

Hb S/ β^+ -thalassemia due to Hb sickle and a novel deletion of DNase I hypersensitive sites HS3 and HS4 of the β locus control region

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Supplemental Data

12.4 kb Mediterranean Deletion of HS3, HS4, and HS5 Core Elements of the β -Globin Locus Control Region

A 5-year Canadian boy of Italian descent was referred for investigation of possible α -thalassemia trait (Hb 109 g/L, RBC $5.25 \times 10^{12}/L$, MCV 65.0 fL, MCH 20.9 pg, Hb A 97.3%, Hb A₂ 2.7%, negative Hb H preparation). The initial molecular tests included deletion-specific gap-PCR analysis for ten different α -thalassemia deletions (---^{SEA} , ---^{FIL} , ---^{THAI} , $\text{---}^{\text{MED-I}}$, $\text{---}^{\text{MED-II}}$, $\text{---}^{\text{---}(\alpha)^{20.5}}$, ---^{BRIT} , ---^{SA} , $\text{---}^{\alpha^{3.7}}$ kb, $\text{---}^{\alpha^{4.2}}$ kb). In addition, the multiplex ligation-dependent probe amplification assay was used to screen for other deletions and rearrangements of the α -globin gene cluster (SALSA MLPA probemix P140 HBA, MRC-Holland, Amsterdam, The Netherlands). Lastly, the *HBA2* and *HBA1* genes were analyzed by locus-specific PCR and direct nucleotide sequencing. All of these tests were negative, largely excluding α -thalassemia as a cause of the microcytic anemia.

The β -globin gene cluster was screened for mutations that could give rise to β -thalassemia trait with normal levels of Hb A₂. The nucleotide sequence of the β -globin gene was normal, apart from heterozygosity for a common non-pathogenic sequence polymorphism (HBB:c.315+74T→G, rs7480526). MLPA analysis of the β -globin gene cluster (SALSA MLPA probemix P102-B2 HBB, MRC-Holland, Amsterdam, The Netherlands) demonstrated that the proband is heterozygous for a deletion involving five MLPA probes. Identical MLPA results were obtained for the proband's father, who also had thalassemia indices (Hb 139 g/L, RBC $6.68 \times 10^{12}/L$, MCV 66.6 fL, MCH 20.7 pg). The junction fragment was amplified using forward primer 5'-AGATG AAGTG SCTAC TAACT GACA GAGG-3 (NG_000007.3 positions 2325-2352) and reverse primer 5'-CTTAC CCTCT CATTCC ACCCA CCTCT-3' (NG_000007.3 positions 15313-15337), and sequenced using the forward primer. Comparison of the sequence of the junction fragment to those of the normal 5' and 3' regions established that the deletion spans a total of 12,364 bp, beginning at position 2,798 and ending at position 15,161 (reference sequence NG_000007.3). At the deletion junction, there is an insertion of 8 "orphan" nucleotides (Figure 1B). The HGVS nomenclature for the deletion is NG_000007.3:g.2798_15161delinsAGAGCCCT. The deletion removes DNase I hypersensitive sites HS3, HS4, and HS5 of the β -globin locus control region.

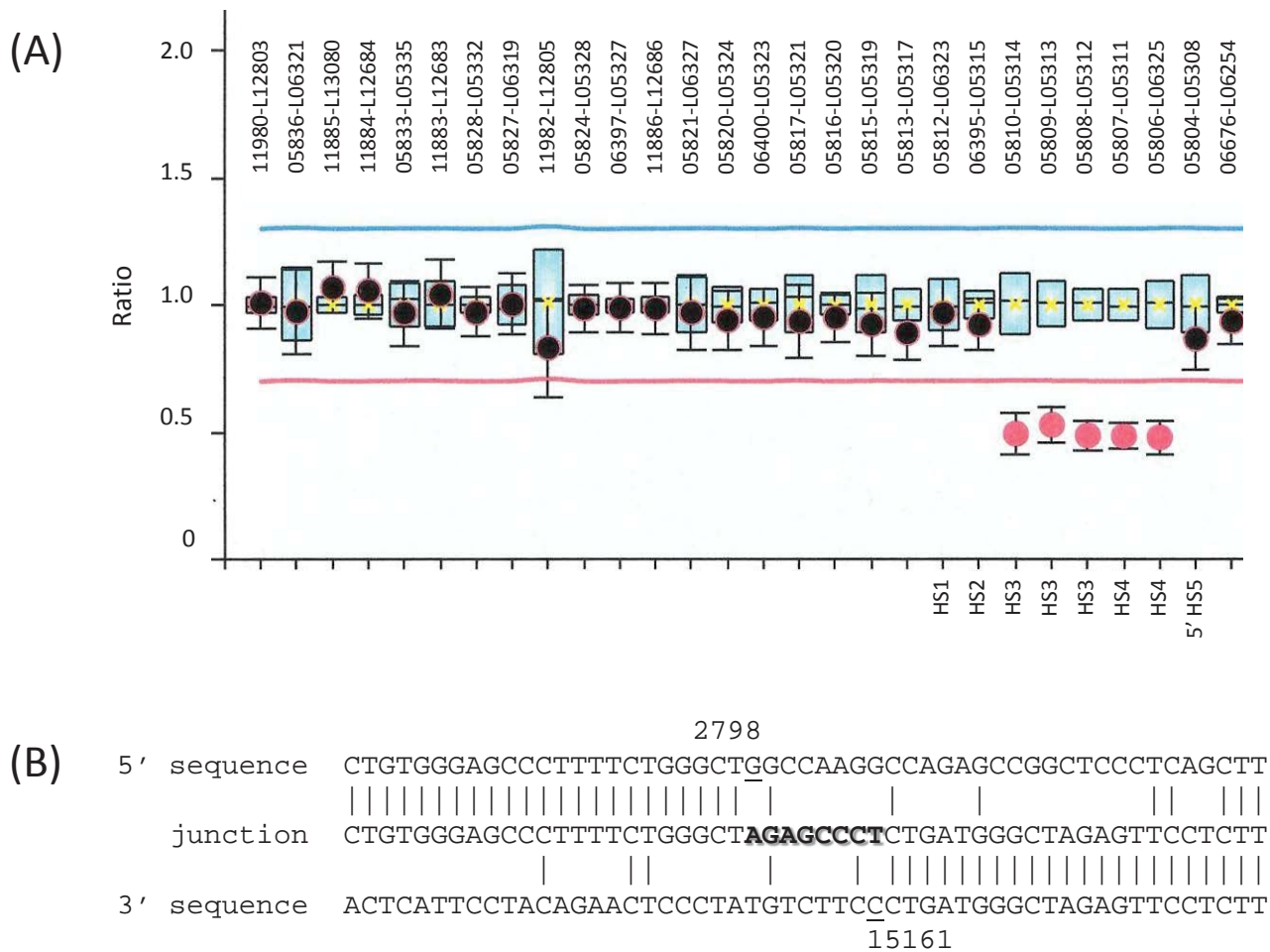


Figure 1: Identification of the deletion endpoints of the 12.4 kb Mediterranean β -LCR deletion. (A) MLPA analysis showing the relative probe signals across the β -globin gene cluster. The MLPA probes that define the deletion are indicated. (B) Sequence of the deletion junction fragment compared to the normal 5' and 3' sequences. The first and last nucleotides of the deleted region are underlined, and the 8 bp orphan sequence is highlighted in bold font.