Abstract β-thalassemia is the most-common genetic disorder of hemoglobin synthesis in Malaysia, and about 4.5% of the population are heterozygous carriers of the disorder. Prenatal diagnosis was performed for 96 couples using the Amplification Refractory Mutation System and Gap-Polymerase Chain Reaction. We identified 17 β-globin defects—initiation codon for translation (T-G), -29 (A-G), -28 (A-G), CAP +1 (A-C), CD 8/9 (+G), CD 15 (G-A), CD 17 (A-T), CD 19 (A-G), Hb E (G-A), IVS1-1 (G-T), IVS1-5 (G-C), CD 41/42 (-CTTT), CD 71–72 (+A), IVS2-654 (C-T), poly A (A-G), 100-kb Gγδβ° and 45-kb Filipino deletions. The 192 β-alleles studied comprised Chinese (151 patients), Malay (21), Orang Asli from East Malaysia (15), Filipino (1), Indian (1), Indonesian Chinese (2), and Thai (1). In the Chinese, 2 β-globin defects at CD 41/42 and IVS2-654 were responsible for 74% of β-thalassemia. β-mutations at CD 19, IVS1-1 (G-T), IVS1-5, poly A, and hemoglobin E caused 76% of the hemoglobin disorders in the Malays. The Filipino 45-kb deletion caused 73.3% of β-thalassemia in the Orang Asli. Using genomic sequencing, the rare Chinese β-mutation at CD 43 (G-T) was confirmed in 2 Chinese, and the Mediterranean mutation IVS1-1 (G-A) was observed in a Malay β-thalassemia carrier. The β-globin mutations confirmed in this prenatal diagnosis study were heterogenous and 65 (68%) couples showed a different globin defect from each other. The use of specific molecular protocols has allowed rapid and successful prenatal diagnosis of β-thalassemia in Malaysia.

Key words Prenatal diagnosis • β-thalassemia • β-mutations • DNA amplification • Ethnic groups

Introduction

The thalassemias in southeast and east Asia form a heterogeneous group of inherited disorders of hemoglobin synthesis. The β-globin defects along the β-globin gene complex result in the absence or reduction in the synthesis of β-globin chains. β-Thalassemia is a public health problem in Malaysia and the disorder is common in the Malaysian Chinese and Malays [1]. In addition, Hb E, which is a structural hemoglobin variant, has been commonly reported in the Malaysian Malays [1–3]. The implementation of successful and effective prenatal diagnosis programs for the hemoglobin disorders in any country depends on the diversity of the ethnic groups, as over 200 molecular defects have been confirmed in the β-globin gene complex [4]. The large heterogeneity of β-mutations in a population also...
often leads to β-thalassemia major children being compound heteryozygous for the disorder. In a study of 50 β-thalassemia carriers in Singapore, 64% of the affected couples carried a different β-mutation each [5].

It is fortunate that despite great allelic heterogeneity of the β-globin locus, β-thalassemia is in any one ethnic group or population is caused by a few common mutations together with a variable number of rare mutations [6]. This has allowed effective and rapid prenatal diagnosis programs for β-thalassemia to be established in each ethnic group or population.

Molecular characterization of the β-globin mutations has been previously reported in the Malay and Chinese ethnic groups in Malaysia [7, 8]. The investigators confirmed the presence of nine β-globin defects – IVS1-1 (G-T), IVS1-5 (G-C), Hemoglobin E (Hb E), -28, CD 15, CD 17, CD19, CD 41/42, and IVS2-654.

Molecular analysis and prenatal diagnosis for β-thalassemia and Hb E has been successfully carried out since 1997 in the University Malaya Medical Center (UMMC). The objectives of the molecular services in UMMC are to confirm the mutations in β-thalassemia carriers so as to offer rapid, sensitive, and cost-effective prenatal diagnosis to families at risk of producing a β-thalassemia major child. A total of 96 couples requested prenatal diagnosis for β-thalassemia between 1997 and 2002. The couples were not only from the predominant Chinese and Malay ethnic groups in Malaysia, but were also from the aboriginal group (Orang Asli) in East Malaysia, and from Filipino, Indian, Indonesian Chinese, and Thai populations. The β-globin mutations confirmed during prenatal diagnosis during the 5-year period will provide more-complete information on the heterogeneity of β-globin defects in the different ethnic groups/populations seeking prenatal diagnosis. These data will greatly assist in genetic counseling for affected families in the different races/populations and thus improve the quality of prenatal diagnostic programs in Malaysia.

**Materials and methods**

**Patient samples**

Ninety-six couples requested prenatal diagnosis for either β-thalassemia or Hb E between 1997 and 2002. Peripheral blood samples with EDTA were collected as anticoagulant. The β-thalassemia/Hb E status of each couple had previously been identified either by erythrocyte indices/hemoglobin electrophoresis or because they already had a β-thalassemia major child. However, the β-mutations in each patient had not been characterized. Whenever possible, the β-globin mutation responsible for the β-thalassemia in each carrier was characterized using DNA amplification techniques before chorionic villi (CV) sampling. This study was approved by the Medical Ethics Committee of the UMMC in accordance with the Declaration of Helsinki. Informed consent was obtained from the β-thalassemia carriers prior to blood collection.

**CV sampling**

CV were obtained by the transabdominal approach using ultrasound guidance at about 10–14 weeks' gestation. CV were separated from decidual tissue with the aid of a dissecting microscope and the use of very fine sterile forceps. CV were repeatedly washed in sterile 0.85% sodium chloride. All CV samples were processed immediately.

**DNA extraction and purification**

DNA from CV and blood samples were extracted in TRIS-EDTA (pH 8) using sodium dodecyl sulfate and proteinase K, and digested overnight at 37°C. DNA was purified using phenol-chloroform-isoamyl alcohol and precipitated in 4 M sodium acetate and ethanol.

**DNA analysis using the Amplification Refractory Mutation System**

In the UMMC, 15 common and rare β-mutations reported in the Malay, Chinese, and Indian ethnic groups can be confirmed using the Amplification Refractory Mutation System (ARMS). The β-mutations confirmed with ARMS are at the initiation codon for translation (T-G), -29 (A-G), -28 (A-G), CAP +1 (A-C), CD 8/9 (+G), CD 15 (G-A), CD 17 (A-T), CD 19 (A-G), Hb E (G-A), IVS1-1 (G-T), IVS1–5 (G-C), CD 41/42 (-CTTT), CD 71/72 (+A), IVS2–654 (C-T), and poly A (A-G). The primer sequences to detect the 15 β-mutations were paired with common ARMS primers to amplify each β-mutation as a specific molecular weight product [9–11].

**DNA amplification across specific deleted β-sequences (Gap-PCR)**

β-Thalassemia can also be due to large deletions along the β-gene complex. The β-thalassemias caused by a large 45-kb Filipino deletion [12, 13] and a Chinese 100-kb (βγδβ)°-specific deletion were confirmed by DNA amplification across the deleted regions of the β-globin gene complex (Gap-PCR) using specific primers that flank the deleted sequences [14, 15].

**Analysis of maternal contamination by DNA amplification**

The presence of maternal contamination in fetal DNA was detected by DNA amplification of a Variable Number of Tandem Repeats (VNTR) locus DIS80. DNA amplification of DIS80 was carried out on DNA extracted from the parents' blood and CV using primers 5'-GAAAATGCGCTCCAAGACATGCCCAGCG-3' and 5'-GTCTTTGTTGGAGATGCACGTGCCCCTTGC-3' [16].