPA	yes	10	nt
56	c.1241A>G	p.Tyr414Cys	c.194T>C	p.Ile65Thr	8	260	Classical	no	5.1	nt
57	c.842C>T	p.Pro281Leu	c.1208C>T	p.Ala403Val	10	1400	HPA	yes	9.8	nt
58	c.842C>T	p.Pro281Leu	c.1208C>T	p.Ala403Val	10	1700	HPA	yes	9.8	nt
59	c.441+5G>T		c.1208C>T	p.Ala403Val	9	2400	HPA	yes	9.8	nt

Table 1. Cont.

	Allele 1 cDNA	Protein	Allele2 cDNA	Protein	SUM AVs	Phe Tolerance mg/day	Phenotype	(C)	GPVs	BH4 response
60	c.829T>G	p.Tyr277Asp	c.1208C>T	p.Ala403Val	12	1500	HPA	yes	9.8	nt
61	c.1208C>T	p.Ala403Val	c.194T>C	p.Ile65Thr	12	1500	HPA	yes	9.8	nt
62	c.1162G>A	p.Val388Met	c.143T>C	p.Leu48Ser	8	600	Mild	yes	2	nt
63	c.781C>T	p.Arg261Ter	c.1162G>A	p.Val388Met	5	550	Mild	yes	1.8	yes
64	c.1162G>A	p.Val388Met	c.194T>C	p.Ile65Thr	8	500	Mild	yes	1.8	no
65	c.473G>A	p.Arg158Gln	c.1162G>A	p.Val388Met	8	290	Classical	no	1.8	nt
66	c.473G>A	p.Arg158Gln	c.473G>A	p.Arg158Gln	2	320	Moderate	yes	0	nt
67	c.473G>A	p.Arg158Gln	c.1169A>G	p.Glu390Gly	9	1500	HPA	yes	7	nt
68	c.473G>A	p.Arg158Gln	c.194T>C	p.Ile65Thr	5	500	Mild	yes	1.1	no
69	c.473G>A	p.Arg158Gln	Not found			330	Moderate			yes
70	c.473G>A	p.Arg158Gln	c.781C>T	p.Arg261Ter	2	290	Classical	yes	0	nt
71	c.143T>C	p.Leu48Ser	c.143T>C	p.Leu48Ser	8	1200	HPA	yes	2	yes
72	c.527G>T	p.Arg176Leu	c.143T>C	p.Leu48Ser	12	2500	HPA	yes	9.7	nt
73	c.527G>T	p.Arg176Leu	c.143T>C	p.Leu48Ser	12	2500	HPA	yes	9.7	nt
74	c.143T>C	p.Leu48Ser	c.1045T>C	p.Ser349Pro	5	450	Mild	yes	2	nt
75	c.143T>C	p.Leu48Ser	c.1045T>C	p.Ser349Pro	5	450	Mild	yes	2	yes
76	c. 809G>A	p.Arg270Lys	c.143T>C	p.Leu48Ser	5	400	Moderate	no	2	no
77	c.143T>C	p.Leu48Ser	c.204A>T	p.Arg68Ser	8	500	Mild	yes	5.4	nt
78	c.781C>T	p.Arg261Ter	c.1184C>G	p.Ala395Gly	5	2000	HPA	no	5	nt
79	c.781C>T	p.Arg261Ter	c.1184C>G	p.Ala395Gly	5	1500	HPA	no	5	yes
80	c.1315+1G>A		c.916A>G	p.Ile306Val	9	1300	HPA	yes	9.8	nt
81	c.1315+1G>A		c.916A>G	p.Ile306Val	9	1300	HPA	yes	9.8	nt
82	c.809G>A	p.Arg270Lys	c.1169A>G	p.Glu390Gly	9	600	Mild	no	7	nt
83	c.809G>A	p.Arg270Lys	c.1169A>G	p.Glu390Gly	9	600	Mild	no	7	yes
84	c.592_613del	p.Tyr198SerfsTer136	c.204A>T	p.Arg68Ser	5	400	Moderate	no	5.4	yes
85	c.592_613del	p.Tyr198SerfsTer136	c.204A>T	p.Arg68Ser	5	400	Moderate	no	5.4	yes
86	c.842C>T	p.Pro281Leu	c.754C>T	p.Arg252Trp	3	300	Classical	no	0	nt
87	c.829T>G	p.Tyr277Asp	c.441+5G>T		5	280	Classical	no	0.4	nt
88	c.781C>T	p.Arg261Ter	c.612T>G	p.Tyr204Ter	2	250	Classical	yes	0	nt
89	c.47_48delCT	p.Ser16Ter	c.183_187delCCTGA	p.Asn61AsnfsTer4	?	320	Moderate	?	0	no
90	c.526C>T	p.Arg176Ter	c.842C>T	p.Pro281Leu	3	300	Classical	no	0	nt
91	c.745C>T	p.Leu249Phe	Not found		?	380	Moderate	?	?	nt
92	Dup I5 /del E6		c.847A>T	p.Ile283Phe	?	390	Moderate	?	0	nt
93	c.441+5G>T		c.1045T>C	p.Ser349prol	2	600	Mild	no	0	nt
94	c.727C>T	p.Arg243Ter	c.194T>C	p.Ile65Thr	5	290	Classical	no	1.1	yes
95	c.734T>C	p.Val245Ala	c.1042C>G	p.Leu348Val	12	1400	HPA	yes	10	nt
96	c.1199+17G>A		c.168+19T>C		9	900	HPA	yes	?	yes
97	c.1157A>G	p.Tyr386Cys	c.1169A>G	p.Glu390Gly	9	1000	HPA	yes	7	yes
98	c.162A>T	*p.Leu54Phe	c.1042C>G	p.Leu348Val	?	500	Mild	?	?	nt
99	c.451delG	*p.Asp151IlefsTer44	c.1249T>A	p.Tyr417Asn	?	500	Mild	?	?	no
100	c.1169A>G	p.Glu390Gly	c.1089delG	p.Lys363Asnfs	9	440	Mild	no	7	yes
101	c.665A>G	p.Asp222Gly	c.727C>T	p.Arg243Ter	5	400	Moderate	no	6	nt
102	c.824C>G	p.Pro275Arg	c.441+5G>T		6	340	Moderate	no	5	nt
103	c.809G>A	p.Arg270Lys	c.833C>A	p.Thr278Asn	2	330	Moderate	YES	0	yes

nt: Not tested. ?: Unknown. *: not previously described

tolerance. This happened in 19/23 patients, 14 of which harbored p.Arg261Gln or p.Val388Met mutations and represented 74% of discordant moderate PKU patients .

Discussion

In this study, we describe for the first time in Argentina, a large number of *PAH* pathogenic variants in a cohort of PKU patients to characterize their genotype and phenotype. As molecular testing is not routinely included in Argentina neither in the NBS programs nor in the follow-up, these are the first results of a large cohort of PKU patients studied.

Argentina is an extense country with a complex and varied immigration pattern.Our cohort represents the first study on unselected patients detected through neonatal screening regardless of their residence. So, many of the patients are from Buenos Aires City and Province, and some of them came from the rest of the country where the colonization may differ. Due to this situation, some population subsets included may vary depending on the studied area: *e.g.* the area of Buenos Aires due to the river access to the country by different European colonizers was more influenced by immigration than inner regions of the country. Unfortunately, genealogical data were not retrieved.

We identified in 103 patients, 54 different PAH mutations with 90.5% of compound heterozygous. Accordingly, PKU phenotypes in our patients were highly heterogeneous and covered all the spectrum of PAH activity with a distribution that shifts to a milder pattern than the one communicated for central Europe and resembling the Mediterranean ethnic composition.[16,17] Despite heterogeneity, the genotype-phenotype correlation found was strong and would have allowed in 2/3 of the patients the precise prediction of the clinical form. Exon 7, 12, and 11 were the more affected sites (26%, 12%, and 11% respectively).

In agreement with Desviat and coworkers our population resembles the genetic diversity of PAH deficiency in the Spanish population with a predominance of c.1066-11G>A, a frequently reported PKU mutation in PAHdb affecting Mediterranean patients.[17] The variants p.Ala403Val, p.Ile65Thr, and p.Val388Met found in the Spanish population were also present in ours explaining a milder phenotype.

The p.Arg261Gln variant, prevalent in Italian, Spanish and Portuguese PKU, was the most prevalent in our cohort pointing out the predominant influence of these immigration waves. [17,18,19] Nevertheless, p.Arg408Trp, prevalent in central and northern Europe was also common in our patients leading to all the phenotype spectrum with severe classic forms when homozygous.[16]

Despite the shared history of Spanish colonization, our findings differ with other Latinamerican published data on PKU genotypes. In a recent Chilean report, Hamilton et al. also described a high heterogeneity and the presence of the already known prevalent p.Val388Met, c.442-?_509+?del and c.1066-11G >A in their patients. [20,21] Also, Santos et al. in Southeastern Brasil communicated the prevalence of p.Val388Met, p Arg261Gln, IVS10-11G>A and p. Ile65Thr. [19] So, our coinciding findings underscore the influence of the Iberian Peninsula migration to our continent. Nevertheless, the proportion of affected alleles was different in our study and p.Arg261Gln was nor so frequent in their cohorts . Similarly, p.Val388Met reported by Brasil, Mexico and Chile as prevalent (21.2%, 8,3%, and 17,3%) accounted for only 5% of our patients.[19,21,22]

The phenotype was also heterogeneous in our patients ranging from HPA to classical PKU, probably due to allelic complementation. Thus, p.ArgA261Gln assigned with intermediate levels of activity showed different behavior depending on the other present allele but keeping residual activity when homozygous.Similarly p.Arg408Trp, although proven with no residual activity, was present all through the phenotype spectrum of our cohort with classical forms in homozygous and milder forms in patients carrying less deleterious mutations in the other allele.

Regarding p.Ala403Val, p.Arg158Gln, p.Leu48Ser, p.Ileu65T and, pTyr414Cys, these changes kept their enzyme activities that were reflected, as described, in the phenotype.[23]

Substantial evidence is available on the utility of genotype-phenotype correlation. [24,25,26]

Although European consensus points out the arbitrarity of phenotype classification[27], a relatively good correlation with predicted phenotype was found in our cohort.

As observed by others, discordant predictions were found mainly in the moderate group or the border of categories assigned. [21,23] Intrinsic conformational changes in the mutated enzymes driven by interallelic interaction may explain to some degree the inaccurate prediction in this group of patients[28,29,30].

As Hamilton et al. in Chile, some mutations found in our cohort showed a weak correlation with the expected phenotype. [21] Thus, the p.Arg261Gln,and p.Val388Met changes were present in two-thirds of our moderate PKU discordant patients. These variants are described with residual activity but affecting the folding and the active tetramer formation of the enzyme [3, 30].

Another issue that may affect the concordance estimation is the accurate assessment of Phe intake allowance (Phe tolerance). True Phe tolerance in PKU patients is not so easy to evaluate in clinical practice. Although in our cohort, a comprehensive evaluation of tolerance was performed at an age where the child is more stable than in the first year of life, sometimes it is difficult to be aware of over restriction or poor adherence to diet and higher Phe intake. The Guldsberg´s phenotype classification adopted for our work classifies PKU forms accurately but leaves HPA definition without a higher boundary . In a milder population like ours, HPA patients are also included in a continuum that goes from those that will never require therapeutic intervention to others that have to be followed carefully and may require diet or even low protein substitutes. Moreover, the regular Argentine diet includes plenty of protein, and even patients with HPA, are at risk of protein overload if the diet is not guided and recommendations are not specified. So, consequently, genotype information would allow establishing individualized treatment rectifying over or under restriction.[31,32,33]

Several patients in this cohort were tested for response to dihydrochloride BH_4, the synthetic cofactor of the enzyme [11]. It is now accepted that a subset of patients would benefit from using this drug.[15,34] As the genotype determines PAH activity and the metabolic phenotype, it is also useful to select patients to be tested or treated. Moreover, the presence of two null mutations makes the testing unnecessary.[11,15]

In Argentina, diet is the first treatment offered to PKU patients. Nevertheless, sapropterin has become available, and BH4 testing if possible, is performed in the neonatal period mainly because it provides the possibility of assessing BH4 deficiencies that are difficult to exclude rapidly because of unavailable biochemical studies.[15] In this context, phenotype would complement the information in each patient helping to select the adequate treatment .Thus, though it may seem in some way sophisticated in our medium and still not available for every patient, genotype indeed allows for personalized treatment and follow-up.[32]

Our results highlight the utility of genotype in the provision of appropriate genetic counseling for PKU patients. The report of PAH mutation spectrum benefits the whole PKU community, sharing patients´ clinical manifestations and providing useful data to select therapeutic options, and design individualized therapy.Other tools that help to understand better the residual activity of the inherited PAH such as activity landscapes or the measure of isotopic breathed dCO2 would also be useful for the patient's management.[35]

Moreover, recently published evidence on genotype/phenotype efficient correlation with the GPVs score, would also help in the patient characterization. Unfortunatelly in our cohort , although calculated and provided in the table for each patient, GPV was not used as suggested because the data on pretreatment Phe levels could not be retrieved accurately.[14]

Summing up,we studied a large cohort of PKU Argentine patients to asses their genotype and genotype-phenotype correlation. Our population is highly heterogeneous with milder clinical forms and predominant Mediterranean influence (mainly Spanish), but with differences with other Latin-American countries.

The most frequent pathogenic variants found were p.Arg261Gln, c.1066-11G>A, p.Arg408Trp, p.Tyr414Cys, p.Ala403Val, p.Val388Met, p.ArgR158Gln, p.Leu48Ser, and p.Ile65Thr. 90.3% of the patients studied were compound heterozygous. Predicted and observed phenotype were overall concordant (62.5%) except in moderate patients where genotype predicted a milder form than the one seen in the clinical ground. The majority of discordant moderate patients carried the p.Arg261Gln, and p.Val388Met variants. The genotype is a useful tool to manage PKU patients, mainly because of alternative therapies and allowing individualized treatment.

Disclosure

A. Chiesa is a researcher from the Health Research Council of the Government of the City of Buenos Aires.

Declaration of Conflicting Interests

The authors declare that there is no conflict of interest.

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