ORIGINAL ARTICLE

LPA Gene Copy Number Variation and *APOE* Gene Polymorphism in Young Acute Myocardial Infarction

Mohd Zamri II[a], Abdullah NZ[a], A. Talib N[a], Muhammad Musa NA[b], Abdullah A[b]

[a]Department of Pathology and Laboratory Medicine, Kulliyyah of Medicine, International Islamic University Malaysia
[b]Department of Basic Medical Sciences, Kulliyyah of Medicine, International Islamic University Malaysia

ABSTRACT

INTRODUCTION: An early onset of acute myocardial infarction (AMI) and a strong family history suggest the possibility of its genetic predisposition. Lipoprotein(a) (*LPA*) and Apolipoprotein E *(APOE*) genes are known to be involved in lipid metabolism which may contribute to the development of atherosclerosis leading to AMI. This study aims to assess the association between *LPA* gene copy number variation (CNV) and *APOE* gene polymorphism in young AMI patients. **MATERIALS AND METHODS:** A total of 40 DNAs were extracted from the buffy coat. *APOE* genotyping and detection of *LPA* gene CNV were performed using multiplex PCR technique and digital PCR. After tabulation of the results of the current study, meta-analyses were performed from selected studies among Asian populations using the Comprehensive Meta-analysis version 3 software program. **RESULTS:** No significant association was found between CNV of the *LPA* gene and the polymorphism of the *APOE* gene with Young AMI patients in the current study. However, our meta-analysis confirmed that the E4 allele increased the risk for CAD with the E3/E4 genotype [p=0.000, OR= 1.60 (95% CI: 1.41-1.83) significantly increased risk of CAD and individuals with E3/E3 genotype [p=0.000, OR=0.73 (95% CI: 0.66-0.81) were protective against CAD. The gain of *LPA* CNV was higher in YAMI [n=5 (25%)] than in control [n=2 (10%)] but they are not significant. **CONCLUSION:** There is no association between the *LPA* gene CNV and the presence of *APOE* polymorphism in young AMI, but our meta-analysis confirmed that the E4 allele increased the risk for CAD.

Keywords
Acute myocardial infarction (AMI); Copy number variation (CNV); Single nucleotide polymorphism (SNP); Lipoprotein (a) (LPA) gene; Apolipoprotein E (APOE) gene

Corresponding Author
Assoc. Prof. Dr. Nor Zamzila Abdullah
Department of Pathology and Laboratory Medicine, Kulliyyah of Medicine, International Islamic University Malaysia, Pahang, Malaysia. Malaysia

E-mail : zamzila@iium.edu.my

Received: 23rd November 2023; Accepted: 1st January 2024

Doi: https://doi.org/10.31436/imjm.v23i02

INTRODUCTION

Acute myocardial infarction (AMI) is defined as the necrosis of myocardial tissue caused by a sudden loss of blood flow and ischaemia to the heart muscle due to the spasm or complete occlusion of the artery by a thrombus that is characterised by non-ST elevation MI (NSTEMI) and ST-elevation MI (STEMI).[1,2] AMI continues to be the principal cause of morbidity and mortality in both developing and developed countries[3] and retains a substantial footprint on global health which affect more than 7 million individuals worldwide each year.[4] Ischaemic heart disease which includes AMI remained as one of the primary causes of death in Malaysia.[5] There are several risk factors for AMI which include modifiable and non-modifiable risks. Risk factors that can be changed are termed modifiable which include hypertension, hyperlipidaemia, and obesity, while those that cannot be changed fall under non-modifiable factors which include age, gender, genetics, and family history. Genetic factors play a more significant role in causing acute myocardial infarction (AMI) in younger patients.[6] A higher risk of AMI in individuals with a strong family history indicates the possibility of a genetic predisposition for AMI. This is supported by various evidence from twin studies.[7–11] The complex genetic inheritance of AMI can be explained by one of the major twin studies in Sweden involving 2,341 first-episode cases of AMI which concluded the contribution of genetic variants to the disease, while additional genetic factors contribute to the disease phenotypes.[12] An underlying genetic predisposition is much more relevant to those who develop AMI at a young age.[13]

Although the genetic background of AMI is complex, one of the consistent candidate genes of AMI by its involvement in the pathogenesis of atherosclerosis is the apolipoprotein E (*APOE)* gene.[14] APOE is involved in lipid metabolism by promoting hepatic and extrahepatic uptake of plasma lipoproteins.[15] *APOE* gene consist of six different genotypes (e2/2, e2/3, e2/4, e3/3, e3/4, and e4/4) generated by three major isoforms of alleles (E2, E3 and E4).[16] Of these alleles, E4 has been consistently found to be the susceptible allele for AMI.[17] Interestingly, the prevalence of E4 allele is much more common in Malays compared to Chinese and Indian ethnic groups.[18]

Another important gene attribute to atherosclerosis formation is the lipoprotein(a) (*LPA*) gene. *LPA* encodes for a protein that inhibits the activity of tissue-type plasminogen activator I, thus promoting thrombogenesis in atherosclerotic lesions making the gene one of the most potent monogenic risk factors for CAD.[19] There was a report of inverse correlation between copy number variation at the locus of Kringle IV of the *LPA* gene with plasma Lp(a).[20] Thus, indicates possible roles of copy number of *LPA* gene and AMI.

Separately, Lp(a) and APOE are involved in the regulation of lipid metabolism, thus contributing to the pathogenesis of atherosclerosis[21,22] and hence AMI. The current study aims to perform a meta-analysis on the association of *APOE* genotypes with coronary artery disease and to assess the association of *LPA* gene copy number variations in the presence of *APOE* polymorphism in Young acute myocardial infarction.

MATERIALS AND METHODS

Subject

The AMI patients were recruited from the emergency department at Hospital Tengku Ampuan Afzan Kuantan (HTAA) and Sultan Ahmad Shah Medical Centre (SASMEC) @ IIUM. The healthy control subjects were recruited from primary health care clinics, Klinik Kesihatan Bandar Kuantan, and among IIUM staff at the IIUM campus. The participants who fulfilled the inclusion and exclusion criteria were enrolled in the study after informed consents were taken. This study is part of a larger study on acute myocardial infarction in young adults. It was conducted in adherence to the Declaration of Helsinki and guidelines from the Ethical Committee of Kulliyyah of Medicine, IIUM (IIUM/305/20/4/1/7) and Medical Research and Ethical Committee (MREC), Kementerian Kesihatan Malaysia (NMRR-16-2572-32869 (IIR)).

The sample size for the remaining study objectives was determined using OpenEpi Software version 3.0, focusing on comparing two means. In this research, a power of 80% was selected to ensure a substantial effect size within a 95% confidence interval.[23] Based on a referencing study conducted by Wu et al. in 2014, the initial calculation suggested a requirement of 280 samples for each group. However, due to a limited number of myocardial infarction cases in our dataset, the study was conducted as a pilot study. As recommended by Birkett and Day in 1994, an internal pilot study requires 20 samples for each arm. Consequently, our study utilised 20 samples for each group.[24]

A total of 40 male participants were enrolled and subsequently divided into two (2) groups; 20 Young AMI and 20 healthy controls. The inclusion criteria for the Young AMI group were Malaysian with an episode of ST-elevation myocardial infarction (STEMI), aged ≤ 45 years. The exclusion criteria for the Young AMI group were patients who had received streptokinase or percutaneous coronary intervention (PCI). For the healthy control group, the inclusion criteria were healthy Malaysians aged 18-45 years old. The exclusion criteria were subjects with chronic illness, on medication including over-the-counter or herbal medication, regular alcohol consumer, and age above 45 years old.

Sample collection and human buffy coat isolation

Four (4) ml peripheral blood was collected into EDTA tubes from all 40 subjects and centrifugated at 2500 rpm for 10 minutes at room temperature for the isolation of the buffy coat. The buffy coat was aliquoted into a microcentrifuge tube and stored at -80°C until further genetic analysis.

DNA extraction

Genomic DNA was extracted using QIAmp DNA Blood Midi Kit (Qiagen, Germany), a purification kit with a final elution of 300 µL with RNase-free water following the manufacturer's protocol. The quantity and quality of the purified DNA were measured using a SimpliNano spectrophotometer (GE Healthcare Life Sciences, USA).

APOE genotyping

Two SNPs (rs 429358, rs 7412) that were located at 19q13.2 in Exon 4 of the *APOE* gene were selected based on the previous study. The identification of six *APOE* genotypes was performed using multiplex polymerase chain reaction (PCR) based on the dual priming oligonucleotide, Seeplex Apo E ACE Genotyping Assay (Seegene, Seoul, South Korea). PCR was performed by remixing 10 µL of reaction mix comprised of 2.5uL of 5X AP PM, 2uL of 8-MOP Solution, and 5.5uL of 2X Multiplex Master Mix with 5 µl of genomic DNA. After the preheating step at 94◦C for 15 minutes, the denaturation at 94◦C for 30 seconds, annealing at 65◦C for 30 seconds, extension at 72◦C for 60 seconds with a total of 35 reaction cycles, and final extension at 72◦C for 10 minutes were performed using Thermocycler (Eppendorf Vapo.protect Mastercycler Pro). The PCR products were electrophoresed on a 2% agarose gel.

LPA gene CNV detection

The detection of copy number variation of the *LPA* gene was conducted using digital polymerase chain reaction (DPCR). The QIAcuity Nanoplate 8.5K 24-well (QIAgen, German) was used for a small sample size and low input volume. The procedure was performed in 12 µL reaction volume for 1X reaction that consisted of 4µl of 3x EvaGreen PCR Master Mix (green channel), 0.48 µl 25x dPCR Copy Number Assay (*LPA*), 0.25 µl EcoR1, 2.27 µl RNase-Free Water and 5 µl (15ng/µl) of DNA. For the reference assay, 0.48 µl of 25x dPCR Copy Number Assay (Human Multicopy Reference R6) was used. The preheating step at 95◦C for 1 minute which was followed by 40 reaction cycles (denaturation at 94◦C for 15 seconds, annealing at 60◦C for 15 seconds, and extension at 72◦C for 15 seconds) was performed, and cooling down at 42◦C for 5 minutes using QIAcuity digital PCR (Qiagen, Germany). The analysis of copy numbers was performed using QIAcuity Software Suite version 1.2 (QIAgen, Germany).

Literature search for meta-analysis

We conducted a comprehensive literature search published before 2023 from the English database, PubMed. The search was performed using various combinations of keywords including "APOE" OR "APOE" AND "polymorphism" OR "variant" OR "variation" OR "genotype" AND "myocardial infarction" OR "coronary heart disease" OR "CHD" OR coronary artery disease" OR "CAD". The studies were considered potentially eligible when they met the inclusion criteria: 1) *APOE* gene polymorphism and risk of CAD/CHD; 2) clearly describe control selection, study design, genotypes, and allele frequencies; 3) sufficient data to calculate the odd ratio (OR) with its 95% confidence interval (CI). Eight (8) studies conducted on Asian populations were selected in this meta-analysis.

Statistical analysis

The statistical analysis is carried out using SPSS version 27.0. The normally distributed data were reported as mean (SD) and the non-normally distributed data were reported as median (IQR). The chi-square test was used to assess the association of *APOE* gene polymorphism and *LPA* gene CNV with AMI and controls and 95% CI was applied. Meta-analysis was performed among Asian populations using the Comprehensive Meta-analysis version 3 software program. The *p*-value <0.05 is considered as statistically significant.

RESULTS

Baseline Clinical Characteristics of the Study Subjects

The baseline clinical characteristics of 40 participants are presented in Table I.

Table I: Baseline clinical characteristics of cases and controls

Variable	YAMI (n=20)	Controls (n=20)	*p*-value
Age (years)	39.50 (8)	27.50 (12)	<0.001*
BMI (kg/m²)	28.705 (5.48)	24.531 (6.09)	0.002*
TC (mmol/L)	5.775 (2.36)	5.150 (1.25)	0.041*
TG (mmol/L)	1.575 (0.92)	1.070 (0.52)	0.002*
HDL-C (mmol/L)	0.990 (0.33)	1.160 (0.44)	0.040*
LDL-C (mmol/L)	4.015 (1.72)	3.235 (1.35)	0.113
Smoking (%)	17 (85%)	6 (30%)	0.001*

Data are presented as median (IQR), except for smoking, which is presented as a number of samples (percentage). Kruskall-Wallis and $\chi 2$-test were used to analyse the data. $p < 0.05$ is taken as statistically significant at a 95% confidence interval *significant difference.

Genotypes analysis

The target region of *APOE* gene involving codon 112 and 158 SNPs was successfully amplified following the manufacturer's protocol as described before. Figure 1 shows the representative photographs of the gel electrophoresis.

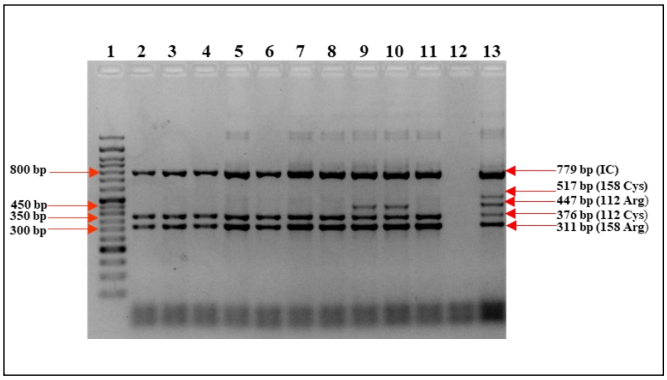

Figure 1: Agarose gel electrophoresis of PCR products of *APOE* amplicons (Lane 1; DNA ladder 50 bp, Lane 2-8; E3/E3 genotypes, Lane 9&10; E3/E4 genotypes, Lane 11; E3/E3 genotypes, Lane 12; NC, Lane 13; AP Marker)

Association of *Apo E* genotypes between YAMI and healthy controls

E3/E3 and ε3 were the most frequent *APOE* genotypes and alleles in both study groups respectively. However, there were no significant differences in *APOE* genotypes and alleles between YAMI and healthy controls. Detailed results are described in Table II.

Table II: Genotypes and alleles in YAMI and controls

APOE genotypes	YAMI (n=20) N (freq)	Controls (n=20) N (freq)	Chi-square	p-value
E2/E2	1 (0.05)	0 (0)	4.471	0.484
E2/E3	0 (0)	2 (0.1)		
E2/E4	1 (0.05)	1 (0.05)		
E3/E3	14 (0.7)	11 (0.55)		
E3/E4	4 (0.2)	5 (0.25)		
E4/E4	0 (0)	1 (0.05)		
Alleles				
ε2	3 (0.075)	3 (0.075)	0.8398	0.657
ε3	32 (0.8)	29 (0.725)		
ε4	5 (0.125)	8 (0.2)		

Note: $\chi 2$ -test, $p < 0.05$ is taken as statistically significant at 95% confidence interval

*significant difference

Meta-analysis of APOE gene in coronary artery disease

Our literature search found a total of 25 studies on the association of APOE genotypes with coronary artery disease. Eight studies from Asian populations with similar methodology were selected for the meta-analysis with total samples of 4,369 and 3,196 for CAD and healthy controls respectively. This meta-analysis discovered that individuals with E3/E4 genotype [p=0.000, OR=1.60 (95% CI: 1.41-1.83) had a significantly increased risk of CAD while individuals with E3/E3 genotype [p=0.000, OR=0.73 (95% CI: 0.66-0.81) were protective against CAD. The E2/E3 genotype [p=0.206, OR= 0.91 (95% CI: 0.78-1.06) was not found to be protective against CAD.

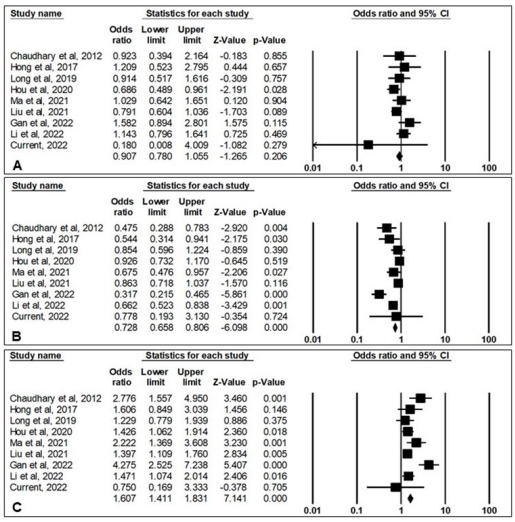

Figure 2: Meta-analysis findings *APOE* genotypes of E2/E3 (A), E3/E3 (B) and E3/E4 (C)

A subsequent meta-analysis on *APOE* alleles affirmed the CAD risk of ε4 allele [p=0.000, OR=1.56 (95% CI: 1.40-1.74)], [p=0.000, OR=1.60 (95% CI: 1.36-1.89)] as compared to ε3 and ε2 alleles respectively. The ε2 allele was not found to be protective against CAD [p=0.76, OR=0.98 (95% CI: 0.86-1.118)] as compared to the ε3 allele.

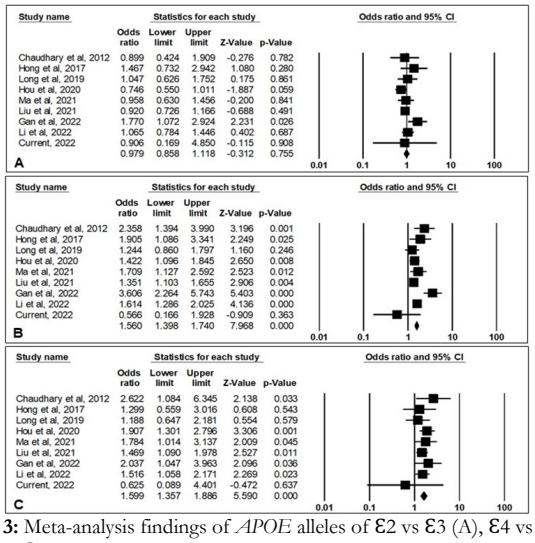

Figure 3: Meta-analysis findings of *APOE* alleles of ε2 vs ε3 (A), ε4 vs ε3 (B) and ε4 vs ε2 (C)

Detection of *LPA* gene Copy Number Variations (CNV)

Figure 4 shows the representative photographs of the 1D scatterplot for absolute quantification.

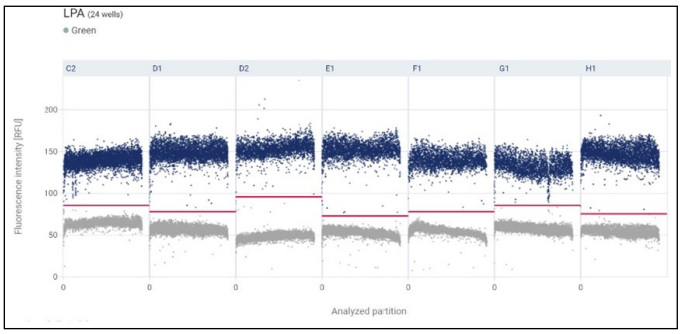

Figure 4: 1D scatterplot view for LPA probe and green channel which concentrates the diagrams for each selected well in a horizontal way separated by a vertical line (column indicator). A header indicates good coordination on each well diagram. The red line indicates the current threshold intensity value (decimal value) to distinguish positive/negative partitions. Fluorescence values below the threshold are shown in grey, and above the threshold in blue.

Association of *LPA* CNV between YAMI and controls

Our study found diverse *LPA* CNV in both groups from one copy to four copies in which two copies were the most frequent *LPA* CNV detected. The copy numbers of the *LPA* gene were grouped into normal (copy number=2), loss (copy number <2), and gain (copy number >2). Although gain *LPA* CNV was found higher in YAMI [n=5 (25%)], no significant difference was found (p=0.459). Detailed results are presented in Table III.

Table III : Analysis of the association of *LPA* CNV between Young AMI and control

a) LPA copy number before grouping.

COPY NUMBER STATUS	YAMI (n=20) n (%)	Control (n=20) n (%)	Chi-square	p-value
1 (n=7)	5 (25%)	6(30%)		
2 (n=22)	10 (50%)	12(60%)	3.94	0.268
3 (n=6)	5 (25%)	1(5%)		
4 (n=1)	0 (0%)	1 (5%)		

b) LPA copy number after grouping.

LPA CNV	YAMI n (%)	Control n (%)	Chi-square	p-value
1 (n=7)	5 (25%)	6(30%)		
2 (n=22)	10 (50%)	12(60%)	3.94	0.268
3 (n=6)	5 (25%)	1(5%)		
4 (n=1)	0 (0%)	1 (5%)		

Note: $\chi 2$ -test, $p < 0.05$ is taken as statistically significant at 95% confidence interval *significant difference

Association of combined *LPA* CNV and *APOE* genotypes between YAMI and controls

Normal *LPA* CNV and E3/E3 genotypes were the most frequent variant combinations in both groups. Although combined gain *LPA* CNV and E3/E3 were more frequent in the YAMI group, we found that there was no significant association between these two groups. The detailed results are shown in Table IV.

Table IV: Association of *LPA* CNV status and *APOE* genotypes between YAMI and controls

CNV STATUS/ GENOTYPES	YAMI (n=20)	CONTROL (n=20)	P-VALUE
NORMAL + E2/E2	0	0	
NORMAL + E2/E3	0	2	
NORMAL + E2/E4	0	1	0.381
NORMAL + E3/E3	7	5	
NORMAL + E3/E4	3	3	
NORMAL + E4/E4	0	1	
LOSS + E2/E2	1	0	
LOSS + E2/E3	0	0	
LOSS + E2/E4	0	0	0.491
LOSS + E3/E3	3	5	
LOSS + E3/E4	1	1	
LOSS + E4/E4	0	0	
GAIN + E2/E2	0	0	
GAIN + E2/E3	0	0	
GAIN + E2/E4	1	0	0.214
GAIN + E3/E3	4	1	
GAIN + E3/E4	0	1	
GAIN + E4/E4	0	0	

Note: $\chi 2$ -test, $p < 0.05$ is taken as statistically significant at 95% confidence interval *significant difference

DISCUSSION

CAD is a main cause of morbidity and mortality worldwide where the risks are contributed by both environmental and genetic factors. The inheritable susceptibility to CAD is estimated to be 40–60%.[25] The increasing prevalence of AMI in young adults of less than 40 years old triggers an insight towards the preponderance of genetic contribution in the pathogenesis of AMI. Since *APOE* and *LPA* genes are one of the important genetic determinants of CAD[26,27] that are involved in lipid metabolism and the development of atherosclerosis, the present study highlighted their association in young adults.

In our study, it was anticipated that there would be no significant differences between the AMI and control groups at baseline. However, observed variations

appeared in certain baseline characteristics, including age, BMI, smoking status, and lipid profile. These differences might have potentially influenced our study outcomes, introducing a confounding factor. These differences may affect the levels of LPA and APO E, which are important aspects of our study.

In the current study, the YAMI group has higher BMI, TC, TG, and LDL-C with lower HDL-C as compared to healthy controls. This indicates that abnormal lipid profiles are an important risk factor for AMI in our study population.

When *APOE* genotypes and alleles were analyzed for their association between YAMI and healthy controls, no associations were found. This study also revealed that the commonest genotypes and alleles are E3/E3 and ε3 respectively. This is in line with previously reported studies among Asian[28-30] and Caucasian[31] populations. In most studies, the ε4 allele especially the E3/E4 and E4/E4 genotypes were reported to be associated with an increased risk of CAD across different populations.[29,32,33] However, in this current study, the frequencies of *APOE* ε4 allele, E3/E4 genotype, and E4/E4 genotype were noted to be higher in healthy controls than in YAMI patients. The differences in these findings could be due to our small sample size.

Further meta-analysis was performed to improve the statistical power of this study by combining the data from a few previous studies performed on Asian populations. In this meta-analysis, we compared the allele frequencies for ε2, ε3, and ε4 with other Asian populations previously published. Our meta-analysis confirmed that the ε4 allele carries a risk of CAD [Figure 3 (B)] but there is no evidence of the ε2 allele being protective against CAD [Figure 3 (A)]. The finding of the ε4 allele as a risk of CAD is in agreement with the meta-analysis conducted previously.[16,34-37] ε4 allele increases the cholesterol level by enhancing the transfer of cholesterol ester from HDL to TG-rich lipoproteins which promotes the hepatic remnant clearance by APOE receptors and decreases LDLR.[38] Furthermore, the meta-analysis by Xu et al.[35] reported that ε4 allele had a 46% higher risk of CAD (OR = 1.46, 95% CI = 1.28– 1.66).

In most studies and meta-analyses, the ε2 allele was reported to be protective against CAD[36,38-41] where *APOE* ε2 binds LDLR poorly which increases the LDLR numbers, therefore lowering the cholesterol level.[38] However, our meta-analysis finding is somehow the opposite of this. It could be due to the higher number of CAD cases as compared to controls in their studies.[25,42-44] In the meantime, our study on other CAD lipid-associated risk factors revealed that *LPA* gene copy number >2 (gain) was higher in YAMI patients while 1 copy number (loss) of the *LPA* gene was higher in controls although there was no significant association. Similarly, a study from [45] reported that 3 copy number of the *LPA* gene is associated with an increased risk of CAD and 1 copy number reduced the risk of CAD. The low copy number of the *LPA* gene can alter its binding affinity for fibronectin and glycosaminoglycans as well as reduce the impairment of fibrinolytic function. When the *LPA* CNV was analysed for its association with the *APOE* gene, no statistically significant associations were found. The non-significant association found in this study could be due to our small sample size. Meta-analysis also cannot be conducted for this parameter due to the limited number of studies found. More studies on *LPA* CNV with a larger sample size are needed to confirm this finding which enables more comprehensive studies and a deeper understanding of the genetic basis behind this disease and condition.

In conclusion, coronary artery disease (CAD) remains a significant global health concern, influenced by both genetic predisposition and environmental factors. The increased incidence of acute myocardial infarction (AMI) in young adults has sparked interest in the genetic fundamentals of this condition. The study focused on APOE and LPA genes, crucial determinants associated with lipid metabolism and atherosclerosis, aiming to understand their connection in young adults.

The current meta-analysis supports the notion that the ε4 allele significantly increases the risk of CAD, whereas the ε2 allele neither increases nor decreases the risk of CAD. However, there is no association between the copy number of the *LPA* gene and the presence of *APOE* polymorphism in young acute myocardial infarction.

Further investigation with larger sample sizes is essential to validate the observed associations, particularly regarding LPA gene copy number variations and their interaction with APOE gene polymorphisms. In addition to a larger sample size, it is crucial to match or stratify participants based on age groups between patients and healthy controls to minimise the impact of age-related differences on the outcomes. This can be accomplished by selecting controls within comparable age ranges as patients or by categorising the analysis into distinct age brackets. Furthermore, future studies should account for gene-environment interactions to effectively assess the genes' etiological role in coronary artery disease (CAD) development.

ACKNOWLEDGMENTS

The author would like to thank Dr. Wan Fatein Nabeila Wan Omar and Dr. Norbaiyah Mohamed Bakrim from Department of Basic Medical Sciences, Kulliyyah of Medicine, International Islamic University Malaysia (IIUM) as well as the Head of Department, and all medical officers in the Emergency Department of Hospital Tengku Ampuan Afzan and the Emergency Department of Sultan Ahmad Shah @ IIUM (SASMEC@IIUM), Kuantan Pahang for their helpful contribution in this study. This study was funded by the Fundamental Research Grant Scheme (FRGS/1/2019/SKK08/UIAM/02/3) from the Ministry of Education Malaysia. The authors also declare no conflicts of interest regarding the publication of this article.

REFERENCES

1. Wu JC. Acute Myocardial Infarction. In: Essential Echocardiography. Elsevier; 2019; 195-199.e1
2. Mendis S, Thygesen K, Kuulasmaa K, Giampaoli S, Mahonen M, Blackett KN, et al. World Health Organization definition of myocardial infarction: 2008 -09 revision. Int J Epidemiol. 2011;40(1):139–46
3. Dan KK, Rahman M, Saha S, Das J. Prevalence of Acute Myocardial Infarction in Young Adults and Conventional Risk Factors. Scholars Journal of Applied Medical Sciences. 2021; 1:172–8. http://doi.org/10.36347/sjams.2021.v09i01.032
4. Reed GW, Rossi JE, Cannon CP. Acute myocardial infarction. The Lancet. 2017; 389(10065):197–210. http://doi.org/10.1016/S0140-6736(16)30677-8
5. Department of Statistic Malaysia. Statistic on Cause of Death, Malaysia, 2022 [Online]. Available at : www.dosm.gov.my. Accessed December 2022
6. Yandrapalli, S., Nabors, C., Goyal, A., Aronow, W. S., & Frishman, W. H. (2019, Feb 12). Modifiable Risk Factors in Young Adults With First Myocardial Infarction. J Am Coll Cardiol, 73(5), 573-584. https://doi.org/10.1016/j.jacc.2018.10.084
7. Smith MC, Baker JR, Gleaves E, Singh A, Kazimuddin M. Twinning: Coronary Artery Disease in Monozygotic Twins. Cureus. 2021; 13(7): e16139. https://doi.org/10.7759/cureus.16139
8. Patil SS, Ramalingam R, Subramanyam K, Pujar D, Manjunath CN, Gajiwala N, et al. Non-identical yet similar: presentation of coronary artery disease in dizygotic twins. Cardiovasc Diagn Ther. 2015; 5 (2):145–9. https://doi.org/10.3978/j.issn.2223-3652.2015.01.11
9. Mangino M, Spector T. Understanding coronary artery disease using twin studies: Table 1. Heart. 2013; 99(6):373–5. https://doi.org/10.1136/heartjnl-2012-303001
10. Evans A, Van Baal GCM, McCarron P, DeLange M, Soerensen TIA, De Geus EJC, et al. The Genetics of Coronary Heart Disease: The Contribution of Twin Studies. Twin Research. 2003; 6(5):432–41. https://doi.org/10.1375/136905203770326439
11. Zdravkovic S, Wienke A, Pedersen NL, Marenberg ME, Yashin AI, De Faire U. Heritability of death from coronary heart disease: a 36-year follow-up of 20 966 Swedish twins. J Intern Med. 2002; 252 (3):247–54. https://doi.org/10.1046/j.1365-2796.2002.01029.x
12. Zdravkovic S, Wienke A, Pedersen NL, de Faire U. Genetic Susceptibility of Myocardial Infarction. Twin Research and Human Genetics. 2007; 10(6):848–52. https://doi.org/10.1375/twin.10.6.848
13. Incalcaterra E, Caruso M, Balistreri CR, Candore G, Lo Presti R, Hoffmann E, et al. Role of genetic polymorphisms in myocardial infarction at young age. Clin Hemorheol Microcirc. 2010; 46(4):291–8. https://doi.org/10.3233/ch-2010-1353
14. Marais AD. Apolipoprotein E and Atherosclerosis.

Curr Atheroscler Rep. 2021; 23(7): 34. https://doi.org/10.1007/s11883-021-00933-4
15. Tudorache IF, Trusca VG, Gafencu AV. Apolipoprotein E - A Multifunctional Protein with Implications in Various Pathologies as a Result of Its Structural Features. Comput Struct Biotechnol J. 2017; 15:359–65. https://doi.org/10.1016/j.csbj.2017.05.003
16. Zhang MD, Gu W, Qiao SB, Zhu EJ, Zhao QM, Lv SZ. Apolipoprotein E Gene Polymorphism and Risk for Coronary Heart Disease in the Chinese Population: A Meta-Analysis of 61 Studies Including 6634 Cases and 6393 Controls. PLoS One. 2014; 9 (4): e95463. https://doi.org/10.1371/journal.pone.0095463
17. Tanguturi P, Pullareddy B, Sampath Kumar P, Murthy DK. Association Between Apolipoprotein E Gene Polymorphism and Myocardial Infarction. Biochem Genet. 2013; 51(5–6): 398–405. https://doi.org/10.1007/s10528-013-9572-2
18. Tan CE, Tai ES, Tan CS, Chia KS, Lee J, Chew SK, et al. APOE polymorphism and lipid profile in three ethnic groups in the Singapore population. Atherosclerosis. 2003; 170(2):253–60. https://doi.org/10.1016/s0021-9150(03)00232-6
19. Tsimikas S. A Test in Context: Lipoprotein(a): Diagnosis, Prognosis, Controversies, and Emerging Therapies. J Am Coll Cardiol. 2017; 69(6): 692–711. https://doi.org/10.1016/j.jacc.2016.11.042
20. Wang Y, Wang L, Liu X, Zhang Y, Yu L, Zhang F, et al. Genetic variants associated with myocardial infarction and the risk factors in Chinese population. PLoS One. 2014; 9(1): e86332. https://doi.org/10.1371/journal.pone.0086332
21. Gudbjartsson DF, Thorgeirsson G, Sulem P, Helgadottir A, Gylfason A, Saemundsdottir J, et al. Lipoprotein(a) Concentration and Risks of Cardiovascular Disease and Diabetes. J Am Coll Cardiol. 2019; 74(24): 2982–94. https://doi.org/10.1016/j.jacc.2019.10.019
22. Hou J, Deng Q, Guo X, Deng X, Zhong W, Zhong Z. Association between apolipoprotein E gene polymorphism and the risk of coronary artery disease in Hakka postmenopausal women in southern China. Lipids Health Dis. 2020;19(1): 139. https://doi.org/10.1186/s12944-020-01323-6
23. Dean, A., Sullivan, K., Soe, M., & Mir, R. (2017). OpenEpi: open source epidemiologic statistics for public health. 2013. OpenEpi. com.
24. Birkett, M. A., & Day, S. J. (1994). Internal pilot studies for estimating sample size. Statistics in medicine, 13(23-24), 2455–2463.https://doi.org/10.1002/sim.4780132309
25. Long Y, Zhao XT, Liu C, Sun YY, Ma YT, Liu XY, et al. A Case-Control Study of the Association of the Polymorphisms of *MTHFR* and *APOE* with Risk Factors and the Severity of Coronary Artery Disease. Cardiology. 2019;142(3):149–157. https://doi.org/10.1159/000499866
26. Kharrazi H, Vaisi Raygani A, Sabokroh AR, Pourmotabbed T. Association between apolipoprotein E polymorphism and coronary artery disease in the Kermanshah population in Iran. Clin Biochem. 2006; 39(6): 613–6. https://doi.org/10.1016/j.clinbiochem.2006.02.002
27. Coassin S, Schönherr S, Weissensteiner H, Erhart G, Forer L, Losso JL, et al. A comprehensive map of single-base polymorphisms in the hypervariable LPA kringle IV type 2 copy number variation region. J Lipid Res. 2019 Jan; 60(1): 186–99. https://doi.org/10.1194/jlr.m090381
28. Zhong Z, Wu H, Wu H, Zhao P. Analysis of apolipoprotein E genetic polymorphism in a large ethnic Hakka population in southern China. Genet Mol Biol. 2018; 41(4): 742–9. https://doi.org/10.1590/1678-4685-gmb-2017-0301
29. Ma W, Ren X, Zhang L, Dong H, Lu X, Feng W. Apolipoprotein E Gene Polymorphism and Coronary Artery Disease Risk Among Patients in Northwest China. Pharmgenomics Pers Med. 2021 Dec; 14:1591–1599. https://doi.org/10.2147/pgpm.s338285
30. Chaudhary R, Likidlilid A, Peerapatdit T, Tresukosol D, Srisuma S, Ratanamaneechat S, et al. Apolipoprotein E gene polymorphism: Effects on plasma lipids and risk of type 2 diabetes and coronary artery disease. Cardiovasc Diabetol. 2012; 11(1): 36. https://doi.org/10.1186/1475-2840-11-36
31. Larifla L, Armand C, Bangou J, Blanchet-Deverly A, Numeric P, Fonteau C, et al. Association of APOE

gene polymorphism with lipid profile and coronary artery disease in Afro-Caribbeans. PLoS One. 2017; 12(7): e018162. https://doi.org/10.1371/journal.pone.0181620
32. Lin Y, Yang Q, Liu Z, Su B, Xu F, Li Y, et al. Relationship between Apolipoprotein E Genotype and Lipoprotein Profile in Patients with Coronary Heart Disease. Molecules. 2022; 27(4): 1–14. https://doi.org/10.3390/molecules27041377
33. Prakash D. Zende PSK. Association between apolipoprotein E polymorphism and lipid profile in patients of myocardial infarction. The Medical Journal of Basrah University. 2020; 38(2): 8–14
34. Zhang Y, Tang HQ, Peng WJ, Zhang BB, Liu M. Meta-analysis for the association of apolipoprotein e ε2/ε3/ε4 polymorphism with coronary heart disease. Chin Med J (Engl). 2015; 128(10): 1391–8. https://doi.org/10.4103/0366-6999.156803
35. Xu M, Zhao J, Zhang Y, Ma X, Dai Q, Zhi H, et al. Apolipoprotein E Gene Variants and Risk of Coronary Heart Disease: A Meta-Analysis. Biomed Res Int. 2016; 2016: 3912175. https://doi.org/10.1155/2016/3912175
36. Shao A, Shi J, Liang Z, Pan L, Zhu W, Liu S, et al. Meta-analysis of the association between Apolipoprotein E polymorphism and risks of myocardial infarction. BMC Cardiovasc Disord. 2022; 22(1): 126. https://doi.org/10.1186/s12872-022-02566-0
37. Ashiq S, Ashiq K. The association of apolipoprotein -E (APOE) gene polymorphisms with coronary artery disease: a systematic review and meta-analysis. Egyptian Journal of Medical Human Genetics. 2021; 22: 16. https://doi.org/10.1186/s43042-021-00135-2
38. Wang YL, Sun LM, Zhang L, Xu HT, Dong Z, Wang LQ, et al. Association between Apolipoprotein E polymorphism and myocardial infarction risk: A systematic review and meta-analysis. FEBS Open Bio. 2015; 5: 852–8. https://doi.org/10.1016/j.fob.2015.10.006
39. Koch W, Hoppmann P, Schömig A, Kastrati A. Apolipoprotein E gene ε2/ε3/ε4 polymorphism and myocardial infarction: Case-control study in a large population sample. Int J Cardiol. 2008; 125(1): 116–7. https://doi.org/10.1016/j.ijcard.2007.01.012
40. Xu H, Li H, Liu J, Zhu D, Wang Z, Chen A, et al. Meta-analysis of apolipoprotein e gene polymorphism and susceptibility of myocardial infarction. PLoS One. 2014; 9(8): e104608. https://doi.org/10.1371/journal.pone.0104608
41. Zhang Y, Tang HQ, Peng WJ, Zhang BB, Liu M. Meta-analysis for the association of apolipoprotein e ε2/ε3/ε4 polymorphism with coronary heart disease. Chin Med J (Engl). 2015; 128(10): 1391–8. https://doi.org/10.4103/0366-6999.156803
42. Liu Q, Wu H, Yu Z, Huang Q, Zhong Z. APOE gene ε4 allele (388C-526C) effects on serum lipids and risk of coronary artery disease in southern Chinese Hakka population. J Clin Lab Anal. 2021; 35(9): e23925. https://doi.org/10.1002/jcla.23925
43. Gan C, Zhang Y, Liang F, Guo X, Zhong Z. Effects of APOE gene ε4 allele on serum lipid profiles and risk of cardiovascular disease and tumorigenesis in southern Chinese population. World J Surg Oncol. 2022 Sep 3; 20(1): 280. https://doi.org/10.1186/s12957-022-02748-2
44. Li Y, Chen L, Shao Y, Zhang M, Zhi L, Lu Y. The effect of apolipoprotein E gene polymorphism and Lp(a) levels on coronary artery disease with atrial fibrillation. Journal of International Medical Research. 2022; 50(7): 3000605221109387. https://doi.org/10.1177/03000605221109387
45. Wu Z, Sheng H, Chen Y, Tang J, Liu Y, Chen Q, et al. Copy number variation of the Lipoprotein(a) (LPA) gene is associated with coronary artery disease in a southern Han Chinese population. Int J Clin Exp Med. 2014; 7(10): 3669–77.