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SHORT COMMUNICATION

IDENTIFICATION OF A NEW HBA1 GENE MUTATION (HBA1:c.301-2A>T) IN *CIS* WITH Hb RICCARTON (HBA1:c.154G>A) [α51(CE9)Gly→Ser]

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□ *We report two point mutations found in a heterozygous state on the HBA1 gene of an 88-year-old Argentinean patient with an* α^+*-thalassemia (*α^+*-thal) phenotype: Hb Riccarton HBA1:c.154G>A) [α51(CE9)Gly→Ser] and a novel mutation, HBA1:c.301-2A>T that affects the splicing acceptor site of the second intron and leads to a non functional α-globin chain. Cloning of the HBA1 PCR (polymerase chain reaction) product and direct sequencing of the clones revealed that both mutations were in* cis.

Keywords α-Thalassemia (α-thal), HBA1 Nondeletional mutation, Genetics

α-Thalassemia (α-thal) (OMIM 604131; HBA2: 141850, HBA1: 141800) is one of the most common single gene disorders in humans, and is characterized by the absence or reduced synthesis of α-globin chains. As hematological diagnosis is hindered by the absence of specific markers, the molecular characterization of the primary defect provided diagnostic confirmation.

In Argentina, deletions of one (−α/) or both (− −/) of the α-globin genes are the major molecular cause of the disease. In a previous report, the prevalence of the $-\alpha^{3.7}$ (rightward) deletion, the most common single gene deletion predominant in Mediterranean, African and Asian individuals, was analyzed in a series of 310 newborns and showed a frequency of 1.94% in our country (1). In our laboratories, from a total of 140 patients with

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hematological phenotypes compatible with α-thal, 60 (42.9%) presented the $-\alpha^{3.7}$ mutation in a heterozygous state (genotype $-\alpha^{3.7}/\alpha\alpha$), and 19 (13.6%) in a homozygous (genotype $-\alpha^{3.7}/-\alpha^{37}$) state. Individuals with hematological data compatible with α-thal, in whom the most common deletions were ruled out, should be screened for nondeletional mutations.

In this report we describe, for the first time, a novel mutation that affects the splicing acceptor site of the second intron on the HBA1 gene (HBA1: c.301-2A>T) in association with the HBA1:c.154G>A mutation, which gives rise to Hb Riccarton [α1 51(CE9)Gly→Ser]. An 88-year-old Argentinean female patient with North Italian ancestry, presented hematological data compatible with an α^{+}-thal phenotype [RBC 5.05×10^{12}/L; PCV 0.37 L/L; Hb 12.5 g/dL; MCV 74.2 fL; MCH 24.80 pg; MCHC 33.4 g/dL; Fe 93 μg/dL; ferritin 291.5 μg/L; Hb A_2 2.7%; Hb F 1.0% and no abnormal hemoglobin (Hb) fractions on alkaline electrophoresis].

The most common causative mutations in Mediterranean individuals [the $-\alpha^{3.7}$/ and $-\alpha^{4.2}$/ (leftward) deletions, the $\alpha\alpha\alpha^{anti\ 3.7}$/ insertion, and the $\alpha^{Nco\ I}\alpha$/ and $\alpha^{IVS\text{-}I(-5\ nt)}\alpha$/ nondeletional mutations] (2) were excluded by gap-PCR (gap-polymerase chain reaction) or PCR-RFLP (restriction fragment length polymorphism) (3–5). In order to investigate the presence of other nondeletional mutations, specific PCR products of the complete HBA2 and HBA1 genes were sequenced in both directions, on the ABI PRISM™ 3130XL genetic analyzer (Applied Biosystems, Seoul, Korea).

The only change present in the HBA2 gene sequence was the T>G transversion corresponding to the *rs2362746* SNP (single nucleotide polymorphism), in a heterozygous state. However, the sequence of the HBA1 gene displayed two distinct heterozygous changes: the first, HBA1:c.154G>A, leads to the replacement of a glycine residue with a serine at codon 51 that gives rise to Hb Riccarton and was previously described in New Zealand (6) and in The Netherlands (7); the other, HBA1:c.301-2A>T, was a novel mutation that affects the normal splicing patterns; the A>T transversion in the splicing acceptor site AG abolishes its recognition by the splicing machinery thus altering this process, which results in no normal mRNA being produced.

There are only a few mutations reported that affect the position analogous to the mutation found in our laboratories on the HB genes; in the same position, in 2003, Harteveld *et al.* (8) reported the mutation HBA1:c.301-2A>G in the study of the molecular spectrum of α-thal in the Iranian population of Hormozgan. Two mutations have been described that affect the first base of the splicing acceptor site of the HBB gene, HBB:c.316-2A>C and HBB: c.316-2A>G, HbVar ID 939 and 940 (9,10) that induce a β^{0}-thal phenotype. So far, there are three other nondeletional mutations described on the HBA genes that disrupt the splicing acceptor sites: HBA2:c.96-2A>G, HbVar ID 1066, HBA2:c.301-1G>A and HBA1:c.96-1G>A, HbVar ID 1067 that induce an α^{+}-thal phenotype (11–13).

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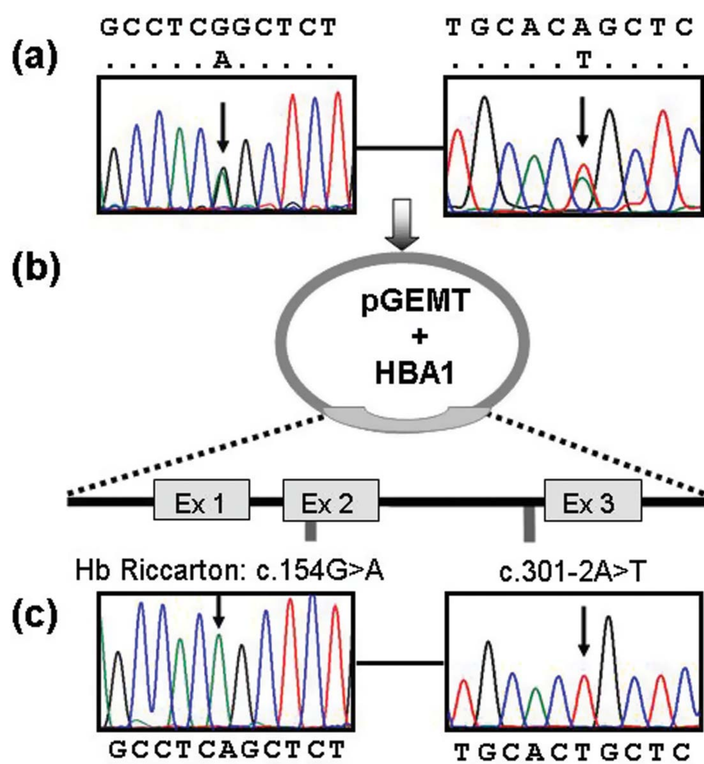

FIGURE 1 Identification of mutations by sequencing of the HBA1 gene. (a) The electropherogram showed the presence of two different changes in a heterozygous state: c.154G>A (Hb Riccarton) and c.301-2A>T. (b) Strategy for cloning the HBA1 PCR product (1534 bp from c.–149 to c.*308) in the pGEM®-T Easy Vector. (c) Direct sequencing of one clone confirmed that both mutations were on the same allele.

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In order to determine whether the mutations were in *cis* or in *trans*, the PCR product of the HBA1 gene (1534 bp, from c.–149 to c.*308) of the patient was cloned in the pGEM®-T Easy Vector Systems (Promega, Madison, WI, USA) and transformed into *Escherichia coli* strain DH5α; plasmids were purified with the Wizard® Plus SV Minipreps DNA Purification System kit (Promega) and sequenced, revealing that both mutations were on the same allele (Figure 1). The change in the splicing site leads to the expression of a non functional α-globin: the computer program NNSPLICE 0.9 (01–97) (14) predicts a loss of the splicing acceptor site of the second intron, indicated by the score of 0 when this variant is present *vs.* a score of 0.95 for the wild type allele.

Given the fact that Hb Riccarton cannot be separated from Hb A using conventional electrophoresis or high performance liquid chromatography, the lack of this variant in the patient is not all that helpful in the interpretation. Although this variant is present in *cis*, the splice acceptor mutation that is

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predicted to disrupt normal splicing, should prevent expression of the abnormal α chain, thus being responsible for the α-thal phenotype of the patient. This study highlights the importance of investigating thoroughly the presence of nondeletional mutations not only on the HBA2 gene, where most point mutations are described, but also on the HBA1 gene, in patients with an α-thal phenotype and absence of deletional mutations.

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