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Blood 142 (2023) 5273–5275

The 65th ASH Annual Meeting Abstracts

ONLINE PUBLICATION ONLY

113.SICKLE CELL DISEASE, SICKLE CELL TRAIT AND OTHER HEMOGLOBINOPATHIES, EXCLUDING THALASSEMIAS: BASIC AND TRANSLATIONAL

Hemoglobin Monza: A New Variant of Unstable Hemoglobin

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Introduction:
Unstable hemoglobins are variants with structural abnormalities; while not hindering protein formation, they lead to protein destabilization and can cause hemolytic crises. Most unstable hemoglobins are caused by point mutations that result in the premature dissociation of heme from the globin chain and usually involve His residues (Weatherall et al. 2001 Nat.Rev.Genetics; Scheps et al. 2018 EJH). Here a new unstable hemoglobin, named Hb-Monza is presented, exhibiting a long duplication in the HBB gene without apparent alterations in α-β subunit interaction or involvement of specific His residues. The variant was identified in a family, with four out of six members involved and is associated with mild chronic hemolysis and acute severe hemolytic crises during febrile infections.

Methods:
The presence of erythrocyte membrane abnormalities was tested by glycerol lysis, Pink, osmotic resistance and by EMA binding test. G6PD and PK activity levels were tested both during the hemolytic crisis and at resolution. Abnormal variants were screened by HPLC and electrophoresis. Stability of the Hb variant was assessed by the Isopropanol Precipitation Test. DNA was extracted from peripheral blood leukocytes, followed by PCR amplification of HBB, HBA1 and HBA2 genes. Sequencing was performed using Genetic Analyzer 3500. A 3D model of the variants was produced by SWISS-MODEL using the crystal structure of the deoxy human hemoglobin (PDB 1A3N) as template and refined by Maestro Suite.

Results:
The index patient (pt) was a 13-year-old girl of Chinese origin; she was the firstborn of 4 children (2 sisters and 1 brother) and came to ER with fever and hyperchromic urine. Blood tests revealed severe anemia (Hb 5.2 g/dL) and signs of hemolysis. Direct Coombs test was negative both for warm and cold antibodies. She tested positive for Mycoplasma Pneumoniae, Epstein Barr Virus (EBV) and Parvovirus B19 (IgG and IgM) antibodies. The child's infection cleared during hospitalization, and hemoglobin levels increased without transfusion. Her hemoglobin further rose to 11 g/dL in outpatient follow-up although signs of low-level chronic hemolysis persisted. All affected family members exhibit mild chronic hemolytic anemia, with all, except the mother, experiencing at least one acute severe hemolytic state triggered by infection. HPLC showed the presence of an abnormal fraction of hemoglobin with slow migration speed in alkaline electrophoresis and extremely slow elution in HPLC (4.8 min). The abnormal Hb fraction in the proband, mother, brother and sister was 20.8%, 17.7%, 28.1%, and 25.6% respectively. The father and the youngest sibling did not show this band. Sequencing of HBB demonstrated an in-frame duplication of 69 bases corresponding to 23 amino acids located in exon 2 (c.176_244dup; p.Pro59_Asn81dup); this was

present in all pts exhibiting the abnormal fraction at HPLC. A functional validation of Hb Monza utilizing the isopropanol test confirmed the unstable nature of the variant (Figure 1). The 3D-model analysis (Figure 2) showed that the duplication is situated amidst helix E and F of HBB resulting in a disordered conformation protruding from the Hb structure The binding of the heme group remains unaffected and the coordination of the heme group with His residues of both the E and F helix is preserved. The tertiary structure of the Hb beta chain is scarcely affected.

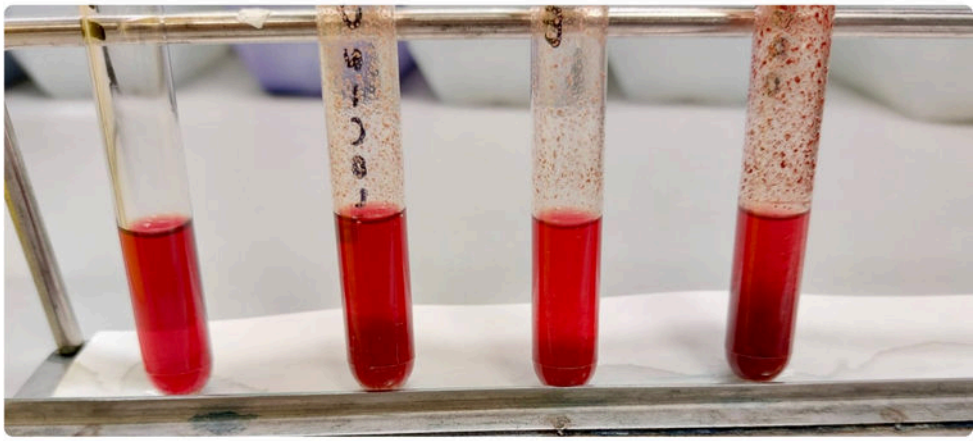
Conclusions:

This report introduces a novel HBB gene variant, Hb Monza (HBB: c.176_244dup), associated with subclinical chronic hemolysis and acute hemolytic crises during fever-inducing infections. Only a few HBB duplications exceeding 20 bp have been documented, all leading to Beta-Thalassemia clinical features through production of a truncated beta globin chain or failure of HBB to bind the alpha chain (e.g. Frischknecht et al. 2007 Haematologica). However, 3D-modeling of Hb Monza indicates minimal tertiary structure alteration. The inclusion of 23 additional amino acids results in a disordered conformation protruding from the Hb structure possibly facilitates direct bonding between the iron molecule in the heme group and internal amino acid side chains due to increased structural flexibility. This could lead during thermal or metabolic stress, to Hb precipitation. Further data from molecular dynamic experiments will be presented.

Dr. Civettini and Dr. Corti contributed equally to this article

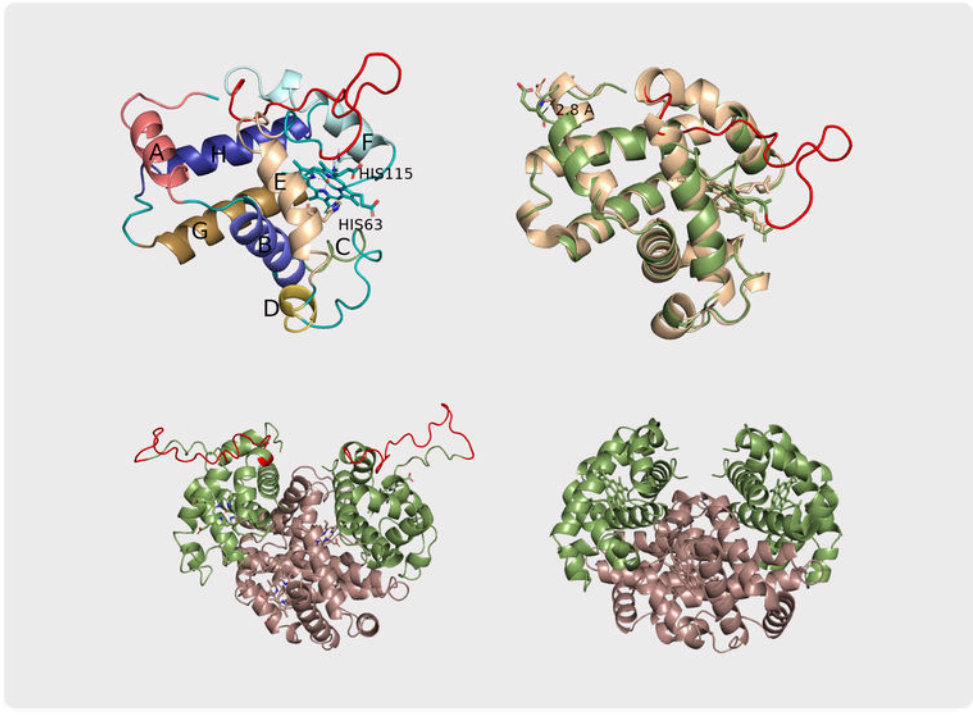
Disclosures Biondi: *Colmmune:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *Galapagos:* Membership on an entity's Board of Directors or advisory committees; *Novartis:* Speakers Bureau; *BMS:* Membership on an entity's Board of Directors or advisory committees; *Agmen:* Speakers Bureau. **Piperno:** *Prothya Biosolutions:* Research Funding.

Figure 1:



The isopropanol test: from left to right, the blood samples analyzed included those obtained from a healthy donor, cord blood, proband's mother, and proband. Flocculation was observed within 5 minutes at the bottom of the test tubes and on the walls of the tube in the proband, the proband's mother, and the cord blood sample which served as positive control.

Figure 2:



This panel compares 3D models of Hb Monza and native Hb, highlighting the 23-amino acid insertion in Hb Monza's beta chain. Upper left: 3D model of Hb Monza beta chain with the insertion highlighted in red. Upper right: superposition of native (bronze) and mutant (green with red insertion) Hb beta chain structures. Lower panel: comparison of quaternary structures in Hb Monza (lower-left) and native Hb (lower-right).

Figure 1

https://doi.org/10.1182/blood-2023-184846