Identification of a Novel Termination Codon Mutation (TAA → TAT, Term → Tyr) in the α2 Globin Gene of a Laotian Girl With Hemoglobin H Disease

To the Editor:

α-Thalassemia is a common hereditary anemia resulting from the deficient expression of the functional α1- and/or α2-globin genes. Although a small minority of cases involve point mutations of the α-globin genes, α-thalassemia is usually caused by deletions that remove one or both α-globin genes. Individuals in whom one or two α-globin genes have been deleted or inactivated are mildly anemic, but otherwise healthy (α-thalassemia trait). However, the loss of three α-globin genes gives rise to anemia of variable severity known as hemoglobin (Hb) H disease. This condition is most common in Southeast Asia, where an estimated 13,000 to 16,000 affected infants are born annually and as many as 680,000 affected individuals presently exist.

Studies of Hb H disease in Southeast Asia have shown that most patients are compound heterozygotes for the Southeast Asian α-thalassemia 1 deletion and single α-globin gene deletions (−/−SEA/−α3.7) or −/−SEA/−α4.2 or Hb Constant Spring (−/−SEA/−αCS). Hb Constant Spring is caused by a mutation of the termination codon of the α2-globin gene, and represents the most common form of nondeletional α-thalassemia. This mutation is associated with reduced mRNA stability such that the resulting Hb variant (Hb Constant Spring) constitutes only a minor proportion (1% to 2%) of the total hemoglobin.

In this report, we describe an 11-year-old Laotian girl who presented with severe microcytic anemia and hematologic indices consistent with Hb H disease (Hb, 81 g/L; mean corpuscular volume [MCV] 65.1 fl; Hb A, 89.2%; Hb A2, 0.8%; Hb F, 1.2%; Hb H, 8.8%; Hb H inclusion bodies present). The proband’s father was born in the city of Paksé (Laos) and has the hematologic profile of Hb E trait with concomitant α-thalassemia (Hb, 143 g/L; MCV, 83.5 fl; Hb A, 72.7%; Hb F, 0.5%; Hb E, 26.8%; Hb H inclusion bodies present). The proband’s mother was born in Northern Laos and appears to have α-thalassemia trait (Hb, 132 g/L; MCV, 65.0 fl; Hb A, 97.8%; Hb A2, 1.8%; Hb F, 0.4%; Hb H inclusion bodies present).

Southern hybridization analysis of the ζ-α globin gene cluster showed that both the proband and her mother carry the Southeast Asian α-thalassemia 1 deletion and have one intact α-globin gene cluster (−/−SEA/−αα) (data not shown). The proband’s father has no
apparent deletions of the \( \zeta \)-\( \alpha \) globin gene cluster. However, his reduced level of Hb E (26.8%) and the presence of Hb H bodies in the blood smear are consistent with concomitant nondeletional \( \alpha \)-thalassemia (\( \alpha'\alpha/\alpha\alpha \) or \( \alpha\alpha/\alpha\alpha \)). Because the proband has the hematologic profile of Hb H disease, it is likely that she inherited a nondeletional \( \alpha \)-thalassemia mutation from her father.

To investigate the possibility that the proband and her father carry the Hb Constant Spring mutation, a fragment spanning the termination codon of the \( \alpha2 \)-globin gene was amplified by polymerase chain reaction (PCR) using primers P1 [5'-CCTGGGCCGCACTGA CCCTCTT-3', IVS-2 region of the \( \alpha2 \)-globin gene] and P2 [5'-CCATTGTTGGCACATTCGGG-3', 3' to the poly-A region of \( \alpha2 \)-globin gene]. The PCR products were dot blotted to nylon membranes and hybridized to allele-specific oligonucleotide (ASO) probes against the normal termination codon [5'-CAAATACCG TTAAGCTGGAGC-3'] and the Hb Constant Spring mutation [5'-CAAATACCGTCAAGCTGGAGC-3'].\(^{19}\) The test results show that the proband and her father do not carry the Hb Constant Spring mutation (Fig 1). However, the proband’s sample failed to hybridize to either the normal or the mutant ASO probe, indicating that she carries a different mutation of the termination codon or the adjacent sequences.

Direct nucleotide sequence analysis of the PCR products showed that the proband carries a novel mutation of the termination codon of the \( \alpha2 \)-globin gene (TAA \( \rightarrow \) TAT) (Fig 2). This mutation changes the termination codon to the codon for tyrosine (Term \( \rightarrow \) Tyr), giving rise to an elongated mRNA that would code for an \( \alpha \)-globin chain of 172 amino acid residues instead of the normal 141 residues. The proband’s father also carries the mutation (data not shown), thereby excluding the possibility of a de novo mutation in the proband. The proband is a compound heterozygote for the Southeast Asian \( \alpha \)-thalassemia 1 deletion and the novel termination codon mutation (\( \alpha \rightarrow T\alpha \)), and exhibits the phenotype of Hb H disease. Her father, who has Hb E trait and carries the nondeletional \( \alpha \)-thalassemia allele, has markedly reduced levels of Hb E (26.8%) as well as Hb H inclusion bodies. These observations are fully consistent with the TAA \( \rightarrow \) TAT (Term \( \rightarrow \) Tyr) mutation causing nondeletional \( \alpha \)-thalassemia.

Before this investigation, four different mutations had been reported for the termination codon of the \( \alpha2 \)-globin gene: TAA \( \rightarrow \) CAA, Term \( \rightarrow \) Gln (Hb Constant Spring);\(^{6,7}\) TAA \( \rightarrow \) AAA, Term \( \rightarrow \) Lys (Hb Icaria);\(^{12,13}\) TAA \( \rightarrow \) TCA, Term \( \rightarrow \) Ser (Hb Koya Dora); and TAA \( \rightarrow \) GAA, Term \( \rightarrow \) Glu (Hb Seal Rock).\(^{14,15}\) These mutant alleles code for \( \alpha \)-chain variants of 172 amino acid residues that are capable of forming stable Hb tetramers. However, the elongated mRNA molecules are quite unstable and the corresponding Hb variants comprise only a small proportion of the total Hb. In this case, we were unable to detect an abnormal Hb using cellulose acetate and citrate agar Hb electrophoresis. We speculate that the TAA \( \rightarrow \) TAT (Term \( \rightarrow \) Tyr) mutation causes reduced mRNA stability, as has been shown for the other termination codon mutations. It is also possible that the variant \( \alpha \)-chains and/or Hb tetramers are unstable. We have designated the predicted Hb variant [\( \alpha2\beta^\alpha_\text{Paks} \)] as Hb Pakse to indicate the birth city of the proband’s father.

Fig 2. Direct nucleotide sequence analysis of the \( \alpha2 \)-globin gene PCR product of the proband. The analysis is restricted to the allele that contains the termination codon mutation (TAA \( \rightarrow \) TAT), because the Southeast Asian \( \alpha \)-thalassemia 1 deletion removes the sequences required for amplification of the other allele.


9. Leibhaber SA, Kan YW: Differentiation of the mRNA transcripts originating from the \(a_1\) and \(a_2\) globin loci in normals and \(\alpha\)-thalassemics. J Clin Invest 68:439, 1981


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