CORRESPONDENCE

MOLECULAR CHARACTERIZATION OF HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN IN A PATIENT FROM CUBA

To the Editor:

Hereditary persistence of fetal hemoglobin (HPFH) syndrome is associated with heterogeneous deletions within the β-globin gene cluster. In the last years the breakpoints of several deletions have been exactly defined and sequenced to investigate the molecular mechanisms underlying their formation. However, since cloning and sequencing are time-consuming, data are available only for single cases.

We report the results obtained studying a black female from Cuba, compound heterozygote for β-thalassemia and HPFH. The patient, aged 35, has slight microcytic-hypochromic anemia (hemoglobin [Hb] 10 g/dL), 98% Hb F, no Hb A, and normal Hb A₂. The two conditions segregate within the family. HPFH heterozygous subjects are hematologically normal, having 15% to 25% Hb F homogeneously distributed among the red blood cells, both of γ and A₃γ type.

DNA was obtained from a peripheral blood buffy coat of the patient and of her relatives, and analyzed by Southern blot to characterize the type of HPFH. The restriction map obtained with different enzymes (BamHI, HindIII, EcoRI, HpaI, BglII, BclI) in the ψ₈-globin gene region and at the level of the 3D probe overlaps that described in black HPFH type I (data not shown). In

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Fig 1. (A) Schematic representation of Black HPFH-1 deletion. The position of primers a and b used to amplify the breakpoint and their sequence is indicated. Results of the amplification reaction on two HPFH carriers are shown in the lower part. (B) Nucleotide sequence across the breakpoint. The insertion of five nucleotides is indicated on the right.

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particular, the 5' breakpoint is located approximately 3.5 kb 5' to the δ gene, and the 3' end occurs more than 100 kb away in the proximity of the 3D probe (Fig 1A).

The nucleotide sequence across the deletion junction of black HPFH type I has been recently reported. Assuming that our deletion was indeed of the latter type, we took advantage of the possibility of amplifying the breakpoint in this condition. We synthesized two oligonucleotide primers, a = 5' ACAGTGTGCAGTGATTATT 3' (located within the RIH sequence) and b = 5' CTAGAGAGAAGGAACTGCTA 3', corresponding to nucleotide 72-90 of the sequence 3' to the breakpoint in black HPFH type I.

Polymerase chain reaction was performed using TaqI polymerase (2.5 U) on 1 pg of DNA in automated equipment previously described for 35 cycles. A fragment of the expected size (approximately 520 bp) (Fig 1A) was obtained only in HPFH carriers.

To confirm the identity of our deletion as black HPFH type I, we directly sequenced the amplified fragment using primer b (Fig 1B). The nucleotide sequence through the breakpoint corresponded exactly to that recently reported by Feingold and Forget in black HPFH type I. In particular, the same insertion of five nucleotides AAATA observed in the case originally investigated was also present in our case. The data were confirmed by also sequencing the opposite strand. Because the endpoints of the deletion in these cases are more than 100 kb distant and their joining region is identical, it is unlikely that this deletion occurred independently in two different occasions. Thus, our data suggest a unique origin for the most common HPFH deletion present in blacks. Moreover, we show that the amplification of the fragment encompassing the deletion junction may be useful for the rapid molecular diagnosis of this condition in the black population.

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Molecular characterization of hereditary persistence of fetal hemoglobin in a patient from Cuba [letter]

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