

**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

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LIPOPROTEIN IN SEMARANG**

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ABBREVIATION LIST

<i>APOA1</i>	: Apolipoprotein A1
<i>ABCA1</i>	: Adenosine triphosphate-binding cassette transporter subfamily A member 1
<i>ABCG1</i>	: Adenosine triphosphate-binding cassette transporter subfamily G member 1
<i>ABCG4</i>	: Adenosine triphosphate-binding cassette transporter subfamily G member 4
<i>CAD</i>	: Coronary artery disease
<i>CETP</i>	: Cholesterylester transfer protein
<i>CM</i>	: Chylomicron
<i>dsDNA</i>	: Double stranded deoxyribonucleic acid
<i>EL</i>	: Endothelial lipase
<i>FHA</i>	: Hypoalphalipoproteinemia
<i>HL</i>	: Hepatic lipase
<i>HDL-C</i>	: High density lipoprotein cholesterol
<i>HRM</i>	: High resolution melting
<i>IDL</i>	: Intermediate density lipoprotein
<i>IHD</i>	: Ischemic heart disease
<i>LCAT</i>	: Lecithincholesterol acyltransferase
<i>LDL</i>	: Low density lipoprotein
<i>LPL</i>	: Lipoprotein lipase
<i>PON1</i>	: Paraoxonase I
<i>PLTP</i>	: Phospholipid transfer protein
<i>RCT</i>	: 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 cellular 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 (T_m) 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* mempunyai 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.
