Hb Plasencia [α2 125(H8)Leu→Arg] is a frequent cause of α^+-thalassemia in the Portuguese population

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ABSTRACT

Hb Plasencia is a thalassemic hemoglobin (Hb) caused by a Leucine to Arginine replacement at residue 125 of the alpha 2 globin chain [α125(H8)Leu→Arg (α2)] (HBA2:c.377T>G). This variant was first described in the heterozygous state associated with a very mild alpha thalassemic phenotype in three members of a Spanish family from Plasencia.

Reviewing the molecular characterization of 308 Portuguese individual suspect of having alpha thalassemia, we found Hb Plasencia as the second most frequent mutation, after the $-\alpha^{3.7}$ deletion.

Alpha plus thalassemia is often associated with *HBA* genes deletions, most frequently the 3.7kb rightward deletion (RW) and the 4.2kb leftward deletion (LW). *HBA* point mutations were rarely described. However, with the increased accessibility to automatic sequencing, the number of known alpha thalassemic Hb variants has increased (1,2).

Hb Plasencia is a thalassemic hemoglobin variant caused by a Leucine to Arginine replacement at residue 125 of the alpha 2 globin chain [α125(H8)Leu→Arg (α2)] (HBA2:c.377T>G). The replacement of a non polar amino acid residue (leucine) by the positively charged amino acid (arginine) changes the $\alpha 1\beta 1$ contact and, therefore, the dimer formation is impaired. No abnormal Hb fractions are detected by electrophoretic or chromatographic methods. This variant was described for the first time in the heterozygous state in three members of a Spanish family from Plasencia, associated with a very mild alpha thalassemia phenotype (3).

Homozygous state for Hb Plasencia has been described in a Portuguese female, from a consanguineous couple, with a clinical presentation suggestive of a type II congenital dyserythropoietic anemia (CDA II) (4).

We reviewed the molecular characterization of 308 samples from individuals attending our hematology clinic or referred from other centers in Portugal, with mild microcytosis (mean cellular volume < 80 fL) and/or hypochromia (mean cellular Hb < 27 pg) and normal Hb A_2 (< 3,5%) and Hb F (< 2%) levels. Iron deficiency anemia was excluded. None of the samples showed abnormal hemoglobin fractions (including Hb H) by high performance liquid chromatography (HPLC) on a VARIANT II™ with the β-Thalassemia Short Program (Bio-Rad Laboratories, Hercules, CA, USA). Informed consent was obtained from all individuals included.

$-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions were screened by multiplex gap-polymerase chain reaction (Gap-PCR) according to Chong et al. (5).

HBA2:c.377T>G mutation was screened by *Msp*I restriction enzyme digestion of the selectively amplified *HBA2* gene. The digestion products were visualized on a 3% agarose gel stained with SYBR® Safe DNA Gel Stain (Invitrogen, Carlsbad, CA, USA) (Figure 1).

Results are summarized in Table I: Among the 308 samples with mild microcytosis and/or hypochromia, 166 had, at least, one of the $-\alpha^{3.7}$ or $-\alpha^{4.2}$ deletions and 23 (from 21 families) were carriers of Hb Plasencia. The $-\alpha^{3.7}$ deletion was found in 119 individuals in the heterozygous state, 30 homozygous and 3 compound heterozygous ($-\alpha^{3.7}$ / $-\alpha^{4.2}$).

Hb Plasencia carriers identified were mainly from Central Portugal and the island of S. Miguel in Azores.

The 23 carriers of Hb Plasencia presented Hb, MCV and MCH values similar to those observed in the heterozygous for $-\alpha$ deletions. Data are summarized in Table II.

As the homozygous state is associated with a CDA II-like phenotype we also screened 22 other patients with severe anemia and dyserythropoesis, but none presented the HBA2:c.377T>G mutation.

Although in the Portuguese population alpha thalassemia is commonly associated with the $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions (6), two novel alpha thalassemic Hb variants have already been described in the Portuguese population, Hb Evora (HBA2:c.106T>C; p.Ser36Pro) (7) and Hb Iberia (HBA2:c.313T>C; p.Cys105Arg) (C. Bento et al, Hemoglobin, *in press*).

Hb Plasencia can be easily identified by restriction enzyme digestion. It is important to remember that this Hb variant, when in the homozygous state, is associated with important dyserythropoiesis.

This work revealed a frequency of the alpha thalassemic hemoglobin variant Hb Plasencia higher than expected. Therefore, we propose that individuals with moderate hypochromic/microcytic anemia, in the absence of iron depletion, and normal Hb A2 and Hb F, in first place should be investigated for the common $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions and than for Hb Plasencia by restriction enzyme digestion.

If these screening turns out to be negative, point mutations in the HBA genes should be investigated by direct sequencing. Alpha-thalassemia individuals heterozygous for large deletions in the alpha globin-cluster have more prominent hipocromic/microcytic anemia than the heterozygous for the $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions or for point mutations. Multiplex ligation-dependent probe amplification (MLPA) is used for large deletions detection (8). Hematological parameters, namely the degree of the hypochromia/microcytosis, the presence or absence of Hb H and familiar history are important data to guide the investigation and the selection for appropriate methodologies.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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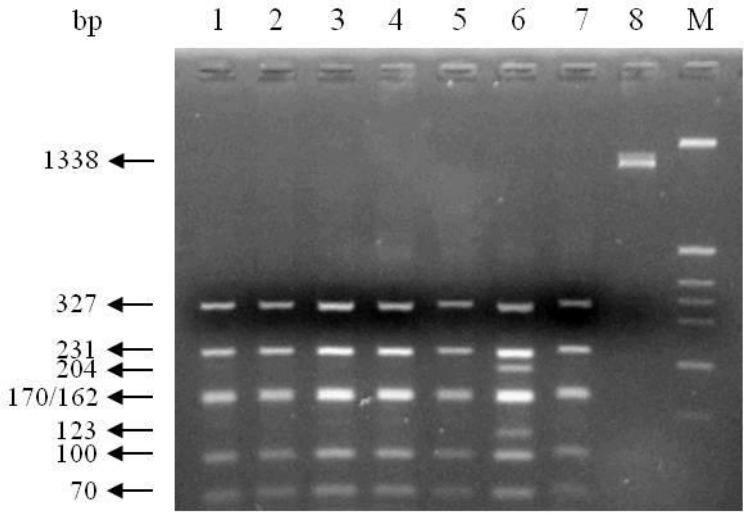

Figure 1. *Msp*I restriction enzyme digestion results. A 1338 base pairs (bp) amplicon was digested with the restriction enzyme *Msp*I. The mutation creates a new cutting site for this enzyme, the 327 bp fragment was split in two additional fragments with 204 bp and 123 bp. Lanes: 1-5,7 – Normal individuals; 6 – Heterozygous α2 CD 125 (CTG/CGG); 8 – Non digested PCR fragment; M – DNA molecular weight marker pBR322 DNA/*Hinf* I

Table I – Total of individuals with mild microcytosis and/or hypochromia presenting α^{+}-thalassemia caused to $-\alpha^{3.7}$ or $-\alpha^{4.2}$ deletions or Hb Plasencia.

Genotype	**Number**	**Percentage (%)**
$(-\alpha^{3.7}/\alpha\alpha)$	119	38.64
$(-\alpha^{3.7}/-\alpha^{3.7})$	30	9.74
$(-\alpha^{4.2}/\alpha\alpha)$	13	4.22
$(-\alpha^{4.2}/-\alpha^{4.2})$	1	0.32
$(-\alpha^{3.7}/-\alpha^{4.2})$	3	0.97
$(\alpha^{Plasencia}\alpha/\alpha\alpha)$	23	7.47
Total individuals studied	308	100

Table II – Hematological parameters observed in Hb Plasencia carriers (Two samples from 2 children were excluded). N = normal values.

	Erythrocytes ($10^6/\mu L$)	Hb (g/dL)	MCV (fL)	MCH (pg)
Female (n=11)	5.36 - 5.95 N (3.8 - 4.8)	12.5 -15.2 N (12 - 16)	70.6 - 79.1 N >80	23.0 - 25.6 N >27
Male (n=10)	5.64 - 6.19 N (4.5 - 5.5)	13.1 -15.9 N (13 - 17.5)	73.4 - 81.4 N >80	23.1 - 24.8 N >27