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24TH ANNUAL MEETING SOCIEDADE PORTUGUESA DE GENÉTICA HUMANA

NOV. 20TH 2020
(VIRTUAL MEETING)

Mutational Spectrum and Geographic Distribution of Alpha-thalassemia in an Adult Microcytic and/or Hypochromic Population Living in Portugal: Results from the First National Health Examination Survey (INSEF 2015)

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Introduction

- **Alpha-thalassemia** (α-thal) is one of the most common human genetic diseases worldwide. It is caused by a deficit in the production of the α-globin chains of hemoglobin.

- The molecular basis of the disease is frequently a **C**opy **N**umber **V**ariation at 16p13.3, which reduce the normal number of functional α-globin genes (αα/αα) (Fig. 1 and 2).

- The disease's clinical severity varies from silent or almost asymptomatic, mild microcytic and/or hypochromic anemia, to a lethal hemolytic condition, depending on the number of affected α-globin genes (1 to 4) (Fig. 2).

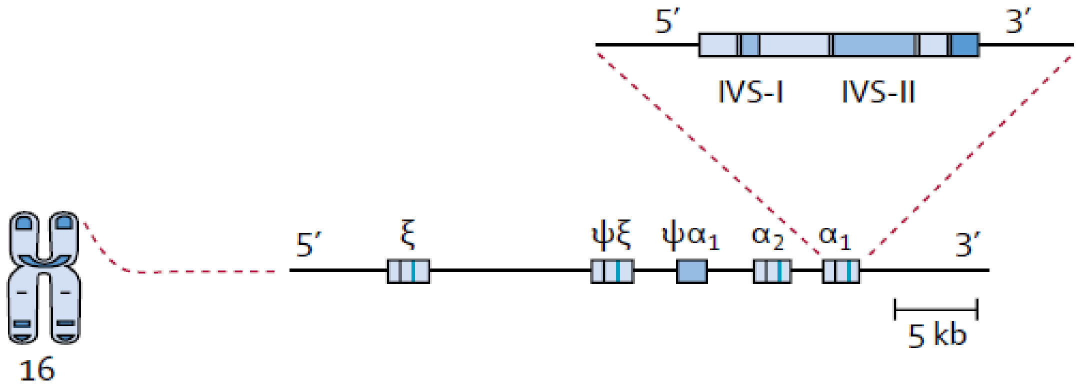
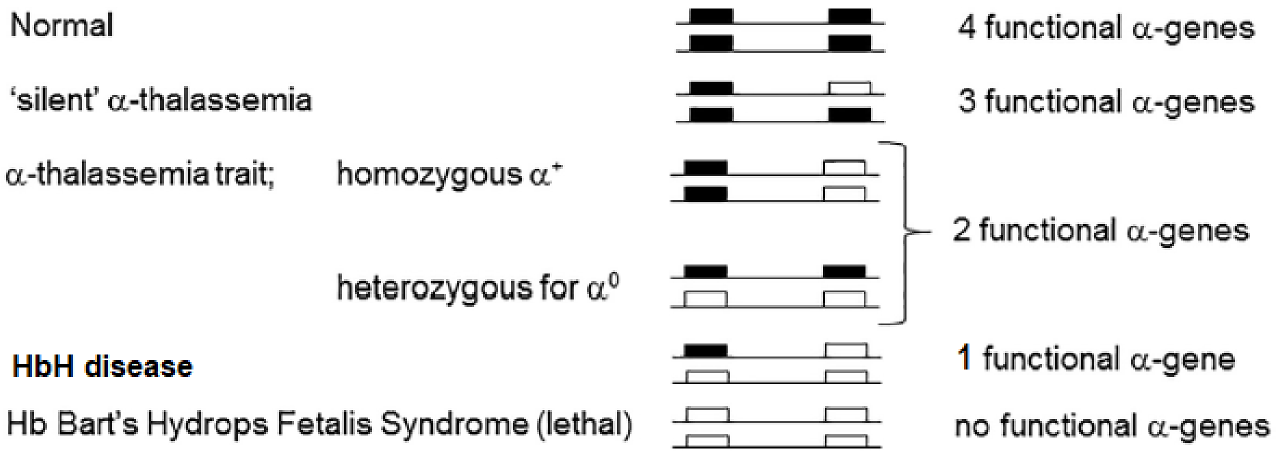


Fig. 1. Schematic presentation of the human α-globin gene cluster on chr. 16. Each normal individual has 4 α-globin genes. Adapted from [1,2].



Normal — 4 functional α-genes

'silent' α-thalassemia — 3 functional α-genes

α-thalassemia trait; homozygous α^+

heterozygous for α^0

2 functional α-genes

HbH disease — 1 functional α-gene

Hb Bart's Hydrops Fetalis Syndrome (lethal) — no functional α-genes

Fig. 2. Different number of affected α-globin genes and the corresponding phenotypic expression. Adapted from [1,2].

Aim

To identify the molecular basis, geographic distribution and prevalence of mild forms of α-thalassemia in Portugal.

Materials and Methods

Red Blood Cell characterization

INSEF

Inquérito Nacional de Saúde com Exame Físico 2013-2016

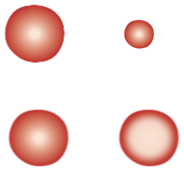
4812 participants

living in Portugal

Complete Blood Count analysis

Microcytosis

(MCV <80fL)



Hypochromia

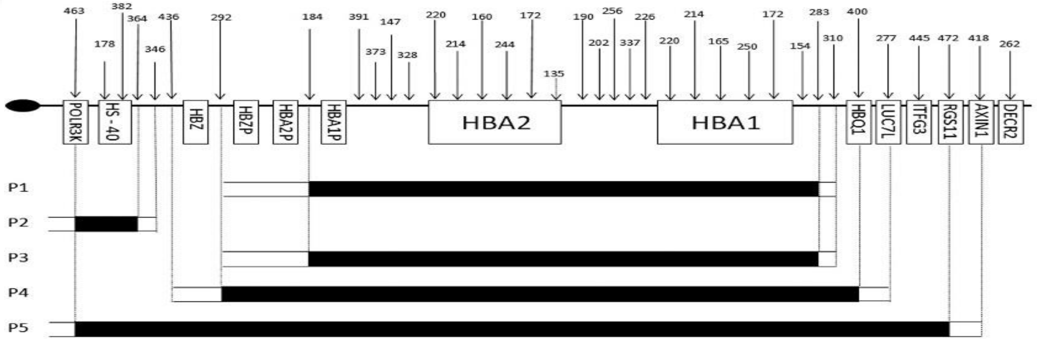
(MCH <27pg)

204 participants

DNA

HBA cluster molecular characterization

Screening for the most common α-thal deletion ($-\alpha^{3.7kb}$) by **Gap-PCR** [3]



Search for CNV by Multiplex Ligation-dependent Probe Amplification (**MLPA**) *SALSA MLPA HBA kit , MRC Holland*

Results

54 out of the 204 INSEF participants analysed have α-thal (26.5%)

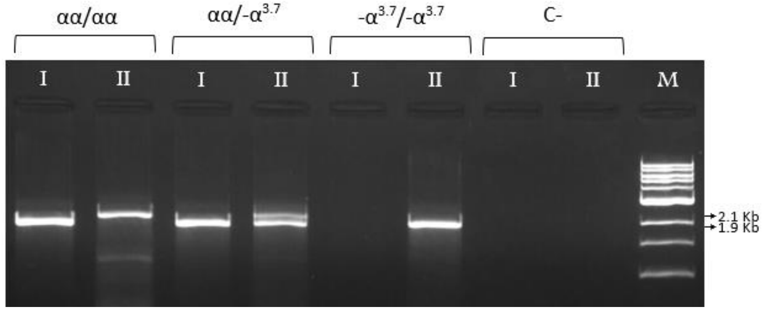


Fig. 3. Gap-PCR analysis. Electrophoresis in agarose gel (1%, w/v) showing the 3 possible genotypes resulting from the $-\alpha^{3.7kb}$ deletion.

We found 52 heterozygous individuals for the 3.7kb deletion and 1 homozygous individual for the same deletion.

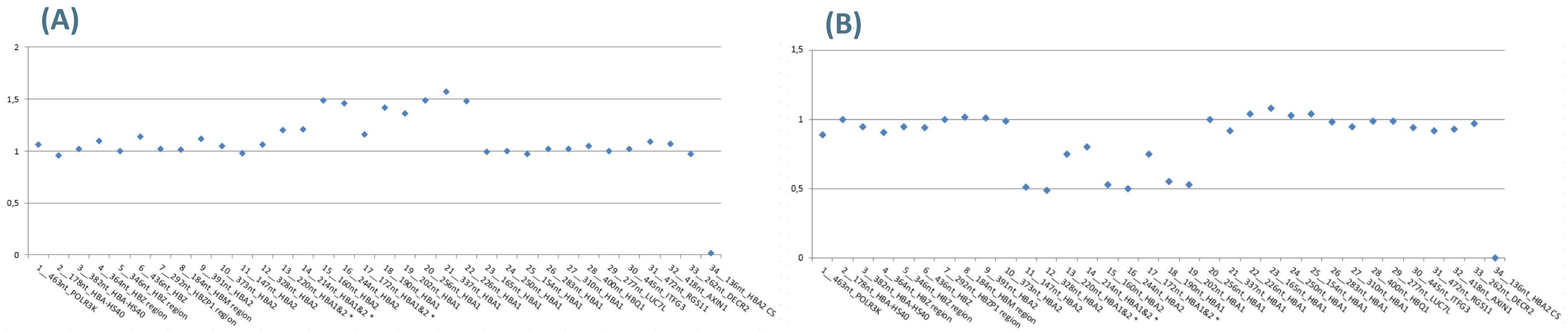


Fig. 4. MLPA analysis. (A) MLPA results for one individual with triplicated α-globin genes (ααα anti$^{3.7}$). **(B)** MLPA results for one individual heterozygous for the $-\alpha^{4.2}$ deletion.

We found 2 individuals with $\alpha\alpha/\alpha\alpha\alpha^{anti3.7}$ and 1 heterozygous individual for the $-\alpha^{4.2kb}$ deletion. MLPA did not reveal other rare CNVs.

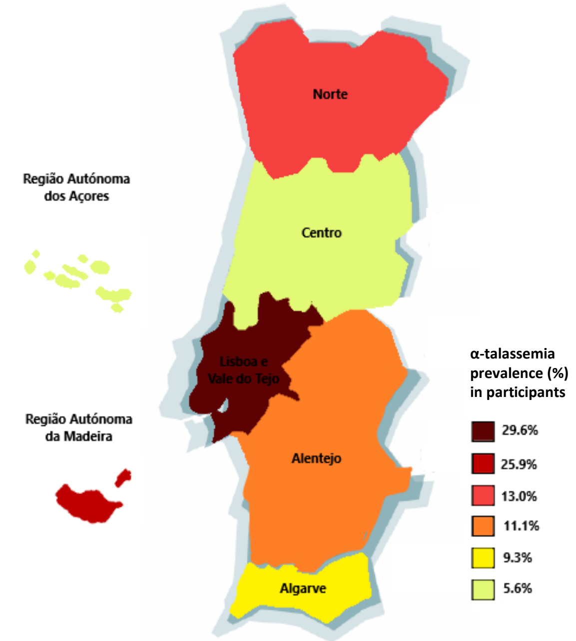


Fig. 5. Geographic distribution of INSEF participants with α-thal associated with hypochromic and/or microcytic red blood cells. Results revealed the regions with the highest prevalence of α-thal: "*Lisboa e Vale do Tejo*" (29.6%) and "*Região Autónoma da Madeira*" (25.9%).

Discussion and Conclusion

- In Portugal, the main cause of α-thal is the **$-\alpha^{3.7kb}$ deletion**.
- We observed a **26.5% prevalence of α-thalassemia in the 204 analysed individuals**, which constitutes a **1.1% prevalence of symptomatic α-thalassemia individuals in Portugal** (based on the 4812 INSEF participants). However, it should be noted that our approach did not consider the silent α-thalassemia cases that are hematologicaly indistingible from the normal population.
- The regions with the highest prevalence of symptomatic α-thalassemia among INSEF participants are **Lisboa e Vale do Tejo (29.6%)** and **Região Autónoma da Madeira (25.9%)**.
- Although the mild forms of α-thal alone are of no clinical significance, they play a major role in modifying the effect of various severe forms of other hemoglobinopathies, such as β-thalassemia and sickle cell disease.
- The α-thal trait can be confused with iron deficiency anemia as the hematological parameters are quite similar. Therefore, the iron status should be properly assessed to distinguish between the two conditions. Additionally, α-thal confirmation at the DNA level is necessary for a definitive diagnosis.

References

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Funding

ICELAND
LIECHTENSTEIN
NORWAY
eea
grants