Genes & Diseases (2016) 3, 289–293

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/gendis/default.asp

SHORT COMMUNICATION

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

Miryanti Cahyaningtias [a,e], Mohammad Saifur Rohman [a], Widodo [b,c,*], Andi Wahjono Adi [a], Rina Yuda [a], Yanna Indrayana [a], Jayarani Fatimah Putri [b], Rusdianto [b], Mifetika Lukitasari [d], Dadang Hendrawan [a]

[a] *Department of Cardiology and Vascular Medicine, Faculty of Medicine, Brawijaya University, Malang, Indonesia*
[b] *Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, Indonesia*
[c] *Pusat Studi Biosystem, LPPM, Brawijaya University, Malang, Indonesia*
[d] *Nursing Science, Faculty of Medicine, Brawijaya University, Malang, Indonesia*
[e] *Department of Cardiology and Vascular Medicine, Brawijaya University Hospital, Malang, Indonesia*

Received 27 May 2016; accepted 29 August 2016
Available online 17 September 2016

KEYWORDS
Acute myocardial infarction;
AMI predictor;
Atherosclerosis;
Lp-PLA_2;
Val279Phe

Abstract Lipoprotein-associated phospholipase A_2 (Lp-PLA_2), a member of the phospholipase A_2 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-PLA_2 levels and coronary heart disease. Lp-PLA_2 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-PLA_2 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

* Corresponding author. Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, Indonesia.
E-mail address: widodo@ub.ac.id (Widodo).
Peer review under responsibility of Chongqing Medical University.

http://dx.doi.org/10.1016/j.gendis.2016.08.002

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.

Introduction

Cardiovascular disease causes 30% of deaths in the world.[1] One cardiovascular disease is coronary heart disease (CHD), that caused one in six death cases in America in 2008.[2] About half a million people in China have had myocardial infarction, and 2 million people have suffered from a stroke.[1] 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.[3]

Atherosclerosis is a chronic, progressive, and fundamental process that causes CHD.[1,4–9] The expression of lipoprotein-associated phospholipase A_2 (Lp-PLA_2) triggers atherosclerosis. Therefore, Lp-PLA_2 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-PLA_2 binds with low-density lipoprotein (LDL) and 15–20% of it binds with high-density lipoprotein (HDL).[5,6,10,11] Lp-PLA_2 is also known as platelet-activating factor acetylhydrolase (PAF-AH) because it hydrolyses platelet-activating factor (PAF).[5–7,10] PAF is a substrate that correlates with thrombogenesis and increases the risk of a coronary event. Lp-PLA_2 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.[4–8,10,12]

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-PLA_2. The 279Phe variant increases the risk of vascular disease and stroke.[10] 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.[13] 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.[10,13–18] 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.[19] A peripheral blood sample was put into a vacutainer with EDTA. DNA isolation was done using a Qiagen QIAmp Whole DNA Isolation Mini Kit® corresponding to the manufacturer's instructions. Isolated DNA was amplified using polymerase chain reaction (PCR) with forward primer 5′CCCCATGAAATGAACAATTATAT and reverse 5′GGGGGCAAAAGAATAGCCTTATAA at an annealing temperature of 53 °C. RFLP was performed using the PCR product with restriction enzyme BST4Ci 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

(abnormal numeric distribution data). Significant differences between the AMI and non-AMI groups were analysed to calculate the odds ratio (OR) between GG, GT, and TT. Statistical analysis was done using SPSS version 17 (SPSS Inc).

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 those of non-AMI patients (38.86 ± 11.29 vs. 44.23 ± 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). Triglyceride levels were significantly higher in non-AMI patients than AMI patients (174.6 ± 148.82 vs. 124.78 ± 68.11; p = 0.009). There were no significant differences between AMI and non-AMI patients for age, total cholesterol, and LDL cholesterol levels (Table 1). Genotype frequencies (GG, GT, and TT) in AMI and non-AMI patients did not differ. Both the AMI and non-AMI groups had higher GG genotype frequency than GT or TT (Table 2; Fig. 1). 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).

Discussion

Non-AMI patients had higher BMI and triglyceride levels than AMI patients. This may be caused by non-AMI 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).

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.[14] 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-PLA_2 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-PLA_2 activity in a Japanese population.[20,21]

Lp-PLA_2 activity is high in rupture-prone plaques that have a significant role in the formation and progressivity of atherosclerosis in coronary events. Lp-PLA_2 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-PLa_2 activity could be used to evaluate cardiovascular risk in the future. On the other hand, inhibition of Lp-PLA_2 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-PLA_2.[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-PLA_2 was

Table 1 Baseline characteristic and risk factors in AMI and non-AMI patients.

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 (%)			0.000
Yes	90 (81.8)	20 (50)	
No	20 (18.2)	20 (50)	
Dyslipidemia (%)			0.000
Yes	22 (19.8)	24 (60.0)	
No	89 (80.2)	16 (40.0)	
Hypertension (%)			0.000
Yes	57 (51.4)	35 (87.5)	
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 Genotype distribution in AMI and non-AMI patients.

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)	

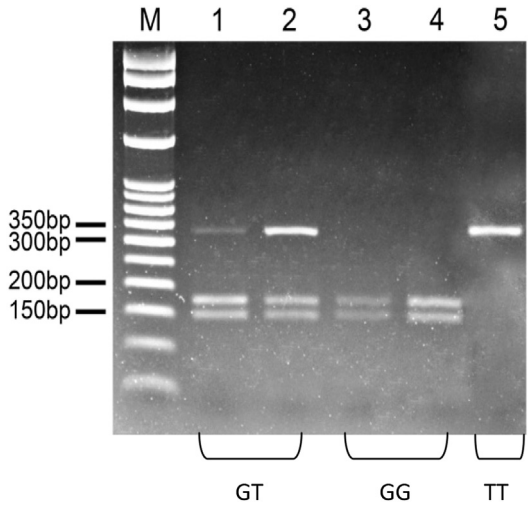

Figure 1 The RFLP genotyping of Lp-PLA2. Substitution of guanine (G) for thymine (T) at position 994 leads to the alteration of amino acid from valine into phenylalanine on residue 279 (Val279Phe) of Lp-PLA_2 could be used to determine the genotype using RFLP metode. The one band for TT, two bands for GG and Three bands for GT genotype. M is DNA marker, 1—5 were DNA samples.

Table 3 Odds ratio of GG to GT/TT in AMI and non-AMI patients.

	AMI	Non AMI
GG	108	37
GT/TT	3	3
Odds ratio	2.9	

found in white people and intermediate activity was found in Hispanic and African-American populations, while the lowest activity was in the Asian population.[23]

According to the American College of Cardiology Foundation (ACCF) and American Heart Association (AHA) guidelines for assessment of cardiovascular risk in asymptomatic adults, Lp-PLA_2 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-PLA_2 is a biomarker that can describe its relation to lipoprotein metabolism, vascular inflammation, and plaque rupture.[24,25] Therefore, the Val279Phe variation of Lp-PLA_2 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.

Competing interests

The authors declare that they have no conflict of interests.

Acknowledgement

We thank the Ministry of Research and Higher Education (No. 007/Add/SP2H/PL/DIT.LITABMAS) for providing the 'Unggulan Universitas, PUPT' research fund for conducting this study.

References

1. Organization WH. *Cardiovascular Diseases (CVDs)*. Fact sheet 317. 2011.
2. Roger VL, Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics—2012 update a report from the American heart association. *Circulation*. 2012;125(1):e2—e220.
3. Kesehatan D, RI KK. *Riset Kesehatan Dasar*. Jakarta: Badan penelitian dan Pengembangan Kesehatan; 2007.
4. Münzel T, Gori T. Lipoprotein-associated phospholipase A2, a marker of vascular inflammation and systemic vulnerability. *Eur Heart J*. 2009:ehp311.
5. Cojocaru M, Cojocaru IM, Silosi I. Lipoprotein-associated phospholipase A2 as a predictive biomarker of sub-clinical inflammation in cardiovascular diseases. *Maedica*. 2010;5(1):51.
6. Silva IT, Mello AP, Damasceno NR. Antioxidant and inflammatory aspects of lipoprotein-associated phospholipase A2 (Lp-PLA2): a review. *Lipids Health Dis*. 2011;10(170):1—10.
7. Madjid M, Ali M, Willerson JT. Lipoprotein-associated phospholipase A2 as a novel risk marker for cardiovascular disease: a systematic review of the literature. *Tex Heart Inst J*. 2010;37(1):25.
8. Tsimikas S, Tsironis LD, Tselepis AD. New insights into the role of lipoprotein (a)-associated lipoprotein-associated phospholipase A2 in atherosclerosis and cardiovascular disease. *Arterioscler Thromb Vasc Biol*. 2007;27(10):2094—2099.
9. Garza CA, Montori VM, McConnell JP, et al. Association between lipoprotein-associated phospholipase A 2 and cardiovascular disease: a systematic review. *Mayo Clin Proc*. 2007: 159—165. Elsevier.
10. Ballantyne CM. *Clinical Lipidology: A Companion to Braunwald's Heart Disease*. Elsevier Health Sciences; 2009.
11. Rosenson RS, Stafforini DM. Modulation of oxidative stress, inflammation, and atherosclerosis by lipoprotein-associated phospholipase A2. *J Lipid Res*. 2012;53(9):1767—1782.
12. Zalewski A, Macphee C. Role of lipoprotein-associated phospholipase A2 in atherosclerosis biology, epidemiology, and possible therapeutic target. *Arterioscler Thromb Vasc Biol*. 2005;25(5):923—931.
13. Li L, Qi L, Lv N, et al. Association between lipoprotein-associated phospholipase A2 gene polymorphism and coronary artery disease in the Chinese Han population. *Ann Hum Genet*. 2011;75(5):605—611.
14. Jang Y, Kim OY, Koh SJ, et al. The Val279Phe variant of the lipoprotein-associated phospholipase A2 gene is associated with catalytic activities and cardiovascular disease in Korean men. *J Clin Endocrinol Metab*. 2006;91(9):3521—3527.
15. Mallat Z, Lambeau G, Tedgui A. Lipoprotein-associated and secreted phospholipases A2 in cardiovascular disease roles as biological effectors and biomarkers. *Circulation*. 2010;122(21): 2183—2200.
16. Jang Y, Waterworth D, Lee J-E, et al. Carriage of the V279F null allele within the gene encoding Lp-PLA2 is protective from coronary artery disease in South Korean males. *PLoS One*. 2011;6(4):e18208.

17. Ishihara M, Iwasaki T, Nagano M, et al. Functional impairment of two novel mutations detected in lipoprotein-associated phospholipase A2 (Lp-PLA2) deficiency patients. *J Hum Genet*. 2004;49(6):302—307.
18. Ninio E, Tregouet D, Carrier J-L, et al. Platelet-activating factor-acetylhydrolase and PAF-receptor gene haplotypes in relation to future cardiovascular event in patients with coronary artery disease. *Hum Mol Genet*. 2004;13(13):1341—1351.
19. Stafforini DM, Satoh K, Atkinson DL, et al. Platelet-activating factor acetylhydrolase deficiency. A missense mutation near the active site of an anti-inflammatory phospholipase. *J Clin Invest*. 1996;97(12):2784—2791. http://dx.doi.org/10.1172/JCI118733.
20. Yamada Y, Ichihara S, Fujimura T, et al. Identification of the G 994→ T missense mutation in exon 9 of the plasma platelet-activating factor acetylhydrolase gene as an independent risk factor for coronary artery disease in Japanese men. *Metabolism*. 1998;47(2):177—181.
21. Ichihara S, Yamada Y, Yokota M. Association of a G994→ T missense mutation in the plasma platelet-activating factor acetylhydrolase gene with genetic susceptibility to nonfamilial dilated cardiomyopathy in Japanese. *Circulation*. 1998;98(18):1881—1885.
22. Cai A, Zheng D, Qiu R, et al. Lipoprotein-associated phospholipase A2 (Lp-PLA 2): a novel and promising biomarker for cardiovascular risks assessment. *Dis Markers*. 2013;34(5):323—331.
23. Lee KK, Fortmann SP, Varady A, et al. Racial variation in lipoprotein-associated phospholipase A2 in older adults. *BMC Cardiovasc Disord*. 2011;11(1):38.
24. Greenland P, Alpert JS, Beller GA, et al. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines developed in collaboration with the American Society of Echocardiography, American Society of Nuclear Cardiology, Society of Atherosclerosis Imaging and Prevention, Society for Cardiovascular Angiography and Interventions, Society of Cardiovascular Computed Tomography, and Society for Cardiovascular Magnetic Resonance. *J Am Coll Cardiol*. 2010;56(25):e50—e103.
25. Davidson MH, Corson MA, Alberts MJ, et al. Consensus panel recommendation for incorporating lipoprotein-associated phospholipase A 2 testing into cardiovascular disease risk assessment guidelines. *Am J Cardiol*. 2008;101(12):S51—S57.