Rapid detection of an α-thalassemia variant (Hb Quong Sze)

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Alpha thalassemia (thal) is a group of hereditary anemias which are commonly due to the deletion of one or more of the α globin genes on chromosome 16. The α globin genes in human are duplicated and loss of one or two α genes on the same chromosome produces α^+ thal (-α/αα) and α° thal (--/αα), respectively, and does not manifest with any clinical symptoms.[1] Loss of three genes results in Hb H disease (--/-α) with a thal intermedia phenotype and complete deletion of all four α globin genes (--/--) results in severe anemia in utero (Hb Bart's hydrops fetalis).1 In South-East Asia and South China, the majority of cases of Hb H disease are due to gene deletion and about 20-40% of cases are due to compound heterozygosity of α° thal and non-deletion α thal (--/αTα) which result in a more severe phenotype.[2-4] Most of the non - deletion α thal in this region is Hb Constant Spring (Hb CS, codon 142, TAA → CAA). The molecular diagnosis of Hb Pakse' (codon 142, TAA → TAT) makes this α thal variant share the common cause of non deletion α thal.[5,6] Hb Quong Sze (Hb QS) is an another non - deletion α thal or α thal variant with the missense mutation at codon 125 of the α2 globin gene (CTG → CCG or Leu → Pro) making this hemoglobin highly unstable and undetectable by routine hemoglobin electrophoresis.[7] We have developed a simple and rapid method for the detection of Hb QS by allele specific PCR and restriction enzyme digestion. A Thai girl was seen at the age of 3 years who had pallor after high fever since the age of 5 months. She was first transfused with packed RBC at the age of 10 months after high fever with a prior-transfusion hematocrit value of 19%. Her physical examination findings are within normal limits except for mild pallor. Her parents are of Thai origin. Her hematologic data at the age of 3 years showed Hb 9.4 g/dL Hct 31%, RBC 4.45 x 10^{12}/L, MCV 71 fL, MCH 19.3 pg, reticulocyte 13.0% and 95% of the RBC contained Hb H inclusion bodies. Her hemoglobin analysis by cellulose acetate electrophoresis (pH 8.5) showed Hb A 68.7%, Hb A2 1.6% and Hb H 29.7%. Her father is healthy without any clinical symptoms:his hematologic data showed Hb 11.2 g/dL, Hct 36%, RBC 5.85x10^{12}/L, MCV 62 fL, MCH 19.1 pg and his hemoglobin analysis showed Hb A 84.3%, Hb A2 1.0% and Hb H 14.7%. Her mother is healthy: her hematologic data showed Hb 12.4 g/dL, Hct 39%, RBC 4.79x10^{12}/L, MCV 82 fL, MCH 25.9 pg and her hemoglobin analysis showed Hb A 96.9% and Hb A2 3.1%. Genomic DNA was extracted from peripheral blood leukocytes obtained from the patient and her parents using EDTA as anticoagulant. Standard α globin genotypes were analyzed by Southern blotting as previously described.8 Identification of the α° thal, SEA type (-- ^{SEA}) and Hb CS genes were performed using the allele-specific PCR method as previously described.[9] The common α^+ thal [3.7 kb deletion ($\alpha\alpha^{-3.7}$) and 4.2 kb deletion ($\alpha\alpha^{-4.2}$)] were examined using a PCR method described elsewhere.10 A non-deletion α thal was characterized by direct genomic DNA sequencing of the PCR-amplified ·2 and α1 globin gene.[8]

The α globin genotypes by Southern blotting and PCR showed that the proband's father has deletional Hb H disease (--/-α) or compound heterozygosity of α° thal (-^{SEA}) and α+ thal ($\alpha\alpha^{-3.7}$). The common α° thal (--^{SEA}), α+ thal ($\alpha\alpha^{-3.7}$and $\alpha\alpha^{-4.2}$) and Hb CS alleles were absent in her mother. α° thal (--^{SEA}) was detected in the proband but the common α+ thal ($\alpha\alpha$-$^{3.7}$and $\alpha\alpha^{-4.2}$) and Hb CS were not detected indicating the presence of an unidentified non-deletion α thal. Sequencing analysis of the α2 and α1 globin genes of the proband revealed a missense mutation at codon 125 of the α2 globin gene (CTG → CCG or Leu → Pro) (data not shown). This mutation is named Hb Quong Sze (Hb QS) as previously described.[7]

In an approach for rapid diagnosis of Hb QS, we designed a set of primers to amplify exon 3 of the α2 globin gene (Figure 1.). A 5' primer located within the IVS-2 of the α2-globin gene is αG17 (5' AGATGGCGC-CTTCCTCTCAGG 3', nt 34,231-34,251 in NG·000006 of GenBank) and the 3' primer is C3 (5' CCATTGTTG-GCACATTCCGGGACA 3', nt 34,598-34,621 in NG·000006 of GenBank). Each PCR reaction (25 μL) contained 1 μg of DNA template, 200 μM dNTPs, 30 pmol of each primer, 0.75 M betaine, 5% dimethylsulfoxide (DMSO), 1 unit *Taq* polymerase (Promega Co., USA) in a buffer containing 12 mM Tris-HCl pH 8.3, 50 mM KCl, 3 mM $MgCl_2$ and 0.01 % gelatin. PCR amplification was performed in a DNA Thermal Cycler 480 (Perkin-Elmer Cetus Corp., USA) with an initial heat activation step of 3 min at 94oC followed by 10 cycles of 94oC for 30 seconds, 60oC for 30 seconds and 68oC for 2 minutes and 20 cycles of 94oC for 30 seconds, 60oC for 30 seconds and 68oC for 2 minutes (plus an additional extension of 20 seconds per cycle in every cycle). Ten microliters of the PCR product (391 bp) were incubated for 3-4 hours at 37oC with 5 U of *Msp* I enzyme (Promega Co., USA) according to the manufacturer's recommendation. The digested products were electrophorosed on 2.5 % agarose gel followed by ethidium bromide staining and visualized on a UV transilluminator. The PCR product was digested into two fragments of 303 and 88 bp in the wild type and three fragments of 223, 180 and 88 bp in Hb QS (Figure 1.). The Figure 1. demonstrates an agarose gel electrophoresis of this analysis. As expected, Hb Quong Sze allele was detected in the proband (lane 1) and her mother (lane 2) but not in the normal control (lane 3). Normal allele was detected in the mother and normal control but not in the proband. This result indicates that the proband is a compound heterozygote of α° thal (--^{SEA}) and Hb QS and her mother is Hb QS heterozygote. Here, we describe a patient with compound heterozygosity for α° thal (--^{SEA}) and Hb QS, producing a Hb H disease with more severe clinical symptoms than that

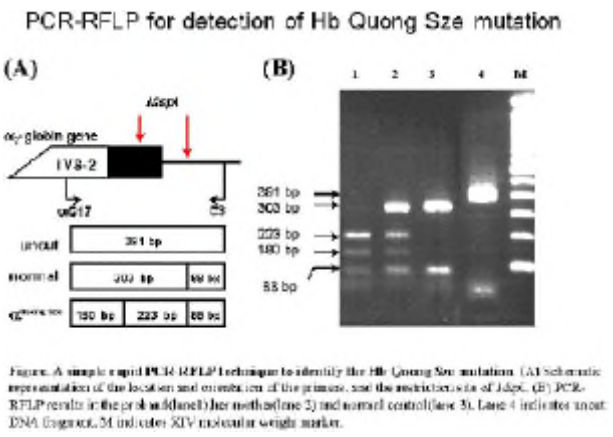

Figure 1.

found in her father with deletional Hb H disease. The Hb H level in this patient was very high, about two-fold higher than that found in her father. The diagnosis of Hb QS is impossible by hemoglobin electrophoresis because HbQS is highly unstable. DNA diagnosis of Hb QS by the simple and rapid method which we propose here will be useful for screening for the Hb QS gene in non-deletion α thal in South China and South East Asia.

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