Thesis Title	Characterization of Some β-Globin Mutations in Thailand and Their Associations
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ABSTRACT

A combination of β^0 thalassemia and HbE was observed in a twenty-four-year-old Thai female (Proposita S.S.), who was first diagnosed as homozygous HbE (HbEE) by routine automated HPLC. The hematological profile showed that the proposita S.S. had hypochromic microcytic anemia with some target cells and slightly elevated HbF. However, the result of dot blot hybridization suggested that she was actually not homozygous for HbE. Thus, detection for β^E mutation by HRP- oligonucleotide probes revealed that the proposita's amplified DNA could hybridize with both normal probe (β–normal) and mutant probe (β^E-mutant). Hb typing by cellulose acetate electrophoresis under alkali conditions showed one intense band with the same mobility as HbE. A single band was also found with the IEF. Globin typing by cellulose acetate electrophoresis under denaturing condition also showed two major bands with the same mobility as α^A and β^E chains. DEAE and CM-cellulose

column chromatography could not separate any other abnormal hemoglobin from HbE. Trypic peptide mapping by HPLC and protein sequencing indicated that the β^{E}-globin chain showed replacement of glutamic by lysine at position 26. Direct DNA sequencing showed the mutation of codon 17, where the normal AAG (Lysine) has been mutated to TAG, a chain termination codon, giving rise to the well-known β^{17}-thalassemia and at the codon 26 showed both GAG (Glu) and AAG (Lys), indicating the β^{E} mutation. It was concluded that the proposita S.S. actually was compound heterozygote for β^{17} thalassemia and HbE.

Hb J-Bangkok was found in a twenty-one-year-old Thai female (proposita S.W.). Hematological data showed slight anemia with elevated HbF. Hb typing by automated HPLC showed an abnormal Hb peak eluted before HbA. Cellulose acetate electrophoresis under native conditions showed 2 bands of Hbs; Hb A and an abnormal Hb moving towards the anode faster than HbA. Under denaturing conditions, proposita S.W.'s hemolysate contained 3 bands: β^{SW}, β^{A} and α^{A} chains. Abnormal Hb was separated by DEAE-cellulose column chromatography. The abnormal β^{SW} chain, prepared by CM-cellulose column chromatography, was subjected to tryptic peptide mapping by HPLC. Amino acid analysis and protein sequencing of peptide βTp5 showed the replacement of glycine by aspartic acid at residue 56 of β-globin chain, which indicated that the abnormal Hb was Hb J-Bangkok. DNA sequencing showed GGC as well as GAC at codon 56 of the β-globin gene. Therefore, it was concluded that the proposita S.W. was heterozygous for Hb J-Bangkok which was first discovered in Thailand in 1966.