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WANNA THONGNOPPAKHUN: IDENTIFICATION OF RNA-PROCESSING ABNORMALITIES OF THE *PKD1* GENE IN THAI FAMILIES WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE. THESIS ADVISORS : PRAPON WILAIRAT, Ph.D., PA-THAI YENCHITSOMANUS, Ph.D., CHINTANA SIRINAVIN, M.D., M.R.C.P., MATHUROSE PONGLIKITMONGKOL, Ph.D. 228 P.
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Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common life-threatening human genetic disorders, characterized by the progressive development of multiple abnormal fluid-filled cysts in the kidneys. The major cause of ADPKD, accounting for approximately 85% of all cases, is due to mutations of polycystic kidney disease 1 (*PKD1*) gene. *PKD1* is mapped to 16p13.3 with the size of 54 kb, containing 46 exons and transcribing a 14-kb mRNA. It encodes, polycystin-1, an integral membrane protein with possible functions in cell-cell or cell-matrix interaction and in signal transduction involved in normal tubulogenesis. Isolation and analysis of *PKD1* have encountered a great difficulty since approximately three-fourth at its 5' end is reiterated and at least three highly homologous genes are present at another region on the same chromosome. The attempts to isolate the entire *PKD1* coding sequence have never previously been successful, delaying the progression in analysis of *PKD1* mutations.

The study in this thesis was initiated by addressing this problem. The fact that information about *PKD1* mutations in Thai ADPKD patients has not been available inhibits the understanding of its molecular pathogenesis. A method of long reverse transcriptase and polymerase chain reaction (RT-PCR) to selectively amplify the entire *PKD1* coding sequence from its mRNA transcripts, prepared from peripheral blood lymphocyte, has successfully been developed in this study. The principle of this method is based on the use of a pair of PCR primers, one primer specific to the sequence in the 3' unique region of *PKD1* and the other to the sequence in the homologous region at a distance of about 13.6 kb apart. The amplified full-length *PKD1* cDNA was fractionated into nine overlapping fragments by nested PCRs, appropriate for further analysis.

This procedure was applied to analyze RNA samples prepared from 10 unrelated Thai ADPKD patients. The important advantage of this procedure for the mutation analysis was realized by the discoveries of two cases with defective RNA processing, in which the resulting defective RNA processing could be demonstrated *ex vivo*. The RNA splicing defects and the causative *PKD1* mutations in these two families were characterized. In addition, an unusual alternative *PKD1*-RNA splicing observed in many patients was also studied. The results showed that in the first family (PK015) the observed 74 nucleotide (nt) deletion of exon 14 in the *PKD1*-mRNA transcripts was most likely to occur from a nucleotide substitution (A->T) at a splice acceptor site in IVS13, IVS13-2A>T. This splice acceptor site was abolished and a cryptic splice acceptor site present in exon 14 was used, leading to the deletion of *PKD1* mRNA. This deletion would cause frameshift and truncation of polycystin-1, comprising only 1,074 amino acids. Allele-specific amplification (ASA) was developed for direct detection of this mutation in the PK015 family, which was found to segregate with the disease. In the second family (PK009), the 291-nt deletion in *PKD1*-mRNA transcripts was found to result from exon 43 skipping caused by deletion of 20-bp in intron 43, IVS43(+5 to +14)del20. This skipping might occur from inadequate length of the deleted intron 43 for spliceosome formation, since the deletion did not disrupt the donor or acceptor splice site or branch site in this intron. As the result of exon 43 skipping, the translated polycystin-1 would have in-frame deletion of 97 amino acids in the transmembrane (TM) and loop regions, confirming the importance of this domain. The intronic deletion in this family could directly be detected by genomic DNA amplification, which was found to inherit with the disease. These two mutations are likely to cause loss of polycystin-1 function, which would support the two-hit model of PKD1 pathogenesis. The intensive studies of *PKD1* mutations will provide a better understanding of polycystin-1 function and molecular pathogenesis of PKD1.