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WATIP BOONYASRISAWAT: ANALYSIS OF *REGIA* GENE IN THAI PATIENTS WITH FIBROCALCULOUS PANCREATIC DIABETES. THESIS ADVISORS: NAPATAWN BANCHUIN, M.D., Ph.D., PA-THAI YENCHITSOMANUS, Ph.D., SATHIT VANNASAENG, M.D., Dip Thai Board of Int. Med., M.Sc., 170 P. ISBN 974-662-705-8.

Fibrocalculous pancreatic diabetes (FCPD) or fibrocalculous pancreatopathy is a unique form of diabetes secondary to chronic pancreatitis found in developing countries of the world. The condition is associated with either overt protein-calorie malnutrition or. more usually. with deficiency of certain micronutrients. FCPD affects young individuals and runs an aggressive course to reach the endpoints of diabetes, pancreatic calculi and exocrine pancreatic dysfunction (steatorrhoea) in the majority of cases. Although the aetiology of FCPD is still unclear, the role of lithostathine or regenerating (Reg) protein could possibly be one of the mechanisms responsible for FCPD. Human lithostathine or Reg protein is a pancreatic secretory glycoprotein encoded by the *Reg* gene, which is expressed both by regenerating beta cell, in which it may promote growth and regeneration, and by pancreatic exocrine tissue, as a secretory protein that may inhibit precipitation of calcium carbonate. It has been suggested that the deficiency of lithostathine in pancreatic secretion may play role in lithiasis in FCPD.

In order to study the role of *Reg1A* gene in the pathogenesis of FCPD, the entire coding region of *Reg1A* gene and its mRNA were analysed for mutation in 10 patients with FCPD, 10 normal control subjects, 4 and 3 patients with type 1 and type 2 diabetes mellitus, respectively. To search for mutation, genomic DNA amplification was performed to obtain the coding sequence of *Reg1A* gene including promoter, exon-intron boundaries, and polyadenylation signal. RT-PCR of illegitimate *Reg1A* transcripts obtained from the peripheral blood lymphocytes was carried out to produce *Reg1A* cDNA. The PCR products were digested with appropriate restriction enzymes to generate smaller sizes of DNA fragments, suitable for detection by single strand conformation polymorphism (SSCP) analysis. DNA sequencing was subsequently used to identify the nucleotide sequence of the DNA fragment revealed as the band shift in gel electrophoresis in the detection of SSCP. The result of SSCP detection showed the mobility shift of exon 1 fragments, at both room temperature and 4°C condition of electrophoresis. DNA sequencing of the shifted fragments showed the nucleotide change from T→C at position –10 of nucleotide sequence. This nucleotide change of *Reg1A* gene caused the mobility shift of DNA banding patterns in SSCP analysis which was found in both diabetic patients and normal controls. The significance of this polymorphism is still unknown. However, no mutation of the *Reg1A* gene was found in any groups of the diabetic patients (FCPD, IDDM, and NIDDM), indicating that the pathogenesis of diabetes mellitus may not be associated with *Reg1A* gene mutation.