**Low *PLA2G10* gene expression level tend to make a higher risk of angina pectoris**

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**Abstract**

Angina pectoris is diagnosed by chest pain and considered as one of the symptoms which are triggered by coronary heart disease (CHD). CHD is marked by atherosclerotic plaque formation in the coronary artery and brings on the vascular narrowing which obstructs blood flow. Group IIA and group V of sPLA2 are one of the enzyme groups which play the main role of atherosclerotic plaque formation. On the other hand, group X sPLA2 which is encoded by PLA2G10  has anti-atherogenic properties by inhibits Th1 cell activation in LDLr-/- mice, which is pro-atherogenic. CHD is exacerbated by the presence of risk factors possessed by sufferers. This study aims to examine the proportion of patients with certain metabolic syndromes with their susceptibility to CHD as a cause of angina pectoris linked to PLA2G10 gene expression level. The samples of this research are 133 deposit biological materials from National Cardiovascular Center Harapan Kita’s collection, from angina pectoris patients with a plaque and no plaque in the coroner. The relative quantification of PLA2G10 gene expression shows no difference between the plaque group and no plaque group (p-value = 0,494) which is measured by TaqMan® Gene Expression Assay, but as many as 44% of the group with a plaque and 69% of the group with diabetes mellitus had low levels of *PLA2G10* gene expression. The group with risk factors has the greatest percentage having low levels of PLA2G10 gene expression. This indicates that more or less *PLA2G10* plays a role in reducing the risk of plaque formation and the severity of risk factors that cause angina pectoris.

Keywords: pla2g10, angina pectoris, antiatherogenic, plaque

**Introduction**

Phospholipases are known to be involved in the pathogenesis of various types of diseases mediated by inflammatory lipids such as atherosclerosis (Curfs et al., 2008). One of its members, namely the phospholipase A2 (PLA2) is a group of lipolytic enzymes that hydrolyze the ester bond group at the sn-2 glycerophospholipids then produce free fatty acids and lisofosfolipid. Among the 10 types of enzymes secreted by PLA2 exist in mammals, sPLA2 Group X (sPLA2-GX) is the enzyme phospholipase which has the highest capacity of hydrolysis against phosphatidylcholine (major phospholipid in cell membranes and LDL) (Bezzine et al., 2000; Karabina et al., 2006). sPLA2 (and Lp-PLA2) are the biomarkers of vascular inflammation and play an important role in atherosclerosis (Santoso et al., 2019). This enzyme has the greatest potential hydrolysis of the mammalian membrane cells in vitro (Bezzine et al., 2000). Besides, its expression is increased in foam cells in the arterial wall which suffered atherosclerosis in animal models (Saiga et al., 2001; Yamamoto et al., 2003)**.**

LDL Hydrolysis by sPLA2-GX produces modified particles which then induces lipid accumulation in macrophages differentiated from monocytes. LDL modified by group X then activates MAP kinase pathway, which leads to an increased release of arachidonic acid and improves adhesion of monocytes to the monolayer of endothelial to enter into the intima, internalize lipid-modified, and then form foam cells that accumulate in the inner layer of the intima (Saiga et al., 2001; Karabina et al., 2006) concluded that LDL modified by sPLA2-GX has a more pro-inflammatory and pro-atherogenic effect in the propagation of atherosclerosis compared to sPLA2-GX itself. Besides, the concept of inflammation involved in the pathogenesis of atherosclerosis has been known since the 1800s (Wong et al., 2012). Various pathways in the inflammatory cause of atherosclerosis are now clearly referring to inflammation as the key regulators and the main character that connects various risk factors in atherosclerosis and its complications in changing the arteries (Libby et al., 2002, 2009; Spagnoli et al., 2007). Some studies suggest that sPLA2-GX has an anti-inflammatory role. CD68 macrophages isolated from PLA2G10 transgenic mice produce large amounts of anti-inflammatory cytokines interleukin-10 (IL-10) and less proinflammation interleukin-6 (IL-6) (Curfs et al., 2008). sPLA2-GX also releases DHA which are precursors of lipid mediators Ω-3-derived anti-inflammatory (Mitsuishi et al., 2007).

In addition to the role of sPLA2-GX that atheroprotective in animals test based on the study above, (Ait-Oufella et al., 2013a) suggest that the expression of sPLA2-GX in bone marrow cells restrict the development of atherosclerosis and can control the immune response of proatherogenic Th1 in experimental animals. In PLA2G10 knocked out mice, the accumulation of atherosclerotic collagen plaque increased, and the size of the necrotic core in atherosclerotic plaque rose to four-fold compared to control (PLA2G10 + /+). Based on that, sPLA2-GX later attributed as an anti-atherogenic agent which inhibits plaque formation by 50% (Ait-Oufella et al., 2013a).

As we know, patients with stable coronary artery disease and angina pectoris have higher rates of future cardiovascular events compared with patients without angina (Eisen et al., 2016). This kind of disease has new powerful predictors such as male sex, reduce EF, diabetes, prior myocardial infarction and high C-reactive protein (Barbero et al., 2016), in addition to elevated serum cholesterol, high blood pressure, smoking and high physical activity as early predictors to angina pectoris (Hagman et al., 1987). This study aims to correlate between the elevating PLA2G10 gene expression levels and the severity level of predictors among the patients with angina pectoris in Jakarta, Indonesia. As an addition, we studied the single nucleotide polymorphism (SNP) of PLA2G10 gene to find whether the polymorphisms alter the gene expression.

**Materials and Methods**

A total of 133 samples of deposit biological material of patients with cardiovascular disease (CVD) under the care of Harapan Kita Cardiovascular Hospital (RSJPDHK) were divided into two groups: a control group without the occurrence of plaque (a sample of the patient's coronary heart disease or CHD), and the group with the plaque in the coronary arteries, which is known through CT coroner. RSJPDHK’s PBMC sample collection was extracted to obtain DNA by using the High Pure PCR Template Preparation kit (Roche®). RNA extractions were done using the High Pure RNA Isolation Kit (Roche®). RNA then synthesized into cDNA for gene expression test using TaqMan® Gene Expression Assay (Applied Biosystems®). The gene expression level was calculated using relative quantification. ​​ΔΔCT values were obtained to be used in the Livak method to define gene expression (Livak and Schmittgen, 2001). The data obtained and analyzed using a proportional test.

**Result and Discussion**

The results showed that at least 44% of subjects with coronary artery plaque and 69% of subjects with diabetes mellitus had low levels of *PLA2G10* gene expression, although showing a low correlation (Fig. 1). This can be explained by referring Ait-Oufella et al (2013) which is said that increase in the expression (overexpression) of *PLA2G10* in bone marrow cells of mice leads to reduced size lesions and plaques to 50% in mice *pla2g10* +/+ *ldlr* -/- compared to controls (*pla2g10* -/-*ldlr* -/-).

Fig 1. The level of *PLA2G10* gene expression among the samples in correlation to risk factor

PLA2G10 is predicted as a gene that is atheroprotective because its overexpression in mice ldlr -/-, resulted in plaque growth reduction to 50% (Ait-Oufella et al., 2013b). PLA2G10 also limits the Th1 cell response that is atherogenic thus activating the protective effects of the growth of plaque in ldlr-/- mice. The decreased Th1 cell response is characterized by a decrease in the production of interferon-gamma. This event causes the activation of monocytes and dendritic cells which previously actively migrated into the endothelial layer and form plaque. On the other hand, the production of TNF alpha also decreased along with the decrease in Th1 cells. The number of macrophages that are undergoing apoptosis dropped and reduce the production of foam cell plaque (Ait-Oufella et al., 2013b; Mallat et al., 2009).

Table 1. Frequency of genotype *PLA2G10* gene in patients with angina pectoris  
The number of alleles detected genotype (n = 133)

|  |  |  |
| --- | --- | --- |
| **Genotype** | **Allele (n=133)** | |
| **T512C**  **rs36072688 (T/C)** | **T123/in1C**  **rs4003232 (T/C)** | |
| **Wildtype** | TT  133 (100%) | TT  0  6(0%) | |
| **Heterozigot** | TC  0 (0%) | TC  133 (0%) | |
| **Homozigot Mutan** | CC  0 (0%) | CC  0 (0%) | |

These two SNPs are observed too in other populations, in this study the whole sample has a wildtype genotype (TT) of T512C, and heterozygous (TC) for the T123/in1C. PLA2G10 gene polymorphism on those two points, according to previous research have a different role from each other. Gora *et al.* (2009) state that the SNP T512C (rs36072688) located in the 5 'untranslated region has been linked with a reduced risk of the patient to re-exposed to cardiovascular disease during follow-up phase so that the mutation at this point are more common in the control group even though it requires a lot of repetition in other cohort studies with other populations. On the other side, the same data show that T123/in1C even more commonly occurs in the case group which had coronary heart disease (CHD) history.

*PLA2G10* relative gene expression data in this study showed no significant difference between the two groups of non-plaque and plaque findings. If this finding is associated with the polymorphism data which is obtained in this study, it becomes more rational. Polymorphisms in the T123/in1C which is showed heterozygosity in the whole sample allows an increased risk of CHD in a sample. This is due to the emergence of mutant alleles in the genotype of each sample that can cause loss of function of genes concerned (Gora et al., 2009). When added with functional mutations of T512C which is atheroprotective, the T512C absence of mutation in this study population may be one reason why the *PLA2G10* relative gene expression in both groups was not different. In addition to other factors that can affect the expression of genes, for example, is the diversity profile lipid levels in each sample.

Lipoprotein is an independent risk factor in the development of the cardiovascular disease. Individuals with concentrations of Lp (a) plasma of more than 20 mg/dL are at risk of developing cardiovascular disease more than twice as large. The component of Lp (a); LDL/apoB-100 and apolipoprotein (a), could interact with molecular components of coagulation in the blood, smooth muscle cells in the walls of blood vessels, endothelial cells, and activate inflammatory pathways (Riches & Porter, 2012). Although the physiological role of Lp (a) is unclear, in pathophysiological, Lp (a) is undoubtedly a decisive factor causing cardiovascular disease (D. L. McCormick, 2017).

Lipoprotein A trigger platelet aggregation, thrombosis, inflammatory cell recruitment, and adhesion are the beginning of atherosclerosis. Therefore, the high PLA2G10 gene expression in the group which has a high-risk factor for Lp (a) adds the evidence that PLA2G10 is more proatherogenic if it is associated with the concentration of Lp (a) plasma (S. P. A. McCormick, 2004).

Deposit biological material of patients with cardiovascular disease, metabolic syndrome, or type 2 diabetes mellitus, abnormal lipid levels not only lead to increased levels of LDL but also lowered plasma levels of HDL and triglycerides. Even patients who had LDL levels at optimal levels still have a high residual risk for vascular complications associated with atherogenic dyslipidemia (Pedro-Botet et al., 2014).

This likely explains why PLA2G10 relative gene expression in patients who had lipid profiles in the low-risk group was tend to also higher. If we observed from the role of sPLA2-GX which it couldn’t be described as pro-atherogenic enzyme or anti-atherogenic yet in this study. (K. Yamamoto et al., 2011) mentions that the mechanistic action of sPLA2-GX in atherosclerosis can not be explained solely by changes in lipoprotein and lipid profile modification in a simple way. It is also reinforced by considering that sPLA2-GX also showed anti-inflammatory function by producing polyunsaturated fatty acids (PUFAS) and other metabolites (Curfs et al., 2008) which also acts as an immunomodulator (Calder, 2008).

Relative gene expression in the sample of patients with angina pectoris who have a history of diabetes mellitus (DM) and non-diabetes mellitus (non-DM) (Figure 10), did not show a significant difference (*p*-value = 0.376), but the group with diabetes still have PLA2G10 gene expression which was higher than in a non-DM group. This is consistent with the results of Shridas et al (2014) which states that sPLA2-GX encoded by the gene PLA2G10 can regulate insulin secretion through a cyclooxygenase mechanism dependent.

One result of hydrolysis of LDL cholesterol and other phospholipids by an enzyme sPLA2-GX is arachidonic acid (AA). AA and other metabolites regulate insulin secretion stimulated by glucose (glucose-stimulated insulin secretion/GSIS) in the pancreas by beta cells by various mechanisms. While AA is an activator GSIS, another metabolite, prostaglandin E2 is an inhibitor of GSIS. Research results by (Shridas et al., 2014) showed that sPLA2-GX can suppress the GSIS in a way that inhibits PGE2 producing beta cells secrete insulin when glucose in the blood is high. This results in increased levels of glucose in the blood without the presence of secreted insulin, causing diabetes.  
PLA2G10 gene is a gene encoding a protein that is expressed at a low level, ie 11.1% of the average of overall gene expression in whole blood samples (NCBI, 2007). It is also shown in this study, which indicates that almost all DNA samples amplified at the end of the cycle of real-time PCR (CT value> 30). Besides the gene’s characteristic which is expressed at a low level, many other factors also affect the relative gene expression between one individual to another individual.

These factors include the ability to penetrate and expressivity. The ability to penetrate (penetrance) is a state that shows how often a gene is expressed. The ability to penetrate the same genes may vary from one individual to another individual and may depend on a person's age. Expressivity is a measure of the extent of a gene could be expressed in a person, which could be expressed as a percentage (Finegold, 2013).

**Conclusion**  
The relative quantification of PLA2G10 gene expression shows no difference between the plaque group and no plaque group (p-value = 0,494) which is measured by TaqMan® Gene Expression Assay, but as many as 44% of the group with a plaque and 69% of the group with diabetes mellitus had low levels of *PLA2G10* gene expression. The group with risk factors has the greatest percentage having low levels of PLA2G10 gene expression. This indicates that more or less *PLA2G10* plays a role in reducing the risk of plaque formation and the severity of risk factors that cause angina pectoris.

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