SHORT COMMUNICATION

Val279Phe variant of Lp-PLA2 is a risk factor for a subpopulation of Indonesia patients with acute myocardial infarction

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Abstract Lipoprotein-associated phospholipase A2 (Lp-PLA2), a member of the phospholipase A2 superfamily, is an enzyme that hydrolyses phospholipids, is found in blood circulation as a sign of inflammation, and takes a role in atherogenesis. There is an epidemiologic relation between increased Lp-PLA2 levels and coronary heart disease. Lp-PLA2 is an enzyme that is produced by macrophages and takes a role as an independent predictor of a coronary event. A genetic variant of Val279Phe on the Lp-PLA2 gene has been reported with various results in Japan, China, Korea, and Caucasian populations. This study aims to analyse the influence of the Val279Phe genetic variant on acute myocardial infarction (AMI) at Saiful Anwar Hospital, Indonesia. This study was conducted on 151 patients (111 AMI patients and 40 non-AMI patients). The genetic variant of Val279Phe was identified through a genotyping method. There were no significant differences in age, total cholesterol level, LDL-C (low-density lipoprotein cholesterol) level, and family history data between AMI and non-AMI patients. However, AMI patients had low HDL-C (high-density lipoprotein cholesterol), triglyceride levels, dyslipidaemia, and hypertension risk factors compared to non-AMI patients. The frequency of the GG genotype (279Val) was dominant in both AMI and non-AMI groups. Further analysis suggested that the GG genotype has a 2.9 times greater risk of AMI compared to the GT/TT genotype (279Phe). This study concluded that the

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Val279Phe genetic variant undoubtedly influenced AMI risk, which is a warrant for further development of early detection and improving strategy to prevent AMI in patients. Copyright © 2016, Chongqing Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Cardiovascular disease causes 30% of deaths in the world. One cardiovascular disease is coronary heart disease (CHD), that caused one in six death cases in America in 2008. About half a million people in China have had myocardial infarction, and 2 million people have suffered from a stroke. A survey of the Indonesia Health Department in 2007 showed that CHD was in third position as a cause of death disease after stroke and hypertension. Atherosclerosis is a chronic, progressive, and fundamental process that causes CHD. The expression of lipoprotein-associated phospholipase A2 (Lp-PLA2) triggers atherosclerosis. Therefore, Lp-PLA2 is used as a biomarker for cardiovascular events in the future. This enzyme is produced by monocytes as an effect of inflammation mediator stimulation. In blood circulation, 80% of Lp-PLA2 binds with low-density lipoprotein (LDL) and 15–20% of it binds with high-density lipoprotein (HDL). Lp-PLA2 is also known as platelet-activating factor acetylhydrolase (PAF-AH) because it hydrolyses platelet-activating factor (PAF). PAF is a substrate that correlates with thrombogenesis and increases the risk of a coronary event. Lp-PLA2 catalyses the degradation of PAF to the biologically inactive products LYSO-PAF and acetate. On the other hand, the enzyme hydrolyses oxidized polyunsaturated fatty acids, producing lysophosphatidylcholine (lysoPC) that upregulates inflammatory mediators, which have potential to be disruptive.

Substitution of guanine (G) for thymine (T) in exon 9 at position 994 leads to the alteration of amino acid synthesis from valine into phenylalanine on residue 279 (Val279Phe) of Lp-PLA2. The 279Phe variant increases the risk of vascular disease and stroke. Moreover, Han’s study indicated that the 279Phe allele was more often found in coronary artery disease (CAD) patients (13.5%) than in healthy controls (9.3%; p = 0.024) in a Chinese population. The severity of atherosclerosis was greater in Val279Phe carriers. This condition was different from observation data in the Korean population which showed that the heterozygous mutation has a lower risk of cardiovascular disease. Enzyme activity of the heterozygous mutation was 23% lower than the homozygous (V/V) mutation in non-acute myocardial infarction (AMI) patients. The aim was to investigate the influence of the Val279Phe genetic variant in men with AMI at Saiful Anwar Hospital, Malang, Indonesia.

Methods

Sample collection

This study was an observational case-control design. Samples were taken consecutively from 151 Indonesian men with age range 30–80 years in Saiful Anwar Hospital, Malang, Indonesia. Patients were divided into two groups, i.e. AMI and non-AMI groups. The AMI group consisted of 111 patients who were diagnosed as AMI patients with or without revascularization using thrombolytics. Levels of cardiac enzymes such as CPK, CKMB, and troponin I were measured and showed an increase in the first 6 h after AMI onset that was higher than normal. Electrocardiogram results showed ST segment deviation in both elevation and depression of anterior, anteroseptal, extensive anterior, inferior, right ventricular, posterior, and new left bundle branch block (LBBB) patterns. Patients were taken care of in the Cardiovascular Care Unit (CVCU), Dr. Saiful Anwar Hospital. The non-AMI group consisted of 40 patients who came to the outpatient cardiology clinic with AMI risk factors without ischaemic symptoms and ECG abnormality. The non-AMI group had traditional risk factors such as smoking, hypertension, dyslipidaemia, and normal treadmill test results. Stratification of risk factors was done to predict cardiovascular events for the next ten years, which grouped patients based on Framingham risk stratification score into low risk, moderate risk, and high risk. Patients who were diagnosed with diabetes mellitus, had had statin therapy for 12 days, had vascular disease (e.g. peripheral artery disease, PAD), infection, malignancy, or were unable to perform the treadmill test were excluded from the study. This study has followed the Code of Ethics of the world Medical Association (Declaration of Helsinki) for experiments in humans and approved by Brawijaya University- Dr. Saiful Anwar Hospital Ethics Committee.

PCR-RFLP

Genotyping was done by using the PCR-RFLP method according to a previous report. A peripheral blood sample was put into a vacutainer with EDTA. DNA isolation was done using a Qiagen QIAamp Whole DNA Isolation Mini Kit corresponding to the manufacturer’s instructions. Isolated DNA was amplified using polymerase chain reaction (PCR) with forward primer 5’CCCCATGAAATGAACAATTAT and reverse 5’GGGGGCAAAAGAATAGCCTTATA at an annealing temperature of 53 °C. RFLP was performed using the PCR product with restriction enzyme BST4Cl that recognizes AC’N’GT. The cutting temperature used for RFLP was 65 °C. The size of PCR-RFLP products was 148 bp and 169 bp for the G allele, and 317 bp for the T allele. Then, the samples were analysed by a sequencing method.

Statistical analysis

Data were presented as mean ± SD. The differences between two sample groups and controls were tested using t-test (normal numeric distribution data) and Mann–Whitney test.
Results

Patients in the non-AMI group performed the treadmill test with normal results. Based on Framingham risk score ATP III, non-AMI patients were divided into three criteria: 11 (27.5%) patients had low-risk criteria, 14 (35%) patients moderate-risk criteria, and 15 (37.5%) patients had high-risk criteria. The HDL-C levels of AMI patients were significantly lower than AMI patients (25.7 ± 3.22 vs. 23.42 ± 3.41; p = 0.000). Triglyceride levels were significantly higher in non-AMI patients than AMI patients (23.42 ± 10.82; p = 0.010). The BMI of non-AMI patients was significantly higher than AMI patients (25.7 ± 3.22 vs. 23.42 ± 3.41; p = 0.000). The difference frequency was a known influence on OR in the AMI group; it was 2.9 times higher than in the non-AMI group (Table 3). The 279Phe variants (GT and TT genotypes) were found in 2.7% of AMI patients and 7.5% of non-AMI patients. These data were correlated with a study in Korea by Jang et al. that showed GT in 17.7% and TT in 1.3% (p = 0.005) of AMI patients, while 25.7% of non-AMI patients were found to have the GT genotype and 1.2% the TT genotype. Also, another study in Korea showed GT/TT genotype frequency was lower in AMI compared to non-AMI patients.13,16,17,20

The OR of GG to GT and TT was 2.9 (95% CI 0.564–15), showing that the GG genotype has a 2.9 times higher AMI risk factor than the GT and TT genotypes. This condition was supported by the Jang study in Korea, which stated that genotype variants of GT and TT had an OR of 0.646 (95% CI 0.490–0.850). The OR of GT to GG was 0.71 (95% CI 0.53–0.95) while the OR of TT to GG was 0.6 (95% CI 0.22–1.6).16 These facts supported that Lp-PLA2 plays a role as a proatherogenic, and an early study on a population with intermediate risk factor is suggested because it probably has the GG genotype. This is in accordance with Yamada and Ichihara’s study which stated that the GG genotype was related to increasing Lp-PLA2 activity in a Japanese population.20,21

Lp-PLA2 activity is high in rupture-prone plaques that have a significant role in the formation and progressivity of atherosclerosis in coronary events. Lp-PLA2 in atherosclerotic plaques causes oxidized LDL to be hydrolysed into lysoPC and oxidized non-esterified fatty acids (oxNEFA) which leads to the migration of leukocytes, inflammation cytokines, amplification of oxidation, and matrix metalloproteinase expression in the lesion area. This condition is caused by the expansion of the necrotic core and attenuated plaque fibrous cap that makes it easier to be ruptured. Lp-PLA2 activity could be used to evaluate cardiovascular risk in the future. On the other hand, inhibition of Lp-PLA2 activity will reduce the volume of the necrotic core and the amount of macrophages, and foam cells that cause the atherosclerosis process are inhibited. This condition is related to the theory that missense mutation acts as an antiatherogenic by reducing the activity of Lp-PLA2.12,16,22

Yamada’s study in Japan also supported our findings. The G allele is an independent risk factor in CHD of Japanese men, but it did not have a correlation in women.20 According to Lee et al, the difference between the GG, GT, and TT genotypes in every district and population was influenced by race; the highest activity of Lp-PLA2 was

![Table 1](image)

| Variable              | AMI patients | Non-AMI patients | p-Value |
|-----------------------|--------------|------------------|---------|
| Age                   | 56.88 ± 10.98| 53.27 ± 10.61    | 0.074   |
| Body mass index (kg BW⁻²) | 23.42 ± 3.41 | 25.7 ± 3.22      | 0.000*  |
| Total cholesterol (mg/dl) | 184.05 ± 48.78 | 200.43 ± 40.51   | 0.059   |
| HDL-C (mg/dl)         | 38.86 ± 11.29| 44.23 ± 10.82    | 0.010*  |
| LDL-C (mg/dl)         | 121.28 ± 41.27| 123.72 ± 25.31   | 0.664   |
| Triglycerides (mg/dl) | 124.78 ± 68.11| 174.6 ± 148.82   | 0.009*  |
| Smoking habit (%)     |              |                  |         |
| Yes                   | 20 (98.1)    | 20 (98.0)        | 0.000   |
| No                    | 20 (82.0)    | 20 (80.0)        |         |
| Dyslipidemia (%)      |              |                  |         |
| Yes                   | 22 (98.0)    | 24 (98.0)        | 0.000   |
| No                    | 89 (80.2)    | 16 (40.0)        |         |
| Hypertension (%)      |              |                  |         |
| Yes                   | 57 (51.4)    | 35 (87.5)        | 0.000   |
| No                    | 54 (48.6)    | 5 (12.5)         |         |
| Family history (%)    |              |                  | 0.053   |
| Yes                   | 19 (17.1)    | 2 (5.0)          |         |
| No                    | 92 (82.9)    | 38 (95.0)        |         |

* shows the significance value.

![Table 2](image)

| Genotype | AMI (n = 111) | Non-AMI (n = 40) | p     |
|----------|---------------|------------------|-------|
| GG (%)   | 108 (97.3)    | 37 (92.5)        | 0.191 |
| GT/TT (%)| 3 (2.7)       | 3 (7.5)          |       |

Risk Factor of AMI for a subpopulation of Indonesia patients

having hypertension and metabolic syndrome, whereas education and previous medication caused a low level of BMI and triglycerides in AMI patients. Smoking behaviour in AMI patients was higher than in non-AMI patients. No family history of CAD in either AMI or non-AMI patients was found (82.9% vs. 95.0%; p = 0.000). Non-AMI patients had a higher rate of hypertension history than AMI patients (87.5% vs. 51.4%; p = 0.000).
found in white people and intermediate activity was found in Hispanic and African-American populations, while the lowest activity was in the Asian population.

According to the American College of Cardiology Foundation (ACCF) and American Heart Association (AHA) guidelines for assessment of cardiovascular risk in asymptomatic adults, Lp-PLA₂ might be reasonable for use in measuring cardiovascular disease risk in asymptomatic adult patients that have intermediate risk (IIb class, level of evidence B). Lp-PLA₂ is a biomarker that can describe its relation to lipoprotein metabolism, vascular inflammation, and plaque rupture. Therefore, the Val279Phe variation of Lp-PLA₂ could be used for early detection or prediction of AMI risk in intermediate or high cardiovascular risk patients.

**Conclusion**

The dominant genotype found in AMI patients was GG. The GG genotype had a 2.9 times greater risk of AMI compared to the GT/TT genotype. This result supported the concept of the Val279Phe genetic variant as a proatherogenic, which is a warrant for further development of early detection and improving strategy to prevent AMI in patients.

**Compelling interests**

The authors declare that they have no conflict of interests.

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