UNRAVELLING THE GENETIC BASIS OF COMPLEX CLINICAL CASES OF HEMOGLOBINOPATHIES

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Introduction

Hemoglobinopathies encompass all genetic diseases of hemoglobin (Hb), the iron-containing oxygen-transport protein present in red blood cells[1,2]. They have autosomal recessive transmission and are due to molecular changes in globin genes or in their regulatory regions[3]. Hemoglobinopathies include **Hb variants** (mainly caused by missense mutations) and **Thalassemias** (due to nonsense and frameshift mutations or indels)[4,5]. Globin genes are located in two globin gene clusters and have differential temporal expression (Figure 1).

Aims

We aimed to identify the molecular lesions in the origin of complex cases of hemoglobinopathies and understand the underlying pathophysiological mechanisms.

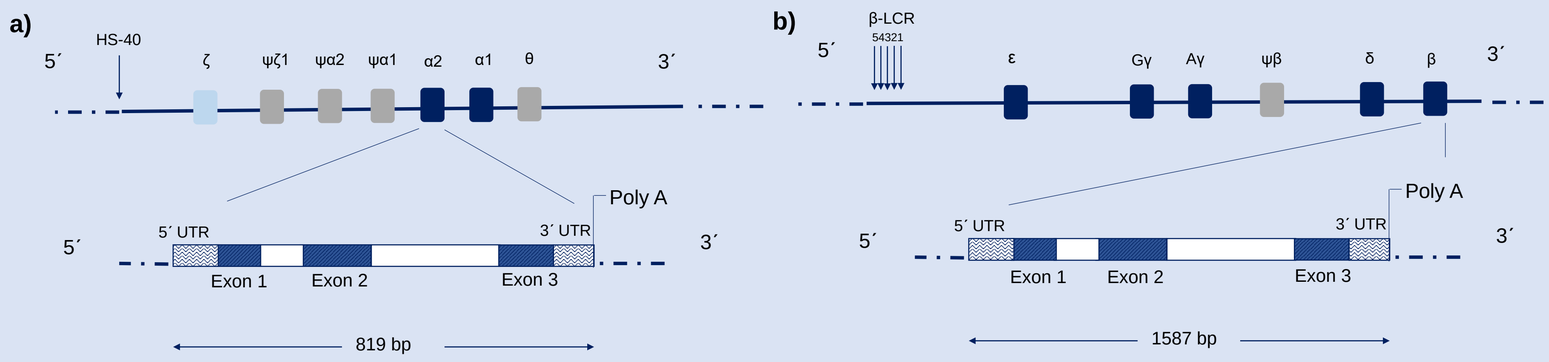


Figure 1. Schematic representation of the human α- and β-globin gene clusters and their distal regulatory elements. **(a)** The α-globin gene cluster on chromosome 16 p13.3 includes the ζ, α2 and α1 genes. The vertical arrow indicates the location of the major regulatory upstream hypersensitive site (HS-40), crucial for the *in cis* gene expression. The α2-globin gene structure is shown below with the three coding exons (striped boxes), the two introns (open boxes), and the untranslated regions (zigzag boxes). **(b)** The β-globin gene cluster on chromosome 11 p15.4 includes the ε, Gγ, Aγ, δ and β genes, arranged in the order of their developmental expression. Upstream of these genes is the Locus Control Region (β-LCR) constituted by five hypersensitive sites indicated by vertical arrows. As in panel a), the β-globin gene is expanded to show its structure[6].

Materials and Methods

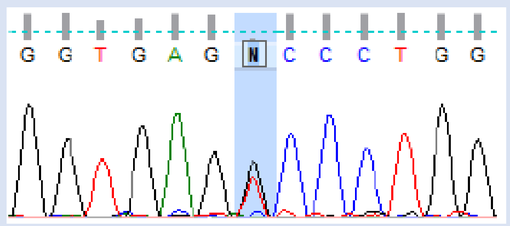
- ✓ We investigated **15 clinical cases** suspected of having one or more hemoglobinopathy, presenting with atypical hematological phenotypes.
- ✓ The study included the search for alterations in β- and α-globin gene clusters by PCR, ARMS, Gap-PCR, Multiplex Gap-PCR, Sanger sequencing, and Multiplex Ligation-dependent Probe Amplification (MLPA).
- ✓ *In silico* analyses were performed using Polyphen-2, SIFT, and varSeak.

Results and Discussion

ABNORMALLY LOW HbA_2 LEVEL IN β-THALASSEMIA CARRIERS

- HbA_2 Yialousa and HbA_2' – Variants of δ-chain

a) *HBD*:c.82G>T



b) *HBD*:c.49G>C

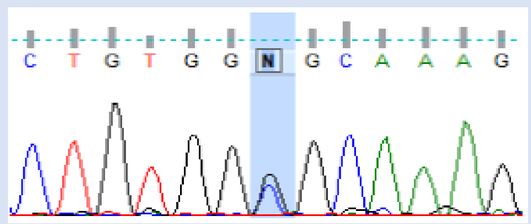
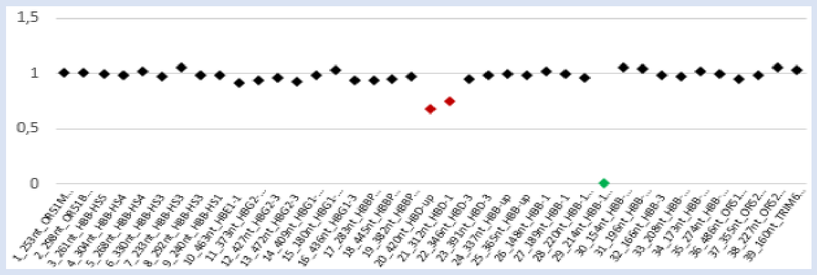


Figure 2. Partial Sanger sequencing electropherograms revealing **(a)** the alteration *HBD*:c.82G>T in heterozygosity responsible for the Hb variant **HbA2-Yialousa**[7] and **(b)** the alteration *HBD*:c.49G>C, also in heterozygosity, responsible for the **HbA2'** variant[7].

- 7.2kb Corfu Deletion – Involving the δ-globin gene

a)



b)

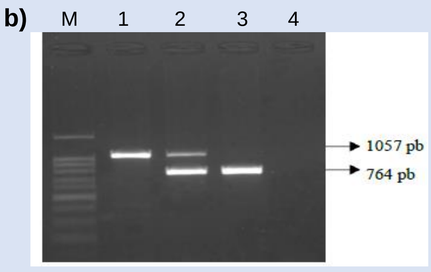
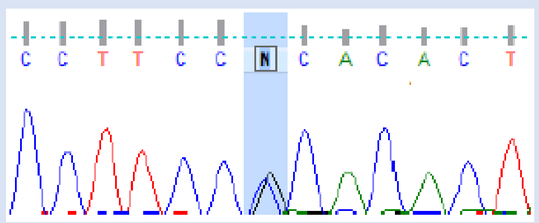


Figure 3. a) MLPA results revealing the presence of a deletion in heterozygosity affecting the δ-globin gene, whose size is suggestive of being the -7.2 kb Corfu deletion[7]. **b)** Electrophoretic profile of the products obtained by the Gap-PCR performed for the confirmation of the diagnosis of the Corfu deletion: M – molecular weight marker; 1 – Normal control sample; 2 – Propositus DNA fragments revealing heterozygosity for the deletion; 3 – Homozygous -7.2 kb Corfu deletion control; 4 – negative control.

ABNORMALLY HIGH FETAL HEMOGLOBIN LEVEL IN β-THALASSEMIA CARRIERS

- Alterations in the promoters of the γ-globin genes

a) *HBG1*:c.-248C>G



b) *HBG1*:c.-228T>C

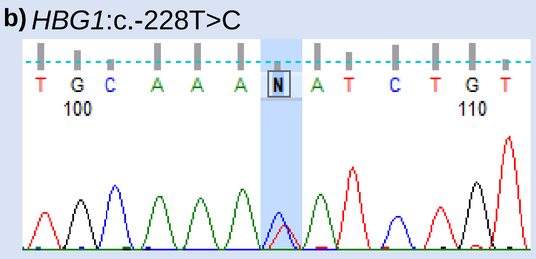
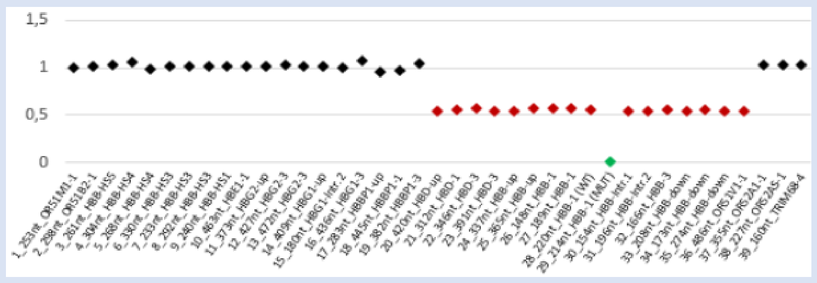


Figure 4. Partial Sanger sequencing electropherograms revealing two alterations in the Aγ-globin gene promoter, which explain the non-deletional Hereditary Persistence of Fetal Hemoglobin (HPFH)[7] in two cases: **(a)** *HBG1*:c-248C>G, responsible for the HPFH Brazilian[7]; **(b)** *HBG1*:c-228T>C, responsible for the HPFH Black[7].

- HPFH-1 and HPFH-2 Deletions – Removing the δ and β-globin genes

a)



b)

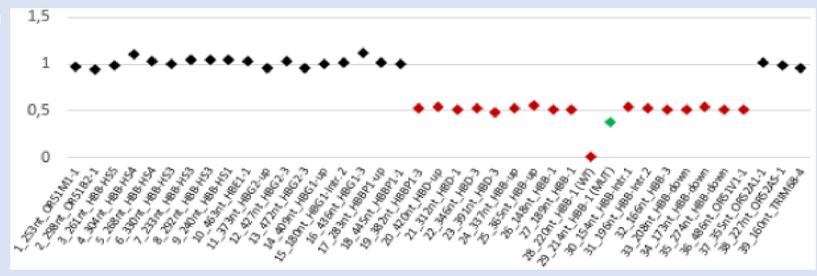
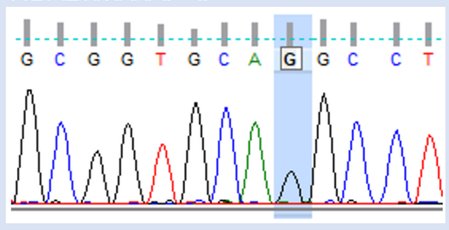


Figure 5. MLPA results revealing two different large deletions, both in heterozygosity, which remove the δ- and the β-globin genes and give rise to high expression of γ-globin genes and very high levels of fetal hemoglobin (HbF):
(a) a deletion similar to the reported **HPFH-1** (84918 bp)[7] found in an individual with HbF=17.5%;
(b) a deletion similar to the reported **HPFH-2** (84281 bp)[7] found in a sickle cell anemia carrier with HbF=24.3%.

A VERY COMPLEX CASE OF TRIPLE HETEROZYGOSITY FOR TWO HEMOGLOBIN VARIANTS AND α-THALASSEMIA

- Hb Westmead and HbE – Variants of α- and β-chain

a) *HBA2*:c.369C>G



b) *HBB*:c.79G>A

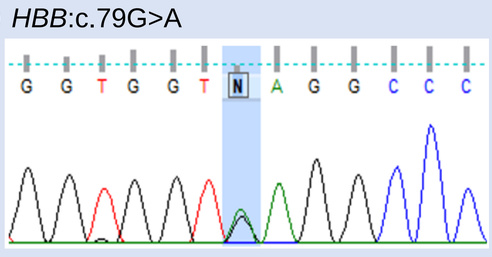


Figure 6. Partial Sanger sequencing electropherograms revealing **(a)** the alteration *HBA2*:c.369C>G in hemizygosity responsible for the **Hb Westmead**[7], an α-chain Hb variant; **(b)** the alteration *HBB*:c.79G>A, in heterozygosity, responsible for the **HbE**[7], a β-chain Hb variant.

- Southeast Asian Deletion – Removing the α2- and α1-globin genes

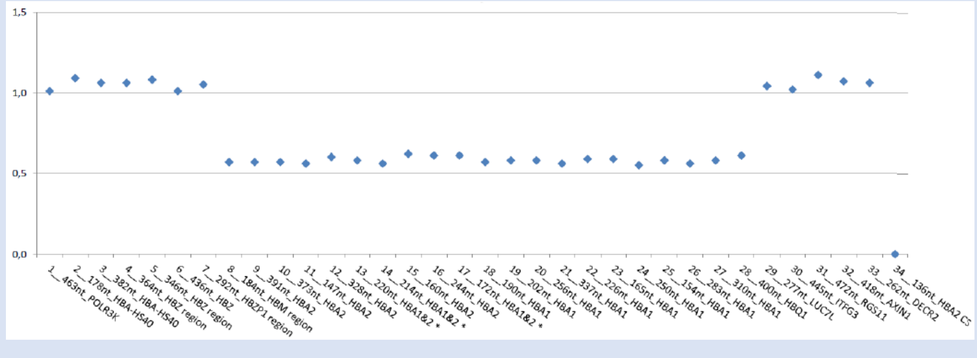


Figure 7. MLPA results showing of a large deletion in heterozygosity, which completely removes the *HBAP1*, *HBA2*, *HBA1*, and *HBQ* genes in the α-globin gene cluster, giving rise to α^0-thalassemia. It was identified by Multiplex Gap-PCR as Southeast Asian deletion[7] with 20.5 kb in length.

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A DELETION THAT REMOVES THE ENTIRE β-GLOBIN GENE CLUSTER

- Deletion $\varepsilon\gamma\delta\beta^0$

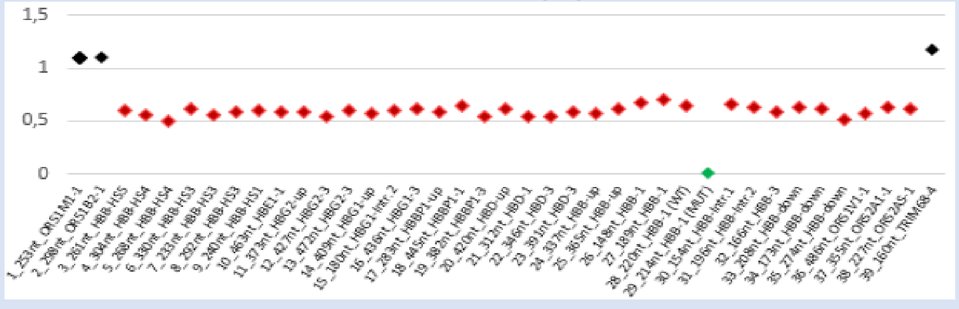

Figure 8. MLPA results showing a probably novel large deletion in heterozygosity, which removes the entire β-globin gene cluster, including the LCR, as well as the olfactory receptor genes, *OR52A1* and *OR51V1*. This deletion may have 196.6 to 730.3 kb in length.

Conclusions

- Individuals presenting abnormal phenotypes due to more than one hemoglobinopathy may be misdiagnosed if not correctly studied.
- Unravelling the genetic basis of complex clinical cases allows a better referral to genetic counselling, improves the understanding of the pathophysiology of the disease and its modifying factors, and may reveal new therapeutic targets.

References

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