

**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**

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**THESIS**  
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**LIPOPROTEIN**

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 Information derived from the published or unpublished work of others has been acknowledged in the text and a list of reference is given.

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## 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

A $\beta$	:	Amyloid beta
<i>APOA1</i>	:	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

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## 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.

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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 mengidentifikasi 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 teridentifikasi pada gen *APOA1* yaitu 756C/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 mempunyai 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.

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