RAPID DIAGNOSIS OF $\alpha$-THALASSEMIA-1 OF SOUTHEAST ASIA TYPE AND HYDROPS FETALIS BY POLYMERASE CHAIN REACTION

To the Editor:

Most of hemoglobin (Hb) Bart’s hydrops fetalis syndrome is due to homozygote $\alpha$-thalassemia-1 in the southeast Asia area, and the defect is the SEA type deletion that loses near 20 kb of DNA at the $\alpha$-globin gene cluster.7 Pregnancy involving Hb Bart’s hydrops fetalis syndrome is associated with an increased risk of maternal complications, such as hydramnios, preeclampsia, antepartum or postpartum hemorrhage, and difficult vaginal delivery.7 There is also considerable emotional strain for the mothers and their family members. We use polymerase chain reaction (PCR) and prenatal diagnosis from chorionic villi biopsy samples to identify couples at risk of conceiving fetuses afflicted with Hb Bart’s hydrops fetalis.

DNA was obtained from the cord blood of six hydrops fetalis

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**Fig 1.** (A) Schematic representation of $\alpha$-thalassemia-1 of Southeast Asia type. The positions of primer A and B are used to amplify the normal area, A and C amplify the breakpoint, and their sequence are indicated. (B) Results of the amplification reaction on four hydrops fetalis (lanes 1 through 4), one heterozygote $\alpha$-thalassemia-1 (lane 5), and one normal control (lane 6). a, primer A + B; b, primer A + C; M, pGEM DNA markers (Promega).

A = 5' - GCGATCTGGGCTCTGTGTTCT-3'
B = 5' - GTTCCCTGAGCCCCGACACG-3'
C = 5' - ACTGCAGCCTTGAACTCCTG-3'
cases and the peripheral blood of their parents, and analyzed by Southern blot hybridization to characterize the type of gene mutations. The restriction map was obtained with three different enzymes (BamHI, BgII, EcoRI) and two different probes (Vc and a). The results showed that all the hydrops fetalis were caused by homozygote of alpha-thalassemia-1 of Southeast Asia type (data not shown).

The nucleotide sequence across the deletion junction of alpha-thalassemia-1 of Southeast Asia type has recently been reported. Assuming that our deletion was indeed of the same type, we took advantage of the possibility of amplifying the breakpoint in this condition. We synthesized three oligonucleotide primers: A = 5'-GGATCTGGGCTCTGTTTCT-3', located at nucleotides 120 to 140 of the 5' to the breakpoint of alpha-thalassemia-1 of Southeast Asia type. B = 5'-GTTCCCTGAGCCCAGACAG-3' corresponding to nucleotides 293 to 314 of sequence 3' to the beginning base of primer A. C = 5'-ACTGCAGCCTTGAAC-T CCTG-3', corresponding to nucleotide 35 to 54 of the sequence 3' to the breakpoint. PCR was performed using pairs of primer (A + B and A + C) and Taq I polymerase 2.5 U (Promega) on 1 μg of DNA in automated equipment, previously described, for 35 cycles. A fragment of 314 bases was obtained in normal or alpha-thalassemia-2, and one of 195 bases was found only in alpha-thalassemia-1 of Southeast Asia type (Fig 1).

To confirm the identity of our deletion as alpha-thalassemia-1 of Southeast Asia type, we directly sequenced the amplified fragment using primer D (5'-CTCGGGACTCGGTG-3', corresponding to nucleotides 72 to 53 5' to the breakpoint (Fig 2). The nucleotide sequence through the breakpoint corresponded exactly to that recently reported. Thus, our data suggest a unique origin of the alpha-thalassemia-1 of Southeast Asia type. Moreover, we show that the amplification of the fragment encompassing the deletion junction and normal sequence is useful for the rapid molecular diagnosis and prenatal diagnosis of alpha-thalassemia-1 and Hb Bart's hydrops fetalis syndrome in Southeast Asia area.

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REFERENCES
Rapid diagnosis of alpha-thalassemia-1 of southeast Asia type and hydrops fetalis by polymerase chain reaction [letter]

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