RESEARCH FINAL REPORT

on

Study of amyloidosis related transtyretin variants in Thai people

by

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Granted by

Thai Government Budget 2008-2009

ABSTRACT

Transthyretin (TTR) is one of the three major thyroid hormone (TH) binding proteins found in plasma and cerebrospinal fluid (CSF) of human. It has been demonstrated as the most abundant protein component of amyloid fibrils which preferentially deposit in peripheral nerve leading to familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC) and senile systemic amyloidosis. More than 80 mutations of TTR have been identified associated to the diseases in which frequency and clinical manifestations vary among populations. In Thailand, no genetic variation of the TTR gene has been studied so far. To accomplish for relevant diagnosis and specific treatment, type and characteristics of TTR mutation distribute in population is essential. To reveal, whole blood (48 samples), lymphoblast cells (44 samples), purified genomic DNAs (158 samples) and human embedded paraffin postmortem brain tissues (20 samples) from Thai people were screened by using single-stranded conformation polymorphism (SSCP) technique for the mutations of TTR gene. Genomic DNAs were purified and amplified by PCR for TTR exon 1, 2, 3 and 4. Three types of single point mutation could be detected, and these can lead to change in amino acid sequence of TTR subunit and, thus, TTR variants as a consequence. Alignment of the amino acid sequences revealed that the novel variants detected in these Thai people are L110P (leucine at position 110 was changed to proline), Y116N (tyrosine at position 116 was changed to asparagine) and V122D (valine at position 122 was changed to aspartic acid). To elucidate their properties and functions, these recombinant TTR variants were successfully synthesized by using the heterologoug gene expression system of *Pichia pastoris*. The protein showed similar mobilities in native-gel at pH 8.6 to TTR in human plasma. There subunit masses were ~17 kDa, and showed cross-reactivity with the antibody raised against TTR purified from human plasma. Fibril formation of the recombinant TTR at pH 4.2 was monitored by ThT binding assay and it revealed that the variant could form amyloid with lower rate comparing to the native TTR.