




Research Article  
Human and Medical Genetics

## Alpha thalassemia and alpha-MRE haplotypes in Uruguayan patients with microcytosis and hypochromia without anemia

Ana María Soler<sup>1</sup>, Bruna Facanali Piellusch<sup>2</sup>, Lorena da Silveira<sup>1</sup>, Gisele Audrei Pedroso<sup>2</sup>, Pablo López<sup>3</sup>, Enrique Savio<sup>4</sup>, María de Fatima Sonati<sup>2</sup> and Julio da Luz<sup>1</sup> 

<sup>1</sup>Universidad de la República (UdelaR), Centro Universitario Regional (CENUR) Litoral Norte, Departamento de Ciencias Biológicas, Laboratorio de Genética Molecular Humana, Salto, Uruguay.

<sup>2</sup>Universidade Estadual de Campinas (UNICAMP), Faculdade de Ciências Médicas, Departamento de Patologia Clínica, Campinas, SP, Brazil.

<sup>3</sup>Universidad de la República (UdelaR), Facultad de Medicina, Hospital de Clínicas Manuel Quintela, Departamento de Laboratorio de Patología Clínica, Montevideo, Uruguay.

<sup>4</sup>Administración de los Servicios de Salud del Estado (ASSE), Hospital Departamental de Salto, Servicio de Laboratorio Clínico, Salto, Uruguay.

### Abstract

Alpha thalassemia is the most common genetic disorder across the world, being the  $\alpha$ -<sup>3,7</sup> deletion the most frequent mutation. In order to analyze the spectrum and origin of alpha thalassemia mutations in Uruguay, we obtained a sample of 168 unrelated outpatients with normal hemoglobin levels with microcytosis and hypochromia from two cities: Montevideo and Salto. The presence of  $\alpha$ -thalassemia mutations was investigated by gap-PCR, restriction endonucleases analysis and HBA2 and HBA1 genes sequencing, whereas the alpha-MRE haplotypes were investigated by sequencing. We found 55 individuals (32.7%) with  $\alpha$ -thalassemia mutations, 51 (30.4%) carrying the  $\alpha$ -<sup>3,7</sup> deletion, one with the  $\alpha$ -<sup>4,2</sup> deletion and three having the rare punctual mutation HBA2:c.-59C>T. Regarding alpha-MRE analysis, we observed a significant higher frequency of haplotype D, characteristic of African populations, in the sample with the  $\alpha$ -<sup>3,7</sup> deletion. These results show that  $\alpha$ -thalassemia mutations are an important determinant of microcytosis and hypochromia in Uruguayan patients with microcytosis and hypochromia without anemia, mainly due to the  $\alpha$ -<sup>3,7</sup> deletion. The alpha-MRE haplotypes and the  $\alpha$ -thalassemia mutations spectrum suggest a predominant, but not exclusive, African origin of these mutations in Uruguay.

**Keywords:** Alpha-thalassemia, alpha-MRE, microcytosis, hypochromia, Uruguay.

Received: October 30, 2020; Accepted: February 10, 2021.

### Introduction

$\alpha$ -thalassemia is the most common genetic disorder across the world. It is mainly caused by deletions of one ( $\alpha$ -) or both ( $\alpha\alpha$ -) HBA genes of the  $\alpha$ -globin gene cluster, although small deletions or point mutations also contribute to the  $\alpha$ -thalassemia mutations spectrum (Foglietta *et al.*, 1996; Huisman *et al.*, 1998; Steinberg *et al.*, 2001).

The absence ( $\alpha^0$ -thalassemia) or reduction ( $\alpha^+$ -thalassemia) of  $\alpha$ -globin synthesis produces an imbalance between a and b-globin chains. This imbalance results in a deficient synthesis of hemoglobin in erythroid cells, causing microcytosis (reduction of mean corpuscular volume, MCV) and hypochromia (reduction of mean corpuscular hemoglobin, MCH). Microcytosis and hypochromia with normal levels of HbA2 can also be due to iron deficiency or anemia of chronic disease (Weatherall and Clegg, 1981; Weiss and Goodnough, 2005).

Hematological alterations depend on the number of  $\alpha$ -globin genes affected (Steinberg *et al.*, 2001). They range from almost asymptomatic or mild microcytic anemia in individuals with one or two affected genes to life incompatibility in the case of the hydrops fetalis syndrome due to the loss of the four  $\alpha$ -globin genes. The consequence of losing or carrying alterations in three genes is the presence of a hemolytic anemia with marked variation in phenotypic expression, characterized by the presence of H hemoglobin (b4) (Higgs *et al.*, 1989).

$\alpha$ -thalassemia reaches high frequencies in regions where malaria is or has been endemic, as in African, Mediterranean basin and Southeast Asian populations (Weatherall and Clegg, 2001). Furthermore,  $\alpha$ -thalassemia mutations have spread across the world by immigration and slave trade (Isola, 1975; Sans, 1994; Weatherall and Clegg, 2001).

The  $\alpha^+$ -thalassemia due to a deletion of 3.7Kb ( $\alpha$ -<sup>3,7</sup> deletion) is the most common cause of  $\alpha$ -thalassemia, which affects both HBA1 and HBA2 genes, resulting in a single hybrid gene (HBA2-HBA1). Another frequent cause of  $\alpha^+$ -thalassemia is the deletion of 4.2Kb ( $\alpha$ -<sup>4,2</sup> deletion) which deletes the entire HBA2 gene (Higgs *et al.*, 1989; Foglietta *et al.*, 1996). African and some Asian populations present the highest frequencies of  $\alpha$ -<sup>3,7</sup> deletion, while the  $\alpha$ -<sup>4,2</sup>

Send correspondence to Julio da Luz. Universidad de la República (UdelaR), Centro Universitario Regional (CENUR) Litoral Norte, Departamento de Ciencias Biológicas, Laboratorio de Genética Molecular Humana, Rivera 1350, 50000, Salto, Uruguay. E-mail: [jdal@fmed.edu.uy](mailto:jdal@fmed.edu.uy)

deletion is more frequent in Asian populations (Kattamis *et al.*, 1996; Weatherall and Clegg, 2001). Both deletions are also observed in European and Mediterranean populations at variable frequencies. For example, in Sardinian and Cypriot populations the  $-\alpha^{3.7}$  deletion frequency is 12.6% and 14.0% respectively, whereas in the Basque population it is practically inexistent (Steinberg *et al.*, 2001; Weatherall and Clegg, 2001).

The loss of the two  $\alpha$ -globin genes in *cis* results in  $\alpha^0$ -thalassaemias. In the Mediterranean region, the  $--^{MED}$  and  $--^{20.5}$  deletions are the most frequent mutations, whereas in African populations they are almost absent (Kattamis *et al.*, 1996; Steinberg *et al.*, 2001; Weatherall and Clegg, 2001). On the other hand, the deletion of a pentanucleotide (TGAGG) located at the 5' end of the HBA2 gene IVS-I ( $\alpha^{HpbIIa}$ ), a point mutation at the initiation codon (ATG-ACG) of the HBA2 gene ( $\alpha^{Ncol}\alpha$ ), as well as a point mutation (ATG-GTG) in the HBA1 gene ( $\alpha^{Ncol}$ ), are the most common non-deletional mutations of  $\alpha$ -thalassemia in the Mediterranean region (Higgs *et al.*, 1989; Foglietta *et al.*, 1996; Kattamis *et al.*, 1996; Steinberg *et al.*, 2001).

For a pediatric Uruguayan population, da Luz *et al.* (2013) previously reported that the estimated incidence of  $\alpha$ -thalassemia was 3.3% and that only the  $-\alpha^{3.7}$  deletion was observed. The  $--^{20.5}$  mutation was observed only in one individual from an enriched sample with microcytosis and hypochromia without iron deficiency and normal levels of HbA2. Interestingly, the  $-\alpha^{3.7}$  deletion was observed mainly in Afro-descendants, classification based on their ancestor's origin (da Luz *et al.*, 2013). Soler *et al.* (2016) reported for the first time in Latin America the  $-\alpha^{5.2}$  deletion, a mutation observed in Greek and Italian populations (Pressley *et al.*, 1980; Fortina *et al.*, 1994).

Previous studies, have shown that the Uruguayan population is tri-hybrid, with a greater genetic contribution of European populations (~84%, mainly Spaniards and Italians) followed in smaller proportions by Native Americans (~10%) and sub-Saharan populations (~6%) (Sans, 1994; Hidalgo *et al.*, 2005; Sans *et al.*, 2006).

In order to contribute to knowledge of  $\alpha$ -thalassemia mutations in the Uruguayan population, we analyzed 168 outpatients from Montevideo and Salto (117 and 51 respectively) who showed normal Hb levels and reduced levels of VCM and MCH. Additionally, to investigate the  $-\alpha^{3.7}$  deletion origin, we sequenced the alpha-MRE region in individuals with and without this mutation.

## Subjects and Methods

A total of 168 unrelated individuals among 1 and 84 years old were analyzed, 117 outpatients from Hospital de Clínicas - Universidad de la República (UdelaR), Montevideo and 51 from Hospital Regional Salto - Administración de Servicios de Salud del Estado (ASSE), recruited in 2015. The protocol and procedure employed in this research were in compliance with the Helsinki Declaration. Each subject and the children's parents gave written informed consent to participate in the study.

All individuals present normal levels of Hb and diminished levels of VCM and MCH. In adults ( $\geq 15$  years old) the Hb levels were  $\geq 13$  g/dL for men and  $\geq 12$  g/dL for

women. For both sexes, the VCM and MCH levels were  $\leq 80$  fl and  $\leq 27$  pg respectively. For individuals between 12 and 15 years old, we considered Hb levels  $\geq 12$  and VCM and MCH levels  $\leq 78$  fl and  $\leq 26$  pg respectively. In children under 12 years old, the levels of Hb were greater than 11 g/dL. In the range between  $\geq 5$  and  $< 12$  years old the VCM and MCH levels were  $\leq 77$  fl and  $\leq 25$  pg respectively, whereas for children under 5 years old VCM and MCH levels were  $\leq 75$  fl and  $\leq 24$  pg respectively. The Hb thresholds were according those established by World Health Organization (de Benoist *et al.*, 2008) and the VCM and MCH levels according to Besses *et al.* (2007).

Genomic DNA was extracted from venous peripheral blood by the salting out method (Miller *et al.*, 1988). All individuals were analyzed for the presence of deletional and non-deletional  $\alpha$ -thalassemia mutations. The seven most common deletional  $\alpha$ -thalassemia ( $-\alpha^{3.7}$ ,  $-\alpha^{4.2}$ ,  $--^{SEA}$ ,  $--^{FIL}$ ,  $--^{MED}$ ,  $-\alpha^{20.5}$ , and  $--^{THAI}$ ) were checked by multiplex gap polymerase chain reaction (gap-PCR) and agarose gel electrophoresis, according to conditions already described (Tan *et al.*, 2001). The most common non-deletional alpha thalassaemia mutations were analyzed by restriction fragment length polymorphism (RFLP) from products amplified by PCR ( $\alpha^{Hpb}\alpha$ ,  $\alpha^{Ncol}\alpha$  and  $\alpha^{Ncol}$ ) (Hall *et al.*, 1993). Additionally, HBA1 and HBA2 genes were sequenced to analyze the presence of point mutations or small deletion (Pedroso *et al.*, 2018; Dodé *et al.*, 1990).

The  $\alpha$ -MRE haplotypes were determined in 157 individuals: 78 individuals with the  $-\alpha^{3.7}$  deletion and 79 without  $\alpha$ -thalassemia mutations. A 310bp DNA fragment was amplified and sequenced according to conditions previously described (Harteveld *et al.*, 2002). Haplotypes were constructed by assuming that the presence of two common haplotypes was more probable than the combination of one common and one rare haplotype or two rare haplotypes (Long *et al.*, 1990; Castro de Guerra *et al.*, 1997).

## Statistical methods

The genotypic and allelic frequencies were estimated by gene counting. The Hardy-Weinberg equilibrium, the estimation of heterogeneity among the samples by the exact test of population differentiation, and the pairwise  $F_{ST}$  were evaluated using Arlequin software package v 3.5.2.2 (Excoffier and Lischer, 2010).  $F_{ST}$  distances between Uruguayan population and other populations were represented in two dimensions by multidimensional scaling (MDS) using the software SPSS 22.0. Data of other populations were obtained from Harteveld *et al.* (2002), Ribeiro *et al.* (2003) and from the 1000 Genomes Database.

## Results

$\alpha$ -thalassemia mutations were found in 55 individuals (32.7%). The distribution and frequencies of  $\alpha$ -thalassemia mutations are showed in Table 1. The  $-\alpha^{3.7}$  deletion was the most frequent mutation observed, 49 individuals heterozygous  $-\alpha^{3.7}/\alpha\alpha$  and two homozygous  $-\alpha^{3.7}/-\alpha^{3.7}$ , whereas the  $-\alpha^{4.2}$  deletion was observed in only one individual. Three individuals were heterozygous for the point mutation HBA2: c.-59C>T. The mutations distribution and frequencies do not differ

significantly between the two cities (Montevideo and Salto) analyzed.

The number of different  $\alpha$ -MRE genotypes and  $\alpha$ -MRE haplotypes are showed in Table 2 and Table S1. The distribution of  $\alpha$ -MRE genotypes and  $\alpha$ -MRE haplotypes were significantly different between individuals with and without the  $-\alpha^{3.7}$  deletion ( $p < 0.05$ ).

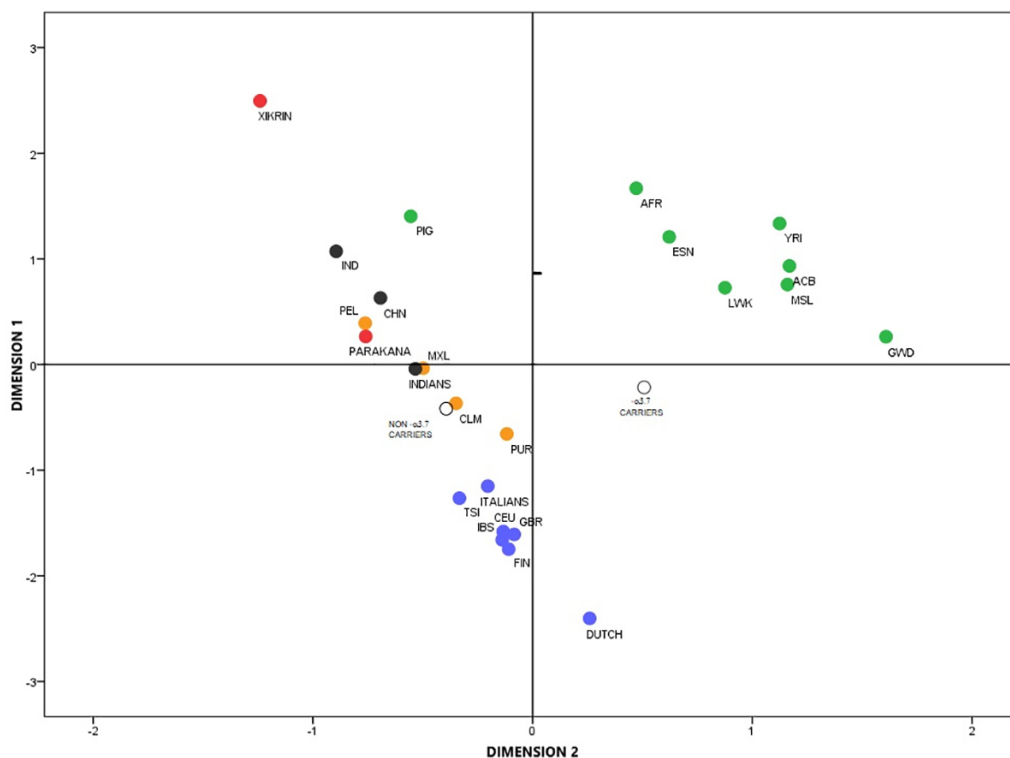
Multidimensional scaling (MDS) showed the subsample without  $-\alpha^{3.7}$  deletion grouped with other Latino-American populations whereas, the sample with the  $-\alpha^{3.7}$  deletion was split from these in the second dimension and was found more related to African populations (Figure 1). The lower genetic distances from the subsample without the  $-\alpha^{3.7}$  deletion were with Latin-American populations from Peru, Colombia, Puerto

**Table 1** – Alpha thalassaemia mutations found in the Uruguayan microcytic and hypochromic sample.

Genotype	Montevideo	Salto	Total
	N (%)	N (%)	N (%)
$\alpha\alpha/\alpha\alpha$	77 (65.0)	36 (70.7)	113 (67.3)
$-\alpha^{3.7}/\alpha\alpha$	36 (30.8)	13 (25.5)	49 (29.2)
$-\alpha^{3.7}/-\alpha^{3.7}$	1 (0.9)	1 (1.9)	2 (1.2)
$-\alpha^{4.2}/\alpha\alpha$	1 (0.9)		1 (0.6)
HBA2:c.-59C>T	2 (1.7)	1 (1.9)	3 (1.7)
Total	117	51	168

**Table 2** –  $\alpha$ -MRE genotypes in Uruguayan population with and without  $-\alpha^{3.7}$  deletion.

Population	$\alpha$ -MRE genotypes						N	p
	AA (%)	AB (%)	AD (%)	BB (%)	BD (%)	DD (%)		
$\alpha^{3.7}$ deletion	23 (29.5)	31 (39.7)	10 (12.8)	5 (6.4)	8(10.3)	1 (1.3)	78	0.0053
$\alpha\alpha/\alpha\alpha$	32 (40.5)	33 (41.8)	1 (1.3)	11 (13.9)	2 (2.5)		79	



**Figure 1** – Multidimensional scaling (MDS) based on pairwise  $F_{ST}$  genetic distances calculated from alpha-MRE genotypes.

Stress: 0.01390; RSQ: 0.99913.  $-\alpha^{3.7}$  CARRIERS & NON  $-\alpha^{3.7}$  CARRIERS: Uruguayan populations. Green: African and African derived populations. YRI: Yoruba in Ibadan, Nigeria. ACB: African Caribbeans in Barbados. GWD: Gambian in Western Divisions in the Gambia. MSL: Mende in Sierra Leone. ESN: Esan in Nigeria. LWK: Luhya in Webuye, Kenya. AFR: Bantu-speaking Africans. PIG: Pygmies from the Central African Republic. Orange: Admixed Latin-American populations. PEL: Peruvians from Lima, Peru. PUR: Puerto Ricans from Puerto Rico. CLM: Colombians from Medellin, Colombia. MXL: Mexican Ancestry from Los Angeles USA. Blue: European populations. IBS: Iberian Population in Spain. TSI: Toscani in Italia. CEU: Northern and Western European Ancestry. FIN: Finnish in Finland. ITALIANS. DUTCH. GBR: British in England and Scotland. Black: Asian populations. IND: Indonesians from Java. CHN: Southern Chinese. INDIANS: Andra-Pradesh, India. (1000genomes; Hartevelde *et al.*, 2002; Ribeiro *et al.*, 2003).

Rico and Mexico. However, the subsample with the  $-\alpha^{3.7}$  deletion presented the lowest genetic distances with Africans and Afro-Latin-American populations (Table S2).

## Discussion

Our results show that  $\alpha$ -thalassemia mutations explain an important percentage (32.7%) of the microcytosis and hypochromia observed in the Uruguayan population without anemia. Furthermore, the  $-\alpha^{3.7}$  deletion was the most frequent mutation. The only non-deletional  $\alpha$ -thalassemia mutation observed (HBA2 c.-59C>T), affects the last nucleotide of TATA box. This mutation was reported only once in an individual from Australia. In vitro analysis shows a reduction of the transcriptional activity in 53.7% when compared with the normal allele (HBA2 wild type) (Qadah *et al.*, 2014). In our study, this mutation was observed in three non-related individuals from two different regions of Uruguay. This data suggests a possible founder effect in the Uruguayan population.

The  $\alpha$ -thalassemia observed frequency (32.7%) is similar to previously reported for Rio Grande do Sul, Brazil (31.7%) (Wagner *et al.*, 2010). However, it is lower than the one reported for Campinas, Southeastern Brazil (49.9%) (Borges *et al.*, 2001). The high frequency reported for Campinas could be explained by a greater African contribution in this population. Nevertheless, in the Campinas sub-sample self-reported as Caucasian, the  $\alpha$ -thalassemia frequency was also higher (41.5%) than the observed for Rio Grande do Sul and Uruguay (Borges *et al.*, 2001; Wagner *et al.*, 2010; da Luz *et al.*, 2013). We hypothesize that those differences may be due to African genetic ancestry not detected in this subsample. Several studies had showed that Latin-American self-reported as whites present variable levels of African and/or Native American genetic ancestry. For example, in a female population from Brasilia, the percentage of African ancestry in self-reported whites was 17.2% (Lins *et al.*, 2011). Another explanation may be that the Campinas population self-reported as white presents a greater European contribution from populations with higher  $\alpha$ -thalassemia frequencies, as South-Italians (Weatherall and Clegg, 2001).

The observed similarity between Rio Grande do Sul and Uruguay is according with the common historical origin from these populations as well as with genetic data showing a high similarity at DNA mitochondrial level (Marrero *et al.*, 2007).

In relation to alpha-MRE haplotypes, the observed differences between the two Uruguayan subsamples (with and without 3.7 deletion) is due to a high frequency of haplotype D in  $\alpha$ -thalassemia subsample (Table S1). Haplotype D is characteristic of African populations. It is practically absent in others populations and therefore, is a useful marker for African ancestry (Harteveld *et al.*, 2002). Moreover, the haplotype B frequency for both Uruguayan subsamples was lower than the ones observed for European populations and similar to observed for other admixed Latin-American and Asian populations (Harteveld *et al.*, 2002; Ribeiro *et al.*, 2003). With exception of Pigmy populations, the MDS plot of  $F_{ST}$  genetic distances calculated from alpha-MRE haplotypes clearly discriminated among African, European and Asian populations. As expected, Native American populations were grouped with Asian populations. Furthermore, admixed

Latin-American populations were placed at an intermediate position between Asian and European populations according to admixture degree. For example, Peruvian and Mexican populations, who have the greater Native American ancestry, were clustered with Asian populations, whereas Colombian and Puerto Rico populations were placed near European populations. Moreover, the Puerto Rico population, which has the greater African ancestry, was displaced towards the African cluster (Rishishwar *et al.*, 2015; Norris *et al.*, 2018). The position of the Uruguayan subsample without the  $-\alpha^{3.7}$  deletion next to the Colombian population, agrees with previous reports about the genetic ancestry of these two populations. Similar to reported for a Colombian population from Medellin (Norris *et al.*, 2018), the Uruguayan population shows a greater European genetic ancestry (76.2%-84%) followed in smaller proportions by Native Americans (10% - 14.7%) and African ancestry (6% - 9.1%) (Sans, 1994; Hidalgo *et al.*, 2005; Sans *et al.*, 2006; Bonilla *et al.*, 2015). However, the Uruguayan  $\alpha$ -thalassemia subsample is placed near African populations, suggesting a greater African ancestry in this subsample. This is in accordance with previous studies that had shown a greater African ancestry based on self-perception in individuals with the  $-\alpha^{3.7}$  deletion (da Luz *et al.*, 2013).

In summary,  $\alpha$ -thalassemia mutations explain a significant proportion of microcytosis and hypochromia in the Uruguayan population. The alpha-MRE haplotypes and the  $\alpha$ -thalassemia mutations spectrum observed suggest a predominant, but not exclusive, African origin of the  $-\alpha^{3.7}$  deletion in Uruguay.

## Acknowledgments

The authors would like to thank all patients who kindly participated in this study. We also thank Dra. Elza Miyuki Kimura for her technical support. This study received financial support from Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior, Brazil and Universidad de la República, Uruguay (Programa CAPES-UdelaR, grant number no. 2014/00984-3), and Comisión Coordinadora del Interior, Uruguay (CCI-2015).

## Conflict of Interest

The authors report no conflicts of interest that could be perceived as prejudicial to the impartiality of the reported research.

## Authors Contributions

JL and MFS conceived the study. AMS, BFP, LS and GAP conducted the experiments. AMS, BFP and JL analyzed the data, AMS and JL wrote the manuscript. ES and PL collected samples and clinical data: All authors read and approved the final version.

## References

- Besses C, Sans-Sabrafén J and Vives JL (2007) Hematología Clínica. 5th edition. Elsevier, Madrid.
- Bonilla C, Bertoni B, Hidalgo PC, Artagaveytia N, Ackermann E, Barreto I, Cancela P, Cappetta M, Egaña A, Figueiro G *et al.* (2015) Breast cancer risk and genetic ancestry: a case-control study in Uruguay. BMC Women's Health 15:11.

- Borges E, Wenning MRSC, Kimura EM, Gervasio SA, Costa FF and Sonati MF (2001) High prevalence of alpha-thalassemia among individuals with microcytosis and hypochromia without anemia. *Braz J Med Biol Res* 34:759-762.
- Castro de Guerra DC, Hutz MH, Bortolini MC and Salzano FM (1997) Beta-globin gene cluster haplotypes in an admixed Venezuelan population. *Am J Hum Biol* 9:323-327.
- da Luz J, Avila A, Icasuriaga S, Gongora M, Castillo L, Serrón A, Kimura EM, Costa FF, Sans M and Sonati MF (2013) Frequency and spectrum of hemoglobinopathy mutations in a Uruguayan pediatric population. *Genet Mol Biol* 36:316-322.
- de Benoist B, Cogswell M, Egli I and McLean E (2008) *Worldwide prevalence of anaemia 1993-2005*. WHO Press, Genève.
- Dodé C, Rochette JR and Krishnamoorthy R (1990) Locus assignment of human  $\alpha$ -globin mutations by selective amplification and direct sequencing. *Br J Haematol* 76:275-281.
- Excoffier L and Lischer HE (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* 10:564-567.
- Foglietta E, Deidda G, Graziani B, Modiano G and Bianco I (1996) Detection of  $\alpha$ -globin gene disorders by a simple PCR methodology. *Haematologica* 81:387-396.
- Fortina P, Parrella T, Sartore M, Gottardi E, Gabutti V, Delgrosso K, Mansfield E, Rappaport E, Schwartz E and Camaschella C (1994) Interaction of a rare illegitimate recombination event and Poly A addition site mutation resulting in a severe form of alpha thalassemia. *Blood* 83:3356-3362.
- Hall GW, Thein SL, Newland AC, Chisholm M, Traeger-Synodinos J, Kanavakis E, Kattamis E and Higgs DR (1993) A base substitution (T $\rightarrow$ C) in codon 29 of the  $\alpha 2$ -globin gene causes  $\alpha$  thalassaemia. *Br J Haematol* 85:546-552.
- Harteveld CL, Muglia M, Passarino G, Kielman MF and Bernini LF (2002) Genetic polymorphism of the major regulatory element HS-40 upstream of the human alpha-globin gene cluster. *Br J Haematol* 119:848-854.
- Hidalgo PC, Bengochea M, Abilleira D, Cabrera A and Alvarez I (2005) Genetic admixture estimate in the Uruguayan population based on the loci LDLR, GYPA, HBBG, GC and D7S8. *Int J Hum Genet* 5:217-222.
- Higgs DR, Vickers MA, Wilkie AO, Pretorius IM, Jarman AP and Weatherall DJ (1989) A review of the molecular genetics of the human  $\alpha$ -globin gene cluster. *Blood* 73:1081-1104.
- Huisman THJ, Carver MFH and Baysal E (1998) *A syllabus of thalassemia mutations*. Sick Cell Anaemia Foundation, Augusta.
- Isola E (1975) *La esclavitud en el Uruguay desde sus comienzos hasta su extinción (1743-1852)*. Comisión Nacional de Homenaje del Sesquicentenario de los hechos históricos de 1825; Talleres Gráficos Monteverde y Cia, Montevideo.
- Kattamis AC, Camaschella C, Sivera P, Surrey S and Fortina P (1996) Human  $\alpha$ -thalassemia syndromes: detection of molecular defects. *Am J Hematol* 53:81-91.
- Lins TC, Vieira RG, Abreu BS, Gentil P, Moreno-Lima R, Oliveira RJ and Pereira RW (2011) Genetic heterogeneity of self-reported ancestry groups in an admixed Brazilian population. *J Epidemiol* 21:240-245.
- Long JC, Chakravarti A, Bohem CD, Antonarakis S and Kazazian HH (1990) Phylogeny of human beta-globin haplotypes and its implications for recent human evolution. *Am J Phys Anthropol* 81:113-130.
- Marrero AR, Bravi C, Stuart S, Long JC, Pereira das Neves Leite F, Kommers T, Carvalho CMB, Pena SDJ, Ruiz-Linares A, Salzano FM *et al.* (2007) Pre- and post-Columbian gene and cultural continuity: the case of the Gaucho from southern Brazil. *Hum Hered* 64:160-171.
- Miller SA, Dykes DD and Polesky HFRN (1988) A simple salting out procedure for extracting DNA from human nucleated cell. *Nucleic Acids Res* 16:1215-1216.
- Norris ET, Wang L, Conley AB, Rishishwar L, Mariño-Ramírez L, Valderrama-Aguirre A and Jordan IK (2018) Genetic ancestry, admixture and health determinants in Latin America. *BMC Genomics* 19:75-87.
- Pedroso GA, Kimura EM, Santos MNN, Albuquerque DM, Malimpensa D, Jorge SE, Verissimo MPA, Costa FF and Sonati MF (2018) Thalassemia major phenotype caused by HB Zürich-Albisrieden [ $\alpha 2$  59(E8) Gly > Arg (HBA2:C.178G > C)] in a Brazilian child. *Pediatr Blood Cancer* 65:e27413.
- Pressley L, Higgs DR, Aldridge B, Metaxatou-Mavromati A, Clegg JB and Weatherall DJ (1980) Characterization of a new  $\alpha$  thalassemia 1 defect due to a partial deletion of the  $\alpha$ -globin gene complex. *Nucleic Acids Res* 8:4889-4898.
- Qadah T, Finlayson J, Dennis M and Ghassemifar R (2014) Molecular and cellular analysis of three novel alpha2-globin gene promoter mutations [HBA2:c.-59C>T], [HBA2:c.-81C>A] and [HBA2:c.-91G>A] reveal varying patterns of transcriptional and translational activities. *Pathology* 46:46-52.
- Ribeiro DM, Figueredo MS, Costa FF and Sonati MF (2003) Haplotypes of  $\alpha$ -globin gene regulatory element in two Brazilian native populations. *Am J Phys Anthropol* 12:58-62.
- Rishishwar L, Conley AB, Wigington CH, Wang L, Valderrama-Aguirre A and Jordan IK (2015) Ancestry, admixture and fitness in Colombian genomes. *Sci Rep* 5:12376.
- Sans M (1994) *Estudio genético e histórico de la población del departamento de Tacuarembó, Uruguay*. PhD Thesis, Universidad de la República, Montevideo.
- Sans M, Merriwether DA, Hidalgo PC, Bentancor N, Weimer TA, Franco MHL, Alvarez I, Kemp BM and Salzano FM (2006) Population structure and admixture in Cerro Largo, Uruguay, based on blood markers and mitochondrial DNA polymorphisms. *Am J Hum Biol* 18:513-524.
- Soler AM, Schelotto M, de Oliveira Mota N, Dorta Ferreira R, Sonati MF and da Luz JA (2016) The  $-(\alpha)(5.2)$  deletion detected in a Uruguayan family: first case report in the Americas. *Haemoglobin* 40:289-292.
- Steinberg MH, Forget BG, Higgs DR and Nagel RL (2001) Disorders of hemoglobin: genetics, pathophysiology, and clinical management. *J R Soc Med* 94:602-603.
- Tan ASC, Quah TC, Low PS and Chong SS (2001) A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for  $\alpha$ -thalassemia. *Blood* 98:250-251.
- The 1000 Genomes Project Consortium (2015) A global reference for human genetic variation. *Nature* 526:68-74.
- Wagner SC, de Castro SM, Gonzalez TP, Santin AP, Filippon L, Zaleski CF, Azevedo LA, Amorin B, Callegari-Jacques SM and Hutz MH (2010) Prevalence of common  $\alpha$ -thalassemia determinants in south Brazil: Importance for the diagnosis of microcytic anemia. *Genet Mol Biol* 33:641-645.
- Weatherall DJ and Clegg JG (1981) *The thalassaemia syndromes*. 3rd edition. Blackwell Scientific Publications, Oxford.
- Weatherall DJ and Clegg JB (2001) Inherited haemoglobin disorders: An increasing global health problem. *Bull World Health Organ* 79:704-712.
- Weiss G and Goodnough LT (2005) Anemia of chronic disease. *N Engl J Med* 352:1011-1023.

## Supplementary material

The following online material is available for this article:

Table S1 –  $\alpha$ -MRE haplotypes.

Table S2 – FST genetic distances.

*Associate Editor: Filippo Pinto e Vairo*

*License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License (type CC-BY), which permits unrestricted use, distribution and reproduction in any medium, provided the original article is properly cited.*