Ancestry of the α-MRE Associated with the 3.7kb α-Thalassemia Deletion in the Portuguese Population

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Introduction

The **α-major regulatory element** (α-MRE), also known as **HS-40**, is a 350 bp enhancer located upstream of the α-globin gene cluster and has a crucial role in the **long-range regulation of α-globin gene expression**[1,2]. This element is genetically polymorphic and six haplotypes (A to F) have been identified in different populations (Figure 1). **Haplotype D** was primarily described in **African populations** and is nearly absent in other populations[3].

In Portugal, the principal molecular basis of α-thalassemia is the **3.7kb deletion** that removes one α-globin gene per allele[4].

Aims

Identification of the HS-40 haplotypes associated with the common 3.7kb α-thalassemia deletion (-α3.7del) in the Portuguese population, and investigation of its ancestry.

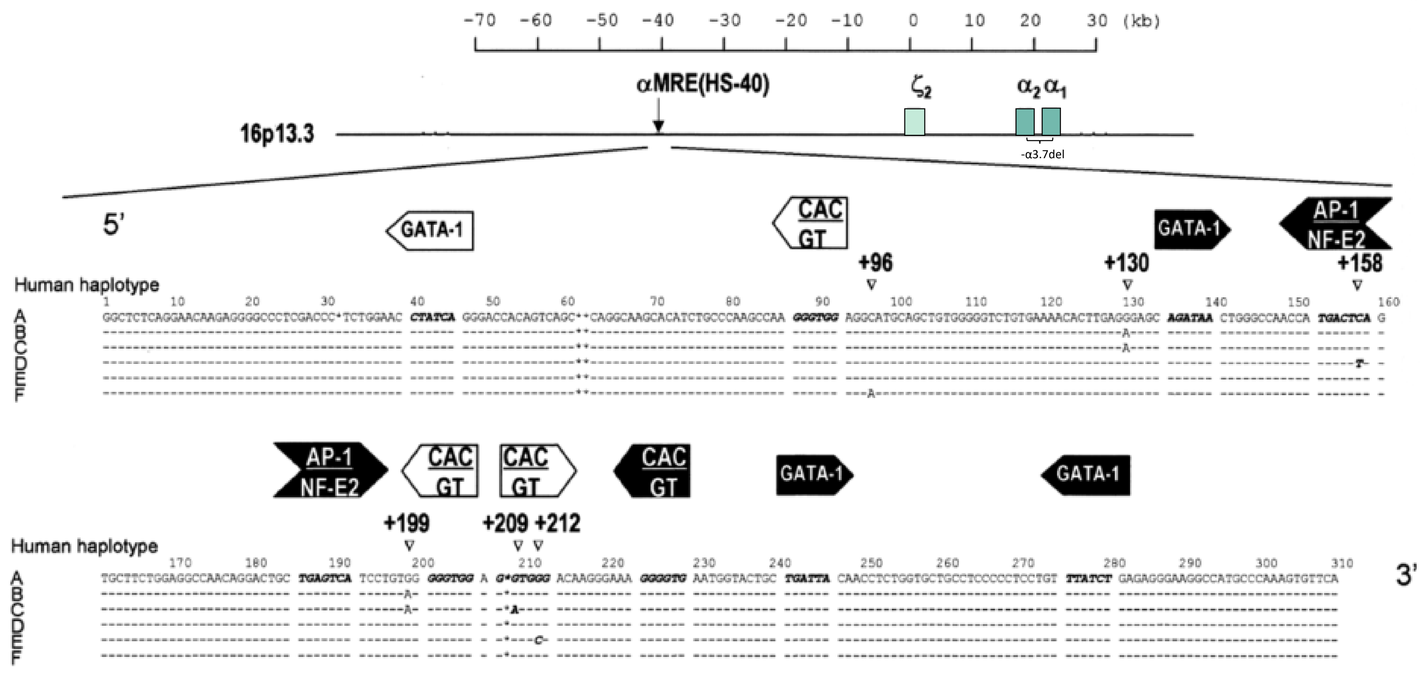


Figure 1 - Schematic representation of the α-globin gene cluster and the upstream regulatory region. Functional genes ζ_2, α_2 and α_1 are represented as blue boxes and the major regulatory element or HS-40 is indicated by a black arrow. The human HS-40 sequence is shown below the α-cluster. The small bars represent sequence similarity, while the asterisks represent gaps in comparison with the human sequence. The position and direction of the binding sites for nuclear factors GATA-1, AP-1/NF-E2, and the CAC/GT binding factors are indicated as arrows. Dark arrows represent the binding sites occupied *in vivo*; white arrows represent binding sites that are not occupied *in vivo*. The human HS-40 haplotypes are indicated next to each sequence as A to F. The numbers indicate the position of the polymorphic sites in the HS-40 sequence. Adapted from [3].

Materials and Methods

1. We selected **111 Portuguese individuals** previously characterized by Gap-PCR for the presence of the **-α3.7del;**
2. A fragment containing the **HS-40** was amplified by PCR and Sanger sequenced;
3. Statistical analysis was performed using *R* software.

Table 1 - HS-40 genotypes in the Portuguese population without the α-thalassemia 3.7kb deletion (αα/αα), with the deletion in heterozygosity ($-\alpha^{3.7}/\alpha\alpha$) and in homozygosity ($-\alpha^{3.7}/-\alpha^{3.7}$)

α-globin genotype	HS-40 genotypes						
	AA	AB	AD	BB	BC	BD	DD
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
αα/αα (n = 50)	18 (36.0)	20 (40.0)	1 (2.0)	8 (16.0)	3 (6.0)	0 (0.0)	0 (0.0)
$-\alpha^{3.7}/\alpha\alpha$ (n = 34)	15 (44.1)	13 (38.2)	3 (8.8)	3 (8.8)	0 (0.0)	0 (0.0)	0 (0.0)
$-\alpha^{3.7}/-\alpha^{3.7}$ (n = 27)	7 (25.9)	7 (25.9)	**10 (37.0)**	0 (0.0)	0 (0.0)	**1 (3.7)**	**2 (7.4)**
Total (n = 111)	40 (36.0)	40 (36.0)	14 (12.6)	11 (10.0)	3 (2.7)	1 (0.9)	2 (1.8)

n= number of individuals

- 71.4% of AD individuals are homozygous for the -α3.7del
- **Genotype AD** is the most common in individuals with the **$-\alpha^{3.7}/-\alpha^{3.7}$ genotype**

Table 2 - HS-40 haplotypes in the Portuguese population without the α-thalassemia 3.7kb deletion (αα/αα), with the deletion in heterozygosity ($-\alpha^{3.7}/\alpha\alpha$) and in homozygosity ($-\alpha^{3.7}/-\alpha^{3.7}$)

α-globin genotype	HS-40 haplotypes			
	A	B	C	D
	x (%)	x (%)	x (%)	x (%)
αα/αα (n = 50; x = 100)	57 (57.0)	39 (39.0)	3 (3.0)	1 (1.0)
$-\alpha^{3.7}/\alpha\alpha$ (n = 34; x = 68)	46 (67.7)	19 (27.9)	0 (0.0)	3 (4.4)
$-\alpha^{3.7}/-\alpha^{3.7}$ (n = 27; x = 54)	31 (57.4)	8 (14.8)	0 (0.0)	**15 (27.8)**
Total (n = 111; x = 222)	134 (60.4)	66 (29.7)	3 (1.3)	19 (8.6)

n= number of individuals; x= number of alleles

- In individuals with the **$-\alpha^{3.7}/-\alpha^{3.7}$ genotype** **haplotype D** is the second most frequent

Distribution of HS-40 haplotypes and genotypes are **significantly different** between groups with and without the -α3.7del ($p<0.001$)

Results and Discussion

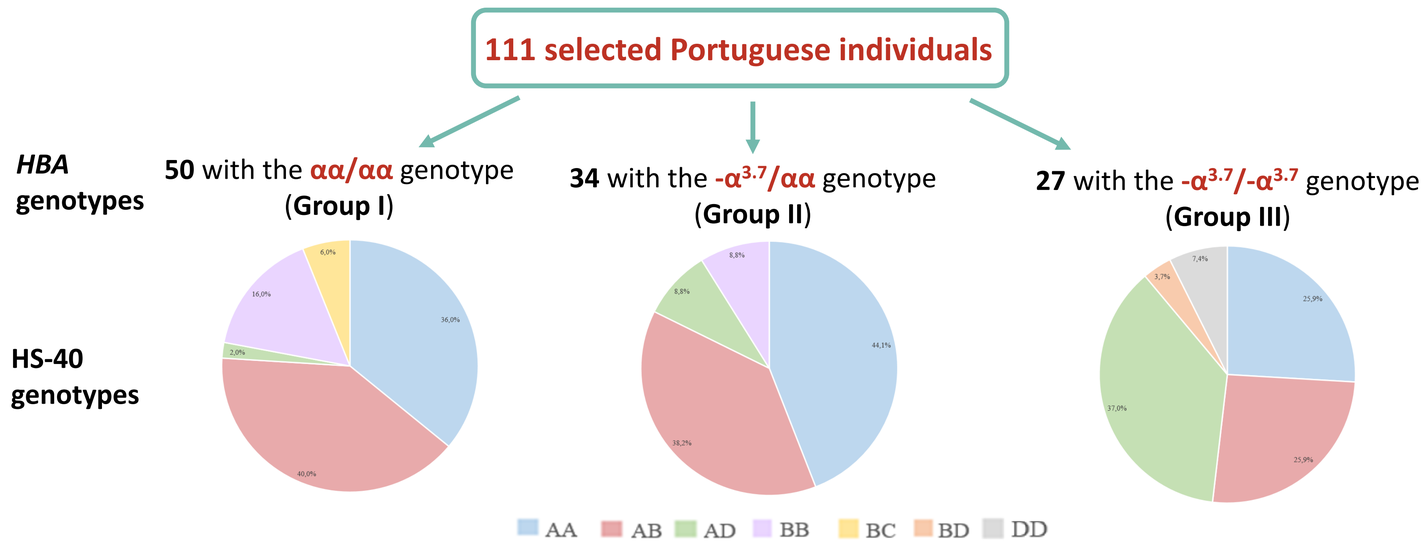
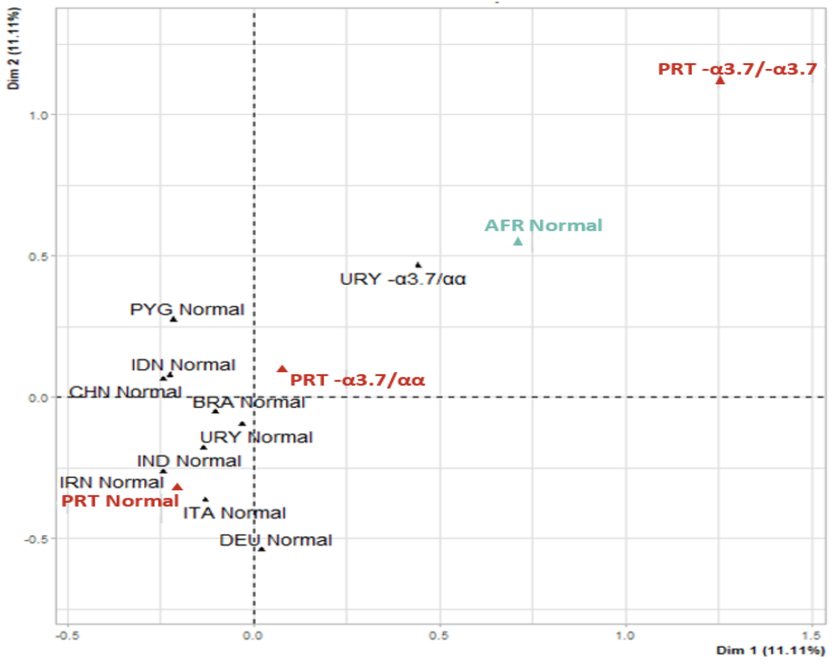


Figure 2 – Graphic distribution of the different HS-40 genotype frequencies (in %) according to the corresponding α-globin genotypes of the studied Portuguese individuals. (I) group without the 3.7kb α-thalassemia deletion (αα/αα); **(II)** group with the 3.7kb deletion in heterozygosity ($-\alpha^{3.7}/\alpha\alpha$); **(III)** group with the 3.7kb deletion in homozygosity ($-\alpha^{3.7}/-\alpha^{3.7}$).



Portuguese individuals **without the -α3.7del** → Grouped with other **European populations**

Portuguese individuals **with the -α3.7del** → More closely related to the **African population**

Figure 3 - Multiple correspondence analysis of the HS-40 genotypes in diverse geographic populations. AFR: African; BRA: Brazilian; CHN: Chinese; DEU: Dutch; IDN: Indonesian; IND: Indian; IRN; Iranian; ITA: Italian; PRT: Portuguese; PYG: Pygmies; URY: Uruguayan. All the genotypes from foreign populations were collected from [3,5-7]. The Portuguese populations investigated in this study are marked as **PRT normal** (group I); **PRT -α3.7/αα** (group II) and **PRT -α3.7/-α3.7** (group III).

Conclusions

- This study revealed for the first time an association of a specific HS-40 haplotype with the common **-α3.7del in the Portuguese population**, and its **likely African ancestry**;
- These results may have clinical importance as **haplotype D** has an alteration in the consensus sequence of the transcriptional factors AP1/NF-E2 binding site and *in vitro* experiments showed a **decrease in its enhancer activity on α-globin genes** [8].

References

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