Two atypical forms of HbH disease in Sardinia

Hemoglobin H disease is usually caused by deletion or inactivation of three α-globin genes, leaving only one α-globin gene intact and active.[1] The most frequent defects responsible for HbH disease in Sardinia are the coinheritance of the --Med deletion in one chromosome and the -$\alpha^{3.7}$ Kb deletion or, less frequently, the α2 initiation codon mutation ATG>ACG ($\alpha2^{NcoI}$) in the other chromosome.[2,3] HbH disease due to deletions including the major upstream regulatory element (MCS-R2) and leaving intact both α-globin genes have also been described.[4,5] We report here two new α^0 deletions, both located on the short arm of chromosome 16, responsible for HbH disease in two different Sardinian families. These unusual deletions were respectively associated with the common $\alpha2^{NcoI}$ mutation and -$\alpha^{3.7}$ deletion *in trans*.

Table 1 shows the hematologic and molecular data of the probands and their family members. All patients had severe microcytic anemia (Hb 2.6-9.5 g/dL, MCV 52.0-75.7 fl). Jaundice, spleen enlargement, sporadic hemolytic and aplastic crisis due to B19 parvovirus infection requiring red blood cell transfusions were detected in patients II-1 and II-2 of Family A. A mild thalassemia-like facies was present only in II-1 of the same family. Molecular screening for the most common α-globin gene deletion and non-deletion defects revealed the apparent homozygosity for the $\alpha2^{NcoI}$ mutation in the proband of Family A and in her sister, and the apparent homozygosity for the -$\alpha^{3.7}$ deletion in the proband of Family B. In spite of that, the $\alpha2^{NcoI}$ mutation was present only in the father of the Family A proband and the -$\alpha^{3.7}$ deletion was present only in the mother of the Family B proband.

In Family A, MLPA analysis, made using MLPA kit (HBA140-B3 MCR-Holland), revealed a deletion of at least 7535 bp beginning in the region between α1-pseudo-globin gene and α2-globin gene and extending to 2.4 kb downstream of α1-globin gene in the proband, in her sister and in their mother. Sequencing analysis of an ~500 bp breakpoint fragment, obtained using specific primers around MLPA deleted probes, allowed us to define the exact deletion breakpoint at position 161276/7 (5') and 170485/6 (3'). This deletion removed a region of 9209 nt involving both α-globin genes and part of the first exon of the θ gene. In addition, an insertion of six nucleotides (ATTAGT) at position 161216 before the 5' breakpoint was detected. No orphan sequence was found. The 5' breakpoint is shifted 2 nt up and the 3' breakpoint is shifted 1032 nt down, as compared to the breakpoints of the α^0 thalassemia deletion found in a recently reported Dutch family.[6]

In Family B, MLPA analysis revealed a larger deletion which removes all the MLPA probes specific for the subtelomeric region, including an α-globin gene cluster with all regulatory elements, in the proband and in her father. CGH-array analysis with oligonucleotides (8x60K Agilent Technologies) and SNP genotyping allowed us to define the 3' breakpoint between the 4th exon of the *NME4* gene and the IVSII of the *DECR2* gene (389660 and 395647 coordinates) (Figure 1).

The greater severity of the $\alpha2^{NcoI}$ non-deletion defect as compared to the -$\alpha^{3.7}$ deletion *in trans* to α^0 deletions, could be the reason for the different phenotypes in the HbH patients of the two families.[2] The different size of alpha^0 deletions and the loss of genes located in the deleted region in Family B do not seem to interfere in the determination of specific phenotype.

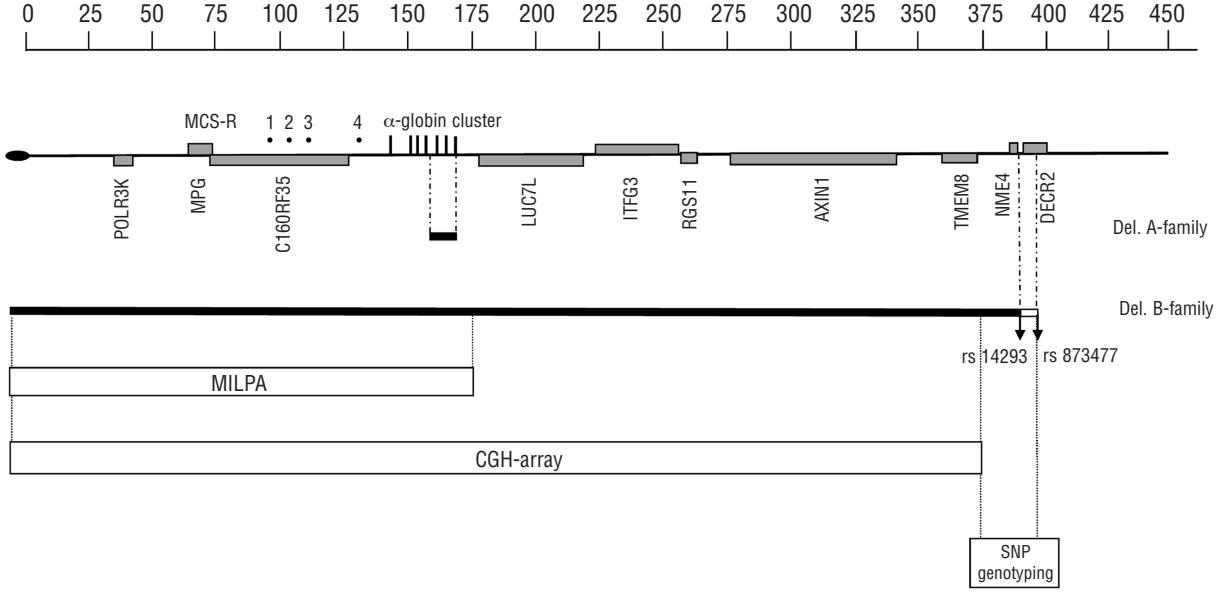

Figure 1. Schematic representation of the short arm of chromosome 16 (16p13.3) and of the α0 deletions in the two families. The α-globin regulatory region (MCS-R 1 to 4) is indicated as black dots. Black bars represent deleted DNA regions. White bar represents the region of uncertainty for deletion breakpoint. In family B the different methods used to detect the deletion are indicated in the boxes. SNP genotyping: loss of heterozygosity at 389660 chromosome position (rs 14293) and presence of heterozigosity at 395647 chromosome position (rs 873477) were detected. The chromosome positions of SNPs are according to GeneBank NT_010393.16.

Table 1. Hematologic data and genotype of the patients and their parents.

	Sex	Age (year)	Hb (g/dL)	MCV (fl)	H%	Hb A2 (%)	Reticulocytes %	α/β (ratio)	Ferritin (ng/mL)	Unconjugated bilirubin (μmol/L)	Alpha globin genotype
Family A											
I-1	M	48	13.1	66.5		2.6					-α/αNcoIα
I-2	F	44	11.7	66.4		2.8					--/αα
II-1	F	11	4.1-8.0	66.9	23.1	0	3.6		36	30.78	--/αNcoIα
II-2	F	10	2.6-8.9	73.2	29.7	0	3.6	0.45	29.9	27.36	--/αNcoIα
Family B											
I-1	M	37	14	67.0		2.2					--/αα
I-2	F	37	12.1	84.4		1.7					-α/αα
II-1	F	5	8.0-9.5	55.8	6.0	0.9	1.0		56.4	8.55	--/-α

Several large deletions involving the α-globin gene cluster have been recently described.[7-10] Although these deletions also remove other genes, affected heterozygotes appear phenotypically normal, apart from α-thalassemia carrier phenotype; however, an HbH patient with a telomeric deletion of ~285 kb associated with the common -$\alpha^{3.7}$ deletion *in trans* presented scoliosis, the severity of which remains unexplained.[8] A region on chromosome 16p for which haploinsufficiency leads to mental retardation typical of ATR16 has been narrowed down to a region ~0.9 and 1.5-1.7 Mb from telomere. Alu-family repeats, frequent in the genome and particularly common in and around the α-globin gene cluster, facilitate DNA strand exchanges during replication and non-homologous recombinations which are a frequent cause of α^0 deletions.[9-11] In addition to the common conventional molecular techniques, recent alternative methods, such as MLPA and CGH, become essential for a correct α-globin genotype definition. The exact identification of uncommon and unknown alpha deletion defects, although rare, allows appropriate genetic counselling to be offered to couples at risk for HbH disease or hemoglobin Bart's hydrops fetalis syndrome, especially in Sardinia were small isolated communities at risk can still be found.

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Key words: α-thalassemia, HbH, deletion, MLPA, a-CGH.

Citation: Paglietti ME, Sollaino MC, Loi D, Sarra F, Zaccheddu E and Galanello R. Two atypical forms of HbH disease in Sardinia. Haematologica 2011; 96(11):1733-1734. doi:10.3324/haematol.2011.048900

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

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