3936703 SIMI/M : MICROBIOLOGY; M.Sc.(MICROBIOLOGY)
KEY WORDS : ENDEMIC DISTAL RENAL TUBULAR ACIDOSIS (EdRTA)/ *ANION EXCHANGER 1(AE1)* GENE/ *BAND 3* GENE/ SINGLE STRAND CONFORMATION POLYMORPHISM (SSCP)

THITIMA TUNGSIRIKUL: ANALYSIS OF *ANION EXCHANGER 1* GENE IN THAI PATIENTS WITH ENDEMIC DISTAL RENAL TUBULAR ACIDOSIS. THESIS ADVISORS: PA-THAI YENCHITSOMANUS, Ph.D., PRIDA MALASIT, M.D., F.R.C.P. (UK) 179 p. ISBN

Endemic distal renal tubular acidosis (EdRTA) is a unique form of distal renal tubular acidosis (dRTA), which is a common health problem in the northeastern region of Thailand. It is characterized by hyperchloremic metabolic acidosis due to the inability of the kidneys to excrete acid to urine, frequently accompanied by generalized muscle weakness, hypokalemia, nephrocalcinosis, and metabolic bone disease. The etiology of EdRTA could be related to either environmental or genetic factors or both. Evidence from past observations shows that many affected individuals were members of the same kindreds, suggesting that the genetic factor alone or in combination with the environmental factor(s) may involve in the pathogenesis of this disease. Although the acid excretion is controlled by several transporter proteins in the type A (acid-secreting) intercalated cells (IC) in the distal and collecting ducts of the kidneys, the defects of Cl^-/HCO_3^- exchanger (kAE1 or band 3), regulating Cl^-/HCO_3^- exchange across the basolateral membrane of type A IC, have recently been found to be associated with both autosomal dominant and autosomal recessive forms of dRTA. *AE1* mutation was therefore hypothesized to be the cause of EdRTA in the northeastern population of Thailand and the study in this thesis was carried out to prove this hypothesis.

Mutations of the *AE1* gene were screened in DNA samples from 10 EdRTA patients from 4 families by radioisotopic polymerase chain reaction and single strand conformation polymorphism (RI PCR-SSCP) technique, and from 7 affected and one unaffected members from a selected EdRTA family by non-RI PCR-SSCP. The analyses were also performed in 11 normal control DNA samples for comparison. From the results of screening of mutations in 18 regions of the *AE1* gene by both RI PCR-SSCP and non-RI PCR-SSCP analyses, mobility shifts were observed in 8 normal control subjects and 14 EdRTA patients in 5 regions including exons 4, 5, 9, 12, and 17. Sequencing analyses were performed in all 5 exons with the mobility shifts in 8 individuals who were selected as representatives of each group.

The results of sequencing analysis showed two nucleotide changes in the non-coding regions (IVS5+27C>T and IVS17+19G>A), two silent mutations (F266F and S438S), and four missense mutations (K56E, D38A, E72D, and G701D). The K56E (band 3 Memphis I) and D38A (band 3 Darmstadt) missense mutations, which were found in normal control subjects and patients, had previously been reported to be *AE1* polymorphisms. E72D is a new *AE1* missense mutation and proposed to be called 'band 3 Siriraj I'. This new mutation is most likely to be a non-disease mutation for the reason that it was present in normal control subjects as well as in only one of the patients resulting in a conservative amino-acid substitution. The G701D (band 3 Bangkok I) missense mutation was found in five patients but not in the normal control subjects. It is a reported disease mutation, which causes autosomal recessive dRTA in children, but its heterozygosity does not result in dRTA. DNA linkage analysis using microsatellite (D17S787) marker mapped close to the *AE1* gene was also carried out in a selected family with several members who were affected by EdRTA. The results of mutation analysis and DNA linkage study did not support the hypothesis that mutation of the *AE1* gene is involved the pathogenesis of EdRTA in the group of the northeastern Thai patients studied.