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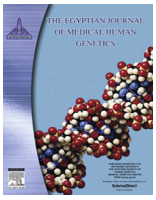

Original article

# The V279F polymorphism might change protein character and immunogenicity in Lp-PLA2 protein

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ABSTRACT

*Background:* Lipoprotein-associated phospholipase A2 (Lp-PLA2) plays a strong role in the occurrence of certain cardiovascular disease processes. Polymorphisms at the protein level are also estimated to correlate with increased risk factors for heart attacks. One such polymorphism is the V279F polymorphism in Lp-PLA2 which results in a change in enzyme performance capability. This in turn implies a reduced risk of acute myocardial infarct (AMI) in Korean and Indonesian subpopulations.
*Aim:* This study aimed to analyze changes in protein properties, structure, energy stability, epitope, and immunogenicity that are due to the substitution of the Valine (V) amino acid at position 279 to Phenylalanine (F) in the Lp-PLA2 protein.
*Methodology:* The role of Lp-PLA2 in the cardiovascular process and in AMI was analyzed based on the protein-protein network according to the BioGRID, MENTHA, and STRING databases. Protein properties and energy stability were examined by FoldX; this was followed by identification of epitope using ElliPro. The immunogenicity was evaluated *in vivo* by injecting the protein into mice and subsequently measuring their antibody production using the ELISA technique.
*Results:* The substitution of Valine for Phenylalanine was predicted to increase protein stability and epitope shifts. Further studies on animal experiments exhibit that the 279V variant is able to induce IgG production better than the 279F variant.
*Conclusion:* Based on these data, it can be concluded that the V279F polymorphism influences the surface structure, energy stability, epitope and immunogenicity of the Lp-PLA2 protein. The changes in the immunogenicity and epitope shift indicated that the protein is valuable as a biomarker for use in acute myocardial infarct. The results of this study provide an opportunity to develop monoclonal antibodies that are specifically able to identify V279F polymorphisms as a predictor of the risk of acute myocardial infarct.


## 1. Introduction

The PLA2G7 gene encodes the Lipoprotein-associated phospholipase A2 (Lp-PLA2) protein, an enzyme of the phospholipase A2 family [1,2] that can degrade oxidized lipids, presumably having an anti-inflammatory enzymatic role [3]. This protein can also reduce the inflammatory response because it is able to normalize the monocyte chemoattractant [2]. However, some reports suggest that this protein is involved in increased inflammation and mineralization in interstitial valve cells [4]. In addition, this protein acts as an enzyme that hydrolyses platelet activating factor and produces atherogenic pro-inflammatory compounds that can affect the risk of cardiovascular disease occurrence. Thus, Lp-PLA2 protein is thought to act as a risk factor for coronary heart disease [5–7], especially in cases of thickening of carotid intimal media [8]. Until recently, there was much debate on whether this protein serves as a pro or anti-inflammatory agent. The role of this essential protein could potentially be developed as a marker for atherosclerosis diagnosis [3,9,10].

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In addition to Lp-PLA2 activity that affects the occurrence of cardiovascular disease, polymorphisms in these proteins also contribute to increased risk factors for atherosclerosis or acute myocardial infarct [11–13]. Several studies have reported the presence of polymorphisms in Lp-PLA2 proteins, including A379V, V279F, and R92H [14,5,15]. The A379V variant of the Lp-PLA2 protein is thought to be associated with ischemic stroke in Han ethnic, China [16]. Generally, genetic variation in the PLA2G7 gene is associated with subclinical manifestations of coronary atherosclerosis. This phenomenon shows a very close relationship between Lp-PLA2 with atherosclerosis in humans, which strongly suggests the use of Lp-PLA2 as a cardiovascular occurrence biomarker [17].

The 279th amino acid change from Valine to Phenylalanine (V279F) on Lp-PLA2 protein is predicted to decrease enzymatic activity due to changes in "folding kinetics" and its substrate binding site [18]. Clinical studies suggest that this mutation is thought to be a risk factor for various inflammatory diseases [19–21]. However, several other studies have shown that this mutation reduces the risk of acute myocardial infarct (AMI) in Korean and Indonesian populations [22]. Given the importance of the Lp-PLA2 function, this study was conducted to perform in depth analysis of its properties, structure, epitope, and immunogenicity which may change as a result of the V279F polymorphism. This information is essential for further studies in developing monoclonal antibodies that specifically identify both variants for early detection of AMI risk factor.

## 2. Research method

### 2.1. Analysis of the protein network and biological processes

Determining the function of Lp-PLA2 in a biological process is crucial to understanding the protein's role in the cardiovascular process and in the development of AMI. The role of the protein in the biological process was examined through the interaction of Lp-PLA2 with other proteins in the cell, based on protein networks. The protein network of Lp-PLA2 was constructed based on three databases, i.e., BioGRID, MENTHA, and STRING [23–25]. The data from the protein network was then used for mapping the role of Lp-PLA2 in a biological process using Cytoscape software. This analysis has often been used as a valid tool for understanding the protein's role in various mechanisms and pathways within cells [26].

### 2.2. Analysis of Lp-PLA2 protein V279F properties and structure

The 279th amino acid change from Valine to Phenylalanine (V279F) in Lp-PLA2 protein is predicted to cause differences in the properties and surface of the protein. Therefore, analysis of hydrophobicity, energy stability, mass and accessible surface area of both variants of this protein was performed. The three-dimensional model of the 279F variance of Lp-PLA2 was constructed using the FoldX software based on the 279V variance of Lp-PLA2 as a template (PDB access code: 5lp1) [27]. Next, the FoldX [28] in Yasara software [29] were used to analyze the protein properties and surface structures of both variants.

The changing amino acids of the V279F polymorphism in the Lp-PLA2 protein are highly likely to cause the changes in surface structure and protein properties that may affect epitope shifts. To anticipate these predictions, "epitope mapping" analysis was then performed based on the three-dimensional structure of the Lp-PLA2 protein in both 279V and 279F variants. This analysis was conducted using the "epitope mapping" software based on a 3D structure, ElliPro [30]. Epitope prediction results were then further analyzed by comparing the position of the present epitope in both protein variants. Protein mapping was performed to determine the existence of a specific epitope for each protein variant. This analysis is particularly important in support of further investigations to identify specific epitopes, especially in developing monoclonal antibodies that are able to recognize both variants of the Lp-PLA2 protein specifically. These monoclonal antibodies can then be employed to mitigate the risk of early AMI based on Lp-PLA2 protein polymorphism.

### 2.3. Immunoprecipitation and SDS-PAGE

Variations in protein are thought to alter the properties, structure, epitope, and immunogenicity of the protein. To date, these studies have proven whether the V279F variation of Lp-PLA2 protein possesses different capabilities in inducing immune system in mice on producing IgG. Both variants of these proteins were isolated from serum of AMI patients with immunoprecipitation methods. Immunoprecipitation was then performed as follows, 50 ul serum was added to 2 ul mouse IgG anti-Lp-PLA2 (RnD system, AF5106). Then, the solution was added PBS (Bio-Rad, 161-0780) up to 100 ul, incubated for one hour at 4 °C, followed by addition of 20 ul A/G PLUS-Agarose bead (Santa Cruz Biotechnology) and incubated overnight at 4 °C. The protein was centrifuged at 10,000 rpm for 10 min at 4 °C. The pellets were washed using PBS 3x, then were resuspended in 100 ul elution buffer (0.1 M glycine-HCl, pH 2.7) (Bio-Rad) to separate antigen and antibody. After centrifugation at 10,000 rpm for 10 min at 4 °C, the supernatant that contains the target protein was then checked by SDS-PAGE [31]. The AMI patients were diagnosed using thrombolysis. The levels of cardiac enzymes such as CPK, CK-MB, and troponin I were measured and showed an increase in the first 6 h after AMI onset which was higher than normal [22]. This work was been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and approved by Brawijaya University- Dr. Saiful Anwar Hospital Ethics Committee.

### 2.4. Immunization and ELISA

Immunization was performed by injecting 100 ul of the Lp-PLA2 proteins (variants 279V and 279F) and supplemented with 100 ul of adjuvant CFA and IFA (InvivoGen, vac-cfa-10, vac-ifa-10). The protein was administered intraperitoneally in each group of mice; groups were injected with Lp-PLA2 variant 279V and 279F, and adjuvant. The adjuvant used in the initial injection contained CFA while on subsequent injections IFA was utilized. A booster is given in the second week after the first injection. The antibody production from the mice was measured by the ELISA method, which was briefly performed as follows: Lp-PLA2 (1 ul) was dissolved with aquadest (9 ul), then 90 μL coating buffer was added, then vortexed. The suspension (100 μL) is distributed into a well and incubated at 4 °C overnight. All fluids are aspirated in the well, then washed using 100 μL PBST (Biorad, Bioworld) for 3 consecutive times. Following this, 100 μL blocking buffer BSA 1% (Promega) was added to the well and incubated for 1 h at room temperature. Afterward, the well was washed using 100 μL PBST 3 times for 3 min. 100 μL of primary antibodies (serum mouse dissolved with PBS of 1: 500) was then poured into the well and incubated for 1 h at 25 °C. The well was then washed using 100 μL PBST at 0.2% 3 times for 3 min. Therefore, 100 μL of secondary antibody (Antibody Goat anti-mouse IgG Antibody HRP conjugated: 1000 × dilution) (Rockland, 610-1319-0500) was added to the well and incubated for 1 h at 25 °C. The post incubation well was washed using 100 μL PBST 0.2% 3 times for 3 min. Subsequently, TMB substrate was added (BioLegend, B218177) into the well and incubated for 30 min at room temperature. The reaction was discontinued by adding 50 μL stop solution (Cusabio, 140652) without removing