EIGHT-BASE DELETION IN EXON 3 OF THE β-GLOBIN GENE PRODUCED A NOVEL VARIANT (KHON KAEN) WITH AN INCLUSION BODY β-THALASSEMIA TRAIT

To the Editor:

During a course of investigation on the molecular basis of β-thalassemia in the hemoglobin (Hb) E-β-thalassemia in the northeastern Thai population,1 we encountered a patient who had an unusually severe β-thalassemia phenotype. The patient was a 3-year-old male who had a history of huge abdomen since 2 years of age. At 2.5 years of age he was pale with a hepatosplenomegaly. The hematologic findings at that time were as follows: Hb 2.7 g/dL, hematocrit (Hct) 11%, and blood film showed a marked anisopoikilocytosis and many erythroblasts. After splenectomy, his Hb level was 8.2 g/dL, Hct 23%, mean corpuscular volume (MCV) 73.1 fl., mean corpuscular Hb (MCH) 25.6 pg., and MCH concentration (MCHC) 35 g/dL. Large inclusion bodies were observed in peripheral blood upon staining with brilliant cresyl blue. Hb electrophoresis showed HbsE and F in which HbE accounted for 40%. The heat and isopropanol stability tests showed no abnormal Hb. Extensive screening for β-thalassemia mutations previously detected in the Thai population2 showed a negative result. In addition, no gross deletion in the α-globin gene cluster was observed when it was

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**Fig 1.** DNA sequencing gel representing sequences in the vicinity of codons 123 to 125 where eight bases (either AGGCCCATT or CCCCACCA) are deleted from the mutant allele of the patient. Ladders represent the nucleotide sequence of the sense strand. The corresponding amino acids and codon numbers are also shown. Deletion of 8 bp in codons 123 to 125 leads to a frame shifting and a synthesis of the β-globin chain variant with 135 amino acid residues as depicted at the bottom. Junction of the cloned fragment and pUC 13 is located to the first nucleotide of codon 121.

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**Codon** | **β Khon Kaen** | **A**
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120 | AAA GAA TTC CCA GTA CCA | GTG CAG
121 | Glu Phe Thr Pro Pro Val Gln | Lys Glu Phe Thr Pro Pro Val Gln
122 | TTT TTT TTT | TAA
123 | Ser | Ser
124 | GCA CCA GGC | Cys Cys Cys
125 | Ala | Ala
126 | GGC | Glu
127 | GTG | Phe
133 | AAA GAA TTC CCA GTA CCA | GTG CAG
134 | Glu Phe Thr Pro Pro Val Gln | Lys Glu Phe Thr Pro Pro Val Gln
135 | TAA
136 | Trp Cys Gly Ter

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The diagram shows the DNA sequencing gel with two DNA sequences compared: one from the β globin gene of the patient (Khon Kaen) and the other from a normal allele (A). The gel reveals a deletion of 8 bp in codons 123 to 125, leading to a frameshift. The corresponding amino acid sequence for this variant is shown at the bottom, indicating the synthesis of a novel β-globin chain variant with 135 amino acid residues. The junction of the cloned fragment and pUC 13 is located to the first nucleotide of codon 121.
tested by the polymerase chain reaction (PCR). All exons, exon-intron boundaries, and the immediate flanking sequences of the β-globin gene of the patient were sequenced directly using various primers. When the exon 3 of the β-globin gene was sequenced, we observed a very complex sequence of ladders due to the heterozygosity of the deletion of several bases between codons 123 and 125 that made sequence interpretation difficult. We therefore cloned the PCR-amplified fragments containing each allele of the β-globin genes in pUC13 and determined the sequences of several clones. By this approach, we could define the β^6 mutation in one clone and a deletion of 8 bp (either ACCCCACC or CCCCCACCA between codons 123 and 125 (Fig 1) in another clone. Because the deletion eliminates the normal HphI recognition site in exon 3 of the β-globin gene, we confirmed this finding by digesting the amplified DNA of exon 3 with this enzyme. Figure 2 demonstrates that the patient was heterozygous for this mutation. Haplotype analysis in the β-globin gene cluster demonstrated that the haplotype (+-----+) was linked to this mutant allele. Either orientation of the 8-bp deletion in this β-globin gene results in the same shift of a reading frame from codon 123 through its c-terminal. As depicted in Fig 1, the β-globin chain synthesized from this mutant allele would consist of 135 instead of 146 amino acid residues with an extremely altered amino acid sequence from residues 123 to 135. Further analyses of the synthetic globin chains by carboxymethyl (CM)-cellulose column chromatography, however, failed to demonstrate abnormal protein, thereby suggesting that this β-globin variant is highly unstable and is likely to be degraded soon after translation. In fact, elimination of normal amino acid residues from codon 123 to 146 of the β-globin chain interferes with H-helix involved in α1β1 contact and α1β2 subunit interactions. The mutant globin chain could not interact with an α-globin chain to form a dimer and thus be removed by proteolysis. Inclusion body was also observed in the peripheral blood of the patient. We have noted previously that extensive alteration or loss of amino acid residues at positions 123 to 131, observed in various inclusion body β-thalassemia traits, might be responsible for inclusion body formation. It has been pointed out that, because of their severe phenotype, these dominant mutations were found mainly in the non-endemic region for malaria infection. The dominant exon 3 mutant described here could be an exceptional case, because this mutation was found in Thailand, one of endemic regions for malaria. The novel β-globin variant described here was named Hb Khon Kaen.

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REFERENCES


Eight-base deletion in exon 3 of the beta-globin gene produced a novel variant (beta khon kaen) with an inclusion body
beta-thalassemia trait

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