MUTATION IDENTIFICATION OF *APOA1* GENE IN SUBJECTS WITH LOW LEVEL OF HIGH DENSITY LIPOPROTEIN

IDENTIFIKASI MUTASI *APOA1* PADA SUBJEK DENGAN KADAR *HIGH DENSITY LIPOPROTEIN* RENDAH

Thesis
Submitted to fulfill the requirement in obtaining Master Degree

Master of Biomedical Science

HESTY WAHYUNINGSIH
22010111400100

FACULTY OF MEDICINE
DIPONEGORO UNIVERSITY
SEMARANG
2014

THESIS
MUTATION IDENTIFICATION OF *APOA1* GENE IN SUBJECTS WITH LOW LEVEL OF HIGH DENSITY LIPOPROTEIN

Arranged by
Hesty Wahyuningsih
NIM: 22010111400100

Has been defended in front of the defense committee on June 30th, 2014
and has been approved by,

Principal Supervisor,	Supervisor,
dr. Bahrudin, M.Si.Med, PhD	dr. Farmaditya E.P. Mundhofir, M.Si.Med, PhD
NIP: 1976 0315 200604 1 001	NIP: 1981 0425 200812 1 0024

Approved by,
Head of Master Degree Program in Biomedical Science
Faculty of Medicine Diponegoro University

Prof. DR. dr. Tri Nur Kristina, DMM, M.Kes
NIP. 19590527 198603 2 001

DECLARATION

I hereby declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education, there are no elements belonging plagiarism forth in Minister Decree No. 17 of 2010. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of reference is given.

Semarang, July 2014

Hesty Wahyuningsih

CURRICULUM VITAE

A. Personal Data

Name	: Hesty Wahyuningsih
Sex	: Female
Nationality	: Indonesian
Place & Date of Birth	: Kendal, February 15th 1987
Last Degree	: Medical Doctor
Address	: Karangmulyo RT 02 RW 03 Pegandon Kendal, 51357
Email	: hestywahyuningsih15@gmail.com

B. Educational Background

1992 – 1998	Elementary School at SD N 2 Karangmulyo
1998 – 2001	Junior High School at SMP Al-Muayyad Surakarta
2001 – 2004	High School at SMA Al-Muayyad Surakarta
2004 – 2010	Faculty of Medicine Sultan Agung Islamic University
2012 – present	Post Graduate Program Diponegoro University, Master in Biomedical Science, Majoring in Genetic Counseling

Training and Courses

2011	Advanced Neurologic Life Support – participant
	Workshop of Evidence Based Medicine – participant
	Training of Tutor for Problem Based Learning Curriculum - participant
	Workshop of Cell Culture and Immunopharmacology – participant
2012	10th Medical Genetic Course: From Basic to Clinic - participant
	Advance Medical Genetic Course: From Basic to Clinic – participant
	Workshop on Neurogenetics – participant

	Cilliary Dysfunction in Developmental Abnormalities an Diseases Symposium – participant
	Workshop on Immunohistochemistry and Western Blot – participant
2013	11th Medical Genetic Course: From Basic to Clinic – committee
	Seminar on Cardiogenetic – participant
	Workshop of Instructor of Patient Simulation for Objective Structured Clinical Examination – participant
	3rd International Seminar on Autism and Fragile-X Syndrome – committee
	Workshop on Behavioural Therapy and Home Training for Autism – committee
2014	Workshop of National Examiner of Objective Structured Clinical Examination – participant

C. Working Experiences

July 2010 – February 2011	General Practitioner (GP) at Emergency Unit, Muhammadiyyah Islamic Hospital, Kendal
September 2010 – February 2011	GP at Emergency Unit, Darul Istiqomah Islamic Hospital, Kendal
March 2011 – present	Staff at Department of Biochemistry, Faculty of Medicine, Sultan Agung Islamic University, Semarang

ACKNOWLEDGEMENT

It is a pleasure to express my gratitude to everyone who give me not only the opportunity but also supports I need to complete my study and this thesis. I am deeply grateful to the great teacher, Prof. dr. Sultana MH Faradz, PhD, for allowing me to do this study and for her support in guiding and teaching me. Thank you for dr. Bahrudin, M.Si Med, PhD for giving guidance, endless encouragement and constant concern in this research. Thank you for dr. Farmaditya E.P. Mundhofir, M.Si.Med, PhD for his time, patience, and guidance in guiding me to complete this thesis.

This research was conducted in CEBIOR. It would have not been possible without the research grant from PNBP UNDIP. I wish to thank dr. Shodiqur Rifqi, Sp.JP(K), dr. Bahrudin, M.Si.Med, PhD, dr. M. Ali Sobirin, PhD, dr. Ferdy Kurniawan C, M.Si.Med and dr. Isna Rahmia Fara for for their helps and supports during this research. I wish to thank Prof. Ichiro Hisatome for allowing us to do sequencing at the Division of Functional Genomics Research Center for Bioscience and Technology Tottori University Japan. I also thankful to dr. Nani Maharani, MSi.Med for helping us to do sequencing process.

My utmost gratitude to Prof. Laode M. Kamaludin, M.Sc., dr. Iwang Yusuf, M.Sc. and Dr. dr. Taufiq R. Nasikun, SpAnd, M.Kes for giving me the opportunity to continue my study.

I would also like to thank all the staff of the Center for Biomedical Research (CEBIOR), Semarang, Central Java, Indonesia, Dwi Kustiani, Intus Apriasa, Lusi

Suwarsi, Rita Indriati, Wiwik Lestari, Evi Nurwulan, for their assistance during my study.

I wanted to thank all the students of Batch 6 of Master in Biomedical Science majoring in Genetic Counseling, Almira Zada, An Nisaa Utami Tihnulat, Ariestya Indah Permata Sari, Donny Nauphar, Gara Samara Brajadenta, Isna Rahmia Fara, Stefani Harum Sari, Venty Muliana Sari, Ziske Maristka and Zukhrofi Muzar. Your companion, support, and help have been a source of inspiration for me to complete my study and finish this thesis.

My utmost and heartfelt gratitude goes to my dearly beloved parents, Ngatno and Mukhayanah for all the unconditional love and limitless support. Countless thanks to my dearest siblings; Susilo Hadi and Untung Prasetyo.

I would like to thank all of the patients that were participated in this research. This research would not be possible without your involvement and cooperation.

This opportunity to join the Master's Degree would have not been possible without the scholarship from Biro Perencanaan Kerjasama Luar Negeri (BPKLN), Ministry of Education, Indonesia, especially DR. AB Susanto, MSc. As the Beasiswa Unggulan program coordinator. I would also like to thank all of the Master's Degree coordinators, especially Prof. dr. Sultana MH Faradz, PhD, Dr. dr. Tri Indah Winarni, M.Si.Med and Ms. Ardina Aprilani for all their hardwork.

CONTENTS

GLOSSARY

Exon	:	A part of gene which has function as coding sequence.
Heterozygous	:	A dissimilar sequence in both of the double stranded DNA.
High density lipoprotein	:	A particle consist of protein and lipid which carry cholesterol from peripheral cell to the liver.
High resolution melting	:	A post-PCR technique for variant scanning and genotyping based on the different melting point of DNA fragment.
Homozygous	:	A similar sequence in both of the double stranded DNA.
Intron	:	A part of gene which has function as non coding sequence.
Mutation	:	The alteration of DNA sequence which cause pathologic.
Sequencing	:	A method to determine the nucleotide order of a given DNA fragment.
Wild type	:	The most common phenotype in a species particular natural population.

ABBREVIATION

Aβ	:	Amyloid beta
APOA1	:	Apolipoprotein A1
ApoAII	:	Apolipoprotein AII
ApoA IV	:	Apolipoprotein AIV
ApoCI	:	Apolipoprotein C1
ApoCII	:	Apolipoprotein CII
ApoCIII	:	Apolipoprotein CIII
ApoD	:	Apolipoprotein D
Apo E	:	Apolipoprotein E
ApoJ	:	Apolipoprotein J
ApoL	:	Apolipoprotein L
ApoM	:	Apolipoprotein M
ABCA1	:	Adenosine triphosphate-binding cassette transporter subfamily A member 1
ABCG1	:	Adenosine triphosphate-binding cassette transporter subfamily G member 1
ABCG4	:	Adenosine triphosphate-binding cassette transporter subfamily G member 4
CAD	:	Coronary artery disease

CEPT	:	Cholesterylester transfer protein
CM	:	Chylomicron
dsDNA	:	double stranded deoxyribonucleic acid
HDL-C	:	High density lipoprotein cholesterol
HRM	:	High resolution melting
IDL	:	Intermediate density lipoprotein
IHD	:	Ischemic heart disease
LCAT	:	Lecithincholesterol acyltransferase
LDL	:	Low density lipoprotein
NGS	:	Next generation sequencing
PON 1	:	Paraoxonase I
RCT	:	Reverse cholesterol transport
SR-BI	:	Scavenger receptor class B member 1
ssDNA	:	single stranded deoxyribonucleic acid
VLDL	:	Very low density lipoprotein

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Background: High density lipoprotein cholesterol (HDL-C) is the densest and smallest lipoprotein. The *APOA1* mutation shows more acceleration in atherosclerosis process than other gene mutation which encode the main protein of reverse cholesterol transport. This study aimed to identify the predicted pathogenic mutation of *APOA1* gene in subject with low level of HDL-C.
Methods: Forty two subjects with low HDL-C level were included. Mutation screening of *APOA1* was done by using high resolution melting (HRM) technique. The aberrant graph samples were confirmed by sequencing.
Results: Subjects consisted of 24 males (57.1%) and 18 females (42.9%). The risk factors which may influence the level of HDL-C were as follows age more than 60 years old (26.2%), male (57.1%), smoking (45.2%), obesity (50%) and diabetes mellitus (45.2%). Statistical analysis showed that HDL-C level did not associate with age (p=0.32), gender (p=0.79), smoking (p=0.86), diabetes mellitus (p=0.87) and body mass index (p=0.67). Two polymorphisms were identified in *APOA1* gene *i.e.* 756C/T and 84T/C. The genotypes of 756C/T were C/C, C/T and T/T in 21 (50%), 18 (42.86%) and 3 (7.14%) subjects, respectively. Those genotypes were not significantly associated with HDL-C level (p=0.55).
Conclusion: Two reported polymorphisms *i.e.* 756C/T and 84T/C were found in *APOA1* gene in subjects with low HDL-C level.

Keywords: Low HDL-C level, *APOA1* gene, HRM, Sequencing

ABSTRAK

Latar Belakang: *High density lipoprotein cholesterol* (HDL-C) merupakan lipoprotein terkecil dan terpadat. Mutasi *APOA1* menunjukkan percepatan proses aterosklerosis dibandingkan dengan mutasi pada gen lain yang mengkode pembentukan protein yang berperan pada proses *reverse cholesterol transport* (RCT). Tujuan penelitian adalah mengidentifakasi mutasi yang diprediksi patogenik pada gen *APOA1* pada subjek dengan kadar HDL-C rendah.
Metode: Empat puluh dua subjek dengan kadar HDL-C rendah dilakukan skrining mutasi dengan menggunakan teknik *high resolution melting* (HRM). Sampel-sampel yang mempunyai kurva abnormal dikonfirmasi dengan sekuensing.
Hasil: Subjek penelitian ini terdiri dari 24 laki-laki (57,1%) dan 18 perempuan (42,9%). Faktor resiko yang dapat mempengaruhi kadar HDL-C adalah usia di atas 60 tahun 26,2%, laki-laki 57,1%, merokok 45,2%, obesitas 50% dan diabetes melitus 45,2%. Analisis statistik menunjukkan bahwa kadar HDL tidak berhubungan dengan usia (p=0,32), jenis kelamin (p=0,79), merokok (p=0,86), diabetes mellitus (p=0,87) dan index masa tubuh (p=0,67). Dua polimorfisme teridenfikasi pada gen *APOA1* yaitu756C/T dan 84T/C. Polimorfisme 756C/T ditemukan pada 21 subjek (50%) dengan genotif C/C, 18 subjek (42,86%) dengan genotif C/T dan 3 subjek (7,14%) dengan genotif T/T. Genotif C/C, C/T dan T/T mepunyai kadar HDL-C yang tidak berbeda secara signifikan pada tiap kelompok (p=0,55).
Kesimpulan: Dua polimorfisme gene *APOA1* yang pernah dilaporkan yaitu 756C/T dan 84T/C ditemukan pada subjek dengan kadar HDL-C yang rendah.

Kata kunci: kadar HDL-C rendah, gen *APOA1*, HRM, Sekuensing