ANALISIS GEN *DPYD* PADA PASIEN TUMOR SOLID DENGAN TERAPI 5-FU

Studi *Cross-Sectional* Gen *DPYD* pada Populasi Malaysia

ANALYSIS OF DPYD GENE IN PATIENTS WITH SOLID TUMOR WHO WERE TREATED BY 5-FU

A Cross-sectional Study of DPYD Gene in Malaysian Population

Thesis

A thesis submitted for the degree of Master of Biomedical Science majoring on Genetic Counseling

I Gusti Kamasan Nyoman Arijana

G4A0080041

BIOMEDICAL SCIENCE POST GRADUATE
MAJORING GENETIC COUNSELING
DIPONEGORO UNIVERSITY
SEMARANG
2011

APPROVAL SHEET

THESIS

ANALYSIS OF DPYD GENE IN PATIENTS WITH SOLID TUMOR WHO WERE TREATED BY 5-FU

By

I Gusti Kamasan Nyoman Arijana
G4A008041

Have been defended in front of The Defend Committee on 2nd December 2011

Verified by,

Supervisor Committee

Malaysian Supervisors

Main Supervisor

Prof. DR. A Rahman A Jamal

Indonesian Supervisors

Main Supervisor

C Suharti, MD PhD SpPD-KHOM FINASIM
NIP. 194711251974012001

2nd Supervisor

Prof. Sultana MH Faradz, MD PhD
NIP. 19540202 197901 2001

Approved by,
Chairman of Biomedical Science Magister Study Program
Magister Program of Diponegoro University

DR. Winarto, MD SpMK Sp.M(K)
NIP. 19490617 197802 1001

DECLARATION

Hereby I declared that this thesis was in my own work and the content was not result from others work which had been proposed in other university or institution. The knowledge that resulted from publication which not yet or unpublished, was referred in manuscript and references.

Semarang, December 2011

I Gusti Kamasan Nyoman Arijana

ACKNOWLEDGMENT

Praise to the God because on His behalf, the thesis entitles “Analysis of DPYD Gene in Patients with Solid Tumor Who were Treated by 5-FU” can be finished. The thesis is proposed in order to fulfill the requirement for Magister of Biomedical Science Concentration in Genetic Counseling in Medical Faculty of Diponegoro University. In this moment I would like to express my gratefulness for:

Prof. Dr. Sultana M.H. Faradz, PhD as the Chairman of Genetic Counseling Study Program, Chairman of CEBIOR, and second supervisor for giving opportunity to increase our knowledge, and to give supervision in my thesis. Prof. Dato DR. A. Rahman A. Jamal as Director of UMBI and Malaysian main supervisor, for giving opportunity in advanced research and supervision during my research. Dr. C. Suharti, PhD, SpPD-KHOM, FINASIM as the first Indonesian supervisor for giving supervision in this thesis. DR. Dr. Winarto, Sp.MK, Sp.M (K) as the Chairman of Biomedical Science Magister Program and the staff for giving opportunity to follow the course. My deepest gratitude also expressed for Dr. Tri Indah Winarni, MSi.Med, PA as Secretary of Genetic Counseling Study Program and my examiner for giving us many help in the course.

I also express my gratitude to The Rector of Diponegoro University for giving opportunity to increase my knowledge and Dr. Abe Susanto, MSc from Bureau of Planning and International Cooperation, Ministry of National

Education The Republic Indonesia, and internal funding of UKM Medical Center's Genomic Unit, University Kebangsaan Malaysia for providing funding for my research.

Prof. DR. dr. I Ketut Swastika, SpPD-KEMD as the Dean of Faculty Medicine of Udayana University and Dr I G N Mayun, PHK as Head of Histology Department for giving recommendation, permission, and support to follow the course. Dr. Wayan Suwitra, PHK for giving recommendation and support to follow the course and all fellows in Histology Department for their supports.

Our beloved mother Ni Ketut Sriati and sister-brother Ni Gusti Kamasan Ayu Intan Utami, I Gusti Kamasan Made Wanarta for giving permission and support to follow the course.

All other person who cannot be mentioned one by one, for giving support in the course, research and thesis. In the end, hopefully the God always shine the sunshine to all of us.

Semarang, December 2011

Author

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

5-FU	:	5-Fluorouracil
A (base)	:	Adenine
C (base)	:	Cytosine
CDS	:	Coding sequence
DNA	:	Deoxynucleic acid
DPYD	:	Dihydropyrimidine dehydrogenase
DPD	:	Dihydropyrimidine dehydrogenase.
dbSNP	:	Database Single Nucleotide Polymorphism
dUMP	:	deoxyuridine monophosphate
dTMP	:	deoxythymidine monophosphate
ELISA	:	Enzyme Linking Immunoabsorbent Assay
FUTP	:	5-Fluoro-Uridine-50-Triphosphate
FdUMP	:	5-Fluoro-20-Deoxyuridine-50-Monophosphate
G (base)	:	Guanine
HPLC	:	High Performance Liquid Chromatography
IVS	:	Intervening Sequence (intron)
NCBI	:	National Center for Biotechnology Information
ND	:	Not Determined
PCR	:	Polymerase Chain Reaction
PBMC	:	Peripheral blood mononuclear cell
RNA	:	Ribonucleic acid
RBCs	:	Red Blood Cells
SDS	:	Sodium Dodecyl Sulfate
SNP	:	Single Nucleotide Polymorphism
T (base)	:	Thymine
TE	:	Tris EDTA
TK	:	Thymidine kinase
TP	:	Thymidine phosphorylase
TS/ TYMS	:	Thymidylate synthase

LIST OF SUPPLEMENTS

ABSTRACT

Background: Drug adverse effects contribute to more than 100,000 deaths in the United States each year besides being the fourth leading cause of death following heart diseases, cancer, and stroke. One of the drugs that account for drug adverse effects is 5-FU which was catabolized around 80% in the liver by DPD enzyme that encoded by *DPYD* gene. Variations in *DPYD* gene has lead to adverse effects based on several studies. Although population studies on *DPYD* gene have been carried out in Caucasian, African, Asian population, additional study is needed to identify distribution of *DPYD* gene variants in Malaysian population. The aim of this study is to analyze *DPYD* gene variants in Malaysian solid tumor patients who were treated with 5-FU.

Methods: Blood samples from 24 Malaysian 5-FU solid tumor patients, specifically Malay and Chinese were consecutively collected. The 10 exons which were represented the most common variants of *DPYD* gene in the world were chosen from literature review. The amplification of 10 exon and its flanking regions of *DPYD* gene was performed using PCR. Sanger sequencing was performed for reading the sequence.

Results: The study revealed variations of c.85T>C in exon 2, c.496A>G in exon 6, c.1627 A>G in exon 13; IVS13+39 C>T in intron 13; IVS13+40 A>G in intron 13, c.1896 T>C in exon 14, c.2194 G>A in exon 18, IVS22+55 C>T in intron 22; IVS22+65 C>T in intron 22 with allelic frequency 10.42%, 4.17%, 31.25%, 27.08%, 64.58%, 8.33%, 4.17%, 100%, 2.08% respectively. New unreported heterozygous IVS22+65 C>T in intron 22 was found in 1 Chinese patient.

Conclusions: Distribution of *DPYD* variants in Malaysian population specifically Malay and Chinese had specific pattern.

Keywords: 5-FU, *DPYD* gene, *DPYD* variants, Malaysian population.

ABSTRAK

Latar belakang: Efek samping obat berkontribusi terhadap lebih dari 100.000 kematian di Amerika Serikat setiap tahun dan menempati urutan keempat penyebab kematian setelah penyakit jantung, kanker, dan stroke. Salah satu obat yang berkontribusi pada efek samping obat adalah 5-FU, dimana obat ini dikatabolisme sebanyak 80% di hepar oleh enzim DPD yang dikode oleh gen *DPYD*. Variasi pada gen *DPYD* telah dihubungkan dengan efek samping pada beberapa studi. Meskipun studi gen *DPYD* telah dilakukan pada populasi Kaukasia, Afrika, dan Asia, studi tambahan diperlukan untuk mengidentifikasi distribusi varian gen *DPYD* pada populasi Malaysia. Tujuan studi ini adalah menganalisa varian gen *DPYD* pada pasien tumor solid yang diterapi 5-FU di Malaysia.

Metode: Sampel darah dari 24 pasien tumor solid dengan terapi 5-FU di Malaysia, khususnya etnik Malay dan China dikumpulkan secara konsekutif. Amplifikasi PCR dilakukan pada 10 ekson dan *flanking regions* yang mewakili variasi yang paling umum di dunia berdasarkan tinjauan pustaka. Sekuensing dengan metode Sanger digunakan untuk membaca urutan nukleotida hasil PCR.

Hasil: Studi memperlihatkan variasi dari c.85T>C di ekson 2, c.496A>G di ekson 6, c.1627 A>G di ekson 13; IVS13+39 C>T di intron 13; IVS13+40 A>G di intron 13, c.1896 T>C di ekson 14, c.2194 G>A di ekson 18, IVS22+55 C>T di intron 22; IVS22+65 C>T di intron 22 dengan frekuensi allel 10,42%, 4,17%, 31,25%, 27,08%, 64,58%, 8,33%, 4,17%, 100%, 2,08%. Varian baru yang belum dilaporkan adalah IVS22+65 C>T di intron 22 pada 1 pasien etnik China.

Kesimpulan: Distribusi dari varian *DPYD* di populasi Malaysia, khususnya etnik Malay dan China mempunyai pola tertentu.

Kata kunci: 5-FU, gen *DPYD*, varian *DPYD*, populasi Malaysia.