

Correlation of Apo E Gene Polymorphism with Recurrent Acute Coronary Syndrome

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History

- Submission Date: 01-04-2023;
- Review completed: 19-05-2023;
- Accepted Date: 22-05-2023.

DOI : 10.5530/pj.2023.15.100

Article Available online

<http://www.phcogj.com/v15/i6>

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ABSTRACT

Background: Recurrent cardiovascular disease (CVD) incidence puts patients at higher risk for mortality and morbidity. One of the CVD symptoms is acute coronary syndrome (ACS). Many genetic polymorphisms are CVD risk factors. This study's purpose was to investigate the correlation between recurrent ACS incidence and apolipoprotein E (ApoE) gene polymorphism. **Method:** Case-control design was used in this study. About 90 patients who visited the cardiology and internal medicine clinics at UNAIR Hospital in Surabaya, Indonesia, served as the study's subjects. There were 30 patients with recurrent ACS, 30 patients with a single ACS, and 30 patients with no history of cardiovascular disease. Afterward, using the polymerase chain reaction-restriction fragment length method, the ApoE gene polymorphism examination was carried out. The Tropical Disease Center UNAIR Laboratory conducted all laboratory testing. **Results:** In the recurrent ACS group, ApoE polymorphism genotype patterns were 5 subjects for $\epsilon 2 \epsilon 2$ (16.67%), 23 subjects for $\epsilon 3 \epsilon 3$ (76.66%), and 2 subjects for $\epsilon 4 \epsilon 4$ (6.67%). Meanwhile, in the single ACS group, ApoE polymorphism genotype patterns were 6 subjects for $\epsilon 2 \epsilon 2$ (20%), 22 subjects for $\epsilon 3 \epsilon 3$ (73.4%), 1 subject for $\epsilon 4 \epsilon 4$ (3.33%), and 1 subject for $\epsilon 2 \epsilon 3$ (3.33%). And, in the non-ACS group, ApoE polymorphism genotype patterns were 4 subjects for $\epsilon 2 \epsilon 2$ (13.34%), 25 subjects for $\epsilon 3 \epsilon 3$ (83.33%), and 1 subject for $\epsilon 4 \epsilon 4$ (3.33%). There was no correlation of ApoE gene polymorphism with recurrent ACS incidence by Chi-square analysis ($p > 0.05$). **Conclusion:** ApoE gene polymorphism cannot significantly affect recurrent ACS incidence.

Key words: Acute Coronary Syndrome, ApoE gene, Polymorphism, PCR RFLP, Public Health.

INTRODUCTION

Cardiovascular disease (CVD) ranks as the leading global cause of death. More than 75 percent of CVD fatalities take place in developing nations, including Indonesia.¹ Acute coronary syndrome (ACS) is one of CVD's common clinical signs. ACS prevalence in the Asia Pacific region had risen to 5%.² Stable angina, ST-elevation myocardial infarction (STEMI), and non-STEMI is the three types of ACS.³

Those who survive a first heart attack, particularly those with ACS, are more likely to experience subsequent attacks. 10% of them will experience repeated heart attacks in the first year. If there are no cardiovascular events in the first year, 3.6% of these individuals will experience a repeat attack in the second year and 5.6% in the fourth year.⁴ A subsequent attack has a higher mortality rate than a primary attack.⁵

Numerous efforts have been made to prevent recurrent ACS. These include risk factor management, medication, and non-pharmacological therapy. Age, gender, smoking, hypertension, dyslipidemia, and diabetes mellitus are risk factors for recurrent ACS (DM). For secondary preventive measures to be successful, it is essential to identify risk factors for recurrent ACS. Genetics is one of the aspects that must be taken into consideration.^{6,7}

Apolipoprotein E (ApoE) gene has a polymorphism consisting of three different alleles, namely $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$. This polymorphism is caused by amino acid

substitutions at 112 and 115. Gene $\epsilon 3$ has cysteine and arginine at amino acid positions 112 and 158. This gene is the most common type found in the population. On the other hand, in gene $\epsilon 2$ arginine is substituted by cysteine at amino acid position 158, while cysteine is substituted by arginine at position 112.⁸

Furthermore, ApoE gene polymorphism can affect the structure and quantity of ApoE. Different structures and quantities of ApoE will show different biological activity. Plasma ApoE will decrease caused of ApoE gene polymorphism. Decrement of plasma ApoE leads to higher LDL cholesterol.^{9,10} High LDL cholesterol, ≥ 100 mg/dL, increases the incidence of recurrent ACS.¹¹ Thus, polymorphism of the Apolipoprotein E (ApoE) gene is a potential genetic factor as a risk factor for recurrent ACS.¹² Based on the background above, this study aims to analyze the correlation of ApoE gene polymorphisms with recurrent ACS.

METHOD

A case-control study approach was used to design this study. 90 outpatient cardiac and internal medicine clinics at UNAIR Hospital from October 2021 to January 2022 served as the study's subjects. These 90 participants were split up into three groups: those with recurring ACS, those with single ACS, and those without ACS. Each group had 30 participants. The individuals in the recurrent ACS group experienced at least two ACS incidents between January 2016 and December 2020. The subjects who had previously experienced an ACS attack made comprised the sale

Cite this article: Fuadi MR, Nugraha J, Suryawan IGR, Kahar H, Aryati, Prabowo GI, et al. Correlation of Apo E Gene Polymorphism with Recurrent Acute Coronary Syndrome. Pharmacogn J. 2023;15(3): 450-453.

ACS group. Moreover, no ACS group included patients who had never experienced an ACS incident. All of the chosen subjects were older than 40. The subjects of this study were not those receiving radiation, chemotherapy, or treatment for psychotic disorders.

Next, information was gathered from patient medical records including age, gender, smoking history, blood glucose levels, lipid profiles, and blood pressure. The history of diabetes mellitus was determined based on blood glucose levels. Dyslipidemia was established by lipid profile laboratory tests. Hypertension history was confirmed by blood pressure data.

After that, 3 mL of the patient's blood was drawn when they had routine control checks at the UNAIR Hospital's Outpatient Installation. The blood was subsequently kept in two BD vacutainer tubes, specifically those that contained EDTA anticoagulant (3 mL). The ITD UNAIR Surabaya laboratory subsequently handled and stored the specimens and conducted the laboratory tests.

The buffy coat specimen was then subjected to DNA extraction by the QIAGEN QIAmp DNA Mini Kit (50) Cat insert kit's instructions. No. 51304. The polymerase chain reaction-restriction fragment length polymorphism (PCR RFLP) technique was then used to analyze the polymorphism of the ApoE gene. The primer sequence used in the PCR amplification was 5'-TAA GCT TGG CAC GGC TGT CCA AGG A-3' for the forward primer and 5'-ACA GAA TTC GCC CCG GCC TGG TAC AC-3' for the reverse primer.¹³ A 20 µl mixed solution containing 12.5 µl of PCR master mix (GoTaq Green: Promega), 1 µl of forward primer, 1 µl of reverse primer, 1 µl of DNA template, and 0.5 µl of distilled water was used to accomplish the PCR amplification.

Initial denaturation at 94°Celsius (C) for five minutes was followed by 35 cycles of 30 seconds at 94°C (Denaturation), 30 seconds at 58°C (Annealing), and 30 seconds at 72° C C. (extension). The last extension was set for 5 minutes at 72° C C. The amplification products (3 µl) and Red Safe Nucleic Acid staining solution (2 µl) were electrophoresed in 3% agarose gel using the Mupidexu electrophoresis machine, and the results were seen under ultraviolet light. To identify the ApoE genotype from amplification products, an electrophoresis gel displayed 246 bp fragments.

Each PCR result that remained after gel electrophoresis was digested with the *HhaI* enzyme. 3 µl of PCR product, 1.5 µl of buffer, and 0.5 µl of restriction enzyme were used in the digesting processes. At 37 °C, restriction analysis was carried out for two hours. The digestion products were separated into 3% agarose gels dyed with Red Safe Nucleic Acid, and they were then seen under an ultraviolet light source.

Data analysis

The age disparities between the groups were compared using the ANOVA test. To evaluate the correlation between gender, DM history, hypertension, dyslipidemia, smoking, and ApoE gene polymorphisms with the incidence of ACS, bivariate chi-square or Fisher's Exact analysis was used. Then, using a 95% Confidence Interval (CI), the Crude Odds Ratio (COR) was used to express how the ApoE gene polymorphism affected the incidence of ACS. Hence, a p-value <0.05 is regarded to be statistically significant. The SPSS 26 program was used to conduct each of these analyses.

RESULT

Table 1 displays the characteristics of the study participants. It says there was no age difference between the three groups (p: 0.555). The table also showed the relationship between recurrent ACS and hypertension, diabetes, and dyslipidemia. Age and smoking habits, however, are unrelated to the occurrence of recurrent ACS.

There were four ApoE genotypes in this study, consisting of ε2/ε2, ε3/ε3, ε4/ε4, and ε2/ε3 alleles. ApoE genotype was determined based on

Table 1: The characteristics of research subjects.

Characteristics	Group			P
	Recurrent ACS (n = 30)	Single ACS (n = 30)	Non-ACS (n=30)	
Age (in years)				
Mean ± SD	61.53±8.57	59.10 ± 8.16	59.63 ± 10.33	0.555 ^a
Sex (n. %)				
Male	22 (73.3)	23 (76.7)	16 (53.3)	0.112 ^b
Female	8 (26.7)	7 (23.3)	14 (46.7)	
Hypertension	29 (96.7)	24 (80.0)	20 (66.7)	0.035 ^c
Diabetes Mellitus	14 (46.7)	14 (46.7)	21 (70.0)	0.042 ^b
Dyslipidemia	28 (93.3)	25 (83.3)	15 (50)	0.003 ^c
Smoker	5 (16.7)	6 (20.0)	1 (3.3)	0.140 ^c

Note: ^aANOVA test, ^bChi-square test, ^cFischer's exact test
ACS: Acute Coronary Syndrome, n: number, p: probability, SD: Standard Deviation

Table 2: The genotype prevalence of ApoE polymorphism.

ApoE Genotype	Group			p
	Recurrent ACS (n = 30)	Single ACS (n = 30)	Non-ACS (n=30)	
ε2ε2	5 (16,67%)	6 (20%)	4 (13,34%)	
ε3ε3	23 (76,66%)	22 (73,34%)	25 (83,33%)	0,89 ^a
ε4ε4	2 (6,67 %)	1 (3,33%)	1 (3,33%)	
ε2ε3	-	1 (3,33%)	-	

Note:^aFisher's exact test
ApoE: Apolipoprotein E ACS: Acute coronary syndrome, ε: epsilon, p: probability

Table 3: The OR values of ApoE gene polymorphisms on the incidence of ACS.

ApoE genotype	Non-ACS on Single ACS			Single ACS on Recurrent ACS		
	OR	CI 95%	p	OR	CI 95%	p
ε2ε2	1.71	0.43-6.84	0.5	0.8	0.21-2.29	0.74
ε4ε4	1.14	0.07-19.26	1	1.93	0.16-22.63	1

ApoE: Apolipoprotein E, ACS: Acute Coronary Syndrome, ε: epsilon, p: probability, OR: Odd Ratio, CI: Confident Interval

fragment sizes of PCR RFLP results. The ε2/ε2 allele had 91 bp and 83 bp, ε3/ε3 allele had 91 bp and 48 bp, ε4/ε4 allele had 72 bp and 48 bp, while ε2/ε3 allele had 91 bp, 83 bp, and 48 bp.ε3/ε3 allele is a normal genotype in the population.

Moreover, Table 2 depicts the genotypic distribution of ApoE gene polymorphisms. The recurrent ACS group had genotype patterns, including 5 subjects for ε2ε2 (16.67%), 23 subjects for ε3ε3 (76.66%), and 2 subjects for ε4ε4 (6.67%). Meanwhile, the genotype patterns in the single ACS group were 6 subjects for ε2ε2 (20%), 22 subjects for ε3ε3 (73.4%), as well as ε4ε4 and ε2ε3 as many as 1 subject for each (3.33%). And, the genotype patterns in the non-ACS were 4 subjects for ε2ε2 (13.34%), 25 subjects for ε3ε3 (83.33%), and 1 subject for ε4ε4 (3.33%). Nevertheless, the results of statistical analysis using Fisher's exact test showed that there was no correlation between the ApoE gene polymorphisms with ACS incidence (p > 0.05).

Furthermore, table 3 illustrates the Odd Ratio (OR) values of the ApoE genotype pattern on the incidence of ACS. The ε2ε2 genotype had an OR value of 1.71 (0.43-6.84) for the initial attack, while for the recurrent attack, it had an OR value of 0.8 (0.21-2.99). On the other hand, the OR value of the ε4ε4 genotype was 1.14 (0.07-19.26) for the initial attack, while for recurrent ACS attack, the OR value was 1.93 (0.16 -22.63). However, there was no statistical significance for all OR values of the ApoE genotype (p>0.05).

DISCUSSION

The highest proportion of alleles, $\epsilon 3\epsilon 3$, was found in the non-ACS group (83.33%), followed by the recurrent ACS group (76.66%), and the single ACS group (73.34%). The most common variant genotype found in the polymorphism was the $\epsilon 2\epsilon 2$ allele. Most of the $\epsilon 2\epsilon 2$ allele proportions were found in the single ACS group (20%), followed by the recurrent ACS group (16.67%) and the non-ACS (13.33%). On the other hand, another genotype variant, namely $\epsilon 4\epsilon 4$, was mostly found in the recurrent ACS group (6.67%) followed by the single ACS group and the non-ACS group with the same proportion (3.33%). There was only one subject in the single ACS group with the $\epsilon 4\epsilon 4$ genotype variant.

The proportion of alleles in this research, however, was not much different from previous research conducted in Indonesia, which reveals that the $\epsilon 3\epsilon 3$ allele was the most common allele found in the healthy population in Surabaya. In the previous research, the proportion of $\epsilon 2\epsilon 2$ genotype was 8.5%. However, there was no $\epsilon 4\epsilon 4$.¹⁴ In addition, the results of the Chi-Square analysis in this research showed that there was no correlation between ApoE gene polymorphisms with ACS incidence in both initial and recurrent attacks.

The result above was similar to the study conducted in Gaza Population, Palestine, that there was no correlation between ApoE gene polymorphisms with coronary heart disease.¹⁵ In line with the previous research, meta-analysis research conducted also reported that the $\epsilon 2\epsilon 2$, $\epsilon 2\epsilon 3$, and $\epsilon 2\epsilon 4$ alleles had no significant risk factors for coronary heart disease.¹⁶ Research on genetic analysis in Turkey even revealed the same result that there was no correlation between ApoE gene polymorphisms with the incidence of ACS.^{17,18}

Nevertheless, there were several previous studies showed that there was a correlation between ApoE gene polymorphisms with ACS incidence. For example, Dzimiri *et al.*¹⁹ reported in Saudi that $\epsilon 4$ correlated with an increased risk of coronary artery disease (CAD), whereas $\epsilon 2$ was associated with classic predictors of atherosclerosis, such as elevated serum triglycerides and total cholesterol, as well as hypertension and diabetes. Compared to controls (5%), only $\epsilon 4$ was elevated in CAD patients (20%).¹⁹ A study conducted by Elousa *et al.*¹² in Boston, United States of America, reported that $\epsilon 2$ was associated with lower carotid atherosclerosis in women, and the $\epsilon 4$ allele was associated with higher internal carotid intimal-medial thickness in diabetic men.¹²

Hence, it can be indicated that studies on different populations can show different results. However, the results of this research showed that there was no correlation between ApoE gene polymorphisms with ACS incidence. ACS is a disease caused not only by one factor but also by various factors, including age, sex, genetics, diabetes mellitus, smoking, dyslipidemia, hypertension, and obesity.

Design of the study was unmatched case control because it was not easy to get matched subjects for each group in the population. It was difficult to find ACS subjects without major risk factors such as hypertension, diabetes mellitus, and dyslipidemia. Small size samples in this study can also influence the results.

CONCLUSION

Based on the result above, it can be concluded that $\epsilon 3\epsilon 3$ allele can be considered as the most common allele in all no-ACS, single ACS, and recurrent ACS groups. Moreover, it is also known that there is no statistically significant correlation between ApoE gene polymorphisms with the incidence of single or recurrent ACS. Nevertheless, further research must consider a larger number of subjects and other types of genetic polymorphisms known as potential risk factors for ACS.

DECLARATION OF COMPETING INTEREST

None of the authors have any conflicts of interest of declaration. This study has not involved any third party in research process. This study has also never been published elsewhere.

STATEMENT OF FUNDING

The study has received no financial support from outside. All of the expenses related to the research process were funded by authors' fund.

ETHICAL CLEARANCE

Approval was obtained from the Ethics Committee of Airlangga University Hospital with the code number 119/KEP/2021. Written informed consent for participation was obtained from all subjects.

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Cite this article: Fuadi MR, Nugraha J, Suryawan IGR, Kahar H, Aryati, Prabowo GI, et al. Correlation of Apo E Gene Polymorphism with Recurrent Acute Coronary Syndrome. *Pharmacogn J.* 2023;15(3): 450-453.