

## Effect of MTHFR A1298C Gene Polymorphism on Acute Coronary Syndrome

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### Original Article

#### Abstract

**BACKGROUND:** Cardiovascular disease (CVD) is the leading cause of mortality worldwide. Acute coronary syndrome is a manifestation of CVD. In Indonesia, limited studies have been conducted on genetics as a potential risk factor for acute coronary syndrome (ACS). Consequently, this study aimed to examine the effect of the methylenetetrahydrofolate reductase (MTHFR) A1298C gene polymorphism on the incidence of ACS.

**METHOD:** The study employed a case-control design. Outpatients from the cardiology and internal medicine clinics at the University of Airlangga (UNAIR) Hospital in Surabaya, Indonesia, constituted the study population. The case group comprised 60 patients with a history of ACS, while the control group consisted of 30 patients without a history of cardiovascular complaints. MTHFR A1298C gene polymorphism examination was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR RFLP) method at the Tropical Disease Center UNAIR Laboratory.

**RESULTS:** Among the ACS group, 29 (48.1%), 13 (21.7%), and 18 (30%) of the individuals had AA, AC, and CC genotype patterns, respectively. In the control group, 16 individuals had AA (53.3%), 6 AC (20%), and 8 CC (26.7%). The C allele variant was identified in 41% of the ACS group and 37% of the control group. The odds ratio (OR) for the incidence of ACS was 1.195 (95% confidence interval [CI]; 0.381-3.752), 1.241 (95% CI; 0.481-3.486), and 1.222 (95% CI; 0.381-3.752). Chi-square analysis revealed no association between MTHFR A1298C gene polymorphism and the incidence of ACS ( $P > 0.05$ ).

**CONCLUSIONS:** MTHFR A1298C gene polymorphism did not significantly affect ACS incidence.

**Keywords:** Cardiovascular disease, Risk factors, Genetic, Polymerase chain reaction, Restriction fragment length polymorphism

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#### Introduction

Cardiovascular disease is the number one killer worldwide, including in Indonesia. Deaths caused by the disease reached 17.5 million in 2012. The death rate is estimated to increase dramatically to 22.2 million in 2030. Coronary heart disease (CHD) was the second leading cause of death in 2012, following stroke.<sup>1,2</sup>

Acute coronary syndrome (ACS) is one manifestation of CHD. ACS is characterized by unstable angina and myocardial infarction with or without ST-segment elevation on an electrocardiogram (ECG).<sup>3</sup> Prevalence of ACS in the Asia Pacific is 5%.<sup>4</sup>

Various efforts have been made to prevent ACS, including controlling risk

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factors, pharmacological therapy, and non-pharmacological efforts.<sup>5</sup> Genes and particular DNA (deoxyribonucleic acid) sequence variations have been identified as hereditary risk factors for ACS.<sup>6</sup> The methylenetetrahydrofolate reductase (MTHFR) gene regulates the production of methylenetetrahydrofolate reductase, where the enzyme converts homocysteine to methionine and S-adenosylmethionine.<sup>7</sup> MTHFR A1298C gene is a genetic polymorphism caused by adenine to cytosine substitution at position 1,298. This polymorphism results in substituting Glu-429 with alanine and frequently causes hyperhomocysteinemia.<sup>8</sup>

Homocysteine has been identified as a risk factor for atherosclerosis in vascular disease since the early 1990s, where elevated homocysteine has been associated with hypercoagulable conditions.<sup>9</sup> In addition, there is an association between vascular disease and hyperhomocysteinemia, especially in Asian populations.<sup>10</sup> Consequently, this study aimed to analyze the effect of MTHFR A1298C gene polymorphism on ACS incidence.

### Material and Methods

This research employed an unmatched case-control design. Subjects were selected based on the 1567 history of high-sensitive troponin (hsTn) I test in laboratory information system data at the Universitas Airlangga (UNAIR) Hospital Surabaya, Indonesia. Case group criteria for the ACS group included being over 40 years old and having a history of acute coronary syndrome treatment between January 2016 and December 2020. Patients without a lifetime history of ACS who routinely visited the cardiology and internal medicine clinic at UNAIR hospital from January to December 2021 constituted the control group.

Diagnosis of ACS is based on the patient's clinical symptoms, ECG, and troponin test.<sup>11</sup> The hsTn I test at UNAIR Hospital is conducted using MINI VIDAS. The test is positive if the troponin levels are  $\leq 19$  ng/L.<sup>12</sup> If the result of the hs troponin test is 19ng/L, ACS criteria can be met with 20 min of chest

pain complaint with or without ST elevation on ECG examination.<sup>3,11</sup>

Subjects with chemotherapy and radiotherapy were excluded. Data on age, sex, smoking history, blood glucose, lipid profile, and blood pressure were obtained from the medical record of subjects. UNAIR Hospital's ethics committee provided ethical clearance approval for this research. All subjects signed an informed consent form before participating in the study.

When participants visited the cardiology and internal medicine clinic at UNAIR Hospital, 3 mL of blood was drawn and collected in tubes containing ethylenediaminetetraacetic acid (EDTA) vacutainer (Becton Dickinson). EDTA blood was centrifuged at 2000 rpm for 5 min, and a 200  $\mu$ L buffy coat was pipetted into aliquots. The buffy coat was stored at  $-20$  °C before genomic laboratory testing. All specimens were stored, processed, and analyzed at the Tropical Disease Center UNAIR laboratory in Surabaya, Indonesia.

DNA was extracted from buffy coat specimens per the QIAGEN QIAmp DNA Mini Kit's recommended protocol. MTHFR A1298C gene polymorphism was detected through the polymerase chain reaction-restriction fragment length polymorphism (PCR RFLP) method. Primers used for the 1298 region of MTHFR gene amplification were 5'-CAA GGA GGA GCT GCT GAA GA-3' for forward primer and 5'-CCA CTC CAG CAT CAC TCA CT-3' for reverse primer.<sup>13</sup> PCR amplification was performed with a SelectCycler II Thermal Cycler using 20  $\mu$ L mixed solution containing 12.5  $\mu$ L PCR master mix (GoTaq Green: Promega), 1  $\mu$ L forward primer, 1  $\mu$ L reverse primer, 5  $\mu$ L DNA template, and 0.5  $\mu$ L distilled water.

PCR amplification cycling was set to initial denaturation at 94 °C for 5 min, followed by 35 cycles of 30 sec at 94 °C (denaturation), 30 sec at 54 °C (Annealing), 30 seconds at 72 °C (extension), and followed by 5 min at 72 °C for the final extension. The amplification products (3  $\mu$ L) and Red Safe Nucleic acid staining solution (2  $\mu$ L) were electrophoresed (Mupid-exU electrophoresis system) in 3%

agarose gel and visualized under ultraviolet light. Amplification products showed 128 bp fragments on an electrophoresis gel.

After gel electrophoresis, each remaining PCR product was digested with the restriction *MboII* (New England BioLabs, USA) separately. The digestion reactions contained 3  $\mu$ l of PCR product, 1.5  $\mu$ l of buffer, and 0.5  $\mu$ l of restriction enzyme. Afterward, restriction analysis was performed for 2 h at 37 °C. The digestion products were subsequently separated on Red Safe Nucleic acid-stained 3% agarose gels and visualized under ultraviolet light.

### Statistical Analysis

The ACS and control groups' ages were compared using the independent sample t-test. Chi-square or Fisher's exact test was utilized to examine the relationship between sex, diabetes mellitus (DM), hypertension, dyslipidemia, smoking history, and ACS incidence. AA, AC, and CC genotypes exist for the MTHFR A1298C gene polymorphism. Genotype AA is a normal or wild-type category, whereas genotypes AC and CC are polymorphism categories. Chi-square was utilized to assess the relationship between MTHFR A1298C gene polymorphism and ACS incidence. Fisher's exact analysis was employed when the chi-square test was insufficient. The odds

ratio (OR) value was calculated to determine the relative risk of ACS incidence by MTHFR A1298C gene polymorphism. A P-value < 0.05 was considered to be statistically significant. All statistical analysis was calculated by IBM SPSS (v. 26) software.

## Results

This study included 60 participants in the ACS group and 30 in the control group. Table 1 shows the demographic and clinical characteristics of the subjects. There was no age difference between the ACS and control groups. ACS incidence was correlated with the male sex. There were three subjects with a troponin I-value > 40000. MINI VIDAS's upper detection limit for hs troponin I is 40000 ng/L. Hypertension, DM, dyslipidemia, and smoking history differed significantly between ACS and the control group.

PCR amplification results showed 128 bp (base pair) bands. The PCR-obtained 128-bp fragment contained two recognition sites for the restriction enzyme *MboII*. Following digestion, the wild-type genotype 1298AA originated from three fragments (28, 28, and 72-bp) but was only identified by the 72-bp fragment, as the other two were not retained in the gel. The genotype CC was determined

**Table 1.** Demographic and clinical characteristics of the research subjects

Characteristic	Group		p-value
	ACS (n= 60)	Control (n = 30)	
Age (year)			
Mean $\pm$ SD	60.3 $\pm$ 8.43	59.6 $\pm$ 10.3	0.28*
Median (min-max)	60 (42-84)	62 (41 – 85)	
hs troponin I (ng/L)			
min – max	19 – >40000	NA	NA
Sex (n, %)			
male	45 (75.0)	16 (53.3)	0.040**
female	15 (25.0)	14 (46.7)	
Hypertension (n, %)	54 (90.0)	20 (66.7)	0.040**
Diabetes mellitus (n, %)	29 (48.3)	21 (70.0)	0.010**
Dyslipidemia (n, %)	57 (95.0)	15 (50.0)	0.001***
Smoking history (n, %)	11 (18.3)	1 (3.3)	0.043***

\*Independent samples t-test, \*\* chi-square, \*\*\* Fisher's exact test

ACS: acute coronary syndrome, n: number, SD: standard deviation, hs troponin I: high-sensitive troponin I.

by the presence of the 100-bp fragment alone, whereas the genotype AC was determined by the presence of both the 72-bp and 100-bp fragments in the gel.<sup>13</sup>

The distribution of the MTHFR A1298C polymorphism genotype is shown in Table 2. There were 29 (48.3%) AA genotypes,

13 (21.7%) AC genotypes, and 18 (30%) CC genotypes in the ACS group. Genotype frequency in the control group was 16 (53.3%) AA genotype, 6 (20.0%) AC genotype, and 8 (26.7%) CC genotype. The C allele variant was found in 41% of the ACS population and 37% of the control population. The OR for

**Table 2.** Distribution of MTHFR A1298C polymorphism allele and genotype frequencies

Genotype/allele	ACS (n:60)	Control (n:30)	p-value	OR (CI 95%)	$\chi^2$
AA	29 (48.3%)	16 (53.3%)	Ref.	Ref.	Ref.
AC	13 (21.7%)	6 (20%)	0.09	1.19 (0.38,3.75)	0.903
CC	18 (30.0%)	8 (26.7%)	0.17	1.24 (0.44,3.49)	
A	0.59	0.63	Ref.	Ref.	Ref.
C	0.41	0.37	0.20	1.22 (0.51,2.94)	0.655

ACS: acute coronary syndrome, n: number, OR: odds ratio, CI: confidence interval, A: adenine, C: cytosine

ACS incidence in subjects with genotypes AC, CC, and allele C were 1.195 (95% confidence interval [CI]; 0.381-3.752); 1.241 (95% CI; 0.442-3.486); and 1.222 (0.508-2, 939), respectively. Chi-square statistical analysis revealed no significant association between MTHFR A1298C gene polymorphism with ACS incidence ( $P > 0.05$ ).

## Discussion

This study demonstrated that the proportion of genotype patterns in ACS and the control group was comparable. Mohammad et al. (2021) reported that AA genotypes were higher and AC genotypes were lower in the ACS group in a study conducted in Sudan.<sup>14</sup> Eftychiou et al. (2012) reported no difference in AA, AC, and CC genotype frequency between myocardial infarction patients and the control group.<sup>15</sup>

The present study observed no significant association between MTHFR A1298C polymorphism and ACS incidence. The findings are consistent with a meta-analysis study conducted by Alizadeh et al. (2016), which found no significant relationship between MTHFR A1298C polymorphism and the risk of myocardial infarction.<sup>16</sup> Mohammad et al. (2021), Eftychiou et al. (2012), and Nasiri et al. (2014) reported similar results.<sup>14-17</sup>

ACS is influenced by many factors, including genetic factors. Environmental or other genetic factors can potentially interact with the MTHFR gene; thus, the MTHFR A1298C gene polymorphism and ACS were not correlated in the present study. Other factors, such as diet, nutritional status, hormones, and activity levels, affect the MTHFR gene polymorphism. This study's small sample size could have also affected its results.

MTHFR A1298C polymorphism is one of 20 polymorphisms gene encoding the MTHFR enzyme. These polymorphisms result in a deficiency of the enzyme function associated with homocysteine increment and cardiovascular disease (CVD). The function is still 60% due to A1298C MTHFR gene polymorphism. In comparison, C677T polymorphism decreases enzyme function to only 30%.<sup>18</sup> Future research should investigate the association between the C677T MTHFR gene polymorphism and ACS.

## Study Limitation

The study's design was unmatched case-control because finding matched subjects for each population group was challenging. In addition, it was difficult to locate ACS subjects without significant risk factors such as hypertension,

diabetes, and dyslipidemia.

### Conclusion

A polymorphism in the MTHFR A1298C gene was observed in both ACS and the control population. The Chi-square test revealed no significant association between the MTHFR A1298C gene polymorphism and the incidence of ACS ( $P > 0.05$ ). Finally, it is recommended that future research analyze an additional potential gene polymorphism as an ACS risk factor, particularly the MTHFR C677T gene polymorphism.

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### Conflict of Interests

The authors have no conflict of interest to declare.

### Authors' Contribution

Robiul Fuadi and Jusak Nugraha conceived and designed the study. Robiul Fuadi performed data analysis. Robiul Fuadi wrote the first draft of the manuscript. Jusak Nugraha, IGR Suryawan, Hartono Kahar, Aryati, Gwenny I Prabowo, Budi Utomo, and Reny P'tishom made critical revisions to the manuscript and significantly contributed to the text. All authors approved the final version of the manuscript.

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