MUTATION ANALYSIS OF *PKD1* GENE IN FAMILIAL POLYCYSTIC KIDNEY DISEASE

ANALISIS MUTASI GEN PKD1 PADA PENYAKIT GINJAL POLIKISTIK FAMILIAL

THESIS
Submitted to fulfill the assignment and fit-out requisite in passing Post-graduate Program

Magister of Biomedical Science

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DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgement is made in the text.

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CONTENTS

ABBREVIATIONS

ADPKD	Autosomal Dominant Polycystic Kidney Disease
AQP	Aquaporin
ARPKD	Autosomal Recessive Polycystic Kidney Disease
CACC	Ca^{2+} activated Cl^- channel
cAMP	Cyclic Adenosin Monophosphate
CFTR	Cystic Fibrosis Trans-membrane Regulator
EGF	Epidermal Growth Factor
ER	Endoplasmic Reticulum
ESRD	End Stage Renal Disease
GCKD	Glomerulocystic Kidney Disease
NKCC1	$Na^+/K^+/2Cl^-$ cotransporter1
HNFB1	Hepatocyte nuclear factor 1, homeobox B
HRM	High Resolution Melting
IPR3	Inositol Triphosphate Receptor
PC1	Polycystin1
PC2	Polycystin2
PCP	Planar Cell Polarity
PCR	Polymerase Chain Reaction
qPCR	Quantitavive or Qualitative Polymerase Chain Reaction
PDE	Phosphodiesterase
PI	Proliferation Index

PKA	Protein Kinase-A
PKD	Polycystic Kidney Disease
PKD1	Polycystic Kidney Disease1
PKD2	Polycystic Kidney Disease2
RyR	Ryanodine Receptor
SERCA	Sarco (Endo)Plasmic Reticulum Ca^{2+} ATP ase
SIFT	Sorting Intolerant From Torelant
SNP	Single Nucleotide Polymorphism
SST2	Somatostatin Receptor2
Tm	Temperature Melting
TNF-α	Tumor Necrosis Factor Alpha
TRP	Transient Receptor Potential Channel
TRPP	Transient Receptor Potential Polycystin Channel
TSC2	Tuberous Sclerosis2
UROM	Uromodulin
V2R	Vasopressin2 Receptor

GLOSSARIES

ADPKD	genetically heterogeneous, with mutations of 2 genes (PKD1 and PKD2) responsible for most cases.
Align-GVGD	a freely access website program to predict missense substitutions whether pathogenic or not. This program analyzes the biophysical characteristics of amino acids and protein multiple sequence alignments to predict missense substitutions in genes of interest fall in a spectrum from enriched delterious to enriched neutral.
Cyst	an abnormal structure of tissue which is formed closed sac-like, containing a liquid, gaseous or semisolid substance. Outer membrane of the cyst, the cyst wall, differentiates this structure to the nearby tissue.
CKD	abnormalities of kidney structure or function, present for 43 months, with implications for health.
ESRD	a continuum of CKD, is defined as irreversible kidney failure treated with dialysis or transplantation.
HRM	a post-PCR method to analyze genetic variations (SNPs, mutations, methylations) in PCR amplicons. HRM is good screening method to analyze variations in PCR amplicons before establishment of definit diagnosis using sequencing.
PCR	a biochemical technology in molecular biology based on using the ability of DNA polymerase to amplify a single or few copies of a spesific region of DNA. The result of PCR reaction is thousand to million copies of a particular DNA sequence.
qPCR	a method part of method in Real Time-PCR which is used to amplify and simultaneously detect or quantify a targeted DNA molecule. In qualitative qPCR, the goal is to detect the presence or absence of a certain sequence, allelic discrimination between

	wild type and mutant, between different SNPs or between different splicing forms.
SIFT	predicts whether an amino acid substitution affects protein function based on sequence homology and the physical properties of amino acids.
Mutation taster	a free web-based application for rapid evaluation of the disease-causing potential of DNA sequence alterations. MutationTaster integrates information from different biomedical databases and uses established analysis tools (Supplementary Methods). Analyses comprise evolutionary conservation, splice-site changes, loss of protein features and changes that might affect the amount of mRNA. Test results are then evaluated by a naive Bayes classifier, which predicts the disease potential.

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ABSTRACT

Background: Polycystic kidney disease (PKD) is a genetic disorder. The replacement of normal structure with numerous cysts is potential leading to renal failure. PKD contributed to 2.51% end stage renal disease (ESRD) in Indonesia, but its genetic base has been unknown. The most common form of PKD is autosomal dominant polycystic kidney disease (ADPKD). Mutation in *PKD1* gene is found in the most of ADPKD cases (85%). Pre-screening molecular diagnostic step is useful to minimize the complexity of mutation analysis in *PKD1* gene.

Methods: DNA samples from 27 patients were collected from 13 index families. Fourty six exons of *PKD1* gene were amplified in 59 fragments. qPCR-High Resolution Melting (HRM) analysis was utilized as pre-screening diagnosis steps. Afterwards, variant samples which had aberrant pattern were sequenced.

Results: The clinical evidence of highly suggested familial *PKD1* was found in6 (46.1%) families. Clinical diagnosis of PKD had associated to hypertension (*p*-value: 0.045). The amplification was successfully done in 36(61%) amplicons, the remaining 23(39%) amplicons still need to be optimized. Seven variants were identified, in which four variants previously described in literature (c.1001C>T, c.1140C>T, c.1142G>T, c.12276A>G) and three newly described by this study. The new variants were missense mutation (c.1021G>A and c.1240C>T) and synonymous mutation (c.1161C>T). All new variants were predicted as non pathogenic. Two PKD samples from one family was found to carry variant of c.1001 C>T which predicted as likely pathogenic.

Conclusion: Clinical diagnosis of PKD had associated to hypertension (*p*-value: 0.045).There were 7 variants of *PKD1* gene identified in this study. The identification of pathogenic mutation for *PKD1* gene has not been completed yet. Further optimization at qPCR-HRM method and mutation confirmation study in healthy control are needed.

Keywords: *PKD1* gene, qPCR-HRM analysis, Indonesia

ABSTRAK

Latar Belakang: Penyakit ginjal polikistik (PGP) merupakan suatu kelainan genetik. Perubahan struktur ginjal digantikan dengan struktur kista multipel dapat menyebabkan gagal ginjal. PGP menyumbang 2,51% gagal ginjal terminal (GGT) di Indonesia, namun latar belakang genetik yang mendasari belum diketahui. Jenis PGP yang paling sering ditemukan adalah penyakit ginjal polikistik dominan autosomal (PGPDA). Mutasi pada gen *PKD1* ditemukan pada sebagian besar kasus ADPKD (85%). Penerapan metode diagnosis pre-skrining diharapkan dapat membantu mengatasi kompleksitas analisis mutasi pada gen *PKD1*.

Metode: Sampel DNA dari 27 pasien dikumpulkan dari 13 keluarga indeks. Gen *PKD1* memiliki 46 ekson yang diamplifikasi dalam 59 fragmen. qPCR-HRM digunakan sebagai metode pre-skrining pada diagnosis molekuler. Sekuensing dilakukan pada sampel yang memiliki pola grafik yang berbeda.

Hasil: Kecenderungan adanya pewarisan gen *PKD1* familial secara klinis didapatkan pada 6(46,1%) keluarga. Diagnosis klinis PKD memiliki hubungan dengan hipertensi (*p-value*: 0.045). Amplifikasi berhasil dilakukan pada 36(61%) amplikon, sebanyak 23(39%) sisanya masih memerlukan tahap optimasi lanjut. Terdapat 7 varian yang ditemukan dalam penelitian ini. Empat varian telah dilaporkan pada studi sebelumnya (c.1001 C>T, c.1140 C>T, c.1142 G>T, c.12276A>G) dan 3 varian dilaporkan pertama kali oleh penelitian ini. Klasifikasi mutasi pada varian baru meliputi mutasi *missense* (c.1021G>A and c.1240C>T) dan mutasi sinonim (c.1161C>T). Semua varian diprediksi sebagai non patogenik, kecuali varian c.1001C>T yang diprediksi patogenik pada 2 sampel dari satu keluarga.

Kesimpulan: Diagnosis klinis PKD memiliki hubungan dengan hipertensi (*p-value*: 0.045). Terdapat 7 varian yang ditemukan pada identifikasi mutasi gen PKD1. Identifikasi mutasi pada gen *PKD1* belum selesai sepenuhnya. Optimasi lanjut pada metode qPCR-HRM dan studi konfirmasi mutasi pada kontrol sehat masih diperlukan.

Kata kunci: gen *PKD1*, analisis qPCR-HRM, Indonesia