

## Combine-ARMS: A Rapid and Cost-Effective Protocol for Molecular Characterization of $\beta$ -Thalassemia in Malaysia

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### ABSTRACT

$\beta$ -Thalassemia major patients have chronic anemia and are dependent on blood transfusions to sustain life. Molecular characterization and prenatal diagnosis of  $\beta$ -thalassemia is essential in Malaysia because about 4.5% of the population are heterozygous carriers for  $\beta$ -thalassemia. The high percentage of compound heterozygosity (47.62%) found in  $\beta$ -thalassemia major patients in the Thalassemia Registry, University of Malaya Medical Centre (UMMC), Malaysia, also supports a need for rapid, economical, and sensitive protocols for the detection of  $\beta$ -thalassemia mutations. Molecular characterization of  $\beta$ -thalassemia mutations in Malaysia is currently carried out using ARMS, which detects a single  $\beta$ -thalassemia mutation per PCR reaction. We developed and evaluated Combine amplification refractory mutation system (C-ARMS) techniques for efficient molecular detection of two to three  $\beta$ -thalassemia mutations in a single PCR reaction. Three C-ARMS protocols were evaluated and established for molecular characterization of common  $\beta$ -thalassemia mutations in the Malay and Chinese ethnic groups in Malaysia. Two C-ARMS protocols (cd 41-42/IVSII #654 and -29/cd 71-72) detected the  $\beta$ -thalassemia mutations in 74.98% of the Chinese patients studied. The C-ARMS for cd 41-42/IVSII #654 detected  $\beta$ -thalassemia mutations in 72% of the Chinese families. C-ARMS for cd 41-42/IVSI #5/cd 17 allowed detection of  $\beta$ -thalassemia mutations in 36.53% of  $\beta$ -thalassemia in the Malay patients. C-ARMS for cd 41-42/IVSI #5/cd 17 detected  $\beta$ -thalassemia in 45.54% of the Chinese patients. We conclude that C-ARMS with the ability to detect two to three mutations in a single reaction provides more rapid and cost-effective protocols for  $\beta$ -thalassemia prenatal diagnosis and molecular analysis programs in Malaysia.

### INTRODUCTION

$\beta$ -THALASSEMIA IS A HETEROGENOUS autosomal recessive disorder, which results from the absence or reduction in the synthesis of  $\beta$ -globin chains.  $\beta$ -Thalassemia major patients require regular blood transfusions throughout life with intensive chelating therapies using desferrioxamine (Giardini, 1977). Affected children have a chronic illness, and complications of their condition pose a heavy burden on blood transfusion and pediatric services. The only hope for a "cure" is a successful bone marrow transplantation. About 4.5% of the population in Malaysia are heterozygous carriers for  $\beta$ -thalassemia, and couples with  $\beta$ -thalassemia are at risk of producing a  $\beta$ -thalassemia major child (George *et al.*, 1992).

The total number of reported  $\beta$ -thalassemia mutations is over 160 and the presence of a compound heterozygous state in a

$\beta$ -thalassemia major patient is common (Lin *et al.*, 1991). However, it is fortunate that each population or ethnic group exhibits a few common mutations together with a variable number of rarer mutations, which account for 85–95% of  $\beta$ -thalassemia cases (Thein, 1993).

Molecular analysis and prenatal diagnosis for  $\beta$ -thalassemia have been carried out using various molecular techniques, from restriction fragment length polymorphism (RFLP), allele specific oligonucleotide (ASO) hybridization, reverse dot blots (RDB), to the amplification refractory mutation system (ARMS) (Old *et al.*, 1990; Lindeman *et al.*, 1991; Tan *et al.*, 1993; Thong *et al.*, 1996). Each of the molecular techniques has specific advantages and disadvantages depending on the type and number of  $\beta$ -thalassemia mutations present in a population. The implementation of effective and successful prenatal diagnosis programs in any country depends very much on

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