To the editor:

A novel deletion causing α thalassemia clarifies the importance of the major human alpha globin regulatory element

The α globin gene cluster, which contains embryonic (ζ) and fetal/adult (α) genes, has been highly conserved throughout evolution. Four putative regulatory elements (called MCS-R1 to MCS-R4; Figure 1) corresponding to erythroid-specific DNase I hypersensitive sites (HSS) are found upstream (30–160 kb) of the α genes in most mammalian species, but the relative importance of each of these elements in each species has not been fully elucidated. In humans, characterization of natural deletions, analysis of interspecific hybrids, and studies of transgenic mice all indicate that the most highly conserved element (MCS-2, which corresponds to HS-40 in humans) is the major regulatory element; on their own, the other elements (MCS-1, -3, and -4) cannot drive substantial levels of α globin expression. Targeted deletion of HS-40 in a hybrid containing human chromosome 16 in a mouse erythroleukemia (MEL) background reduces human α globin expression to less than 5% of normal. It was therefore surprising to find that deletion of the highly conserved MCS-R2 element (mHS-26) in mice, using homologous recombination, only reduced α globin expression to approximately 50% of normal, suggesting that the 4 elements may play different roles in different species and casting some doubt on the value of the mouse as a faithful model of human α globin gene expression. At present, the only way to determine the role of individual regulatory elements in situ, in the normal human erythroid environment, is to identify natural mutants in human populations.

Figure 1. Summary of the molecular analysis of a novel interstitial upstream deletion. (A) The (α)2Dv deletion and its relative position within chromosome 16p13.3. Coordinates (in kb) according to GenBank no. NT_037887.4 are depicted above the line. Erythroid DHS are shown as black arrows. Numbered gray boxes represent known genes located in this subtelomeric region in addition to the α globin genes (labeled with Greek symbols). The small gray bar below the line represents the shortest region of overlap (20.4 kb) derived from previously characterized upstream deletions. The (α)2Dv deletion provides a new 3' limit for minimal upstream sequences required for full α globin expression (14.7 kb, dashed line). The small white and gray boxes below the line represent probes used for Southern blotting (white) and MLPA (gray). (I) 757 × 758, (87 755–88 337); (II) 4a, (90 548–90 603); (III) 755 × 756, (96 671–97 389); (IV) 5a (103 695–103 774); (V) 767 × 768, (107 503–107 588); (VI) 6a (113 512–113 591); and (VII) 6a (120 541–120 590). Four small black boxes show the multispecies conserved sequences–regulatory elements (MCS-R1 to -R4). (B) Characterization of (α)2Dv breakpoint. The 3' breakpoint of the (α)2Dv chromosome was characterized after Bgl II, Xba I, Sac I, and Bcl I digestion, hybridized with combined probes V and VI. Breakpoint fragments were identified with probes I and II, using forward (ZW-2F; 5'-AGGCAGACTGCACTTCAT-GCCTAGGGGAAACTGCAGGTG-3') and reverse (ZW-2R; 5'-AGGCAGACTGCACTTCAT-GCCTAGGGGAAACTGCAGGTG-3') primers, the PCR product was amplified in peripheral blood DNA (PB-DNA) in triplicate. The PCR did not amplify a normal chromosome. M indicates 100-bp marker (New England Biolabs, Hitchin, United Kingdom). (C) Direct sequencing of the breakpoint fragment demonstrates the sequence adjoining between coordinates 90 777 and 106 774 (leftward and rightward to the arrow respectively). (E) Marked decrease in mRNA levels from the (α)2Dv chromosome. An RNase protection assay was used to analyze total RNA extracted from chromosome 16 x mouse erythroleukemia, interspecific hybrids. This showed a severely reduced level of human α globin expression (133-bp protected band) in both uninduced (U) and hemin-induced (I) cells containing the mutant (M) but not the normal (N) chromosome 16. The 93-bp protected fragment represents the mouse α globin mRNA as an internal control.
We have previously characterized 9 deletions that remove various combinations of the upstream regulatory elements and cause \( \alpha \)-thalassemia.\(^5\) In all cases, MCS-R1, -R2, and -R3 are removed and in 4 cases, MCS-4 is also deleted. Here we describe a small, approximately 16-kb interstitial deletion that removes only MCS-R1 (HS-48) and MCS-R2 (HS-40) and that caused \( \alpha \)-thalassemia in a Filipino girl. The combination of severely reduced red cell indices (hemoglobin [Hb], 113 g/L [11.3 g/dL]; mean corpuscular volume [MCV], 65 fl; and mean corpuscular hemoglobin [MCH], 19.48 pg), normal Hb analysis (HbF 1% and HbA\(_2\) 2.2%), demonstrable HbH inclusion bodies, and a significantly reduced \( \alpha \)-thalassemia with inactivation of at least 2 of the normal 4 \( \alpha \) globin genes. Analysis of interspecific hybrids confirmed that expression from the affected chromosome is reduced to less than 1% of normal (Figure 1). Although we have confirmed that the \( \alpha \) genes are structurally normal (data not shown), analysis of the upstream region using multiplex ligation–dependent probe amplification (MPLA) analysis identified a deletion removing MCS-R2 (HS-40).\(^10\) DNA sequence across the breakpoint shows that this deletion (\( \alpha \alpha \)) extends for 15 997 bp (coordinates, 90 778-106 773 bp) and that it arose via recombination between 2 Alu repetitive elements (Figure 1), as observed for many other deletions in this region of the genome.

This patient provides the first example of an upstream deletion in which MCS-R3 (HS-33) remains intact (Figure 1A), demonstrating that this element alone or in combination with MCS-R4 has no significant positive effect on \( \alpha \) globin expression. To fully demonstrate that, in humans, in contrast to mice, HS-40 is the dominant \( \alpha \) globin regulatory element, it now only remains to be seen whether MCS-R1 (HS-48) can act alone or in combination with MCS-R2 (HS-40) in human erythroid cells. Future identification of an upstream deletion, including MCS-R2, in which MCS-R1 remains intact, would therefore complete the search for such deletions and help clarify the apparent differences between the regulation of \( \alpha \) globin expression in mice and humans.


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References

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