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High Frequency of Severe Phenylketonuria in Jalisco, Mexico

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ABSTRACT This study reports the epidemiological findings collected during 11.5 years of the genotypes, metabolic and clinical phenotypes, of phenylketonuria (PKU) in twenty-two Mexican children in the state of Jalisco. The phenylalanine hydroxylase (*PAH)* variants were identified in 17/22 PKU cases. Four cases had mild hyper-phenylalanine (MHPA), two had mild PKU, one subject had moderate PKU and ten cases had classic PKU. Twelve variants of the *PAH* gene were identified: c.60+5G>T with 47.1 percent followed by c.441+5G>T, c.508C>G and c.1241A>G with 8.8 percent each; c.106611G>A with 5.9 percent and other variants with 2.9 percent each. A new pathogenic missense mutation is reported in c.791A>G. The researchers' study suggests that the population of Jalisco has a spectrum not found in the rest of the country with a genetic heterogeneity that has shown more severe variants.

INTRODUCTION

Phenylketonuria (PKU; OMIM # 261600) is an autosomal recessive disease caused by mutations in the phenylalanine hydroxylase (*PAH*) gene, conditioning a deficiency of the PAH enzyme, ameliorating the metabolism of phenylalanine (Phe) and tyrosine and consequently increasing serum levels (Blau 2016; Sumaily and Mujamammi 2017).

Within the state of Jalisco, a higher prevalence of PKU has been observed in the geographic region of Los Altos, and it has been associated with a settlement of the Spanish in the XVIII century and a possible founder effect (Velazquez et al. 1996; Blau et al. 2010).

Objective

The study presents Mexican children with PKU from the state of Jalisco as well as with their genotypes, metabolic phenotypes and the severity of the cases and introduces a new variant in this population.

METHODOLOGY

Study Design and Participants

An ambispective study was performed at the Regional Hospital "Lázaro Cárdenas", No. 46,

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Instituto Mexicano del Seguro Social (IMSS) in Guadalajara, Jalisco, México from July 2005 to June 2016. The IMSS health system provides medical care to sixty percent of the population in Jalisco and is a referral hospital center for patients with positive screening for PKU with ninety-eight percent coverage at the Neonatal Screening Program. The Research and Ethics Committee of the hospital approved this study (No. R-2015-1304-20). All parents provided informed consent and assent was obtained from the children.

General Data

General characteristics of the children were obtained by direct examination and by medical records. At 48 hours of birth, neonatal screening was performed. Eight drops of capillary blood were collected on filter paper (Schleicher and Schuell) and dried to measure the concentration of Phe by standard fluorometric assay (Lubenow et al. 1994).

The diagnosis was made according to the clinical characteristics and Phe concentration in plasma. Phe was quantified by high-performance liquid chromatography (HPLC) (Qu et al. 2001).

Metabolic phenotype classification was performed with plasma Phe levels prior to treatment initiation with the following categories: mild hyperphenylalaninemia (MHPA) Phe <600 µmol/l, mild PKU, Phe 600-900 µmol/l, moderate PKU, Phe 901-1200 µmol/l and classic PKU, Phe >1200 µmol/l (Blau 2016). Prediction of responsiveness to tetrahydrobiopterin (BH_4) was made using the criteria for phenotype and genotype (Aldámiz-Echevarría et al. 2016).

Genotyping

To determine the variants of the *PAH* gene, DNA was obtained from 18 samples by standard procedures of peripheral blood.The 13 exons of the *PAH* gene were amplified by polymerase chain reaction (PCR). PCR products were purified from agarose gels. Bidirectional sequencing was done by the Sanger method for each of the 13 exons and their flanking regions. DNA sequences were analyzed and compared with the published gene sequences. The variants were confirmed by repeat sequence analysis.

Variants were identified using the BioPKU database. The prediction of the functional effects of the new mutation was evaluated by the substitution of amino acids in the structure of the human protein. In silico analysis was performed using the program in Polyphen2 http://genetics.bwh.harvard.edu/pph2/. This program has a prediction rate of ninety-two percent in all Hum-Div Data and seventy-three percent (Adzhubei et al. 2013).

Data Analysis

Comparisons were done with χ^2 or Fisher's exact test. The statistics used were live births reported in the health system, which provides coverage to sixty percent of newborns. The incidence was obtained from ninety-eight percent of live births with screening for PKU. The average per year was calculated from January 2005 to May 2016. The incidence reported was per 100,000 live births.

The populations included in the region of Los Altos were the villages of San Juan de los Lagos, Tepatitlán, Atotonilco and Arandas. The central region, it covered the metropolitan area of Guadalajara with the population of Guadalajara, Zapopan, Tlaquepaque, Tonala, Tlajomulco, El Salto and Ahualulco; and the southern region including the population of Ciudad Guzman, Tamazula, Zapotiltic and Tuxpan (Fig. 1).

RESULTS

In Jalisco, the population with coverage by the Mexican social security health system recorded 530,427 live births from January 2005 to May 2016, with 39,943 births corresponding to the Los Altos region. The general incidence of PKU in this health system in Jalisco for the last 11.5 years was 4.78 per year/100,000 live births (1:21,551). Twenty-two children positive for PKU were found during screening.

During the same period, the Los Altos region recorded an average incidence of 20.42 per year/100,000 live births (1:5,706) according to the Mexican health system. The average prevalence of 0.017 percent contrasts with the central region of Jalisco which has an average incidence <3.7 per year/100,000 live births and the prevalence of 0.003 percent of live births (1:25,730).

Based on demographic data of the IMSS, a carrier frequency of 1/35 was estimated in Los

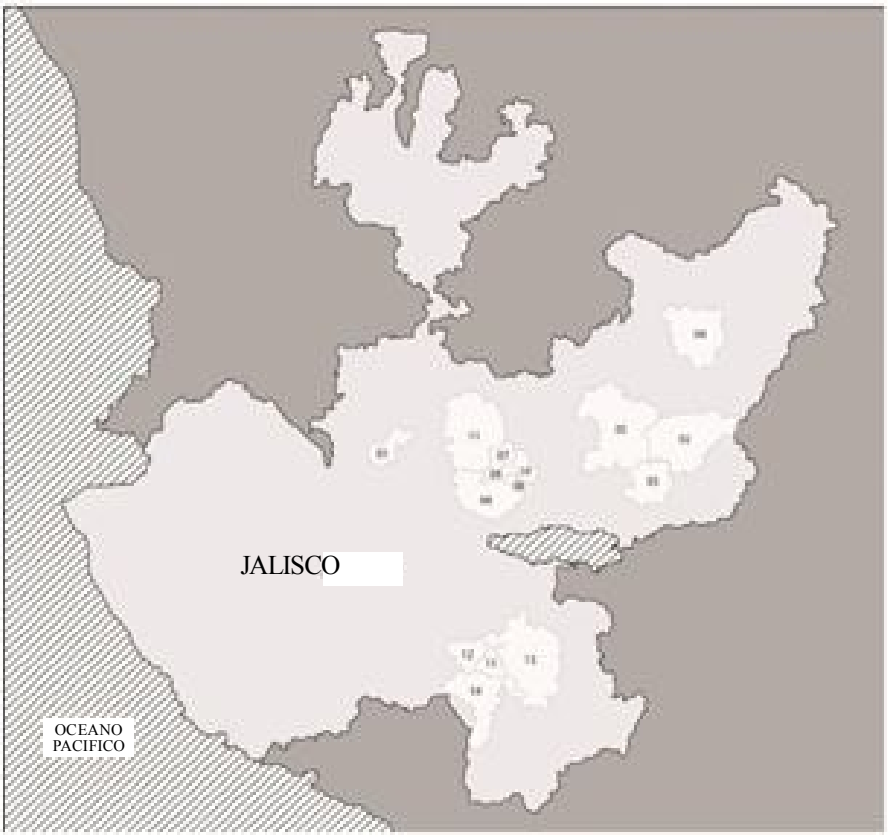

Fig. 1. Map of the state of Jalisco, Mexico showing high-incidence regions for phenylketonuria (PKU) Los Altos region: 02 – Arandas, 03 Atotonilco, 04 San Juan De Los Lagos, 05 – Tepatitlán; Central región: 01 – Ahualulco, 06 – El Salto, 07 – Guadalajara, 08 – Tlajomulco, 09 – Tlaquepaque, 10 – Tonalá, 11 – Zapopan; and South región: 12 – Ciudad Guzmán, 13 – Tamazula, 14 – Tuxpan, 15 – Zapotiltic

Altos (high incidence) in contrast with the general carrier frequency in the state of Jalisco (1/66 including Los Altos) or 1/75 (without Los Altos).

Of the 22 children identified in Jalisco with an initial diagnosis of PKU, genotyping was performed in only 18 children. For the remaining four children, the family did not return for the genotyping.

Among the 18 children with genotyping, one was negative to mutations in *PAH* gene. For the analysis, 17 patients from 3 months of age to 9 years old were included; 11 (64.7%) were boys, and six (35.3%) were girls, with ages of 3.9 ± 3.3 and 3.2 ± 2.7 years, respectively ($p<0.67$). Subjects were from the following regions of the state of Jalisco: central (nine), Los Altos (seven), and south (one).

The frequency of the metabolic phenotype showed four (23.5%) cases with MHPA, two (11.8%) with mild PKU, one (5.9%) with moderate PKU, and 10 (58.8%) with the classic disease. Genotype-phenotype concordance was 58.8 percent. The gene variants at *PAH* and metabolic phenotypes of patients with PKU are shown in Table 1.

The frequency of variants in the *PAH* gene is described in Table 2. Twelve variants were identified in the *PAH* gene; 11 were single-base substitutions, and there was one frameshift deletion. The most frequently found allelic variants were c.60+5G>T with 47.06 percent followed by c.441+5G>T, c.508C>G and c.1241A>G with 8.82 percent each; c.1066-11G>A with 5.88 percent, and other variants with 2.94 percent each (Table 2).

Only one gene variant was not previously reported, which was a heterozygous variation,

Table 1: Gene-protein variants PAH and metabolic phenotype of patients with PKU

Num	*Reg*	*Sex*	*c.DNA*	*Protein variant*	*Loc.*	*Initial diagnostic PKU*	*Phe µmol/ mL baseline*	*Phenotype PAH activity (%)*
2	C	M	c.60+5G>T	IVS1+5 G>T	In 1	Moderate	1329.3	Classic (94%)
			c.165delT	p.Phe55LeufsX6	Ex 2			Classic (100%)
3	A	M	c.441+5G>T	IVS4+5 G>T	In 4	Severe	2754	Classic (92%)
			c.1241A>G	p.Tyr414Cys	Ex 12			Mild (62%)
4	A	W	c.60+5G>T	IVS1+5 G>T	In 1	Moderate	1380.4	Classic (94%)
			c.60+5G>T	IVS1+5 G>T	In 1			Classic (94%)
5	C	M	c.441+5G>T	IVS4+5 G>T	In 4	Mild	445.9	Classic (92%)
			c.969+6T>A	IVS9+6 T>A	In 9			ND
6	A	M	c.60+5G>T	IVS1+5 G>T	In 1	Moderate	1480	Classic (94%)
			c.60+5G>T	IVS1+5 G>T	In 1			Classic (94%)
7	C	W	c.60+5G>T	IVS1+5 G>T	In 1	Moderate	1444.7	Classic (94%)
			c.1066-11G>A	IVS10-11 G>A	In 10			Classic (100%)
8	A	W	c.60+5G>T	IVS1+5 G>T	In 1	Moderate	1783.2	Classic (94%)
			c.60+5G>T	IVS1+5 G>T	In 1			Classic (94%)
9	C	M	c.60+5G>T	IVS1+5 G>T	In 1	Mild	477	Classic (94%)
			c.508C>G	p.His170Asp	Ex 5			MHPA (100%)
10	C	M	c.728G>A	p.Arg243Gln	Ex 7	Moderate	1566.2	Classic (82%)
			c.791A>G	p.His264Arg	Ex 7			ND
13	S	M	c.1066-11G>A	IVS10-11G>A	In 10	Mild	1085.2	Classic (100%)
			c.1241A>G	p.Tyr414Cys	Ex 12			Mild (62%)
14	C	M	c.441+5G>T	IVS4+5 G>T	In 4	Mild	757.7	Classic (92%)
			c.508C>G	p.His170Asp	Ex 5			MHPA (100%)
15	A	W	c.1157A>G	p.Tyr386Cys	Ex 11	Mild	632.5	Classic (94%)
			c.1241A>G	p.Tyr414Cys	Ex 12			Mild (62%)
18	A	M	c.60+5G>T	IVS1+5 G>T	In 1	Severe	2761.7	Classic (94%)
			c.60+5G>T	IVS1+5 G>T	In 1			Classic (94%)
19	C	W	c.60+5G>T	IVS1+5 G>T	In 1	Severe	2095.8	Classic (94%)
			c.60+5G>T	IVS1+5 G>T	In 1			Classic (94%)
21	C	W	c.1315+1G>A	IVS12+1G>A	In 12	Mild	240.9	Classic (97.18%)
			c.527G>T	p.Arg176Leu	Ex 6			MHPA (100%)
22	A	M	c.60+5G>T	IVS1+5 G>T	In 1	Severe	3001	Classic (94%)
			c.60+5G>T	IVS1+5 G>T	In 1			Classic (94%)
17	C	M	c.508C>G	p.His170Asp	Ex 5	Mild	450.2	MHPA (100%)
			c.60+5G>T	IVS1+5G>T	In 1			Classic (94%)

Central (C), Los Altos (A), South (S), male (M), woman (W), Location (Loc), Intron (In), Exon (Ex), Candidate (c)

Table 2: Frequency of variants and mutations in *PAH* gene

Nucleotide aberration	*Allelic variant*	*Alleles (n)*	*Frequency (%)*
c.60+5 G>T	IVS1+5G>T	16	47.06
c.441+5 G>T	IVS4+5G>T	3	8.82
c.508 C>G	p.His170Asp	3	8.82
c.1241 A>G	p.Tyr414Cys	3	8.82
c.1066-11 G>A	IVS10-11G>A	2	5.88
c.969+6 T>A	IVSA9+6T>A	1	2.94
c.1315+1G>A	IVS12+1G>A	1	2.94
c.527G>T	p.Arg176Leu	1	2.94
c.728 G>A	p.Arg243Gln	1	2.94
c.791 A>G	p.His264Arg	1	2.94
c.1157 A>G	p.Tyr386Cys	1	2.94
c.165delT	p.Phe55LeufsX6	1	2.94

c.791A>G (p.His264Arg). This new variant corresponded to a 2-year-old male from the central region of Jalisco and who had a value before treatment of Phe 1566.2 µmol/l, classifying a classic metabolic phenotype. The patient has no family history of inborn errors of metabolism on the side of paternal or maternal second-degree relatives. The current neuropsychological report categorizes that the subject does not demonstrate mental retardation or problems with medical-nutritional therapy. The change of amino acids, histidine for arginine at codon 264, was analyzed using the program Polyphen2. This mutation presented a damaging probability with high specifics, which suggests that it is a harmful or disease-causing mutation (Adzhubei et al. 2013).

DISCUSSION

For the first time, genetic heterogeneity has shown more severe variants in the Los Altos region than the mild genotype reported in Spain (Giżewska et al. 2016).

Also, the incidence and prevalence of PKU in the state of Jalisco are reported, which is recognized as having a high frequency in Mexico, where the incidence is estimated at 1:60,000 to 1:90,000 cases in the central and southern regions of the country. (Alcántara-Ortigoza et al. 2012).

The prevalence of PKU in the region of Los Altos in the state of Jalisco is reported to be much higher compared to Europe, where it is 1:10,000, and Latin America, where the estimated prevalence is between 1:25,000 and 1:50,000 at birth (Loeber 2007; Blau et al. 2010). The prevalence found is also higher than the most prevalent regions of Spain, which reported 1:6,532 births with a predominance of mild phenotypes (Fonnesbeck et al. 2013).

The epidemiological profile of PKU in Mexico has been described with a high frequency in Jalisco (Velázquez et al. 1996), mainly in the region of Los Altos. During the time of the colonization when the Spaniards arrived in this region, there was a high frequency of PKU alterations. This explains the "founder effect genotype" or "genetic drift" described by Velazquez et al. (1996). Vela-Amieva et al. (2015) included cases of PKU from different states of Mexico, finding a high frequency in Jalisco and the states of Guanajuato, Michoacán, and Aguascalientes with genetic heterogeneity similar to the genetic profile of the data of this study.

In this study, most children with classic biochemical phenotype showed the c.60+5G>T variant, corresponding to a severe genotype. Almost all cases from the region of Los Altos were also the classic type (severe genotype), and the central region had a much lower frequency. The only case reported from the southern region did not present a severe genotype, corresponding to the c.1066-11G>A variant.

The new mutation found has a biochemical and clinically severe pattern. This suggests that the pattern of heterogeneous mutation characterizes the region of Los Altos and continues to increase with the same tendency towards higher severity.

Currently, the most frequently described variant in the world is the c.1066-11G>A (BioPKU Database, Blau and Yue 2015, http://www.biopku.org/biopku/), which described a 9.73 percent allelic frequency in Spain (Aldámiz-Echevarría et al. 2016). Pérez et al. (1993) in Mexico described a frequency of 35.7 percent and Vela-Amieva et al. (2015) recently described a lower allelic frequency of 8.3 percent, similar to this study.

In this study, 12 allelic variants were found in Jalisco, the most frequent being c.60+5G>T. This variant was also reported as the most common in the study of Vela-Amieva et al. (2015) with 20.8 percent frequency. However, the frequency of the population of Jalisco is presented to be more than twice the frequency reported by Vela-Amieva (2015), although these differences are not statistically significant ($p>0.05$). This c.60+5G>T variant is more closely related to the more severe phenotype where almost eight of ten patients have a classic phenotype.

Only the cases from the southern region of Jalisco share the Mediterranean c.1066-11G>A variant but not those cases in the region of Los Altos as expected.

None of the variants was found in the Los Altos region, where it has been suggested that the Spanish mixture is equal to that described recently in Spain (Aldámiz-Echevarría et al. 2016). However, migration from the region of Los Altos to the southern region of the state is possible.

Our study population expressed other variants of *PAH* gene mutations, and differences in the variants with the Spanish colonizers are increasing. Further studies are needed to clarify these changes. This new mutation is identified as pathogenic with a high probability and corresponds to the phenotype observed in the patients reported here.

It is possible that the new mutation is a harmful PAH variant protein according to the results of the analysis using the program Polyphen2, which is related to high concentrations of Phe in the cases before treatment and categorized as a classic phenotype.

Other variants of *PAH* were also reported by Vela-Amieva et al. (2015) as follows: c.1241 A>G 9.4 percent, c.1066-11G>A 8.3 percent, c.441+ 5G>T in 5.2 percent of cases, and c.527G>T 3.1 percent, among others. These variants were also found by our research group.

Data obtained from both studies are very like those presented in different regions of Mexico and are in agreement with the idea of heterogeneity of only Mexican variants, which are increasingly independent of Spanish influence (Vela-Amieva et al. 2015).

CONCLUSION

This unique profile in the native population of the state of Jalisco corroborates the high frequency of the disease and the warning that it can be serious. Unfortunately, the most prevalent variant in Mexico does not respond to treatment in this population. In this research, the concordance reported was fifty-eight percent, and only in half of the cases could the genotype potentially predict the phenotype. Given the different admixture conditions in our country and the presence of regions of high consanguinity, regional screening programs are required for identifying genotypic variants to assess the severity trend of the cases.

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