MUTATION IDENTIFICATION OF *ABCA1* GENE IN SUBJECTS WITH LOW LEVEL OF HIGH DENSITY LIPOPROTEIN IN SEMARANG

IDENTIFIKASI MUTASI GEN *ABCA1* PADA SUBJEK DENGAN KADAR *HIGH DENSITY LIPOPROTEIN* RENDAH DI SEMARANG

Thesis
submitted to fulfill the requirement in obtaining Master Degree

Master of Biomedical Science

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THESIS

MUTATION IDENTIFICATION OF *ABCA1* GENE IN SUBJECTS WITH LOW LEVEL OF HIGH DENSITY LIPOPROTEIN IN SEMARANG

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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 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 Information derived from the published or unpublished work of others has been acknowledged in the text and a list of reference is given.

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CONTENTS

ABBREVIATION LIST

APOA1	: Apolipoprotein A1
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
CETP	: Cholesterylester transfer protein
CM	: Chylomicron
dsDNA	: Double stranded deoxyribonucleic acid
EL	: Endothelial lipase
FHA	: Hypoalphalipoproteinemia
HL	: Hepatic lipase
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
LPL	: Lipoprotein lipase
PON1	: Paraoxonase I
PLTP	: Phospholipid transfer protein
RCT	: Reverse cholesterol transport

SR-BI : Scavenger receptor class B member 1

ssDNA : Single stranded deoxyribonucleic acid

UTR : Untranslated region

VLDL : Very low density lipoprotein

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GLOSSARY

Low level of High-Density Lipoprotein : Level of high density lipoprotein cholesterol in the peripheral blood after 8 hours of fasting with level of concentration less than 40 mg/dL

ABCA1 gene : Gene which encodes adenosine triphosphate-cassette binding transporter subfamily A member 1 which is located at chromosome 9q31.1. This gene has 149 kb length and contains 50 exons that works as a cholesterol efflux pump in the celluler lipid removal pathway

DNA : Deoxyribonucleic acid is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms.

Mutation : The alteration of DNA sequence

Exon : A part of gene which has a function as coding sequence

Intron : A part of gene which has a function as non-coding sequence

HRM : High resolution melting (HRM) is a molecular technique which can detect genetic variations in the DNA fragment based on the differences of melting point (Tm) displayed in curve analysis that shows aberrant curve samples

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

Homozygous : A similar sequence in both of the double stranded DNA

Heterozygous : A dissimilar sequence in both of the double stranded DNA

ABSTRACT

MUTATION IDENTIFICATION OF *ABCA1* GENE IN SUBJECTS WITH LOW LEVEL OF HIGH DENSITY LIPOPROTEIN IN SEMARANG

Background: The crucial part of HDL metabolism for protection against development of atherosclerosis is generally attributed to its role in reverse cholesterol transport, and *ABCA1* gene is a key element to this process. *ABCA1* gene mutation comprises about 15% of low HDL-C level cases. The purpose of this study was to identify the predicted pathogenic mutation of *ABCA1* gene in subject with low level of high density lipoprotein.
Methods: Blood samples were taken from 42 subjects with low HDL-C level (<40mg/dL). Analysis of mutation screening for *ABCA1* gene was done by using high resolution melting (HRM) technique. The aberrant samples were confirmed by DNA sequencing. Alamut software was used to predict pathogenic mutation.
Results: Subjects were consisted of 24 (57,1%) males and 18 (42.9%) females. The secondary risk factors which may influence the level of HDL-C were age, gender, smoking, diabetes mellitus, body mass index, menopause, hypertension, and CAD were not significant different to HDL-C level at each group. Nine polymorphisms were identified. One variant found in 5'UTR known was c.-76dup (rs1799777). Three variants were identified in intron, i.e., c.+378G>C (rs1800978), c.814-14dup (rs2067484) and c.1892+24T>A (rs4743763). Four variants found as synonymous substitutions were c.936C>T (rs2274873), c.948G>A (rs2246841), c.2040C>A (rs2853579), and c.5586G>A. A variant c.2311G>A (rs2066718) was predicted deleterious. We found no symptoms or other similar condition from the family in Pedigree analysis of this subject. The c.5586G>A was a novel variant, while the rest were already reported in the SNP database.
Conclusion: Nine of *ABCA1* variant were identified in this study, it consists of 8 reported SNP and 1 novel variant c.5586G>A. One variant known as missense substitution c.2311G>A was predicted pathogenic. No risk factors were significant different to influence the low level of HDL-C.

Keywords: Low HDL-C level, *ABCA1* gene, HRM, Sequencing

ABSTRAK

IDENTIFIKASI MUTASI GEN *ABCA1* PADA SUBJEK DENGAN KADAR *HIGH DENSITY LIPOPROTEIN* RENDAH DI SEMARANG

Latar Belakang: Peranan terpenting metabolisme HDL dalam proteksi terhadap aterosklerosis dihubungkan dengan perannya dalam proses *reverse cholesterol transport*, dan gen *ABCA1* merupakan elemen terpenting pada proses tersebut. Mutasi gen *ABCA1* terjadi sekitar 15% pada kasus kadar HDL-C rendah. Tujuan penelitian ini adalah untuk mengidentifikasi mutasi yang diduga patogenik pada gen *ABCA1* pada subjek dengan kadar HDL-C rendah.
Metode: Sampel darah diambil dari 42 subjek dengan kadar HDL-C rendah (<40mg/dL), lalu dilakukan skrining mutasi gen *ABCA1* dengan menggunakan teknik *high resolution melting* (HRM). Sampel yang mempunyai variasi kurva dikonfirmasi dengan DNA sekuensing. Analisis mutasi patogenik menggunakan software Alamut.
Hasil: Subyek penelitian terdiri dari 24 (57,1%) laki-laki dan 18 (42.9%) perempuan. Faktor resiko yang dapat mempengaruhi terhadap kadar HDL-C adalah umur, gender, merokok, diabetes melitus *body mass index*, menopause, hipertensi dan *CAD* mepunyai kadar HDL-C yang tidak berbeda secara signifikan pada tiap kelompok. Diidentifikasi 9 polimorfisme yaitu, satu varian terdapat di 5'*UTR* c.-76dup (rs1799777), tiga varian ditemukan di intron, c.+378G>C (rs1800978), c.814-14dup (rs2067484) dan c.1892+24T>A (rs4743763), empat varian adalah *synonymous substitutions* c.936C>T (rs2274873), c.948G>A (rs2246841), c.2040C>A (rs2853579), c.5586G>A, dan satu varian c.2311G>A (rs2066718) diprediksi *deleterious*, tidak ditemukan kondisi atau gejala yang mirip pada keluarga lainnya pada analisa *Pedigree*. c.5586G>A merupakan varian baru, sedangkan yang lain telah dilaporkan di *SNP database*.
Kesimpulan: Penelitian ini mengidentifikasi 9 varian *ABCA1* terdiri dari 8 variant SNP yang telah dilaporkan dan 1 varian baru yaitu c.5586G>A. Varian *missense substitution* c.2311G>A diprediksi patogenik. Tidak ditemukan faktor resiko yang berpengaruh secara signifikan pada subjek dengan kadar HDL rendah.