Hb Hekinan in a Taiwanese Subject: A T Substitution at Codon 27 of the α1-Globin Gene Abolishes an HaeIII Site

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SHORT COMMUNICATION

Hb HEKINAN IN A TAIWANESE SUBJECT: A G→T SUBSTITUTION AT CODON 27 OF THE α1-GLOBIN GENE ABOLISHES AN HaeIII SITE

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We recently observed a heterozygote for Hb Hekinan in a Taiwanese subject. The molecular lesion of Hb Hekinan is a substitution of G→T at codon 27 of the α1-globin gene, which abolishes an HaeIII restriction enzyme site. Hb Hekinan [α27(B8)Glu→Asp, GAG→GAC (α2)] has not been found in Taiwan. This variant can be detected by high performance liquid chromatography (HPLC) but not by capillary or cellulose electrophoresis.

Keywords Hb Hekinan, α1-Globin gene, Codon 27, Taiwanese

Hb Hekinan [α27(B8)Glu→Asp, GAG→GAC (α2)] has been reported in Japanese, Thai, Chinese and Guyanan people (1–4). It is due to a substitution of a glutamic acid by an aspartic acid residue at position 27 of the α1- or α2-globin chain. We recently observed this variant in the heterozygous state in a Taiwanese subject. The subject was a 10-years-old boy admitted to the Department of Pediatric Hematology of China Medical University Hospital (Taichung, Taiwan) for anemia. The hemogram showed: Hb 9.9 g/dL, RBC...
4.57 × 10¹²/L, PCV 0.333 L/L, MCV 72.9 fl, MCH 21.7 pg, MCHC 29.7 g/dL and ferritin 2.58 ng/mL. Electrophoresis of freshly prepared hemolysates in cellulose acetate at pH 8.6 or in capillary electrophoresis showed no remarkable change. On electrophoresis by automated high performance liquid chromatography (HPLC) (PRIMUS CLC 385; Primus Company, Kansas City, MO, USA), an abnormal peak that was distinguished from Hb A in the amount of 14.7% was found (Figure 1A). The abnormal hemoglobin (Hb) could not be detected by capillary electrophoresis (Figure 1B).

The α1-, α2- and β-globin gene analyses were performed and DNA was isolated from white blood cells using standard methods. The α1-globin gene was specifically amplified with primers P1 (forward primer, 5′ non coding area): 5′-CTC TTC TGG TCC CCA CAG AC-3′ and P2 (reverse primer, 3′ non coding area): 5′-AGG GGC AAG AAG CAT GGC CA-3′, to amplify the whole coding region and two introns. The α2-globin gene was specifically amplified with primers P1 and P3 (reverse primer, 3′ non coding area): 5′-CAG GAA GGG CCG GTG CAA GGA G-3′, to amplify the whole coding region and two introns. The β-globin genes were amplified with primers P4 (forward primer, 5′ non coding area): 5′-GCT TAC CAA GCT GTG ATT CC-3′, and P5 (reverse primer, 3′ non coding area): 5′-GGA CTT AGG GAA CAA AGG AAC C-3′, to amplify the whole coding region and two introns. The polymerase chain reaction (PCR) conditions were as follows: the amplification was performed in 50 μL which consisted of 500 ng genomic DNA, 50 ng each of the primers (P1 + P2 or P1 + P3 or P4 + P5), 0.3% DMSO, 50 μM of each dNTP, 1 × PCR buffer and 2.5 units of Taq

**FIGURE 1** A) An abnormal peak was distinguished from Hb A by HPLC and amounted to 14.7% of the total Hb. B) The abnormal hemoglobin could not be detected by capillary electrophoresis.
polymerase (Perkin Elmer Corporation, Norwalk, CT, USA), using 35 cycles of 2 min. at 94°C for denaturation, 2 min. at 60°C for annealing, and 3 min. at 72°C for extension, and a final extension of 5 min. at 72°C, using a Perkin Elmer Cetus PCR thermocycler. The PCR products were isolated and sequenced as described previously (5,6). In addition to primers P1, P2, P3, P4, and P5, the sequencing primers for the α-globin gene were as follows: P6 (reverse primer, intron 1): 5′-CAG GAC GGT TGA GGG TGG CCT-3′, P7 (forward primer, intron 1): 5′-ACC CCA CCC CTC ACT CGC TT-3′, P8 (reverse primer, intron 2): 5′-TGC GAG GAA GGC GCC ATC TG-3′ and P9 (forward primer, intron 2): 5′-GCA GAG GAT CAC GCG GGT TG-3′. The sequencing primers for the β-globin genes were: P10 (reverse primer, intron 1): 5′-GCC AGA GAG AGT CAG TGC CTA-3′, P11 (reverse primer, exon 2, near intron 2): 5′-CCT GAA GTT CTC AGG ATC CA-3′ and P12: (forward primer, intron 2): 5′-TGC TAA TCA TGT TCA TAC CT-3′.

The results showed a G→T substitution at the third base of codon 27 of α1-globin gene (GAG→GAT) that resulted in the substitution of a glutamic acid for an aspartic acid residue (Figure 2A). This mutation abolishes an HaeIII restriction enzyme site. We further amplified the mutation area using primers (P1 + P6), and the PCR products were digested with HaeIII. The results are shown in Figure 2B.

Hb Hekinan co-migrates with Hb A on cellulose acetate electrophoresis and capillary electrophoresis, and is difficult to distinguish from Hb A by

![Figure 2](image_url)

**FIGURE 2** The results of direct sequencing of the α1-globin gene showed a G→T substitution at codon 27. Upper case: normal control; lower case: the variant. The results of restriction enzyme HaeIII digestion of PCR products showed that the patient (lane 3) and positive control (lane 1) had fragments of 121 and 65 bp, respectively (the 18 bp fragment was not visible), and the normal control (lane 2) had fragments of 73, 65 and 48 bp, respectively (the 18 bp fragment was not visualized). M: 100 bp ladder.
these methods. The HPLC procedure using a weak cation exchange material with polyaspartic acid can differentiate these two Hbs. The Hb variant can be confirmed by either sequencing analysis of the $\alpha_1$-globin gene product or $Hae$III digestion of the amplified $\alpha_1$-globin gene product.

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