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Expanding diversity within phenylketonuria in ecuadorian patients: genetic analysis and literature review of newborn screenings

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Abstract

Phenylketonuria (PKU) is an autosomal recessive metabolic disorder caused by a deficiency in the phenylalanine hydroxylase (PAH) enzyme, leading to the accumulation of phenylalanine and its metabolites, which are toxic to the central nervous system. Without treatment, PKU can result in severe intellectual disability and neurological issues. This study aims to present the first cohort of clinically described Ecuadorian PKU patients, analyzing genotype-phenotype correlations and comparing these variants with global databases to improve diagnosis and treatment in Ecuador. Detailed clinical histories were collected, and an analysis of genotype versus phenotype (affected protein domain) of the variant was performed. Among the *PAH* genotypes identified, we found 15 distinct variants, with c.[754 C > T](p.Arg252Trp); [754 C > T](p.Arg252Trp) being the most frequent genotype (23.68%), followed by c.[1045T > C](p.Ser349Pro); [1045T > C](p.Ser349Pro) (15.79%) and c.[441 + 5G > T]; [754 C > T](p.Arg252Trp) (13.16%). Additionally, several unique genotypes were identified, such as c.[140G > A](p.Ala47Val); [140G > A](p.Ala47Val) and c.[331 C > T](p.Arg111Ter); [1243G > A](p.Asp415Asn), which are not commonly reported in other populations. Most genotypes were heterozygous (63.2%). The majority of variants were missense variants (66.6%) affecting the catalytic domain (53.3%). The highest phenylalanine levels were found in patients with c.[754 C > T](p.Arg252Trp); [754 C > T] (p.Arg252Trp) (2700 umol/L). Phenotypic data were available for 11 patients, showing 45.45% with classic PKU, 45.45% with mild hyperphenylalaninemia, and 9% with mild PKU. There was a 63.6% concordance with the BIOPKU database. Five low-frequency genotypes not reported in BIOPKU were identified, suggesting unique regional variants. Our study highlights the genetic complexity of PKU in Ecuador, with a high prevalence of unique variants not commonly found in other regions. This underscores the necessity for region-specific genetic analysis to improve PKU diagnosis and treatment. The findings emphasize the importance of tailored therapeutic strategies and continued research to enhance outcomes for PKU patients in Latin America.

Keywords Phenylketonuria, Ecuador, Phenylalanine-hydroxylase, Hyperphenylalaninemia, Metabolic screening

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Background

Hyperphenylalaninemia (HPA) is a broader term that refers to any condition with elevated phenylalanine (Phe) levels in the blood, ranging from mild to severe, including but not limited to phenylketonuria (PKU). PKU, a specific form of HPA, is an autosomal recessive metabolic disorder caused by a deficiency in the function of the phenylalanine hydroxylase (PAH) enzyme, which is normally produced by the liver [1]. The primary role of the PAH enzyme is to convert the aromatic amino acid Phe into tyrosine (Tyr), an essential substrate for neurodevelopment from the fetal period through childhood and adolescence. A deficiency in PAH results in an accumulation of Phe and its toxic metabolites, particularly affecting the central nervous system [2]. Without appropriate treatment, this neurotoxicity can cause irreversible brain damage, leading to profound intellectual disability, seizures, microcephaly, and psychiatric and behavioral issues [3]. While most cases of PKU are related to variants in the *PAH* gene, approximately 2% of cases of HPA are due to defects in tetrahydrobiopterin (BH4), a critical cofactor required for PAH function. It is important to note that BH4 deficiency is distinct from PKU, even though both conditions result in elevated Phe levels [4–7].

The Newborn Screening (NBS) program in Ecuador, introduced in December 2011 and centralized under the Ministry of Public Health, screens for four diseases: congenital hypothyroidism, PKU, galactosemia, and congenital adrenal hyperplasia [8]. In contrast, other Latin American countries like Cuba, Costa Rica, Uruguay, and Chile have had functional screening programs for over 20 years. The scope of disorders tested varies significantly across countries, with no international standard. In Ecuador, the current incidence of PKU reported by the NBS program is 1.53 per 100,000 newborns tested by High-Performance Liquid Chromatography [9]. A study using data from the NBS program between 2012 and 2019 did not provide information on false-negative cases, highlighted discrepancies in coverage due to inadequate case detection in certain provinces and lacked a follow-up strategy for positive cases [8, 10]. Genetic counseling is essential, particularly in populations with higher consanguinity rates [11].

The molecular variants of PKU reveal the mutational background across different populations. Both the United States and European guidelines recommend that *PAH* genotyping be performed for all patients diagnosed with PKU. The European guidelines are more stringent, mandating genotyping as a key part of effective management. In contrast, the ACMG guidelines in the United States suggest that while *PAH* genotyping is important for optimizing therapy, it is not always feasible. Therefore, in the U.S., treatment can still be effectively planned based on blood Phe levels, as has been the traditional approach, while efforts continue to improve access to genotyping [12]. In Latin America, data on genotype analysis is limited, and some countries have yet to report such data. Understanding the genotype-phenotype correlation in PKU—how specific *PAH* gene variants relate to the severity of the disease—is crucial. For example, variants like c.728G>A (p.Arg243Gln) and c.1068 C>A (p.Tyr356Ter) are often linked to severe phenotypes such as classic PKU (cPKU), while variants like c.158G>A (p.Arg53His) are associated with milder forms like mild hyperphenylalaninemia (MHP) [13, 14]. This knowledge is vital for predicting outcomes, customizing treatment, and guiding genetic counseling and prenatal diagnosis.

This study aims to present the first series of cases of clinically described Ecuadorian PKU patients, analyzing the genotype/phenotype correlation and the correspondence of these variants with those represented in different databases from all over the world to improve diagnosis and treatment for patients in Ecuador.

Methods

This study was designed as a descriptive case series and was conducted at the genetic outpatient clinic of the Universidad San Francisco de Quito in Ecuador and included twenty patients diagnosed with PKU through the Ecuadorian NBS program. The inclusion criteria were patients with PKU diagnosed by the NBS program and with a *PAH* deficiency confirmed by mutational studies or by clinical exclusion criteria of differential diagnoses, and patients with serum Phe levels greater than 360 umol/L. Exclusion criteria were patients diagnosed with PKU who are not residents of Ecuador and patients with serum Phe levels less than 360 umol/L pre-treatment.

The Universidad San Francisco de Quito Ethics Committee approved the protocol for this study on April 13th, 2021, with the code 2020-038IN. Written informed consent was obtained from the legal representatives of all patients before the study commenced. After obtaining signed informed consent, detailed clinical history was collected, including prenatal screening, past medical history, laboratory studies, imaging tests, and genetic testing history. We ordered the Innate Error of Metabolism panel from an outsourced laboratory which confirmed all variants by sanger sequencing.

We use NM_000277.3 as the reference PAH transcript. The protein structure of human PAH was retrieved from the Alpha Fold repository (https://alphafold.ebi.ac.uk/entry/P00439) and used as a template for homology modeling for the genotype versus phenotype (affected protein domain) [15, 16]. Structural and functional domains were predicted using the SMART tool [17, 18].

Allelic frequencies were calculated as the number of alleles of each variant per 100/total number of

Table 1 Allelic frequency of the genetics variants. Protein change, classification according to APV and the comparison of the allelic frequency of Ecuador and the global allelic frequency of each genetic variant present in the study

Variant	ACMG classification	Classification according APV	Allele frequency Ecuador	Allele frequency global
c.1066-11G > A	Pathogenic	cPKU	2.63	6.434
c.1161 C > A (p.Tyr387Ter)	Pathogenic	cPKU	2.63	0.012
c.1162G > A (p.Val388Met)	Pathogenic	cPKU	2.63	1.408
c.1208 C > T (p.Ala403Val)	Pathogenic	MHP	2.63	2.133
c.1241 A > G (p.Tyr414Cys)	Pathogenic	mPKU	2.63	2.303
c.1243G > A (p.Asp415Asn)	Pathogenic	MHP	2.63	0.370
c.165T > G (p.Phe55Leu)	Pathogenic	cPKU	2.63	0.114
c.331 C > T (p.Arg111Ter)	Pathogenic	cPKU	2.63	1.034
c.60 + 5G > T	Pathogenic	cPKU	2.63	0.247
c.140G > A (p.Ala47Val)	Pathogenic	mPKU	5.26	0.022
c.842 C > T (p.Pro281Leu)	Pathogenic	cPKU	5.26	3.118
CNV EXON 5 DELETION	Pathogenic		5.26	
c.824 C > G (p.Pro275Arg)	Pathogenic	mPKU or cPKU?	7.89	0.037
c.441 + 5G > T	Pathogenic	cPKU	13.16	1.013
c.1045T > C (p.Ser349Pro)	Pathogenic	cPKU	15.79	0.843
c.754 C > T (p.Arg252Trp)	Pathogenic	cPKU	23.68	1.507

Table 2 Concordance of measured phenotype and the reported phenotype in the BIOPKUdb for each genotype studied

Allele 1	Allele 2	Level of Phe (umol/L)	PKU classification	BIOPKUdb classification	Concordance	BH4 responsiveness
c.754 C > T (p.Arg252Trp)	c.1208 C > T (p.Ala403Val)	242	MHP	MHP	Yes	Not tested
c.140G > A (p.Ala47Val)	c.140G > A (p.Ala47Val)	371	MHP	Not reported	-	Not tested
c.1066-11G > A	c.165T > G (p.Phe55Leu)	381	MHP	MHP	Yes	Not tested
c.60 + 5G > T	c.441 + 5G > T	440	MHP	cPKU	No	No
c.842 C > T (p.Pro281Leu)	c.1161 C > A (p.Tyr387Ter)	620	MHP	Not reported	-	
c.1045T > C (p.Ser349Pro)	c.1045T > C (p.Ser349Pro)	1179	mPKU	cPKU (100%)	No	No
c.754 C > T (p.Arg252Trp)	c.1162G > A (p.Val388Met)	1200	cPKU	cPKU	Yes	No
c.754 C > T (p.Arg252Trp)	c.824 C > G (p.Pro275Arg)	1376	cPKU	Not reported	-	
c.1045T > C (p.Ser349Pro)	c.1045T > C (p.Ser349Pro)	1500	cPKU	cPKU (100%)	Yes	No
c.754 C > T (p.Arg252Trp)	c.441 + 5G > T	1850	cPKU	cPKU	Yes	Not tested
c.754 C > T (p.Arg252Trp)	c.754 C > T (p.Arg252Trp)	2700	cPKU	cPKU	Yes	No
c.60 + 5G > T	c.824 C > G (p.Pro275Arg)	ND		Not reported	-	
c.754 C > T (p.Arg252Trp)	c.60 + 5G > T	ND		cPKU	-	Not tested
c.824 C > G (p.Pro275Arg)	c.1241 A > G (p.Tyr414Cys)	ND		Not reported	-	
c.331 C > T (p.Arg111Ter)	c.1243G > A (p.Asp415Asn)	ND		MHP	-	Not tested
c.754 C > T (p.Arg252Trp)	c.60 + 5G > T	ND		cPKU	-	Not tested
CNV exon 5 deletion (Copy Number Variation)		ND		Not reported	-	
c.754 C > T (p.Arg252Trp)	c.842 C > T (p.Pro281Leu)	ND		cPKU	-	No
c.1045T > C (p.Ser349Pro)	c.1045T > C (p.Ser349Pro)	ND	cPKU	cPKU (100%)	Yes	No

NP = patient did not provide the data

studied alleles. Comparisons of frequencies were performed using one sample proportion test in R, with a p-value < 0.05 considered significant. Global allele frequencies, allelic phenotype value, enzyme activity, Sorting Intolerant From Tolerant tool (SIFT), and Polyphen and BH4 responsive alleles were retrieved from the BioPKU database, which includes a registry of 16,270 *PAH* alleles (Tables 1 and 2, Supplementary Table 1). Abbreviated words in the following section include cPKU (classic phenylketonuria: Phe levels > 1200 umol/L); mPKU (mild phenylketonuria: Phe levels 600–1200 umol/L); and MHP (mild hyperphenylalaninemia: Phe levels 120–600 umol/L). In the bioPKU database, BH4 responsiveness is defined as a significant reduction (typically 30% or more) in blood phenylalanine levels following BH4 administration, indicating that the patient's PAH enzyme activity can be enhanced by BH4 supplementation (Supplementary Fig. 1).

The NBS programs of each country in Latin America were also reviewed using search terms "newborn screening" and "phenylketonuria newborn screening (Supplementary Table 2). A literature review using PubMed and Google Scholar databases was carried out to retrieve molecular variants reported in Latin America. Keywords included "phenylketonuria molecular variants," "Latin America," and "phenylketonuria genetics." The search criteria included each Latin American country and other countries globally. The search yielded 8,404 articles; 4,344 duplicates were removed, 4,005 were excluded after title and abstract screening, 28 were excluded because the full text could not be retrieved, and 2 were removed because they did not include genetic variants. The final number of relevant papers was 25. These records were compared with the variants found in our patients in Ecuador [19] (Supplementary Table 3).

Results

Molecular variants in Ecuadorian patients

Our study focused on the molecular characterization of PKU in Ecuadorian patients and compared the findings with data from other Latin American countries. We analyzed molecular variants in 20 Ecuadorian patients diagnosed with PKU. Among the *PAH* genotypes identified, we found 15 distinct variants, with c.[754 C>T] (p.Arg252Trp); [754 C>T](p.Arg252Trp)being the most frequent genotype (23.68%), followed by c.[1045T>C] (p.Ser349Pro); [1045T>C](p.Ser349Pro) (15.79%) and c.[441+5G>T]; [441+5G>T] (13.16%). Most of the genotypes were heterozygous (63.2%), with three homozygous cases attributed to consanguinity. One case was excluded from the analysis due to the absence of a pathogenic variant in the *PAH* gene. Among the 15 identified variants, most were missense variants (66.6%), with 53.3% affecting the catalytic domain and 20% located in exons 7 and 12. The Allelic Phenotype Value (APV), calculated using data from 10,500 HPA/PKU patients with over 800 different *PAH* variants, showed that most variants were associated with classical PKU (60%), followed by MHP (26.67%) and mPKU (13.3%) (Tables 1 and 2). Predictive analyses using SIFT and PolyPhen-2 indicated that most variants were likely deleterious, except for c.1243G>A (p.Asp415Asn), which was classified as benign.

We recorded the highest and most recent Phe levels for the 20 Ecuadorian patients. The patient harboring the c.[754 C>T](p.Arg252Trp); [754 C>T](p.Arg252Trp) genotype had the highest Phe level at diagnosis (2700 umol/L), followed by the patient with the c.[754 C>T] (p.Arg252Trp); [441+5G>T] genotype (1850 umol/L), and the patient with the c.[1045T>C](p.Ser349Pro); [1045T>C](p.Ser349Pro) genotype (1500 umol/L). Phenotypic data were available for 11 of the 20 patients. Among these, 45.45% had cPKU, 45.45% had MHP, and 9% had mPKU (Supplementary Fig. 1). We found phenotypic concordance of 63.6% (7/11 genotypes) with reports from the BIOPKU database [19]. The reported BH4 (sapropterin) response for each genotype found in our patients was also included .

The PAH protein has two main structural domains: an ACT domain consisting of 65 amino acids at the N-terminal and a Biopterin hydroxylase domain in the catalytic core that spans 330 amino acids. Protein modeling reveals a complete conformation with an oligomerization motif at the C-terminal. The structures of pathogenic variants, when modeled and aligned with the wild-type (wt) isoform, allowed for a comparative analysis of molecular and electrochemical differences (Supplementary Table 4). Pathogenic variants c.60+5G>T, c.441+5G>T, c.1066-11G>A affect the G-quadruplex DNA domain within the 5' and 3' ends of intronic sequences. This GGGG sequence appears to be crucial for the proper processing of the primary transcript and the excision of intron sequences. Studies have shown that the guanine at the fourth position is especially important for maintaining this activity [20, 21]. These variants often lead to intron retention, which may subsequently result in nonsense mediated decay. This process is detrimental to cellular homeostasis and could accelerate cell aging [22].

Five specific genotypes were not reported in the BIOPKU database: c.[140G>A](p.Ala47Val); [140G>A] (p.Ala47Val), c.[331 C>T](p.Arg111Ter); [1243G>A] (p.Asp415Asn), c.[824 C>G] (p.Pro275Arg); [1241 A>G] (p.Tyr414Cys), c.[824 C>G](p.Pro275Arg); [754 C>T] (p.Arg252Trp), and c.[60+5G>T]; [824 C>G] (p.Pro275Arg). Although these variants are globally rare, they occur with higher frequency in the Ecuadorian population. Among these, the genotypes c.[60+5G>T]; [824 C>G](p.Pro275Arg) and c.[331 C>T](p.Arg111Ter); [1243G>A](p.Asp415Asn) were identified as compound heterozygotes, with c.1243G>A (p.Asp415Asn) predicted to be benign. Clinical observations include a patient with the homozygous genotype c.[140G>A] (p.Ala47Val); [140G>A](p.Ala47Val), born to consanguineous parents, who exhibited severe intellectual disability (90%), likely due to delayed nutritional management. This patient also showed significant developmental delays, including impaired motor skills and speech development. Another patient with the compound heterozygous genotype c.[331 C>T](p.Arg111Ter); [1243G>A] (p.Asp415Asn) was diagnosed at one month of age. Early and consistent treatment led to normal cognitive development, normal growth parameters, and no signs of intellectual disability. A patient with the genotype c.[60+5G>T]; [824 C>G](p.Pro275Arg) was diagnosed and treated early, resulting in no intellectual disability or developmental delays. Regular monitoring indicated

normal neurodevelopmental milestones, normal growth patterns, and no neurological abnormalities. In contrast, a patient with the genotype c.[824 C>G](p.Pro275Arg); [754 C>T](p.Arg252Trp) experienced inconsistent nutritional treatment, leading to moderate intellectual disability, severe developmental delays, including gross motor delay and language impairment, as well as behavioral issues such as irritability and attention deficits. Lastly, the patient with the genotype c.[824 C>G](p.Pro275Arg); [1241 A>G] (p.Tyr414Cys) was lost to follow-up.

A one-sample test of proportion was used to compare the allelic frequency of the variants in Ecuador to the global allelic frequency, considering a p-value<0.05 as statistically significant (Supplementary Table 3). Of the genotypes studied, 68.75% (11/16) were associated with cPKU, 18.75% (3/16) with mPKU, and 12.5% (2/16) with MHP. Half of the variants (8/16) in this study were statistically significantly more common in Ecuador (Supplementary Fig. 1). For example, the variant c.165 A>C(p.Phe55Leu), associated with cPKU, had an allelic frequency of 2.63% compared to the global frequency of 0.114%. The c.824 C>G (p.Pro275Arg) variant, associated with mPKU, had an allelic frequency of 7.89% compared to the global frequency of 0.037%. The c.[1243 C>T](p.Asp415Asn) variant, associated with MHP, had an allelic frequency of 2.63% compared to the global frequency of 0.37%. Overall, we identified four variants not previously reported in Ecuador, with a higher prevalence in this population compared to global frequencies. All variants in this study were characterized as pathogenic using ACMG guidelines (Table 1, Supplementary Table 1).

Our literature review identified 663 individuals with PKU across several Latin American countries. The three most frequent variants in Latin America were c.1162G>A (p.Val388Met) with an allele frequency (AF) of 13.9%, c.782G>A (p.Arg261Gln) with an AF of 10.7%, and c.1066-11G>A with an AF of 9.4%, all correlating with cPKU. Brazil reported these three variants in the same order of frequency [19, 23]. In Argentina, c.1222 C>T (p.Arg408Trp) was the most common variant, which is also the most prevalent globally. Mexico's most frequent variant was c.60+5G>T, with a local allele frequency of 23.4% and a rare global frequency of 0.247% [19, 23, 24]. In Chile, the most common variants were c.1162G>A (p.Val388Met) (17.2%), c.442-?_509+?del (14.9%), and c.1066-11G>A (12.7%) [25] (Supplementary Table 3).

These findings provide valuable insights into the genetic landscape of PKU in Ecuador and highlight the need for region-specific genetic analysis and tailored therapeutic approaches.

Literature review of PKU Newborn screening in Latin America

Latin America consists of 33 diverse countries, each facing unique economic and healthcare challenges, especially in diagnosing and treating inborn errors of metabolism. Out of these, 20 countries have established a NBS program, with only a few achieving high coverage. Notably, four countries—Cuba, Costa Rica, Chile, and Uruguay—have optimal NBS fulfillment, with coverage reaching approximately 99% [26]. Other countries, like Honduras and the Dominican Republic, have significantly lower coverage, sometimes less than 5%. This disparity also reflects on infant mortality rates, where countries with nearly full coverage have lower rates (3.7–6.4 per 1,000 live births), compared to those with less developed programs (8.8–49.5 per 1,000 live births) (27,28) (29) (30) (31). PKU screening is included in the national programs of countries like Argentina, Brazil, Chile, Costa Rica, Cuba, Ecuador, Mexico, Panama, Paraguay, Peru, Uruguay, and Venezuela, albeit with varying degrees of coverage. In contrast, countries such as Bolivia, Nicaragua, and the Dominican Republic only offer PKU screening in the private sector, while El Salvador, Haiti, and Honduras do not have established PKU testing programs [26, 27] (29) (Supplementary Table 2, Supplementary Table 5).

Discussion

Our study provides a comprehensive analysis of PKU variants in Ecuadorian patients, contextualizing these findings within global and regional genetic data. The BIOPKU database, which includes data from over 16,900 subjects across 51 countries, reveals that the most common type of variant in PKU is substitution (80.5%), followed by deletions (12.9%) and duplications (2.1%) [25]. Most variants are missense variants (58.3%), with frameshift, splice site, nonsense, synonymous, and in-frame variants also represented [19]. Globally, the most prevalent PKU variants are c.1222 C>T (p.Arg408Trp), c.1066-11G>A, and c.782G>A (p.Arg261Gln) [19] (Tables 1 and 2, Supplementary Table 1).

Our findings align with these global trends, with the most common variants in our Ecuadorian cohort being c.[754 C>T](p.Arg252Trp); [754 C>T](p.Arg252Trp), c.[1045T>C](p.Ser349Pro); [1045T>C](p.Ser349Pro), and c.[441+5G>T]; [441+5G>T]. Notably, these variants are not the most prevalent in other Latin American countries, suggesting a unique genetic landscape in Ecuador. This is further supported by the high incidence of the c.[754 C>T](p.Arg252Trp); [754 C>T](p.Arg252Trp) variant, which is common in Saudi Arabia but less frequent in other parts of South America (Fig. 1, Supplementary Table 3).

The prevalence of specific PKU variants varies significantly across Latin America. Brazil has the highest

A)

Variant	Origin
c.140C>T (p.Ala47Val) c.1045T>C (p.Ser349Pro) c.842C>T (p.Pro281Leu)	Netherlands
c.331C>T (pArg111Ter)	China
c.1243G>A (p.Asp415Asn) c.165T>G (p.Phe55Leu) c.[441+5G>T]	Mexico
c.1162G>A (p.Val388Met)	Portugal
c.1208C>T (p.Ala403Val)	Czechia
c.1241A>G p.[Tyr414Cys]	Denmark
c.754C>T (p.Arg252Trp)	Saudi Arabia
c.1066-11G>A	Lebanon
c.60+5G>T c.1161C>G (p.Tyr387Ter) c.824C>G (p.Pro275Arg)	-

B)

Variants
c.60+5G>T
c.[1222C>T];[1222C>T]
c.[1162G>A];[1162G>A]
c.[782G>A];[782G>A]
c.[1066-11G>A];[IVS10-11G>A]
c.[1162G>A];[1162G>A]
c.[754C>T];[754C>T]
c.[1045T>C];[1045T>C]
c.[441+5G>T];[441+5G>T]

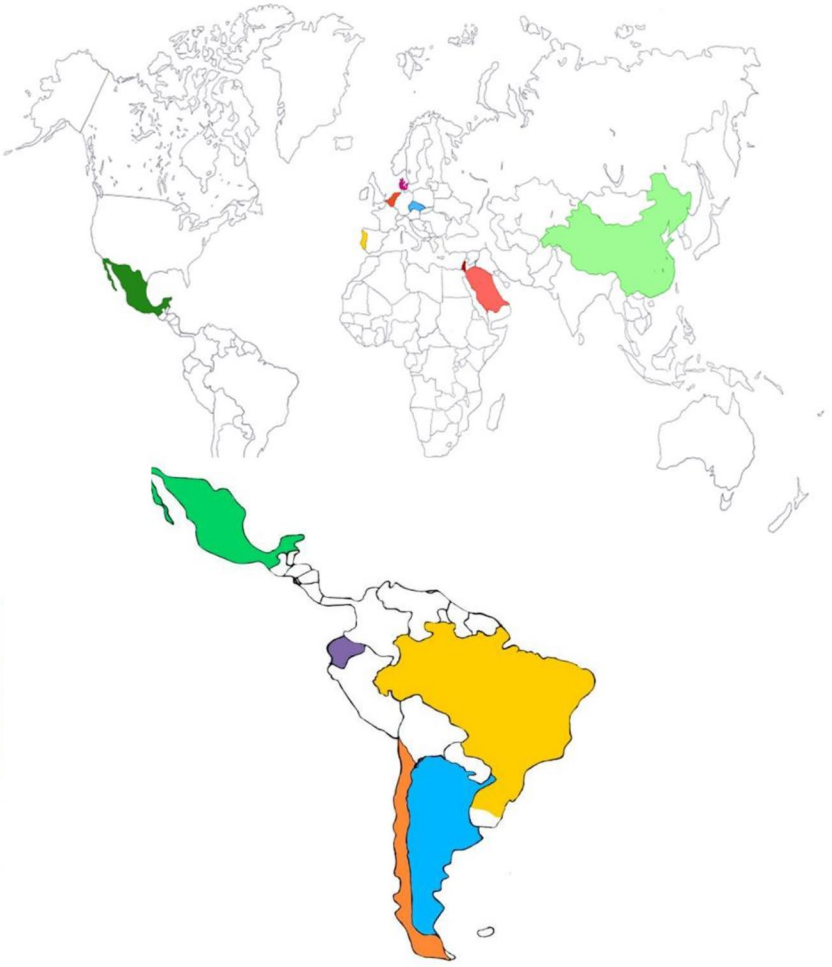

Fig. 1 Countries and origin of PKU variants worldwide and common variants in Latin America. (**A**) Geographical map of the world that demonstrates some of the variants that exist worldwide and are present in Ecuador. In red color are the variants from the Netherlands. In light green color is the variant from China. In green color, the variants from Mexico. In a yellow color variant from Portugal. In a light blue variant from Czechia and the rest from Denmark, Saudi Arabia, and Lebanon. Three variants have no apparent or reported origin. (**B**) Geographical map of Latin America that demonstrates the distribution of the variants of Ecuador in Latin America. In green color, the variants from Mexico. In blue color, the variants from Argentina. In orange color, the variants from Chile. In purple color, the variants from Ecuador

regional prevalence of classic PKU (63%), while Argentina has the lowest (43%). Mexico's prevalence rates for classic PKU, mPKU, and MHP fall between these extremes (Fig. 1).

Our study found that c.1222 C>T (p.Arg408Trp), the "Celtic" variant common in Europe, is absent in our Ecuadorian cohort, highlighting the dominance of indigenous variants or a small number of patients. Ecuador's genetic landscape is influenced by its diverse population, which includes indigenous, African, and Spanish ancestries. The unique genotypes identified in our study, such as c.[140G>A](p.Ala47Val); [140G>A](p.Ala47Val) and c.[331 C>T](p.Arg111Ter); [1243G>A](p.Asp415Asn), underscore the genetic diversity within the country. These findings emphasize the importance of region-specific genetic analysis to improve diagnosis and treatment strategies for PKU [19, 23, 24].

Despite the valuable insights gained from our study, several limitations must be acknowledged. The lack of comprehensive data from many Latin American countries limits the generalizability of our findings. Additionally, the absence of parental studies in our cohort prevents us from confirming variants in trans. Future studies should include haplotype analysis to provide more accurate information on the origin of these variants.

Our sample size was relatively small, and we were unable to obtain detailed phenotypic data for all patients due to loss to follow-up. This limitation may affect the

representativeness of our findings. However, our study significantly contributes to bridging the knowledge gap regarding PKU in Ecuador and highlights the need for continued research in this area.

The estimated worldwide incidence of PKU varies depending on the population In Europe, the incidence of PKU ranges from 1:850 in the Karachay-Cherkess Republic (Russia) to only 1:112,000 live births in Finland [19] (32). In Latin America, the incidence ranges from 1:24,617 to 1:20,775. In Ecuador, the estimated incidence is 1:65,539, which may not be accurate due to incomplete data from the newborn screening program [9]. NBS for PKU started in the US in 1963, leading to early detection and prevention of neurologic damage. This success prompted the expansion of NBS programs globally (25,29). In Latin America, the implementation of NBS for PKU varies significantly, with some countries having established programs for over 20 years, while others lack such programs entirely.

An effective NBS program for PKU requires comprehensive disease panels, widespread coverage, and strong legislative and follow-up frameworks, yet implementation across Latin America varies widely due to financial and infrastructural challenges. Chile's program, established in 1992, stands out with nearly universal coverage and robust support, contributing to the highest reported incidence of PKU in the region. Argentina and Brazil also have national programs, but coverage is uneven, particularly in rural areas. Mexico, despite pioneering NBS in 1974, faced setbacks with a suspension in 1998, leading to gaps in care. Costa Rica and Uruguay, with smaller, centralized healthcare systems, have achieved almost full coverage since the early 2000s and mid-1990s, respectively. Conversely, countries like Bolivia and Nicaragua offer PKU screening mainly through private healthcare, while El Salvador, Haiti, and Honduras lack comprehensive programs, leaving many newborns without access to early diagnosis and treatment. This uneven landscape underscores the urgent need for standardized, region-wide efforts to expand and improve PKU screening, drawing on successful models to ensure all children in Latin America benefit from early detection and care (27,28) (29) (30) (31).

Ecuador's genetic diversity, resulting from historical interactions among African, European, and indigenous American populations, is reflected in the range of PKU variants observed. Our study identified common, rare, and low-frequency variants, highlighting the underrepresentation of indigenous variants from native South American populations. We found five low-frequency genotypes in the PKU database and reported the second case of an exon five **deletion** resulting in a loss of function [20] (33).

The genetic background and type of PKU influence the nutritional management of each patient. Some variants do not respond to cofactor sapropterin supplementation, indicating the need for personalized treatment strategies. Individualized studies for each country are crucial for improving overall patient care, particularly in regions with distinct genetic profiles.

Conclusion

In conclusion, our study underscores the genetic complexity of PKU in Ecuador and the necessity for region-specific approaches to diagnosis and treatment. The unique variants identified in our cohort highlight the diverse genetic background of the Ecuadorian population and emphasize the importance of tailored therapeutic strategies. Continued research and comprehensive genetic studies are essential to improving outcomes for PKU patients in Latin America.

Abbreviations

PKU	phenylketonuria
PAH	phenylalanine hydroxylase
BH4	tetrahydrobiopterin
Phe	phenylalanine
Tyr	tyrosine
NBS	Newborn screening
cPKU	classic phenylketonuria
mPKU	mild phenylketonuria
MHP	mild hyperphenylalaninemia

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12887-024-05140-z.

Supplementary Material 1

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Author contributions

AA: retrieved the medical information, analyzed the data and wrote the manuscript. EH: contacted the individuals, retrieved the medical information, analyzed the data, and wrote the manuscript. AC: contacted the individuals, retrieved the medical information, analyzed the data, and wrote the manuscript. EH: analyzed the data and wrote the manuscript. AC: analyzed the data and wrote the manuscript. BA: performed the protein analysis. AM: analyzed the data and wrote the manuscript. JC: contacted the individuals and reviewed the manuscript. VR: contacted the individuals, analyzed the data, and reviewed the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by the institutional review board of Universidad San Francisco de Quito (USFQ) (code 2020-038IN) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Consent for publication

Written informed consent for publication was obtained from the participant.

Competing interests

The authors declare no competing interests.

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