ERRATA

GENOTYPING OF BETA THALASSEMIA TRAIT BY HIGH-RESOLUTION DNA MELTING ANALYSIS

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In the Southeast Asian Journal of Tropical Medicine and Public Health 2013; 44(6): 1055-1064. There are errata of the above article in the MATERIALS AND METHODS Section on page 1056-57, right column of the subtitle : **Primer design** and on page 1058 left column of the subtitle : **Real-time PCR and high resolution DNA melting analysis**. The corrections are as below:

Primer Design

All primer pairs were designed according to the Primer-BLAST program. Four primer pairs (A, B, C, and D) were designed to cover the four regions of the HBB gene where the common beta thalassemia mutations are located (Fig 1). The A-primers were for the amplification of the promoter DNA segment, the B-primers for exon I, the C-primers for exon II, and the D-primers for the IVSII and exon III DNA segments. The A-forward primer 5′ TAGACCTCACCCTGTGGAGC 3′ (nucleotide number 62025-44) and the A-reverse primer 5′ TGGTGTCTGTTTGAGGTTGC 3′ (62168-87) were used to amplify the promoter region and generated a PCR product 163 base pairs (bp) in length and used to detect -87 C-A, -31 A-G, and -28 A-G mutations. HBB gene exon I was amplified with the B-forward primer 5′ CTGAGGAGAAGTCTGCCGTT 3′ (62203-22) and the B-reverse primer 5′ GTCTCCACATGCCCAGTTTC 3′ (62320-39). The PCR product was 137 bp in length and used to detect CD17 A-T, CD27/28 +C, CD30 G-A, IVSI-1 G-T and IVSI-5 G-C mutations. HBB gene exon II was amplified with the C-forward primer 5′ CTGCTGGTGGTCTACCCTTG 3′ (62410-29) and C-reverse primer 5′ AAAGGTGCCCTTGAGGTTGT 3′ (62555-74). The PCR product was 165 bp and was used to detect CD35 C-A, CD41 –C, CD41/42 –CTTT and CD71/72 +A. The IVSII and exon 3 DNA segment was amplified using the D-forward primer 5′ TATCATGCCTCTTTGCACCA 3′ (63229-48) and D-reverse primer 5′ GCAATATGAAACCTCTTACATCAGTT 3′ (63330-55). The PCR product length was 127 bp and was used to detect the IVSII-654 C-T mutation. To detect the 3.4 kb deletion, three different primer pairs were designed. These was E1-forward, E1-reverse and E2-reverse. The E1-forward primer 5′ GTCACACTTTGGGTTGTAAGTGAC 3′ (61360-83) and E1-reverse primer 5′ TCAATGTGCTCTGTGCATTAGTTAC 3′ (61459-83) were used to amplify the wild type gene at the 5′ DNA break point of the 3.4 kb deletion (U01317; 61417). The product size was 124 bp in length. To verify

the 3.4 kb mutant, the DNA sequence of the deleted junction (U01317, HBB gene; 61417/64902) was amplified with the E1-forward primer and the E2-reverse primer 5′ TGCCATTTCATGGTTCACCTTTCA 3′ (65041-68). The product obtained was 224 bp. All primers were purchased through Invitrogen (Carlsbad, CA).

Real-time PCR and high resolution DNA melting analysis

To identify beta thalassemia mutations, two series of five PCR protocols were carried out simultaneously. One was conducted with a normal DNA sample and the other with an unknown beta thalassemia trait sample. Each test sample contained one of 5 specific primer pairs and PCR mixtures and was performed in two replicates. To detect the 3.4 kb deletion mutant, E1 forward/E1 reverse primers were used to amplify normal control DNA and E1 forward/ E2 reverse primers were used for unknown samples. The 25 μl PCR mixture included 5 μl of DNA, 1.5 μM $MgCl_2$, 200 μM of each deoxynucleotide triphosphate, 3μM of SYTO9, 1 unit of Platinum® *Taq* DNA polymerase, 0.3 μM of each primer (Invitrogen, Carlsbad, CA) and the rest was a 1X PCR buffer. Thermal cycling was performed using the Bio-Rad CFX96 real-time system (Bio-Rad Laboratories, Hercules, CA), starting with an initial step of activating the *Taq* DNA polymerase at 94°C for 2 minutes, followed by 40 cycles of denaturation at 94°C for 15 seconds, annealing at 60°C for 15 seconds and extension at 72°C for 20 seconds. Fluorescence activity was measured on a SYBR Green I channel at the end of each cycle. The HRM melting program started at 95°C for 10 seconds, followed by a melting cycle from 75°C to 95°C (except for 3.4 kb deletion and IVSII-654 that the melting cycle was from 68°C to 85°C) with a transitional rate of 0.2°C per 10 seconds. Fluorescence was monitored continuously during the melting process. HRM analysis was performed using Bio-Rad Precision Melt Analysis Software (Bio-Rad, Hercules, CA). The different fluorescence temperature-shifted curved were compared between the wild type control and the unknown sample with each primer protocol. The positive primer set revealed curves that were not in alignment (Fig 3).