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Haplotype Study in Argentinean Variegate Porphyria Patients

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Key Words
Variegate porphyria · Founder effect · Haplotype analysis · Short tandem repeat

Abstract
Background/Aims: The porphyrias are genetically heterogeneous diseases, and each mutation is exclusive to one or two families. Among the mutations responsible for variegate porphyria in our country, c.1042_1043insT stands out, since it was described only in Argentina and is present in about 40% of genetically diagnosed families. Thus, we hypothesized the possible existence of a common ancestor for the mutation in our population. ***Methods:*** We conducted a study based on microsatellite (short tandem repeats) haplotypes. ***Results:*** We found a common haplotype in all of the patients carrying the common mutation. The age of the mutation was estimated to be about 375 years. ***Conclusion:*** There is a recent founder effect in our population for this particular genetic alteration in variegate porphyria.

Introduction

Porphyrias are a group of metabolic disorders caused by the partial deficiency of one of the enzymes involved in the heme biosynthetic pathway. Particularly, variegate porphyria (VP; OMIM 176200) is one of the acute/cutaneous or mixed hereditary porphyrias and it is the third most prevalent in Argentina (1 in 500,000 to date) [1]. This autosomal dominant disorder results from a partial deficiency of protoporphyrinogen oxidase (PPOX; E.C. 1.3.3.4), the seventh enzyme of the pathway, which catalyzes the oxidation of protoporphyrinogen to protoporphyrin IX (PROTO IX) [2].

Patients with VP can present skin lesions and/or acute neurovisceral attacks. The cutaneous symptoms include photosensitivity leading to blistering in sun-exposed areas, skin fragility, hyperpigmentation and hypertrichosis due to the accumulation of porphyrins. The acute attacks are characterized by abdominal pain, constipation, vomiting, hypertension, tachycardia and various peripheral and central nervous system manifestations due to the accumulation of the neurotoxic precursors, δ-aminolevulinic acid (ALA) and porphobilinogen (PBG). VP shows incomplete penetrance and its expression is highly variable, which is in part due to the exposure to environmental, metabolic and hormonal factors.

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Human *PPOX* gene has been mapped to chromosomal region 1q22-23, and up to now more than 150 different mutations have been described in VP. Most of them are private, restrictive to one or a few unrelated VP families. However, there are two prevalent mutations that have been reported in a large number of families in South African and Dutch populations [3–8] and a Chilean population [9], suggesting the existence of a founder effect.

In Argentina, 36 different families have been diagnosed at a molecular level with VP, and 14 of them (39%) carry the same mutation, the small insertion c.1042_1043insT, described for the first time by our laboratory [10] and never found in any other population. To elucidate if the unusual high prevalence of this mutation was due to a founder effect, we carried out a haplotype analysis on the basis of short tandem repeat (STR) analysis in all the families carrying the small insertion, families carrying other VP mutations and in a control group. Our results indicate that, as expected, the families with the insertion would have a common ancestor.

Materials and Methods

Patients and Controls

VP patients were divided in two groups for haplotype analysis, patients carrying the insertion c.1042_1043insT, which comprised 14 families and their available relatives (ins, n = 26), and VP patients carrying other VP mutations, which included 22 families and their available relatives (other, n = 51). The symptomatology and the mutation carried by each family are shown in online supplementary table 1 (see www.karger.com/doi/10.1159/000445749 for all online suppl. material). For allelic segregation studies those available members of each family without any mutation were included, reaching to 31 and 78 members, respectively. Moreover, 80 normal alleles were assayed to characterize the selected STRs amongst control samples. These samples were obtained from the Centro de Investigaciones sobre Porfirinas y Porfirias (CIPYP) control DNA bank.

The biochemical and genetic VP diagnosis was made as described in Méndez et al. [1]. Written informed consent was obtained from all patients prior to their inclusion in the study. The protocol was approved by the Ethics Committee of CIPYP (Hospital de Clínicas, CONICET) and was performed in accordance with the Declaration of Helsinki (1964).

STR Analysis

Five STRs flanking the PPOX gene were genotyped and characterized. They spanned an interval of 7.1 cM on the Marshfield Genetic Map (http://research.marshfieldclinic.org/genetics/GeneticResearch/compMaps.asp). These markers were selected based on their prior characterization and inclusion in other populations' haplotype VP studies (online suppl. table 2) [5–9].

The genotyping was performed by means of fluorescent PCR and fragment length analysis by capillary electrophoresis on an automated sequencer (ABI3730XL, Macrogen Genescan Service). Every PCR was performed in a final volume of 25 μl with 1.5 mM of Mg^{2+}, 1.5X of BSA, 200 μM of each dNTP, 0.16 μM of each primer, 0.5 U of Platinum Pfx DNA Polymerase (Invitrogen) and 150 ng of gDNA. The amplification profile was 5 min at 94°C followed by 10 cycles at 94°C for 30 s, 60°C for 30 s and 40 cycles at 94°C for 30 s, 55°C for 30 s with a final extension at 68°C for 10 min.

The STRs D1S484, D1S1677 and D1S1679 were assayed using the M13 tailed-primer strategy [11–13], with the following primer concentration: 125 μM of reverse primer, 75 μM of M13 primer and 50 μM of forward primer. In these cases, the amplification profile was 5 min at 95°C, 10 cycles at 95°C for 30 s, 68–63°C for 30 s, 68°C for 30 s; 15 cycles at 95 for 30 s, 63°C for 30 s, 68°C for 30 s; 10 cycles at 95°C for 30 s, 59–54°C for 30 s, 68°C for 30 s; 15 cycles at 95°C for 30 s, 53°C for 30 s, 68°C for 30 s, and a final extension at 68°C for 10 min.

The allele size was obtained using PeakScanner (Applied Biosystems), and each value corresponds to each PCR fragment length, including the primers, in base pairs. Allelic and genotypic frequencies, the total number of alleles and genotypes and observed heterozygosity were calculated employing the R software (The R project for statistical computing, http://www.r-project.org/). The same program was used to calculate the Hardy-Weinberg equilibrium in the control group. Haplotypes were deduced on the basis of allele segregation between each family, and the PHASE program was additionally employed to determine the most probable haplotype of each patient and to compare the haplotypes of each group ($p < 0.95$).

Estimation of the Age of the Mutation

The age of this small insertion was estimated using the approach described by Genin et al. [14] based on the likelihood that uses multilocus data to estimate the age of the most recent ancestor of the mutation and not the age of the mutation itself, which would require assumption about population genetic processes that are usually difficult to validate. The basic assumption of the method is that the **N** affected individuals descend from a common ancestor who introduced the mutation ***n*** generations ago and the problem is to estimate this ***n*** value from the size of the haplotype shared by these **N** individuals on each side of the mutation. So, the method estimates the number of generations between the present time and the most recent ancestor of all the mutation-bearing individuals as a function of a recombination fraction between the mutation and the marker loci, the rate mutation of microsatellite loci and the allele frequencies of the marker loci [14]. The 95% confidence interval (CI) for allele age was estimated according to Schneider-Yin et al. [15].

Results

It can be mentioned that a previous study with about the same VP families on the basis of SNPs analysis indicated that these molecular markers were not informative in our population, since the allele shared by all the patients with the insertion was also found in about 80% of the control individuals, so they could not be used for this

Table 1. Haplotype analysis in the Argentinean VP families carrying the insertion

Marker	VP families													
	I	II	III	V	VI	XXI	XXII	XXIII	XXIV	XXV	XVII	XVIII	XIX	XX
D1S2707	143	143	153	153	153	153	153	153	153	151	153	153	153	143
D1S484	**158**	**158**	**158**	**158**	**158**	**158**	**158**	**158**	**158**	**158**	**158**	**158**	**158**	**158**
D1S2705	**146**	**146**	**146**	**146**	**146**	**146**	**146**	**146**	**146**	**146**	**146**	**146**	**146**	**146**
PPOX c.1402-1043insT mutation														
D1S1679	**180**	**180**	**180**	**180**	**180**	**180**	**180**	**180**	**180**	**180**	**180**	**180**	**180**	**180**
D1S104	160	154	154	156	154	158	154	154	160	154	162	154	156	154

The core haplotype is shown in bold and the extended haplotype is underlined. Numbers represent the size (bp) of each allele considering the length of the PCR product.

Table 2. Haplotype analysis in the Argentinean VP families carrying other mutations

Marker	VP families																		
	I	II	III	V	VI	XXI	XXII	XXIII	XXIV	XXV	XXVI	XXVII	XXIX	XXX	XXXI	XXXII	XXXIII	XXXIV	XXXV
D1S2707	151	151	145	151	151	153	151	147	153	149	153	149	149	151	143	143	141	153	151
D1S484	158	152	156	154	**158**	158	156	154	154	154	154	154	154	152	154	154	154	**158**	154
D1S2705	148	146	146	152	**146**	146	146	150	148	148	146	148	150	154	146	146	152	**146**	146
PPOX mutation																			
D1S1679	180	176	180	180	**180**	176	180	172	176	164	180	180	180	180	188	180	176	**180**	176
D1S104	154	160	160	156	154	164	154	162	164	160	156	160	162	160	154	156	168	154	160

Haplotypes of VP patients with other mutations. The central haplotype shared by VP families with the insertion is shown in bold.

aim [16]. For this reason, the present study was carried out on the basis of STR analysis.

Since the 5 molecular markers used in this work were not previously characterized in the Argentinean population, as a starting point, we analyzed them in 80 normal alleles. We found that all these STRs are in Hardy-Weinberg equilibrium and they were informative due to the fact that they all presented heterozygosity values similar to those found in other populations (The ALlele FREquency Database http://alfred.med.yale.edu/alfred/index.asp; Genethon Genetic Maps http://www.bli.uzh.ch/BLI/Projects/genetics/maps/gthon.html) (online suppl. table 2). We also found up to 9 different alleles, up to 21 different genotypes and a wide range of allelic frequencies (online suppl. table 3).

STRs characteristics for both groups of VP patients are shown in online supplementary table 4. The pedigrees and genotypes for families from both VP groups are shown in online supplementary figures 1 and 2.

Allelic frequencies for the 3 groups analyzed are shown in online supplementary table 5. When we compared them, no significant differences were found between the 3 groups; however alleles D1S2707-153, D1S484-158, D1S2705-146 and D1S1679-180 have a higher frequency in the VP group with the insertion (in bold) than in the other 2 groups, indicating that they likely would be associated with the insertion. This difference is not so significant for the D1S104-154 allele. The allelic frequencies for a European origin group are also shown (http://www.gdb.org).

The haplotypes for the VP Argentinean families carrying the insertion or other mutations are shown in tables 1 and 2, respectively. We found a core haplotype that includes D1S484 allele 158, D1S2705 allele 146 and D1S1679 allele 180, associated to the c.1042_1043insT mutation in all the families included in this study (table 1), while only 10.5% of the families carrying other VP mutations shared this haplotype (table 2). Moreover, an

Table 3. Values used for mutation age estimation

Allele	Mb[1]	θ[2]	Freq[3]
D1S2707-153	84.26	0.0026	0.235
D1S484-158	84.84	0.0011	0.073
D1S2705-146	85.42	0.000	0.165
PPOX c. 1042_1043insT	–	–	–
D1S1679-154	85.42	0.000	0.275
D1S104-154	87.81	0.0093	0.225

[1] Physical distance. [2] Recombination fraction calculated using the Kosambi mapping function (see text). [3] Frequency of the marker allele shared by the patients.

extended haplotype that includes D1S2707 allele 153 was found, which was present in 71.4% of the families carrying the insertion analyzed.

Estimation of the Age of the Mutation

According to the method by Genin et al. [14], we consider two possibilities, the largest haplotype shared by 10 families and the shortest shared by the other 4 families. Since the genetic and physical distances between the two extreme haplotypes are 7.1 cM and 3.5 Mb, respectively (online suppl. table 2), the correspondence between genetic and physical distances over the whole region was estimated to be 2.0 cM for 1 Mb. Recombination fractions between the different markers and the insertion were computed from genetic distances using the Kosambi mapping function (http://statgen.ncsu.edu/qtlcart/manual/node46.html), and the frequency of the marker allele shared by the patients was estimated from a sample of 40 unrelated Argentinean control subjects (table 3). The age of the most common ancestor of the c.1042_1043insT mutation was estimated to be about 15 generations (95% CI 8–21) considering a marker rate mutation equal to zero. This result did not significantly differ if we considered a marker mutation rate of about 0.0006 for dinucleotide microsatellite markers. If we assume that one generation is 25 years, this corresponds to about 375 years.

Discussion

As already stated, porphyrias are genetically heterogeneous diseases, so usually there are a number of different mutations affecting every population, and each mutation is restricted to one or a few unrelated families.

However, there are reports describing mutations with high prevalence for some populations, suggesting then a founder effect for them. Particularly, the p.R59W mutation is carried by about 90% of the South African population, and several studies have demonstrated that all of them share a common ancestor, likely a Dutch couple established in Cape Town at the end of the 17th century. In effect, an extended haplotype analysis in 3 South African and 3 Dutch families confirmed this hypothesis [8]. Moreover, a common ancestor for a VP mutation based on haplotype analysis has been described for Chilean population [9].

In our country, we found that the c.1042_1043insT mutation is present in about 39% of Argentinean VP families, all of them from Buenos Aires. This small insertion was first described in our laboratory and leads to the formation of a stop codon one codon downstream [10]. It has never been found in any other country, although most of them have Spanish or Italian ancestry.

There are two possible explanations for this scenario: the mutation is in a mutational hot spot or its high prevalence is due to a founder effect. Even though there is also a T insertion one base pair away from ours (c.1041_1042insT) reported for the Swiss population [17], which could suggest that, in fact, this region would be a hot spot, we set out to investigate the founder effect hypothesis.

In order to do so, we carried out a haplotype study including 5 molecular markers. These markers were STRs that are flanking the *PPOX* gene, spanning an interval of 7.1 cM and were selected because they were previously characterized and used in similar studies in other populations [5–9].

In this work, we found a core haplotype that is shared by 100% of the families included in this study and an extended haplotype that includes 71.4% of these families (online suppl. table 5). Only 10.5% of the Argentinean VP families carrying other mutations share the core haplotype (table 2).

Taking into account that the STRs used are very variable in our population, the coincidental sharing of an identical haplotype seems highly improbable; therefore, we think that these results would indicate that Argentinean VP patients carrying the recurrent c.1042_1043insT mutation share a common ancestor.

Moreover, we applied the method described by Genin et al. [14] to estimate the age of the mutation, and we found that it could be generated about 375 years, so it seems to be a very recent founder mutation. This would be in accordance with the fact that we have to use extra-

genic microsatellite markers to analyze the founder effect as it has been reported for other mutations in VP patients from South Africa [5]. Considering the ancestry of the population studied, most of them of Spanish or Italian origin, the prevalent European ancestry components of the Argentinean population living in Buenos Aires [18, 19], we would point to a founder effect from European migrants, although this mutation has not been yet found in European populations. The possibility of a Native American ancestry seems, at the moment, less viable, although this is also an important contribution to the biogeographical ancestry of Argentineans [18, 19].

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Disclosure Statement

B.X.G. is a postgraduate student from the University of Buenos Aires. A.B., M.V.R. and V.E.P. are superior and independent researchers from CONICET.

References

1 Méndez M, Granata BX, Jiménez MJ, Parera VE, Batlle A, de Salamanca RE, Rossetti MV: Functional characterization of five protoporphyrinogen oxidase missense mutations found in Argentinean variegate porphyria patients. JIMD Rep 2012;4:91–97.

2 Puy H, Gouya L, Deybach JC: Porphyrias. Lancet 2010;13:375, 924–937.

3 Meissner PN, Dailey TA, Hift RJ, Ziman M, Corrigall AV, Roberts AG, Meissner DM, Kirsch RE, Dailey HA: A R59W mutation in human protoporphyrinogen oxidase results in decreased enzyme activity and is prevalent in South Africans with variegate porphyria. Nat Genet 1996;13:95–97.

4 De Rooij FWM, Minderman G, de Barr C: Six new protoporphyrinogen oxidase mutations in Dutch variegate porphyria patients and the R59W mutation in historical perspective. Acta Haematol 1997;98(suppl 1):103.

5 Groenewald JZ, Liebenberg J, Groenewald IM, Warnich L: Linkage disequilibrium analysis in a recently founded population: evaluation of the variegate porphyria founder in South African Afrikaners. Am J Hum Genet 1998;62:1254–1258.

6 de Villiers JN, Kotze MJ, van Heerden CJ, Sadie A, Gardner HF, Liebenberg J, van Zyl R, du Plessis L, Kimberg M, Frank J, Warnich L: Overrepresentation of the founder PPOX gene mutation R59W in a South African patient with severe clinical manifestation of porphyria. Exp Dermatol 2005;14:50–55.

7 van Tuyll Van Serooskerke AM, Schneider-Yin X, Schimmel RJ, Bladergroen RS, Poblete-Gutiérrez P, Barman J, van Geel M, Frank J, Minder EI: Identification of a recurrent mutation in the protoporphyrinogen oxidase gene in Swiss patients with variegate porphyria: clinical and genetic implications. Cell Mol Biol (Noisy-le-grand) 2009;55:96–101.

8 van Tuyll van Serooskerken AM, Drögemöller BI, Te Velde K, Bladergroen RS, Steijlen PM, Poblete-Gutiérrez P, van Geel M, van Heerden CJ, Warnich L, Frank J: Extended haplotype studies in South African and Dutch variegate porphyria families carrying the recurrent p.R59W mutation confirm a common ancestry. Br J Dermatol 2012;166:261–265.

9 Frank J, Aita VM, Ahmad W, Lam H, Wolff C, Christiano AM: Identification of a founder mutation in the protoporphyrinogen oxidase gene in variegate porphyria patients from Chile. Hum Hered 2001;51:160–168.

10 De Siervi A, Parera VE, Varela LS, Batlle A, Rossetti MV: A novel mutation (1320InsT) identified in two Argentine families with Variegate Porphyria. Hum Mut 2000;16:96–98.

11 Oetting WS, Lee HK, Flanders DJ, Wiesner GL, Sellers TA, King RA: Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared fluorescence and M13 tailed primers. Genomics 1995;30:450–458.

12 Schuelke M: An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 2000;18:233–234.

13 Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF: M13 – tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques 2001;31:24–28.

14 Genin E, Tulio-Pelet A, Begeat F, Lyonnet S, Abel L: Estimating the age of rare disease mutations: the example of Triple-A-syndrome. J Med Genet 2004;41:445–449.

15 Schneider-Yin X, Hergersberg M, Goldgar DE, Rüfenacht UB, Schuurmans MM, Puy H, Deybach JC, Minder EI: Ancestral founder of mutation W283X in the porphobilinogen deaminase gene among acute intermittent porphyria patients. Hum Hered 2002;54:69–81.

16 Granata BX, Marques Serra P, Parera VE, Batlle A, Rossetti MV: Análisis de haplotipos en pacientes Argentinos con porfiria variegata. Medicina 2008;68(suppl II):181.

17 Schneider-Yin X, Minder EI: Swiss patients with variegate porphyria have unique mutations. Swiss Med Wkly 2006;136:515–519.

18 Avena SA, Goicochea AS, Rey J, Dugoujon JM, Dejean CB, Carnese FR: Mezcla génica en una muestra poblacional de la ciudad de Buenos Aires. Medicina (Buenos Aires) 2006;66:113–118.

19 Corach D, Lao O, Bobillo C, van Der Gaag K, Zuniga S, Vermeulen M, van Duijn K, Goedbloed M, Vallone PM, Parson W, de Knijff P, Kayser M: Inferring continental ancestry of Argentineans from autosomal, Y-chromosomal and mitochondrial DNA. Ann Hum Genet 2010;74:65–76.