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SUMALEE ORANWIROON : MUTATIONS OF FACTOR VIII GENE IN THAI HEMOPHILIA A PATIENTS. THESIS ADVISORS : SAKOL PANYIM, Ph.D., VARAPORN AKKARAPATUMWONG, Ph.D., PA-THAI YENCHITSOMANUS, Ph.D. 158 p. ISBN 974-662-065-7

Hemophilia A is a common X-linked bleeding disorder caused by mutations in the coagulation factor VIII (FVIII) gene. Mutation analysis in hemophilia A has been hampered by the large size of the factor VIII gene (which spans 186 kb in Xq28 at ~1 Mb from telomere), by the heterogeneity of molecular defects and by the gene's tissue-specific expression. In order to facilitate the search for mutation, a combination of long reverse transcription-polymerase chain reaction (long RT-PCR) of illegitimate factor VIII transcripts from the peripheral lymphocytes to derive a factor VIII cDNA (9 kb), and genomic DNA amplification was performed to obtain the entire essential sequence of the factor VIII gene fractionated into eight fragments. Four fragments containing the coding sequences of factor VIII gene, except exon 14, were amplified from the factor VIII cDNA. The exon 14 sequence was amplified into two overlapping fragments using genomic DNA as the template. Two fragments corresponding to the putative promotor and the polyadenylation/cleavage signal region were also obtained from genomic DNA amplification. The seven fragments, except the fragment containing the polyadenylation/cleavage signal, were overlapped each other. Single strand conformation polymorphism (SSCP) technique was chosen as a method to screen the mutated sequence in these fragments. The fragments were digested with appropriate restriction enzymes to generate the size suitable for detection by SSCP. DNA sequencing was subsequently used to identify the mutated sequence. Family studies and carrier detection were also included in this study.

SSCP analysis was performed in ten hemophilia A patients from 10 families. The mobility shifts were observed in fragments from six patients. DNA sequencing of the shifted fragments showed that the mutations were: two nonsense mutations (R-5X and R1966X), three missense mutations (D542Y, G1850V, and G2325C), and a 4 bp insertion (ACTA) at codon 2245. All of these mutations were identified in severe cases, except for the R-5X and G2325C mutations which were found in moderate cases. Family studies indicated that the mutation R-5X was not inherited from the mother, therefore this mutation may be a result of *de novo* mutation. For the 5 remaining families, the mutations were also found in patients' mothers and other family members, indicating that the mutations were inherited. This study has shown that the mutations of factor VIII gene in Thai hemophilia A patients seem to be heterogeneous as found in other ethnic groups. Nevertheless, carrier detections in the families in which the mutations have been identified are feasible.