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Hemoglobin Lake Tapawingo [$\alpha 46$ (CE4)Phe→Ser; HBA2:c.140T>C]: a New Unstable α Chain Hemoglobin Variant Associated with Low Systemic Arterial Saturation

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Abstract

A new unstable alpha globin variant was detected in a child with hypoxemia and anemia. The child's mother was found to carry the same mutation. The hemoglobin variant co-eluted with Hb A₂ by cation-exchange high performance liquid chromatography (CE-HPLC) and appeared cathodal to Hb A and anodal to Hb F by isoelectric focusing. It represented less than 20% of the total hemoglobin and was unstable by isopropanol testing. Gene sequencing identified a missense mutation in the $\alpha 2$ gene [HBA2:c.140T>C]. Oxygen dissociation and P₅₀ test results were normal.

Keywords

Hemoglobin (Hb) Lake Tapawingo; α globin variant; unstable hemoglobin (Hb); oxygen saturation

Structural change can alter the affinity of hemoglobin to bind its ligands, resulting in low or high oxygen affinity and unstable heme-globin interactions (1). At present, 1116 hemoglobin variants have been cataloged, 141 of which are categorized as unstable (2). We report the clinical and laboratory features associated with a previously undescribed unstable $\alpha 2$ variant in a clinically well child with low peripheral oxygen saturation.

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A 2-year-old Caucasian male was referred for evaluation of persistently low oxygen saturation by pulse oximetry. During an initial evaluation for bronchiolitis at the age of 11 months, he had a documented oxygen saturation of 92% on room air. Similar oxygen saturations in the low 90% range were noted on follow-up visits with his primary care physician. His past medical history included a patent foramen ovale, detected by echocardiogram, after a heart murmur was heard at one month of age. The patient had normal growth and development without cyanosis, respiratory difficulty, or activity intolerance.

Investigations prior to hematology referral included an arterial blood gas (ABG) which showed abnormally low pO₂ of 68 mmHg on room air. The pO₂ improved to 385 mmHg on 100% FiO₂. Cardiac catheterization revealed a structurally normal heart with no evidence of right to left shunt. Central left atrial and pulmonary venous saturations were 95% but the femoral arterial saturation was abnormally low at 91%. Repeat ABG measurement on room air one month later revealed a persistently low pO₂ of 52 mmHg.

The family history in this patient was significant for a hemoglobin abnormality on the maternal side. A maternal uncle had been previously diagnosed with hemoglobin “Lepore” according to the family’s understanding and past medical records. The mother and maternal grandmother were also known to have a “small amount of abnormal hemoglobin.” All three family members were reportedly asymptomatic.

Laboratory evaluation of the patient revealed anemia with reticulocytopenia and otherwise normal hematological indices (Table I). Hemoglobin (Hb) testing by cation-exchange high performance liquid chromatography (CE-HPLC) using BioRad Variant^R (Hercules, CA) BetaThal column demonstrated Hb A 85.4%, Hb X 14%, and Hb F 0.6%. The heterozygous hemoglobin variant co-eluted with Hb A₂ (retention time 3.56 minutes) and appeared slightly cathodal to Hb A and anodal to Hb F on isoelectric focusing using Resolve^R system (Isolab Inc, Akron Ohio) (Figure 1 a&b). Globin chain electrophoresis confirmed an alpha chain variant with Hb A 86%, Hb X 10.5%, Hb A₂ 3%, and Hb F 0.5%. Hemoglobin absorption spectra from 480 to 660 nm showed a normal pattern with absorbance peaks at 577 nm and 542 nm wavelengths. The variant was unstable by isopropanol testing and stable by heat testing. An oxygen dissociation curve plotted normally with P₅₀ of 30 mmHg. Gene sequencing at Mayo Clinic revealed a missense mutation in the alpha 2 gene, [HBA2:c.140T>C], resulting in a substitution of serine for phenylalanine at codon 46 (Figure 1 c).

The mother of the patient agreed to undergo similar testing and was confirmed to have 16% of variant Hb X + A₂ by CE-HPLC. Isopropanol stability testing was abnormal and heat stability testing was normal on her sample, consistent with the patient’s results. The mother was not anemic and did not have evidence of hemolysis or hypoxemia (Table I). Oxygen dissociation curve and P₅₀ testing were normal. An attempt was made to purify the variant hemoglobin from the wild type hemoglobin, but Hb X co-eluted with Hb A₂ and could not be easily separated. X-ray crystallography of the total hemoglobin was consistent with wild type hemoglobin.

This previously undescribed alpha globin mutation is the third variant reported involving codon α46(CE4) and is the first to describe a phenylalanine to serine substitution at this highly conserved site. It has been entered into the HbVar database (ID 2779) and has been named Hb Lake Tapawingo, after the town of residence of the patient and his mother (2). The previously described hemoglobins with α46 mutations are Hb Rockaway [HBA1:c.139T>C (or HBA2) or 141C>G or 141C>A; Phe→Leu] and Hb Hillingdon [HBA1:c.139T>G (or HBA2); Phe→Val]. Hb Rockaway is a stable variant that co-elutes with Hb A on CE-HPLC and causes no clinical effects (2). Hb Hillingdon is also clinically

asymptomatic and is similar to Hb Lake Tapawingo in that it co-elutes with Hb A₂ on CE-HPLC (3). The Phe→Ser of Hb Lake Tapawingo substitutes an uncharged polar side chain for a nonpolar residue. Two programs, SIFT and PolyPhen, which calculate the likely impact of amino acid substitutions based on sequence conservation and structure, predict this change will affect protein function(4, 5).

We speculate that the change of amino acid Phe→Ser at codon 46 alters the hydrophobic interactions of the CD corner of the distal heme cavity, possibly leading to a change in oxygen affinity that was undetectable by P₅₀ testing, and affecting the overall stability of the molecule. Figure 2 shows the distal residues HisE7 (His58), PheCD4 (Phe46) and LysE10 (Lys61) at the α-heme distal pocket of wild type hemoglobin. HisE7 has been predicted to rotate out of the pocket to allow for access of ligand (6). The benzyl side-chain of PheCD4 guards the ligand channel and may act as a gate to prevent easy rotation of HisE7 and therefore ligand offloading (7). It follows that mutation of Phe46→Ser, a smaller amino acid with a polar side chain, could greatly enhance the movement of HisE7, resulting in weaker ligand affinity and enhanced oxygen delivery with lower observed oxygen saturation. It may also have an effect on the tertiary structure, but this may not affect the overall stability of the structure since it is far removed from either the inter- or intra-dimer interface. It remains unclear why isopropanol induces instability in this molecule.

Though oxygen dissociation testing was normal for this variant, the concentration of Hb Lake Tapawingo was relatively low and therefore oxygen affinity testing may be unreliable. Furthermore, the etiologic impact of Hb Lake Tapawingo on the patient's anemia and hypoxia is purely speculative. We conclude that hemoglobin studies are an important part of the diagnostic evaluation of patients with persistent hypoxemia without a recognized cause, particularly in patients with history of hemoglobin abnormalities in the family.

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Figure 1.

a) HPLC results of the variant hemoglobin, which co-eluted with Hb A₂ at a retention time of 3.56 minutes. b) Isoelectric focusing gel with variant hemoglobin in duplicate (arrows) with isoelectric point cathodal to Hb A and anodal to Hb F. c) DNA sequencing results of exon 2 of the $\alpha 2$ gene (forward strand). A TTC→TCC substitution is present in codon 46, resulting in a Phe→Ser amino acid substitution.

Figure 2. Representation of the distal α -heme pocket of wild type hemoglobin with conserved amino acid residues. The rotation of HisE7 (His58) is thought to open a channel between heme and its ligand. PheCD4 (Phe46) may provide a gate to regulate the ease of this movement.

Table I

Hematologic data [Reference values]

Parameters	Patient (2 years)	Mother (23 years)
RBC ($\times 10^6/\mu\text{L}$)	4.05 [3.90–5.30]	4.7 [4.00–5.20]
Hemoglobin (g/dL)	10.3 [11.5–13.5]	13.9 [12.0–16.0]
MCV (fL)	80.0 [75.0–87.0]	89.8 [82.0–100.0]
MCH (pg)	25.4 [24.0–30.0]	29.6 [26.0–34.0]
Reticulocytes ($\times 10^6/\mu\text{L}$)	0.042 [0.020–0.080]	0.059 [0.020–0.080]
RDW (%)	13.8 [11.5–14.5]	13.2 [11.5–14.5]
Hb A (%)	85.4	83.1
Hb F (%)	0.6	0.3
Hb X + A ₂ (%)	14.0	16.6
HPLC Retention Time (min)	3.56	3.56
Heat stability	Normal	Normal
Isopropanol stability	Abnormal	Abnormal
P ₅₀ (mm Hg)	30 [24–32]	28 [24–32]