Molecular Aspects of α-Thalassemia in Myanmar

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Accepted for publication on April 17, 2001

ABSTRACT. We studied an occurrence of α-thal genes in 191 cases (117 children, age ranged from nine months to 12 years; 69 adults, age ranged from 13 to 66 years; and five parents) from Myanmar. Different racial (Myanmar, Chinese, Indians, Muslims) and ethnic groups (Bamar, Kayin, Mon, Rakhine, Shan, Kachin, Chin) involved. Except the parents, they were diagnosed as thal major and under regular blood transfusion. Polymerase chain reaction (PCR) using primer sets specific for three common α-thal mutations ($-\alpha^{3.7}$, $-\alpha^{4.2}$, and $--^{SEA}$ genes) was applied to DNA samples collected from all of the cases. A total of 33 samples (17%) were found to have α-thal mutations and distributed as follows: (a) 16 were α-thal-2 having $-\alpha^{3.7}/\alpha\alpha$ genotype, (b) 10 were α-thal-1 having $-\alpha^{3.7}/-\alpha^{3.7}$ genotype in two cases and $--^{SEA}/\alpha\alpha$ genotype in eight cases, and (c) seven cases were Hb H disease having $-\alpha^{3.7}/--^{SEA}$ genotype. The $-\alpha^{4.2}$ gene was not detected. Four of these α-thal patients were also Hb E carriers. The findings from this study demonstrated a high prevalence of α-thal and Hb E in Myanmar and, then, increased frequency of Hb E-α-thal should be expected. This work could firstly describe a molecular situation of α-thal occurring in thal major patients of Myanmar.

Key words: PCR — α-thalassemia — Hb E-α-thalassemia — Myanmar

α-Thalassemia (α-thal), a complex clinical syndrome, is likely the most commonly occurring genetic disorder, affecting persons in many parts of the world. It is indicated as an α-thal-2 and α-thal-1 whenever there is a deletion of one α-globin gene ($-\alpha/\alpha\alpha$) and of two α-globin genes ($--/\alpha\alpha$ or $-\alpha/-\alpha$) from α-globin gene arrangement, 5'-α2-α1-3', on the chromosome, respectively. Combinations of α-thal-1 and α-thal-2 alleles, then having only one active α-globin gene, leads to Hb H disease ($--/-\alpha$). In the Southeast Asia region, the most common types of α-thal-2 and α-thal-1 are $-\alpha^{3.7}$ and $--^{SEA}$, respectively.[1-3] We studied an occurrence of α-thal genes in 191 cases from Myanmar in whom 186 cases were thal major patients having regular blood transfusion and remaining five cases were parents of the respective patients.

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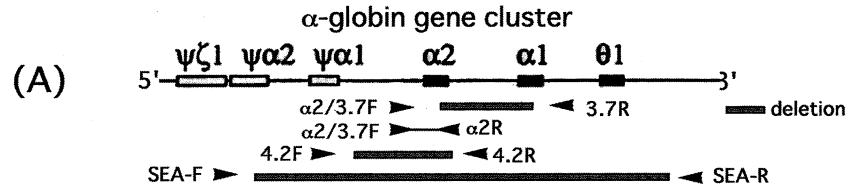
MATERIALS AND METHODS

Subjects: Genomic DNA samples of 186 patients and 5 parents were involved in this study. Different races (Myanmar, Chinese, Indians, Muslims) and ethnic groups (Bamar, Kayin, Mon, Rakhine, Shan, Kachin, Chin) of any age [ranged from 9 months to 66 years (117 were children under 12 years and 74 were adults more than 12 years)] and both sex (108 females and 83 males) were included.

Detection of Hb E or β^E-gene: The β^E-globin gene was detected by the method of PCR/Mnl I restriction enzyme digestion as described previously.[4]

Detection of α-thal mutation: DNA amplification of three α-thal mutants ($-\alpha^{3.7}$, $--^{SEA}$, $-\alpha^{4.2}$) was done according to the method described by Chong *et al.*[5] Oligonucleotides used for the detection of α-thal mutants were shown in Fig 1. PCR reaction was done with 35 cycles of denaturation 95°C for one min, annealing at 55°C for 30 sec, and extention at 72°C for 2 min, in the mixture of 3.2 μL of dNTPs solution, 2 μL of 10×PCR buffer solution, 4 μL of Q-sol, 3.5 μL of primer mixture (5 pmol/L solution), 1 μL of DNA



Primer Names	5' → 3' Sequence	Amplification (size: kb)
α2/3.7F	CCCCTCGCCAAGTCCACCC	$-\alpha^{3.7}$ frag,
3.7R	AAAGCACTCTAGGGTCCCAGCG	(2.02kb)
4.2F	GGTTTACCCATGTGGTGCCTC	$-\alpha^{4.2}$ frag,
4.2R	CCCGTTGGATCTTCTCATTTCCC	(1.6kb)
SEA-F	CGATCTGGGCTCTGTGTTCTC	$--^{SEA}$ frag.
SEA-R	AGCCCACGTTGTGTTCATGGC	(1.35kb)
α2R	AGACCAGGAAGGGCCGGTG	present α2 gene (1.8kb)

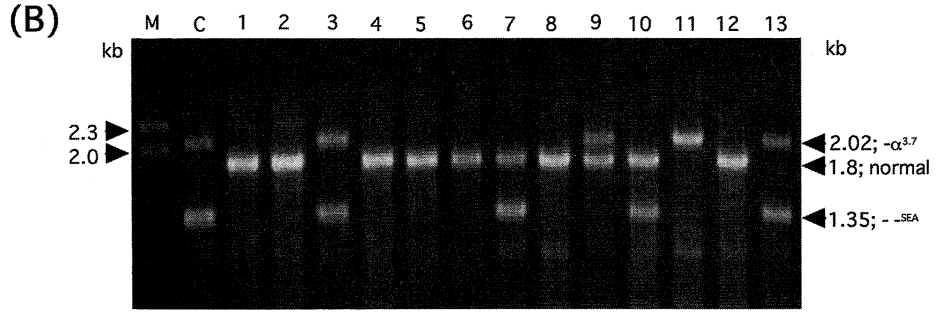


Fig 1. PCR for genotype analysis of common α-thalassemia deletion. A: The relative position and sequence of PCR primers on the α-globin gene cluster and their amplification size (kb). B: Results from DNA samples with various α-genotypes (but not detected the DNA sample with $-\alpha^{4.2}$ gene). M: Molecular marker (λ DNA/Hind III). C: Control with $-\alpha^{3.7}/--^{SEA}$ genotype. Lanes 1, 2, 4, 5, 6, 8 and 12: Samples with normal genotype ($\alpha\alpha/\alpha\alpha$). Lanes 3 and 13: Samples with $-\alpha^{3.7}/--^{SEA}$ genotype. Lanes 7 and 10: Samples with $--^{SEA}/\alpha\alpha$ genotype. Lane 11: samples with only $-\alpha^{3.7}$ gene.

solution, 0.12 μL of Q-Taq (1 unit of DNA polymerase; Qiagen-Japan Inc. Tokyo, Japan.) after an activation at 95°C for 15 min, and distilled water to make 20 μL final volume. PCR products were electrophoresed in 1% agarose gel and photographed under UV after ethidium bromide stains.

RESULTS

Hb E was detected in 115 samples (60%) [as heterozygous (Hb E/Hb A) in 109 samples and as homozygous (Hb E/Hb E) in 6 samples].

A total of 33 samples (17%) have α-thal mutations [16 cases were $-\alpha^{3.7}/\alpha\alpha$ genotype; two $-\alpha^{3.7}/-\alpha^{3.7}$ genotype; eight $--^{SEA}/\alpha\alpha$ genotype; seven Hb H disease ($-\alpha^{3.7}/--^{SEA}$ genotype)] (Fig 1). Among these 33 samples, Hb phenotype Hb A/Hb A was found in 29 samples (88%), Hb A/Hb E (heterozygote) in three samples (9%), and Hb E/Hb E (homozygote) in one sample (3%). All four samples of Hb E (both heterozygotes and homozygote) had $-\alpha^{3.7}/\alpha\alpha$ genotype only. Neither $-\alpha^{3.7}/-\alpha^{3.7}$ nor $--^{SEA}/\alpha\alpha$ nor $-\alpha^{3.7}/--^{SEA}$ was detected.

In the parent group, four of five samples were Hb A/Hb A phenotype and the remaining one had Hb E/Hb A phenotype. Among them, only one samples with Hb A/Hb A phenotype had $--^{SEA}/\alpha\alpha$ genotype.

DISCUSSION

The overall prevalence of α-thal among our study population of Myanmar was approximately 18% either in the form of $-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{3.7}/-\alpha^{3.7}$, $--^{SEA}/\alpha\alpha$ or $-\alpha^{3.7}/--^{SEA}$. The α-thal-2 with a genotype of $-\alpha^{3.7}/\alpha\alpha$ was the commonest at a prevalence of 8.9% followed in order by α-thal-1 of $--^{SEA}/\alpha\alpha$ genotype (4.2%), Hb H disease of $-\alpha^{3.7}/--^{SEA}$ genotype (3.6%) and α-thal-1 of $-\alpha^{3.7}/-\alpha^{3.7}$ genotype (1.0%). The overall frequency of α-thal in this study was higher compared to previous reports.[6,7] The difference in the characteristics of population studied here may be the important cause. With the exception of five parents, all were patients who were under interval transfusion for chronic refractory anemias in our study.

Hb E is the commonest abnormal Hb in Myanmar at a frequency of 1-26%[6] varying with ethnicity. In our study, Hb E was found in approximately 60% of the cases, and so, much higher than the previous report.[6] This might be due to the difference in the type of study and study population involved. Our study was hospital based where as previous studies was community based.

Although it had been reported from many aspects like epidemiology,[6] clinical conditions,[8,9] ethnical heterogeneity,[6,7] malaria endemicity[9] and so on, α-thal of Myanmar has never been described from the molecular aspect. This study was conducted as an attempt to get a complete molecular characterization in combine with β-thal mutation analysis in transfusion dependent thal major patients of Myanmar. The findings from this work provided high incidence of Hb E and α-thal, which in turn suggested a possible high frequency of Hb E-α-thal in Myanmar. The association of α-thal of any type with Hb E-β-thal or β-thal major was an influencing factor for the clinical heterogeneity of these syndromes.[10] Observation of clinical and molecular heterogeneity of transfusion dependent thal patients of Myanmar is the main area of interest of our collaboration work.

ACKNOWLEDGMENT

This work is part of a Japan-Myanmar collaborating project, a Grant-in-Aid (12672259) by Ministry of Education, Science, Culture and Sports, Japan, and also by a Research Project Grant (No. 12-105) from Kawasaki Medical School. We are indebted to Professor Thein Thein Myint of Yangon Children Hospital and Professor Rai Mra of Yangon General Hospital, providing clinical data and allowing to collect blood samples. Special thanks are due to technicians of Pathology Research Division, Department of Medical Research (Lower Myanmar), Yangon, Union of Myanmar, for proper sample collection and preparation.

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