Molecular characterization of thalassemia intermedia with homozygous Hb Malay and Hb Malay/HbE in Thai patients

We describe hematologic and DNA characterization of thalassemia intermedia in Thai adolescents caused by homozygosity for hemoglobin Malay and compound heterozygosity for hemoglobin Malay/hemoglobin E. A simple DNA assay, based on an allele-specific polymerase chain reaction (PCR), for accurate diagnosis of hemoglobin Malay was also developed.

Patients with thalassemia intermedia have a mild anemia and survive without needing regular blood transfusions.[1] Here we report two cases of Thai patients with β-thalassemia intermedia caused by homozygosity of hemoglobin Malay (Hb Malay; $\alpha_2\beta_2^{19\text{Asn-Ser}}$)[2] and compound heterozygosity of Hb Malay and hemoglobin E (Hb E; $\alpha_2\beta_2^{26\text{Glu-Lys}}$). Both patients presented with a history of anemia with marked microcytosis and hypochromia for years. They had normal growth and development and were not blood transfusion-dependent. The results of other blood examinations are listed in Table 1.

DNA analysis with polymerase chain reaction[3] detected homozygosity for the β19; AAC-AGC mutation in patient #1 and a compound β19; AAC-AGC and β26; GAG-AAG for Hb E in patient #2. This β19 mutation leads to a substitution of serine for asparagine at codon 19 of the β-globin chain known previously as Hb Malay.[2] The mutation increases the homology of a cryptic splice site in exon 1 of the β-globin gene to the donor splice site that leads to a β^+-thalassemia phenotype.[4] No α–globin gene deletion causing α-thalassemia 1 or α-thalassemia 2 was found in either case. These findings confirm that, unlike the compound Hb Malay /β^0-thalassemias which are associated with severe thalassemia symptoms,[5,6] homozygosity for Hb Malay and compound Hb Malay/Hb E produces features of thalassemia intermedia. Hb Malay accounts for 15% of Malaysian β-thalassemia genes and 16% of those in Southern Thailand.[2,3,5] It has also been found among Indonesians[7] and Chinese.[8] Identification of Thai β^{Malay} genes on the Malaysian haplotype; (- + - + + +)[2] (Table 1) indicates that Hb Malay appears to have originated only once in the Asian population.

Since Hb Malay is indistinguishable from Hb A on routine hemoglobin electrophoresis and chromatography, it is usually identified as Hb A. It is conceivable that the incidence data observed could be underestimated and Hb Malay needs to be included in the differential diagnosis for patients of Asian descent with β-thalassemia major or intermedia. In order to provide a rapid method of diagnosing Hb Malay, we applied the allele-specific PCR shown in Figure 1. The β^{Malay} specific primer, G28R (5' ACC ACC AAC TTC ATC CAC GC 3') and the β^A specific primer, G27 (5' GCC CTG TGG GGC AAG GTG AA 3') were used with primers S1 (5' TGT CAT CAC TTA GAC CTC AC 3') and S3 (5' T CCC ATA GAC TCA CCC TGA A 3') to produce the 230 bp β^{Malay} specific and the 420 bp normal specific fragments, respectively. In each reaction tube, as an internal control, two additional primers with the sequences (5' G GCC TAA AAC CAC AGA GAG T 3') and (5' C CAG AAG CGA GTG TGT GGA A 3') for amplification of the 578 bp $^G\gamma$–globin gene promotor fragment[10] were also included. The allele-specific PCR reaction mixture (50 mL) contains 0.1 mg DNA, 15 pmol of each primer, 200 mM dNTPs and 1 unit of *Taq* DNA polymerase (Promega Co., USA) in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.01% gelatin and 3.0 mM $MgCl_2$. The amplification reactions were carried out in a DNA Thermal Cycler 480 (Perkin-Elmer Cetus Co., USA). After initial heating at 94°C for 3 min, 30 cycles were performed under the following PCR conditions: 94°C for 1 min and 68°C for 1.5 min. The amplified product was analyzed by 1.5% agarose gel electrophoresis and

Table 1. Results of hematologic and globin genes analyses of the two Thai patients with β-thalassemia intermedia.

	Patient #1	*Patient #2*
Sex	male	female
Age (yr)	19	27
Hb (g/dL)	8.4	8.7
Hct (%)	26.0	27.7
MCV (fL)	54.2	60.1
MCH (pg)	17.5	18.9
MCHC (g/dL)	32.3	31.4
Hb A (Malay) (%)	74.0	34.6
Hb F (%)	18.6	11.8
Hb A_2 / E (%)	7.4	53.6
α-genotype	αα/αα	αα/αα
β-genotype	$\beta^{Malay}/\beta^{Malay}$	β^{Malay}/β^E
β-globin haplotype *	(-/-,+/+,-/-,+/+,+/+,+/+,-/-)	(-/-,+/+,-/-,+/+,+/+,+/+,-/-)

**Including 7 polymorphic sites in the β-globin gene cluster; Hinc II 5' to ε-globin gene, Hind III sites in $^G\gamma$ and $^A\gamma$ globin genes, Hinc II sites in the ψβ-globin gene and its 3' region, Ava II site in the β-globin gene and BamH I site 3' to the β-globin gene.[9]*

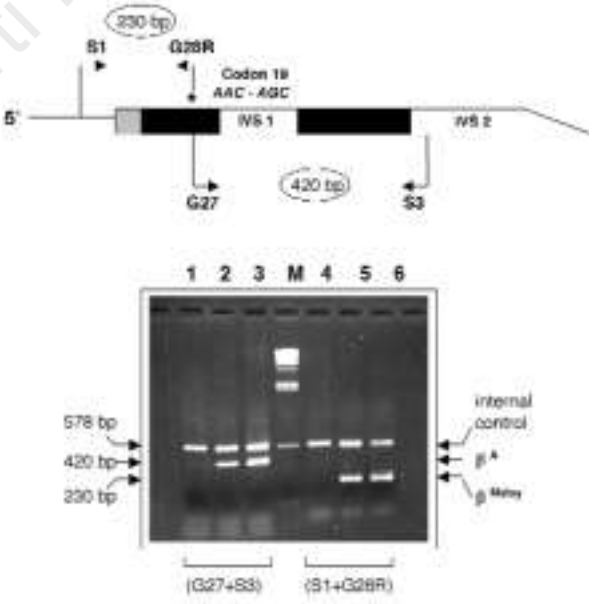

Figure 1. Identification of hemoglobin Malay mutation by allele-specific polymerase chain reaction. The locations and orientations of primers on the α-globin gene and the size of the amplified fragments are depicted. The 578 bp band is an internal control fragment of the $^G\beta$-globin gene promotor. The 420 bp and 230 bp amplified fragments indicate the presence of β^A and β^{Malay} genes, respectively. Lanes 1-3 and 4-6 are the result of amplification with primers (G27+S3) and (S1+G28R). Lanes 1 & 6, 2 & 5, and 3 & 4 indicate patient # 1 (homozygous Hb Malay), patient #2 (Hb Malay/HbE) and a normal subject, respectively. M is the λ/Hind III size marker.

visualized under UV-light after ethidium bromide staining. While the 578 bp internal control fragment was observed in all samples tested, the 230 bp and 420 bp fragments were specifically observed in subjects with and without Hb Malay, respectively. Both homozygous (patient #1) and heterozygous (patient #2) Hb Malay could be readily identified on the gel. The result indicates that this simple DNA test can be used for rapid identification of Hb Malay gene. This procedure will prove a useful complement to routine hemoglobin analysis methods to determine the genotype properly and will facilitate the prevention and control program as well as genetic counselling of thalassemia in the region.

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Key words: Hb Malay, thalassemia intermedia, allele-specific polymerase chain reaction (ASPCR).

Acknowledgments: this work was supported by a grant from the National Science and Technology for Development Agency (NSTDA), Thailand.

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