ularly in countries where a significant proportion of HH patients carry a wild type HFE gene (such as Italy and Greece), and for further characterization of HH cases carrying only one mutated HFE allele.

Maria Pissia, Katerina Polonifi, Marianna Politou, Konstantinos Lilakos, Nikos Sakellaropoulos, George Papanikolaou
First Department of Internal Medicine, School of Medicine, National and Kapodistrian University of Athens
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Correspondence: Marianna Politou, MD, PhD, 23–27 Makrigianni st, 117–42 Athens, Greece. E-mail: mpolitou@hotmail.com

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Red Cell Disorders

Hb Gun Hill: a further de novo observation

Here we report the third observation (the second de novo) of unstable Hb Gun Hill or [β91(β7)–95(β5)Leu–His–Cys–Asp–Lys→0] Hill. The two-year old male carrier showed low mean corpuscular hemoglobin (MCH) and mean hemoglobin concentration (MCHC), 8.5% fetal hemoglobin and ≥30% variant hemoglobin. Mild hemolytic symptoms were detected seven years later. DNA sequencing and functional studies of mRNA and globin chains were performed.


Hb Gun Hill [β91(β7)–95(β5)Leu–His–Cys–Asp–Lys→0] has been described at the protein level in a North–European family1 and as a de novo mutation in a black American female.2 The variant is due to the deletion of five amino acids from the final three residues of F–helix to the first two residues of the FG corner.3 Because of the duplication of Leu–His at codons 91–92 and 96–97 the deletion breakpoints remain ambiguous. The five-residue deletion causes a profound rearrangement of the heme pocket, which impairs heme binding and leads to molecular instability. The variant hemoglobin (Hb) shows lower absorption in visible wavelengths, and consequently a lower total Hb value;4,5 because there are only two heme groups per molecule (linked to the α-chains). Moreover, the alteration of the molecular structure causes functional alterations such as increased oxygen affinity, decreased cooperativity and absence of the Bohr effect.

A two-year old boy from Catania, Sicily, was found to have a low MCH and MCHC associated with slight anisocytosis, and absence of the Bohr effect. The patient showed reticulocytosis, high levels of lactate dehydrogenase and low levels of haptoglobin but had no jaundice, splenomegaly or hepatomegaly (Table 1). These symptoms were milder than those described in the two other patients with Hb Gun Hill who had hemolytic anemia with increased indirect bilirubin and one also had scleral jaundice and splenomegaly (Table 1). This difference could be because our patient is younger than the other two. The mutation in our patient was de novo. Paternity was confirmed by analysis of blood groups and RFLP haplotypes in the β-globin gene cluster (Table 1). Two duplicated
sequences at codons 90/92 and 95/97 (5'-AGCTGCAC-CGT-GACA-AGCTGCAC-3') can mediate a slipped-mispairing, or less probably an unequal crossing over leading to the deletion. Moreover, semi-palindromic sequences such as that of 6 nucleotides with 1 mismatch at codons 92/98 (5'-CAC(T)GTG-ACACAGTCG-CAGGT-G3') could favor the formation of an intrastem strand-loop structure, followed by the synthesis of anomalous sequences.

To conclude, of the three known cases of the unstable Hb Gun Hill this is the second due to a de novo mutation. The high frequency of de novo events leads us to define the mutation as recurrent and indicates that the mutation occurs in a region definable as a deletion hot spot. It is likely that the Hb Gun Hill as well as other variants which impair binding with the heme group should be hypothesized in all patients showing a low MCH and MCHC (due to decreased absorption of the Hb variant) associated with a normal mean corpuscular volume (MCV) and mild hemolytic symptoms.

Maria De Angioletti,* Giuseppina Lacerra,* Rosario Testa,* Angela Flagiello,* Gino Schiöli,* Clementina Carestia*
*Istituto di Genetica e Biofisica "A. Buzzati Traverso", Naples; °Dipartimento di Pediatria, Università degli Studi di Catania; *Dipartimento di Chimica Organica e Biochimica, Università di Napoli Federico II, Naples, Italy

Key words: Hb Gun Hill, unstable hemoglobin, de novo mutation, β-globin gene.

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Correspondence: Clementina Carestia, MD, Istituto di Genetica e Biofisica "Adriano Buzzati Traverso", CNR, Via G. Marconi 10-12, 80125, Naples, Italy. Phone: international +39.081.7257241. Fax: international +39.081.7257243. E-mail: carestia@igl.na.cnr.it

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Figure 1. A) Cation exchange HPLC of the hemoglobins in the proband. B) Cellulose acetate gel electrophoresis: 1: father; 2: patient; 3: Hb C carrier; 4: Hb S carrier. C) Sequence analysis of the coding strand of the β-globin gene of the patient. The wild type (wt) and mutated (GH) DNA sequences are indicated. An arrow indicates the first nucleotide in heterozygosis. D) Semi-quantitative analysis of the mRNA by radioactive RT-PCR. Total RNA was isolated from reticulocyte-enriched peripheral blood cells with Triazol (Life Technologies, NY, USA). The cDNA was synthesized from the RNA using Moloney Murine Leukemia Virus Reverse Transcriptase and an Oligo d(T) primer (GeneAmp, Perkin Elmer, and Foster City, CA, USA). cDNA was amplified with 20 PCR cycles using (α-32P)dCTP and the following primers: GTGCCTTTAGTGATGGCCTG and CTTTGC-CAAAGTGATGGGCCAGC (+390/+409 and +1394/+1372 relative to the β-globin cap site). The cDNA samples were run on a 7.5% polyacrylamide gel at 350 V for 1.5 hours. Four bands were obtained and identified by sequencing as normal (156 bp) or mutated (141 bp) homodimer or heterodimer bands. The ratio between radioactive mutated/wild type (wt) homodimer bands was quantified using a phospho-imager with ImageQuant software (Molecular Dynamics, Sunnyvale, CA, USA). A 50bp ladder is shown. 1: normal control; 2: patient.