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Non-Radioactive Detection of Common β-Thalassemia Mutations in Chinese by Polymerase Clhain Reaction (PCR) Using Dried Blood Spot Specimens

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Codon 17 (A→T), 41/42 frameshift (-TCTT deletion), –28 TATA (A→G), and IVS-II 654 (C→T) have been reported to account for 85%-96% of mutant alleles of -thalassemia in southem Chinese. Codon 17 (A→T) mutation, which create a recognition sequence of Mae I, can be identified by analyzing restriction fragments. However, the other three mutations don't create or alter recognition sequence of endonucleases. To facilitate the detection of these mutations, we designed three mismatch primers to create a recognition sequence on the PCR products of gene carrying 41/42 frameshift, –28 TATA, or IVS-II 654 mutation. Because dried blood spots specimens collected on filter paper are easy to be transported and stored, a method to amplify DNA from it by PCR was developed.

Dried blood spot samples of forty carriers and eight normal cases detected by our β-thalassemia carrier screening program were collected. Among the 96 alleles, there were five codon 17, twelve 41/42 frameshift, sevem –28 TATA, thirteen IVS-II 654, three unknown mutations, and fifty-six normal alleles were found by analyzing the restriction fragments of the PCR products. These results are fullly coincident with that identified by allele specific oligonucleotides probes, which normally requires radioactive label. In this study, dried blood spot specimens, a technique widely used in neonatal screening for congenital metabolic diseases, and analyzing the restriction fragments of the PCR products were used to provide a fast and non-radioactive detection of common -thalassemia mutations in Chinese. This method could be easily adapted on the dried blood spot maternal screening program which has been on trial in the rural areas in Taiwan since 1988 and may be applied on amniotic or chorionic villi samples for prenatal diagnosis.

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