NOVEL AND RARE LARGE DELETIONS IN THE GLOBIN GENE CLUSTERS CAUSING DIFFERENT TYPES OF THALASSEMIA

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INTRODUCTION

The major component of the red blood cells is **hemoglobin A** which consists of 2α- and 2β-globin chains encoded by the α- and β-globin genes, located in two different gene clusters (16p13.3 and 11p.15.5, respectively)[1].

Molecular defects (usually point mutation or short deletion) that give rise to a quantitative reduction of the corresponding globin chain, result in a hereditary hypochromic and microcytic anemia called **thalassemia**[2].

However, rarely, the molecular basis of this pathology can be a large deletion affecting several globin genes and/or their distal regulatory sequences[3,4]. These molecular lesions can abolish all globin genes expression of the affected allele.

In this study, we aimed to screen for **large deletions** in the globin gene clusters on chromosomes 16 and 11, in patients presenting thalassemia phenotypes but in whom no globinic molecular abnormalities had been found by standard diagnostic procedures.

METHODS

Four patients with hematological phenotypes suggestive of thalassemia (MCV<70fL, MCH<25pg, normal or high HbA_2 level), without any globinic molecular lesion identified, were screened for deletions in globin gene clusters on chromosomes 16 and 11, by **Multiplex Ligation-dependent Probe Amplification (MLPA) assay.**

We used the commercial kits Salsa MLPA P140-B3 HBA and Salsa MLPA P102-B1 HBB (MCR-Holland, Amsterdam, The Netherlands) following the manufacturer's instructions.

To further characterize the breakpoints of the deletions found, we employed synthetic MLPA probemixes designed in our laboratory (according to the online manufacturer's instructions), as well as PCR and DNA sequencing.

RESULTS

We identified two cases of α^0-thalassemia (Table 1) caused by two distinct large deletions which remove all α-like structural genes and their distal regulatory sites (Fig.1):

Table 1. Hematological phenotype and type of deletion presented in two patients

Hematological parameters				Deletion
Hb (g/dL)	**MCV (fL)**	**MCH (pg)**	**HbA_2 (%)**	
11.4	71.9	22.8	2.3	**Del I (- -/αα)**
11.2	65.0	20.0	Normal	**Del II (similar to Dutch II)**

α-GLOBIN GENE CLUSTER

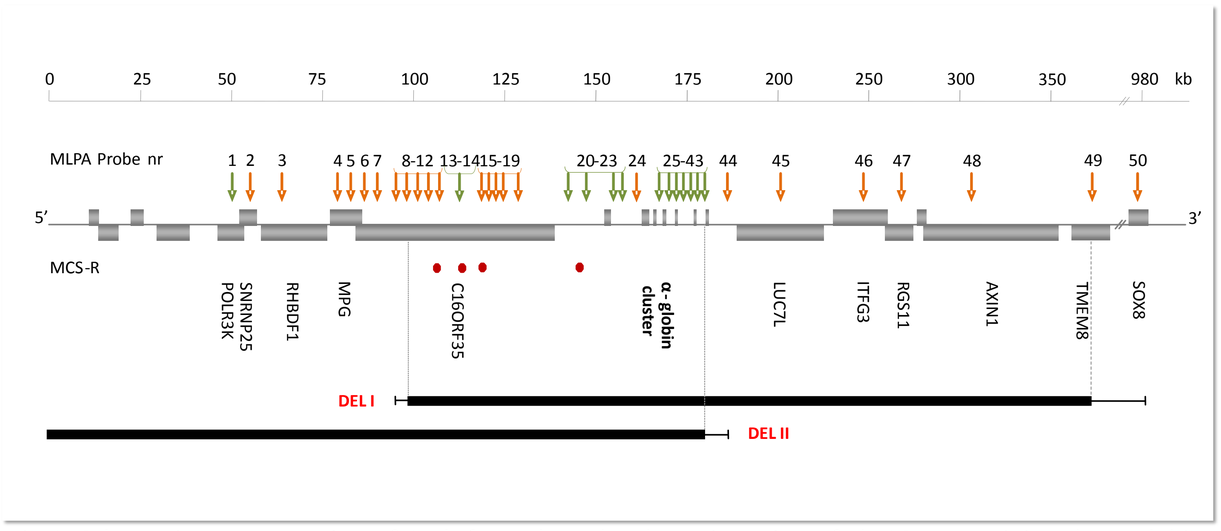

Fig 1. Schematic representation of 1Mb from the sub-telomeric region of chr16p, containing the α-globin gene cluster. MLPA probe hybridization sites are indicated by green and orange arrows referring to commercial and synthetic probes, respectively.

	Del I	Del II
Minimum length*	271.14 kb	231.0 kb
5' breakpoint	Uncertainty region with 4.9kb (removes HS-40, -33 and -10)	Telomeric
3' breakpoint	Uncertainty region with 610.6kb	Uncertainty region with 4,3kb (probably within 3'HVR)

* Length comprising all deleted MLPA probes (without uncertainty regions)

We identified an $\varepsilon^G\gamma^A\gamma\delta\beta^0$-thalassemia and a β^0-thalassemia (Table 2) caused by two distinct deletions. In the first case, a large deletion removes the entire β-globin cluster including the distal regulatory region (LCR). In the second case, a smaller deletion removes the 3' end of the cluster (Fig. 2):

Table 2. Hematological phenotype and type of deletion presented in two patients

Hematological parameters				Deletion
Hb (g/dL)	**MCV (fL)**	**MCH (pg)**	**HbA_2 (%)**	
12.5	64.1	20.7	2.2	**Del III ($\varepsilon^G\gamma^A\gamma\delta\beta^0$)**
12.3	72.1	22.8	5.0	**Del IV (similar to other[4])**

β-GLOBIN GENE CLUSTER

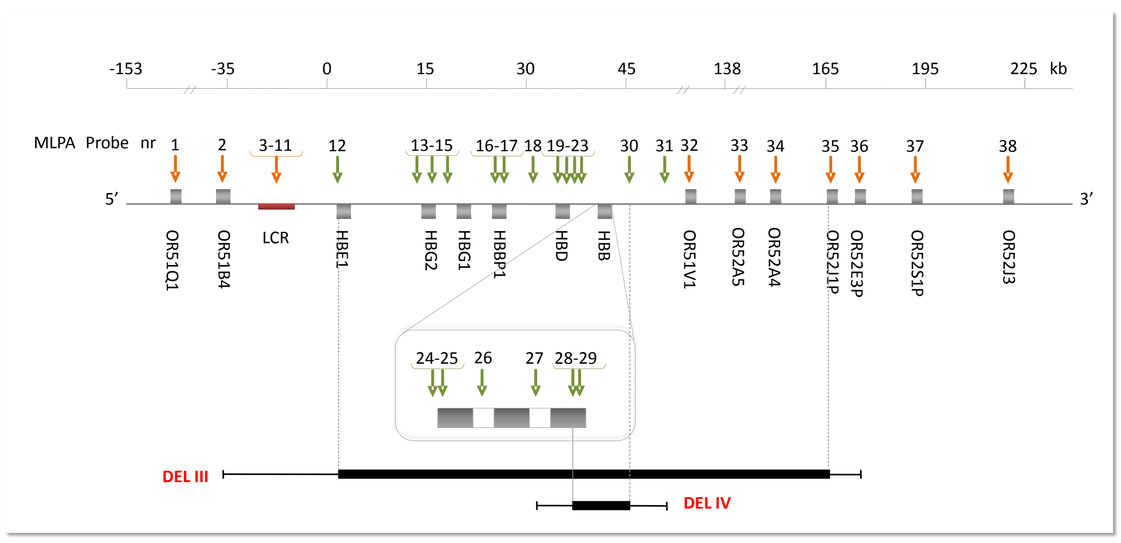

Fig 2. Schematic representation of chr11p, containing the β-globin gene cluster. MLPA probe hybridization sites are indicated by green and orange arrows referring to commercial and synthetic probes, respectively.

	Del III	Del IV
Minimum length*	186.0 kb	3.0 kb
5' breakpoint	Uncertainty region with 6.5 kb	Uncertainty region with 906 bp
3' breakpoint	Uncertainty region with 11.4 kb	Uncertainty region with 7.5 kb (within L1 repetitive region)

* Length comprising all deleted MLPA probes (without uncertainty regions)

CONCLUSIONS

• **Both α- and β-cluster larger deletions are novel (Del I and Del III)** and were named **$--^{CMB}/\alpha\alpha$** (CMB stands for Coimbra the city of patient's origin) and **PORTUGUESE $\varepsilon\gamma\delta\beta^0$-Thal**, respectively.

• The other two smaller deletions (Del II and Del IV), given the uncertainty regarding their breakpoints, might be similar to others already published.

• All deletions were found in heterozygosity in the mentioned patients. Their globinic genotypes were well correlated with the different thalassemic phenotypes presented. Clinician were recommended to study patients' closer relatives. Genetic counselling should be provided to them.

• MLPA proves to be a useful technique to identify known and unknown large deletions affecting globin gene clusters.

References:
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