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VALLAYA SUTTHIKHUM: POLYMORPHISM ANALYSIS IN INTRON 4 AND SCREENING FOR MUTATION IN EXON 4 OF LOW-DENSITY LIPOPROTEIN RECEPTOR GENE IN THAI HYPERCHOLESTEROLEMIA SUBJECTS. THESIS ADVISOR: KLAI-UPSORN PONGRAPEEPORN Ph.D., ATIP LIKIDLILID, M.Sc. 96 p. ISBN 974-663-644-8.

The prevalence of primary hypercholesterolemia is high in the population of Thailand. Mutation in the coding sequences, splice junctions or promoter of the gene for the LDL receptor are known to be the underlying cause of primary hypercholesterolemia. However, minor sequence changes elsewhere in intron can be deleterious as well. In this study, the genotype of *Taq*I restriction site polymorphism in intron 4 of 145 unrelated Thai subjects were investigated by PCR-RFLP technique. The allele frequency of the presence of *Taq*I polymorphic site in intron 4 of LDL receptor gene was 38% which was similar to the Japanese population. The genotype frequencies were 38.6%, 46.2%, and 15.2% for the (-/-), (+/-), and (+/+) genotype, respectively. In this study, the genotype distributions conformed to Hardy-Weinberg Proportions. No statistically significant association between genotypes and lipid phenotypes was observed in these subjects ($p < 0.05$). In addition, screening for mutation(s) in hotspot exon 4 of LDL receptor gene was performed by PCR-RFLP, SSCP and CLEAVASE Fragment Length Polymorphism (CFLP). PCR-RFLP was applied for screening for substitution of Asp200 to Gly that creates the *Msp*I restriction site. All of 45 primary hypercholesterolemia patients in this study contained the normal restriction pattern of *Msp*I. SSCP was applied for screening mutation(s) in the entire fragment of exon 4 and CFLP was applied for screening mutation(s) in amplicons that might be resistant to analysis by SSCP. Six DNA samples from 45 primary hypercholesterolemia patients showed a higher intensity band differing from the reference wild-type. One of these putative mutant alleles was identified by silver-stained direct DNA sequencing and G to T transversion at nucleotide sequence number 514 in the codon of amino acid residue 151 of LDL receptor protein was found. This amino acid residue belongs to the fourth repeat of the ligand binding domain. This nucleotide change resulted in a substitution of uncharge R group tyrosine (TAC) for charge R group aspartic acid (GAC), note D151Y. This novel missense mutation does not create any restriction site. The proposed structural and functional consequences of the mutation have been analyzed by amino acid alignment indicated that the mutation is non-conservative substitution in conserved region and probably affected the protein structure and function. This preliminary study presents a possible molecular mechanism, concerning novel missense D151Y mutation in LDL receptor gene, underlying the primary hypercholesterolemia condition in Thai subjects.