HBB loss of heterozygosity in the hemopoietic lineage gives rise to an unusual sickle-cell trait phenotype

We report, in a constitutionally heterozygous carrier of the sickle-cell trait, an abnormally low HbS level (12%) caused by a somatic deletion of the *HBB* gene (βS allele) that occurred presumably in an early precursor of the hematopoietic lineage.

The propositus was a 39-year old woman originating from the Ivory Coast who came to us for genetic counseling because of a surprisingly low HbS level of 12% at cation-exchange high performance liquid chromatography (CE-HPLC) (Variant II, Biorad). Her HbF level (1.7%) and her hematologic parameters (mean cell volume, MCV, 93 fL, mean cell hemoglobin, MCH, 33 pg) were normal and she had no history of transfusion or vaso-occlusive crisis. After obtaining her written consent, the following genetic abnormalities were searched: the most common α-thal deletions ($\alpha^{-3.7}$, $\alpha^{-4.2}$, SEA, MED and $\alpha^{-20.5}$) by gap polymerase chain reaction (PCR),[1] α- and β-globin point mutations by Sanger direct sequencing (Applied 3130XL) and large α- and β-globin deletions by specific multiplex ligation-dependent amplification (MLPA) kits.[2] Also, the β-globin cluster haplotypes were determined by a FRET Light-Cycler method.[3]

Neither a common α-globin deletion nor a large deletion of the α- or β-globin gene clusters was found and the βS chromosome appeared to be of Benin type. The α-globin sequencing results were normal while β-globin sequencing was in accordance with Hb study, showing the β6 GAG > GTG (HBB:c.20A>T) mutation in heterozygous condition, as well as two intronic polymorphisms, IVSII-74 G>T (HBB:c.315+74G>T) and IVSII-666 T>C (HBB:c.315+666T>C), also in heterozygous condition. However, while interpreting the sequencing data, we noticed that the β^S peak was abnormally weak compared to the β^A peak (Figure 1A). The same profile was also observed for the C allele of IVSII-666 T>C and for the G allele of IVSII-74 G>T (*data not shown*). Allele specific amplification was ruled out since the direct sequencing with another primer set of the regions covered by the initial primers did not reveal any single nucleotide polymorphism (*data not shown*). We, therefore, performed the β-globin sequencing on oral mucosal cell DNA and found the same mutations in heterozygous condition but, this time, the sequencing profiles appeared normal (Figure 1B). Meanwhile, the family study revealed that the son and the mother of the proband were both βS heterozygous with a typical HbS level (34% and 35%, respectively) and with a normal Sanger sequencing profile (similar heights for the β^A and β^S peaks).

Taken together, these results were highly suggestive of an acquired deletion of the *HBB* gene (β^S allele) in one of the early precursors of the hematopoietic lineage leading to mosaicism of blood cells that have either both the β^A and β^S alleles or the β^A allele only (hemizygosity). To definitively confirm this hypothesis, fluorescent *in situ* hybridization (FISH) was performed on metaphases from lymphocytes using, as specific probe, a 3 kb long-range PCR fragment covering the *HBB* gene (primer coordinates and PCR conditions available on request). This probe was fluorescently labeled by nick-translation. Nine out of the 18 mitoses observed showed one HBB signal while 9 mitoses showed two HBB signals (Figure 2), corresponding to a mosaicism level of roughly 50%. We also performed the karyotype that was strictly normal.

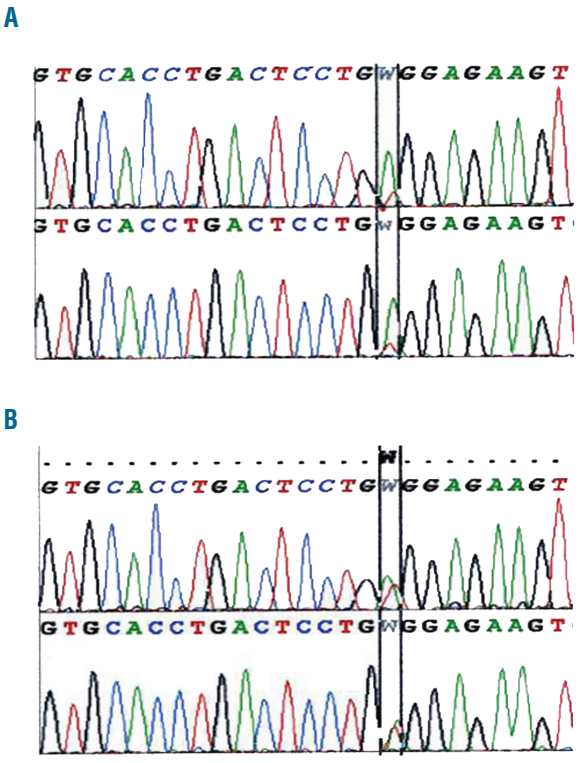

Figure 1. Comparison of the Sanger sequencing profiles with DNA from white blood cells (WBC) or from oral mucosal cells. (A) With WBC DNA, the S peak (nucleotide T) is very small compare to the A peak (nucleotide A). This suggests an important under-representation of the S allele. (B) On the contrary, with oral mucosal cells DNA, the S and the A peaks are of similar heights as is usual for a sickle cell trait carrier.

We report a constitutional sickle-cell carrier who harbors a somatic deletion of the *HBB* gene (β^S allele) in approximately 50% of her blood cells, thus explaining her abnormally low HbS level (12%) at CE-HPLC. But, contrary to what would be expected, no additional microcytosis was observed. A similar mechanism of acquired deletion of the *HBB* gene in the hematopoietic lineage has already been described to explain a thalassemia intermedia phenotype for 3 heterozygous β-thalassemic patients.[4,5] Nevertheless, this is the first time that such a mechanism is described for the sickle cell trait. In contrast to the cases of thalassemia where the deletion regarded the normal allele and worsened the phenotype, the deletion described here occurred on the mutant allele. But if it had occurred on the normal allele, it would have surely given a new genetic form of sickle-cell disease not explained by the classical Mendelian inheritance, just like the recently described case of sickle-cell disease caused by uniparental disomy.[6] As to future clinical follow up of the propositus, it would be interesting to check her HbS level in a few years time to determine if the deleted clone has a proliferative advantage or not compared to the normal clone.

The mosaicism could not be confirmed by PCR on erythroid burst-forming units as our propositus presented no immature cell in her peripheral blood and we considered that it was not ethically correct to ask for a bone marrow aspiration without any clinical benefit. On the other

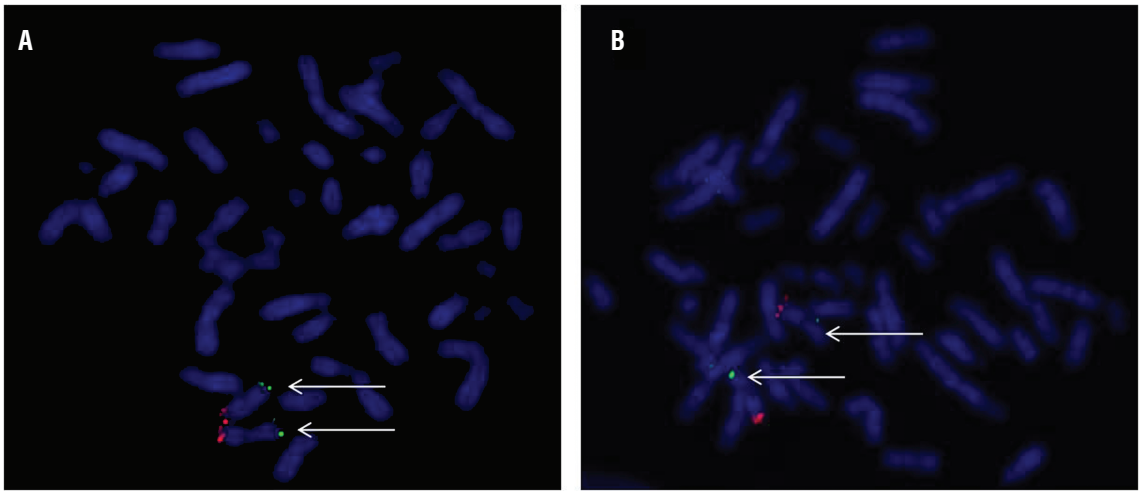

Figure 2. FISH analysis of lymphocytes metaphase spreads with a DNA probe (3 kb) specific of the *HBB* gene. The red spots correspond to an 11qter specific probe (D11S4974, Cytocell, Cambridge, UK) used to easily identify the two chromosome 11 while the green spots correspond to the HBB specific probe. Two types of mitosis were observed: (A) one with two green spots (absence of deletion of the 11p15.4 region); (B) one with one green spot only (deletion of the 11p15.4 region).

hand, neither MLPA nor a secondary performed Comparative Genomic Hybridization (CGH) reaction were sensitive enough to detect this somatic deletion (*data not shown*). Consequently, we could only estimate its extent by performing Sanger sequencing on multiple loci (in a centripetal way) at the 5' and 3' sides of the *HBB* gene, until a heterozygosity without peaks disequilibrium could be found. In this way we were able to determine that the 3'-breakpoint was located 0.5 to 8.5 kb after the 3'-end of the *HBB* gene, more precisely between U01317.1:g.64081 (last identified position with peaks disequilibrium) and U01317.1:g.72044 (first identified position without peaks disequilibrium) (*Online Supplementary Figure S1*). The 5'-breakpoint region could not be precisely determined but we could demonstrate that the deletion extends at least until the HS-region (*Online Supplementary Figure S2*). In other words, it belongs to the $(\varepsilon\gamma\delta\beta)^0$-thalassemia group I. Deletions of this group remove the entire β-globin gene cluster (including the *HBB* gene) and, to date, 10 have been previously described.[7] They are all extremely rare and unique to the families in which they have been identified.

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doi:10.3324/haematol.2012.071167

Key-words: HBB gene, sickle cell, loss of heterozygosity, hemopoietic lineage

Acknowledgments: the authors would like to thank Alain Francina for his methodological advice and Caroline Garcia for her technical assistance.

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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