## South-Italy β°-thalassemia: a novel deletion not removing the γ-globin silencing element and with 3' breakpoint in a hsRTVL-H element, associated with β°-thalassemia and high levels of HbF

Hereditary persistence of fetal hemoglobin (HbF) in adulthood may be due to: mutations in the γ-globin genes promoter, mutations in the transcriptional factors involved in their regulation, polymorphic sequences or large deletions in the β-globin cluster.[1]

Recently, Sankaran clarified the role of the 3.5 kb intergenic region upstream of the δ-globin gene that appears to be required for efficient γ-globin silencing through binding with the transcription factor BCL11A.[2,3]

In this paper, we describe a compound heterozygote with a point β°-thalassemia mutation and a novel β°-thalassemia deletion who suffers from mild thalassemia intermedia with high HbF levels.

A 15-year old boy from Bari, south-east Italy, was referred for mild microcytic anemia associated with a slight enlargement of spleen and liver. In the propositus, the HbA was absent and replaced by HbA2 (4.7%) and HbF (95.3%). The non α/α globin chain biosynthetic ratio was 0.30. Both parents showed a β-thalassemia trait phenotype with increased levels of HbF and HbA2. The

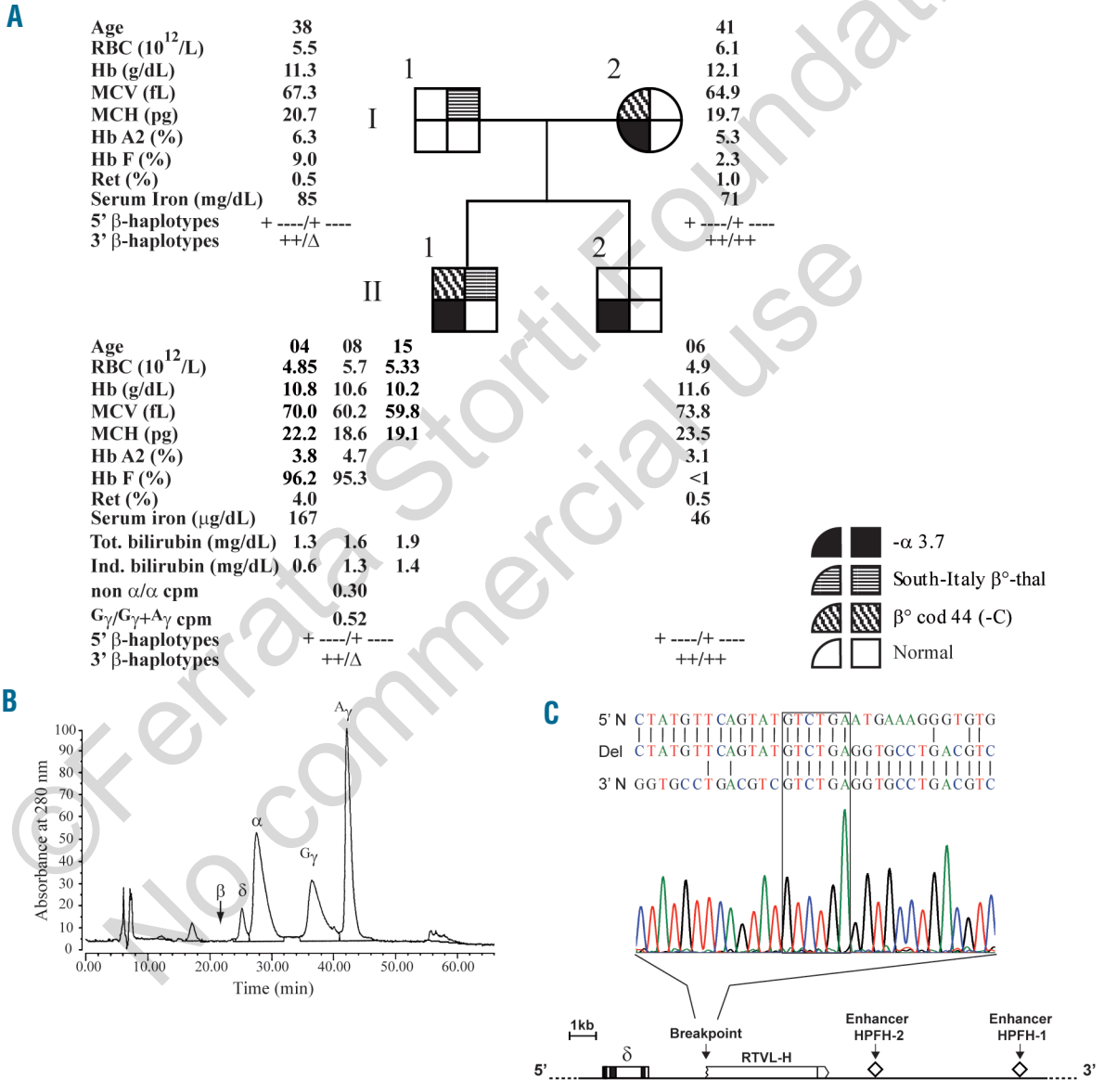


Figure 1. (A) Pedigree of the family. The hematologic and molecular data are reported. (B) Globin chains separation by reverse-phase HPLC in the proband. The α, $^{G}$γ and $^{A}$γ globin chains are indicated. The position of the "missing" β-globin chain is indicated by an arrow. The ratio between $^{G}$γ and $^{A}$γ-globin chains is 52:48. (C) Electropherogram of the sequence of the deletion breakpoint. The sequence from South-Italy β°-thal deletion breakpoint (Del) is aligned with the corresponding normal 5' (5'N) and 3' (3'N) sequences. The region of microhomology (5'-GTCTGA-3') present in the sequences closely flanking the breakpoints is boxed. In the lower part of the figure is reported the structure of the β- globin gene cluster in the South-Italy β°-thalassemia. The RTVL-H element and the HPFH-2 and the HPFH-1 enhancer elements (diamonds) are also reported.

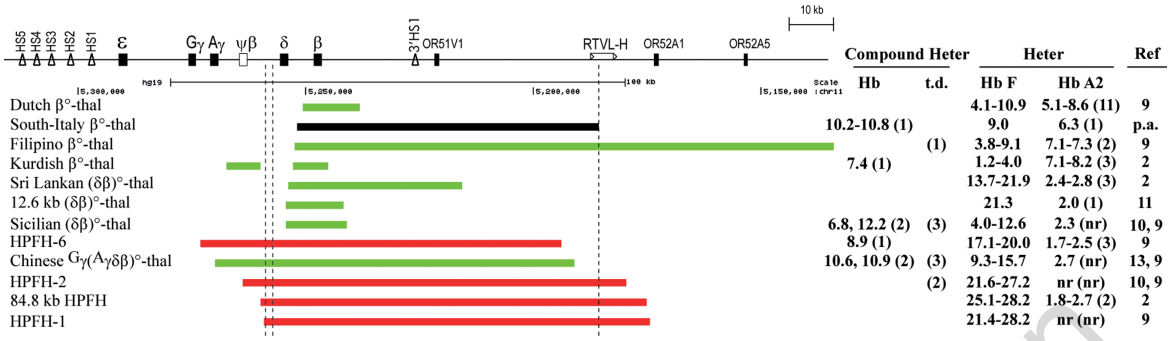


**Figure 2.** Scheme of β-globin gene cluster with the position of the RTVL-H element. Genes are indicated by filled rectangles; pseudogene is indicated by an empty rectangle; DNA Hypersensitive Sites (HS) are indicated by empty triangles. The dashed lines indicate on the left the position of BCL11A binding-site at 5' of δ-globin gene and on the right the 3' breakpoint of the South-Italy β°-thal deletion. In the lower part of the figure the position of various deletions is shown by thick lines: deletions associated to either a β°- or (δβ)°-thal phenotype are in green, deletions associated to an HPFH phenotype are in red and the novel South-Italy β°-thal is in black. On the right side are reported: (Hb) Hb levels in the patients suffering from thalassemia intermedia (the number of thalassemia intermedia patients is indicated in parenthesis); (t.d.) the number of transfusion-dependent patients; the HbF and Hb A2 levels found in the heterozygotes.

propositus, his mother and his brother were carriers of the -α[3.7] deletion (Figures 1A and B).

By sequence analysis, the mother was found to be heterozygous for the β-thalassemia mutation at codon 44(-C), the father and brother appeared normal; the propositus was apparently homozygous for the maternal mutation. This discrepancy suggested the presence of a β-globin gene deletion inherited from the father. By restriction mapping and inverse-PCR[4] (*Online Supplementary Figure S1*) we identified a novel 66,151 bp deletion with the 5' breakpoint at -3,632 bp upstream the β-globin gene (NG_000007.3:g.66902_133052del66151) and the 3' breakpoint at about 62 kb downstream the β-globin gene: the South-Italy β°-thalassemia. The sequences closely flanking the breakpoints present a microhomology (5'-GTCTGA-3') that may have contributed to the non-homologous recombination event (Figure 1C). The 3'-arm of the deletion sequence showed 13 SNP base substitutions (*Online Supplementary Figure S2*).

To explain the molecular basis of the high HbF levels observed in our patient, we analyzed several polymorphisms *in cis* or *in trans* to the β-globin gene cluster, associated with increased HbF levels (*Online Supplementary Table S2*).[5-8]

The mutation β°-thal cod 44(-C), was linked with the Gγ–309(A>G) polymorphism associated with moderate increase of HbF. However, HbF levels in the mother (2.3%) are similar to those found in other heterozygotes for cod 44(-C).[9] Moreover, homozygotes and compound heterozygotes for cod 44(-C) suffer from thalassemia major.[9]

The South-Italy β°-thalassemia was not linked with any polymorphisms associated with high HbF levels (*Online Supplementary Table S2*). All family members were heterozygote for the SNP rs11886868(T>C) in BCL11A, associated with reduced expression of BCL11A and, thus, moderate increased levels of HbF (0.8-5.0%).[7]

Subsequently, we analyzed the rearrangement of the β-globin locus generated by this deletion to identify the factors that could contribute to the high HbF levels in the propositus.

A known mechanism is the reduced competition of the deleted β-globin promoter for the activity of Locus Control Region which could also explain the increased levels of HbA2 (6.3%) observed in the South-Italy β°-thalassemia carrier. The finding is common in complete or partial β-globin gene deletions showing microcytosis with significantly higher HbA2 levels (5.5-9.0%), derived mainly from the δ-globin gene *in cis* to the deletion, and a modest increase in HbF (0.2-11.7%).[9]

Six other β-globin gene deletions retain the 3.5 kb γ-silencing element[2,3,9-11] and have the 5' breakpoint in the same region of the South-Italy β°-thal; compound heterozygotes with a β°-thal mutation suffer from either transfusion-dependent thalassemia (n=4) or severe thalassemia intermedia (n=2) with Hb levels of 7.4 (β⁺-thal) and 6.8 g/dL (β°-thal) (Figure 2).[9-11] The South-Italy β°-thal does not remove the γ-silencing element but the propositus has only a mild anemia (Hb 10.2-10.8 g/dL) with 95.3% of HbF. Thus the 3.5 kb γ-silencing element prevents the high levels of HbF expression in all these deletions of β-globin gene cluster but it is less efficient in the South-Italy β°-thal.

We compared the phenotype of our patient with that of compound heterozygotes carrying β-deletions with similar 3' breakpoints but without the γ-silencing element (Figure 2). All compound heterozygotes with HPFH-2, and 60% of those with Chinese $^{G}\gamma(^{A}\gamma\delta\beta)$°-thal, are transfusion-dependent; the patient with HPFH-6 has thalassemia intermedia phenotype with Hb<9. In contrast, our patient with South-Italy β°-thal is only slightly anemic with 10-11 g/dL of Hb, most of which was HbF. It is intriguing that such a mild clinical phenotype is present in only a fraction of compound heterozygotes with these deletions, specifically in 40% of those with the Chinese $^{G}\gamma(^{A}\gamma\delta\beta)$°-thal.[12,13] It is, therefore, possible that additional genetic variations result in the clinical heterogeneity of these patients. The high HbF levels observed in our patient results from the peculiar configuration of the 3' breakpoint of the South-Italy β°-thal. The putative enhancers of the HPFH-2 and of the HPFH-1 are at 5,332 and 11,287 bp, respectively, downstream the South-Italy β°-thal. More importantly, the 3' breakpoint of the South-Italy β°-thal is within a 6 kb long element that belongs to a human DNA repetitive family, *Homo Sapiens* Retrovirus-like-H (hsRTVL-H), characterized by two 415 bp long terminal repeats (LTRs).[12] This hsRTVL-H element is downstream the Chinese $^{G}\gamma(^{A}\gamma\delta\beta)$°-thal and the HPFH-6; in contrast, the South-Italy β°-thal removes the RTVL-H 5' portion retaining the 3' LTR

(Figures 1C and 2) and this rearrangement could increase its enhancer ability.[14] The level of HbF in our patient is higher than that in the carriers of similar β-globin deletion retaining or missing the γ-silencing element. This phenotype may be explained by some factor that could ameliorate the thalassemic phenotype ($^{G}$γ–309(A>G), BCL11 Ars11886868 C, -α$^{3.7}$ heterozygosity), but more importantly, the South-Italy β°-thal is the only deletion that brings 3 different enhancers (hsRTVL-H, HPFH-2 and HPFH-1) within proximity of the γ-globin genes. Although BCL11A is a key regulator of γ-globin silencing, our data support the notion that other factors, in this case the 3 enhancers downstream the 3' breakpoint of the South-Italy deletion and the potential effect of a large deletion on chromatin organization and remodeling, may overcome the silencing effect of BCL11A, and may allow the persistence of high levels of HbF expression during adulthood.

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*doi:10.3324/haematol.2013.089722*

*Acknowledgments: the authors would like to thank the family for their consent to this study and for their collaboration. We would like to thank Dixie L. Mager for the restriction mapping of the region containing the RTVL-H and for the probe Sst I/Bam HI 1.08 kb. We also thank Rosario Notaro and Tolulase Olutogun for their helpful suggestions and fruitful discussions. This study was supported by grants from Ministero Istruzione, Università e Ricerca (MIUR), legge 488/92 (Cluster C02, Project 2). A very preliminary molecular characterization of the South-Italy β°-thal has been presented at the 6th 'International Conference on Thalassaemia and the Haemoglobinopathies', Malta, April 1997.*

*This article is dedicated to the memory of Clementina Carestia.*

*Key-words: β-globin gene deletion, β-thalassemia, γ-globin gene regulation, HbF*

*Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.*

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