RESEARCH FINAL REPORT

on

Study of the novel cardiac amyloidosis related transthyretin variant that is identified in Thai people

by

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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. Up to date over 90 single point mutations of TTR have been identified and more than 80 are associated to amyloidosis of which frequency and clinical manifestations vary among populations. To accomplish for relevant diagnosis and specific treatment, type and characteristics of TTR mutation distribute in population is essential. By single-stranded conformation polymorphism (SSCP), a novel single point mutation in the TTR gene which led to change valine at position 122 of the mature TTR to aspartic acid (TTR V122D) was detected in an embedded paraffin postmortem brain tissue of Thai patient. Since mutation of valine 122 have been earlier reported to associate with familial amyloidotic cardiomyopathy (FAC), the question arose whether this TTR V122D also associate to FAC. To reveal, synthesis of recombinant TTR V122D was attempted in *Pichia pastoris*, its general properties and ability to form fibril were determined. The recombinant TTR V122D was successfully synthesized in *Pichia* and extracellularly secreted into the culture medium. This protein could be purified from other *Pichia* proteins by preparative native-PAGE. The recombinant TTR showed similar mobility in native-gel at pH 8.6 to TTR in human serum. Molecular weight of its subunit determined by SDS-PAGE was 16422.95 daltons. By Western blot analysis, the recombinant protein specifically interacted with the antibody raised against human TTR purified from plasma. In addition, Thioflavin T (ThT) binding result showed that TTR V122D had higher potential to form amyloid than human TTR wild type.