Platelet-Activating Factor-Acetylhydrolase (PLA2G7) A379V (Exon 11) Gene Polymorphism is Functionally Associated with Coronary Artery Disease Severity but not the Onset of Acute Coronary Syndrome

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Background: Oxidation of low-density lipoproteins is an initial step of atherogenesis that generates pro-inflammatory phospholipids, including platelet-activating factor (PAF). PAF is degraded by PAF-acetylhydrolase (PAF-AH), which is also a risk factor for myocardial infarction. The role of PAF-AH in the onset of acute coronary syndrome (ACS) and its association with atherosclerosis among ACS patients are still unclear.

Methods: PAF-AH encoding gene (PLA2G7) A379V variation was investigated in a cohort of patients having ACS (n = 200) and a sex-age-matched control group (n = 200). The activity of PAF-AH was evaluated by ELISA assay and coronary angiograms were evaluated among 100 ACS subjects for the severity of coronary atherosclerosis.

Results: The V allele of A379V (exon 11) polymorphism on the PLA2G7 gene was more frequent in ACS patients (p = 0.02). This V allele polymorphism was also associated with a lower activity of plasma PAF-AH (VV vs. VA vs. AA: 9.8 ± 5.3 vs. 20.5 ± 7.7 vs. 24.8 ± 5.9 nmol/mL, respectively; p for trend = 0.03) and a more complex coronary atherosclerosis (diffuse score: VV vs. VA vs. AA: 7.3 ± 1.7 vs. 5.2 ± 1.4 vs. 3.4 ± 1.3, respectively; p for trend = 0.04). Multiple logistic regression analysis revealed three independent risk factors: smoking (OR 2.14, 95% CI 1.77 to 8.10, p = 0.02), diabetes mellitus (OR 2.08, 95% CI 1.55 to 5.32, p = 0.007) and hypertension (OR 3.18, 95% CI 1.15 to 7.36, p = 0.002), were all independent risk factors for the onset of ACS. However, this genetic variation did not show significant difference (OR 1.21, 95% CI 0.89 to 5.80, p = 0.18).

Conclusion: We conclude that the PLA2G7/A379V polymorphism on exon 11 of PAF-AH gene is functionally associated with the PAF-AH activity and the severity of coronary atherosclerosis, but not onset of ACS, among Taiwanese population.

Key Words: Platelet-activating factor • Polymorphism • Acute coronary syndrome • Atherosclerosis

INTRODUCTION

Platelet-activating factor acetylhydrolase (PAF-AH), also called PLA2G7, may play important roles in the pathophysiology of thrombosis and atherosclerosis related to its catalytic action in the degradation of PAF and oxidized phospholipids. 1 The role of PAF-AH in atherosclerosis remains controversial. PAF-AH has been detected in human and rabbit atherosclerotic lesions, hence
potentially contributing to the release of lyso-PC and free oxidized fatty acids, although it might also prevent biological activities of PAF-like substances in situ. Experimental data support both the pro- and the antiatherogenic roles of this enzyme. Supporting the proatherogenic role of PAF-AH is the fact that inhibiting PAF-AH reduces atherosclerotic lesion formation in hypercholesterolemic rabbits. In favor of the antiatherogenic role is the fact that recombinant PAF-AH shows anti-inflammatory properties in animal models.

PAF has been considered as a product from the signal transduction pathway via the activation of phospholipase A2 (PLA2). Secretory type II PLA2 (sPLA2) significantly contributes to the pathogenesis of various inflammatory diseases. The plasma levels of sPLA2 were also higher in patients with coronary artery disease (CAD), and acted as a prognostic indicator in these patients. We previously reported that the PAF-AH (PLA2G7) A379V gene polymorphism could functionally decrease the PAF-AH activity and was associated with an increased sPLA2 activity in premature MI patients. We thus speculated that this genetic variation with a lower catalytic activity could putatively increase the levels of PAF, and also be associated with vascular instability and subsequently higher possibility of ACS events.

METHODS

Study participants
Study population
We enrolled 200 patients (mean age, 56.2 ± 4.1 years; 86% men) with survival after their first ACS event. Patients were diagnosed with ST-segment elevation myocardial infarction (STEMI) when they had a new or presumed new ST-segment elevation ≥ 1 mm in any location, or a new left bundle branch block on the index, or a qualifying ECG with at least one positive cardiac biochemical marker of necrosis (including troponin T, creatine kinase, and creatine kinase-MB isoform measurements). In cases of non-ST-segment elevation myocardial infarction (NSTEMI), at least one positive cardiac biochemical marker of necrosis without new ST-segment elevation on the index or qualifying ECG had to be present. Unstable angina was diagnosed when serum biochemical markers were within the normal range. Patients originally admitted because of unstable angina, but in whom MI evolved during their hospital stay, were classified as having an MI. Patients who had a history of percutaneous coronary intervention within 6 months or coronary artery bypass surgery within 1 year were excluded. Patients who had heart failure, cardiomyopathy, or valvular heart diseases were also excluded. Because statin therapy may affect PAF-AH levels, patients taking statins before enrollment were excluded.

Control population
The control group recruited 200 sex-age-matched subjects (mean age, 55.9 ± 5.3 years) from consecutive subjects admitted to our hospital for routine health examinations, the same as our previous study criteria. In brief, they showed no clinical or electrocardiographic evidence of ischemia or cerebrovascular disease. Written informed consents were obtained, and this study was approved by the research committee in our hospital.

Background of population
All participants in this study were Taiwanese from the same geographic area. Demographic data and information about traditional coronary risk factors were collected from all study participants. For all participants, data were taken from the medical records at the time of admission for ACS. They were considered to have systemic hypertension if elevated blood pressure (> 140/90 mmHg) was measured on three occasions, or if they were already being treated with anti-hypertensive agents. The participants were defined as having diabetes mellitus if they had a fasting blood glucose level > 126 mg/dL, or were already being treated for diabetes. The total cholesterol level was determined at the beginning of the study. Since statin therapy can affect PAF-AH levels, patients taking statins before enrollment were excluded. Family history was investigated by reviewing the history for any cases of premature onset MI or sudden
cardiac death among first-degree relatives, i.e. parents, siblings, and children.

**Blood sampling, laboratory, and angiographic methods**

To analyze the association between this genetic variation, PAF-AH and the severity of atherosclerosis, we test the *in vivo* activity of PAF-AH and analyzed patients’ coronary angiograms among different genetic background subgroups. We non-randomly and constitutively collected 100 surviving ACS subjects who received catheterization and blood sampling at least 2 weeks after the onset of their ACS events. PAF-AH activity was measured from plasma stored at -70 °C by the trichloroacetic acid precipitation procedure in 96-well plates as previously described.9 Samples were measured in duplicate. A pool of 10 control plasma samples served as an internal standard for all measurements. The within-assay variability was < 5%. Levels of plasma sPLA₂ were also measured by enzyme immunsorbent assay (EIA) as in our previous works.8 The inter-assay and intra-assay coefficients of variation were < 10%. Serum lipid levels were determined immediately.

Coronary angiography was performed, and the extent of coronary atherosclerosis was evaluated using a modified “diffuse score”, which had been applied and found acceptable for evaluation of coronary atherosclerosis in several study cohorts.18 The overall diffuse score is the sum of the individual segment scores, and the maximum score is 11.5. All angiograms were reviewed by experienced cardiologists blind to the levels of PAH-AH or sPLA₂. The intra- and inter-observer correlations were 0.90 and 0.88, respectively.

**Genomic analysis**

The blood samples were drawn into 5-mL EDTA tubes and centrifuged at 2200 g for 15 minutes to separate plasma. The buffy coat after centrifugation was obtained, and DNA in each sample was extracted by using a Puregene DNA Isolation Kit (Gentra System, Inc., Minneapolis, USA) according to the manufacturer’s instructions. The DNA samples were stored at -70 °C until use.

The primer and amplification methods were applied as in our previous report.11 In brief, we used 5’ CAG ACC AAC AAG ACC AGT AC 3’ as our forward primer and 5’ TAA AGC TGT ATT AAG ATA GAC A 3’ as our reverse one. The PCR program was set as 30 cycles with 94 °C for 45 seconds, 48 °C for 45 seconds and 72 °C for 45 seconds. Genotypes of PLA2G7/A379V (AA, normal; AV, heterozygote; and VV, deficient homozygote) were then determined by SSCP for these 200 patients with ACS and the control subjects. The frequencies of the V allele were calculated and compared.

**Statistical analysis**

Data on age and cholesterol levels were presented as mean ± SD. Differences between the groups were analyzed using the unpaired Student’s *t* test. The differences in frequencies of smoking, hypertension, hyperlipidemia, diabetes mellitus, and PLA2G7/A379V genotypes were analyzed using Fisher’s exact test. The genotypes of PLA2G7/A379V (AA, normal; AV, heterozygote; and VV, deficient homozygote) were determined directly for these 200 ACS patients and the control subjects. The frequency of the V allele was calculated and compared between ACS group and the controls. χ² analyses were used to test deviations of genotype distribution from Hardy-Weinberg equilibrium and to determine allele or genotype frequencies between patients and control groups. Differences of normal variables across three or more groups were evaluated using analysis of variance (ANOVA).

Correlations between plasma levels of PAF-AH or sPLA₂ and the severity of CAD or other factors were evaluated using Spearman’s rank correlation test. Forward stepwise multiple logistic regression analysis was used to elucidate the association between PAF-AH or sPLA₂ levels and complex lesions. The risk factors that appeared to be possible significant predictors (*p* < 0.05) in the single-variant analyses were included in the multiple logistic regression analyses. Adjusted estimations of conditioned relative risk and 95% confidence intervals (CIs) were also determined. All statistical analyses were performed using SPSS Advanced Statistics 10.0 for Windows. Statistical significance was set at *p* < 0.05.

**RESULTS**

**Comparison of traditional coronary risk factors**

We compared the traditional coronary risk factors
Distribution of PAF-AH genotypes

Table 2 shows the distributions of different PLA2G7/A379V genotypes between these 2 groups. For this exon 11 A379V polymorphism, there was a significantly higher prevalence of the AV and VV genotype among patients with ACS compared with control subjects. The homozygous VV genotype also had a statistically significant higher rate in ACS subjects when compared with the controls. However, the genotype distribution was similar among the three subgroups of ACS (STEMI, NSTEMI and unstable angina) by ANOVA test. The allele and genotype distributions between patients and control groups were both compatible with the Hardy-Weinberg equilibrium.

Associations of PLA2G7/A379V polymorphism, PAF-AH activity and the severity of CAD

The plasma levels of PAF-AH were strongly negatively associated with the number of V allele of PLA2G7/A379V genotype (homozygous VV vs. heterozygous AV+VV).
VA vs. homozygous AA: 9.8 ± 5.3 vs. 20.5 ± 7.7 vs. 24.8 ± 5.9 nmol/mL, respectively; *p* for trend = 0.03) (Figure 1A). Besides, the plasma levels of sPLA₂ were associated with the number of V allele of PLA2G7/A379V genotype (homozygous VV vs. heterozygous VA vs. homozygous AA: 277.6 ± 25.7 vs. 219.5 ± 34.8 vs. 146.8 ± 34.5 ng/dL, respectively; *p* for trend = 0.03) (Figure 1B). By using the coronary angiographic scoring, we found that the severity and complexity of CAD among these ACS patients were significantly correlated with the numbers of V allele PLA2G7/A379V genotype (Figure 2A). Those patients carrying VV genotype of PLA2G7/A379V had the most severe and complex CAD when compared with VA or AA genotypes (scores 7.6 ± 2.3 vs. 5.5 ± 2.4 vs. 3.7 ± 2.6, respectively; *p* for trend = 0.04). Complex lesions were found in 45 of 100 ACS patients (45%), 39 of whom had ≥ 2 lesions. The PAF-AH levels were lower in patients who had more complex lesions (score ≥ 5, *n* = 45) than in those with less complex lesions (score < 5, *n* = 55) (16.7 ± 5.2 vs. 33.4 ± 6.5 nmol/mL, *p* = 0.04) (Figure 2B). After being adjusted by age, sex, and traditional risk factors, the levels of PAF-AH in ACS patients had a strong negative association with the complexity and the severity scores of CAD (*R* = -0.52, *p* = 0.03). Plasma levels of PAF-AH or sPLA₂ were similar among the three subgroups of ACS (STEMI, NSTEMI and unstable angina) (Table 1).

**Figure 1.** Gene-phenotype associations between the platelet-activating factor acetylhydrolase polymorphism and plasma activities of (A): platelet-activating factor acetylhydrolase activity and (B): secretory phospholipase A₂. (*n* = 100)

**Figure 2.** Factors associated with the severity or complex of coronary atherosclerosis. (A): Correlation with the V allele mutation numbers of the platelet-activating factor acetylhydrolase PLA2G7/A379V gene. (B): Higher severity score (> 5) indicates lower activity of platelet-activating factor acetylhydrolase. (*n* = 100)
Identification of independent risk factors of ACS

Table 3 shows the results of multiple logistic regression analysis to identify the independent risk factors of ACS. Hypertension, diabetes mellitus, smoking and PLA2G7/A379V genotype were all used as the independent variables. Multiple logistic regression analysis showed that smoking (OR 2.14, 95% CI 1.77 to 8.10, $p = 0.02$), diabetes mellitus (OR 2.08, 95% CI 1.55 to 5.32, $p = 0.007$) and hypertension (OR 3.18, 95% CI 1.15 to 7.36, $p = 0.002$) were all independent risk factors for the onset of ACS. However, PLA2G7/A379V genetic variation did not show significant difference (OR 1.21, 95% CI 0.89 to 5.80, $p = 0.18$) for the onset of ACS.

DISCUSSION

In this gene-phenotype association study, we found that the A379V polymorphism on exon 11 of PLA2G7 gene was significantly associated with reduced PAF-AH activity and, furthermore, diffuse coronary atherosclerosis. The association between this gene variation and the onset of ACS was not significant after multiple logistic regression analysis in this Taiwanese population.

In our current study, we failed to establish this genetic variation as one of the independent risk factor for the onset of ACS in Taiwanese population. Compared with the premature MI cohort in our previous report, we found that the heterogeneity and older age in current study’s population might partially explain this difference. The etiologies for onset of ACS include not only plaque rupture, but also local vasospasm, acute thrombosis or diffuse atherosclerotic changes of coronary arteries. However, the major components of MI are contributed by the vulnerability and instability of the plaque. With advanced aging process, some gene-environment factor played more important role for the pathophysiologic changes of human vasculature. It would be possible that the genetic factor would be less predominant among an aged epidemiologic cohort survey.

Another possible etiology for the result of genetic analysis would be the impact of lipid profile. PAF-AH and sPLA2 have recently been recognized as two important factors in mediating lipid metabolism and the atherogenesis process. Compared with other cohorts tested for the role of this polymorphism in CAD or premature MI, the lipid profiles of our participants were lower, both in diseased and control groups. In fact, PAF-AH was found to modulate the lipid and inflammatory metabolism only among dyslipidemic or lower high-density cholesterol subjects. Under the background of less frequency of dyslipidemia, as in our ACS cohort, it was possible that the roles of covariates like hypertension or diabetes mellitus would be more important than this genetic polymorphism.

Our current study, similar to another prospective cohort study, had results supporting the anti-atherogenic effect of PAF-AH in atherosclerosis. One Japanese ethnic epidemiologic cohort reported a genetic deficiency caused by another locus of genetic variation, G994T on exon 9 of the PLA2G7 gene. This loss-of-function mutation was present in 4% of a Japanese ethnic group, leading to a complete loss of catalytic activity, and also was associated with increased risks of CAD and stroke. Furthermore, PAF-AH has been reported to prevent myocardial ischemia-reperfusion injury in rabbit animal model. These data all support the anti-atherogenic role of PAF-AH.

PAF has been considered as a product from the signal transduction pathway via the activation of sPLA2. sPLA2 can generate lysophospholipids, fatty acids, and precursors of various pro-inflammatory lipid mediators and involve in modifying low-density lipoprotein. sPLA2 is not only higher in patients with CAD, but also plays a role as a prognostic indicator in these patients with or

Table 3. Risk factors of acute coronary syndrome identified by multiple logistic regression analysis

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>OR for ACS (N = 200)</th>
<th>95% CI</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>2.14</td>
<td>1.77-8.10</td>
<td>0.02</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2.08</td>
<td>1.55-5.32</td>
<td>0.007</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3.18</td>
<td>1.15-7.36</td>
<td>0.002</td>
</tr>
<tr>
<td>PLA2G7/A379V genotype</td>
<td>1.21</td>
<td>0.89-5.80</td>
<td>0.18</td>
</tr>
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</table>

Abbreviations: please see Table 1. SE = standard error; OR = odds ratio; CI = confidence interval.
without coronary interventions. Our study demonstrated that plasma sPLA₂ levels were higher in patients with ACS and that they were strongly associated with the severity of CAD. In addition, higher sPLA₂ levels were associated with complex coronary lesions in patients with ACS. Angiographically complex lesions are associated with plaque instability and are predictive of coronary events, such as MI. Our results suggest that higher sPLA₂ levels in patients with ACS or CAD may reflect plaque instability in coronary arteries or may be aimed at destabilizing coronary plaques. CAD patients with higher plasma sPLA₂ levels may be prone to a higher risk of subsequent unstable coronary events. Though limited by the unavailable tissue levels of sPLA₂, our findings could support an important role of sPLA₂ in the pathogenesis of atherosclerosis.

The actual mechanism of PAF-AH or sPLA₂ response to inflammatory or atherosclerotic process, such as MI, is still not clear. Sretlor et al. have reported an in vitro study of inflammatory stimulation on liver cell system. They found that the expression of plasma PAF-AH mRNA and production of plasma PAF-AH protein were increased in the resident macrophages of the liver in response to inflammatory exposure. They also observed an up-regulation response of the plasma PAF-AH expression to be an important mechanism for elevating the local and systemic ability to inactivate PAF, sPLA₂ or oxidized phospholipids. We thus speculate that the PAF-AH may be responsiveness to ACS with highly inflammatory challenge, and thus modify the expression of oxidized phospholipids in the plasma.

PAF-AH was reported to be depressed during the acute phase of MI. It might thus be hypothesized that these changes of PAF-AH or sPLA₂ levels derived secondarily from myocardium necrosis during ACS. We thus surveyed the PAF-AH or sPLA₂ levels during their relatively sub-acute stage: at least 2 weeks after the ACS event. This might have helped us demonstrate their baseline levels and analyze the genotype-phenotype association.

Our study was limited by its retrospective and survival-only cohort design. Due to this limitation, we only enrolled 100 subjects, those who had received angiography and blood sampling after their ACS events, for the biochemistry and angiography association analysis among the whole 200 ACS subjects. Besides, the PAF-AH and sPLA₂ levels in this relatively sub-acute stage may not actually represent their prior baseline levels or predict their subsequent effects. Second, it was difficult to determine whether the plasma PAF-AH and sPLA₂ completely originated in coronary plaque. Because of ethical considerations, we had no angiographic results for our asymptomatic and low-risk control participants. Finally, the exclusion of subjects under statin-using or -receiving procedures before ACS events also caused selection bias such that some high-risk patients were excluded. These selection biases might probably influence the power of prediction for our current results.

CONCLUSION

We conclude that the A379V polymorphism on exon 11 of PLA2G7 gene is significantly associated with the PAF-AH activity and the severity of coronary atherosclerosis. Its association with the onset of ACS is not significant among this Taiwanese population.

ACKNOWLEDGEMENTS

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REFERENCES


血小板激活素乙醯水解脢 Exon 11 A379V 基因多形性变異與急性冠狀動脈症候群病患冠狀動脈嚴重度相關而非其發生率

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背景 低密度脂蛋白的氧化作用會啟動粥狀動脈硬化。而最初的作用便是形成發炎物質，包括血小板激活素。血小板激活素乙醯水解脢能分解血小板激活素，而目前過高的血小板激活素被認為是心肌梗塞的危險因素。血小板激活素乙醯水解脢在急性冠狀動脈症候群病患的功能角色和相關性尚未被清楚地研究。

方法 利用基因分析的方法我們研究了基因多形性在患者有急性冠狀動脈症候群 (n = 200) 的分布比率，並且由一群性別以及年齡相配的相對正常人員當作對照組 (n = 200)。血小板激活素乙醯水解脢的活性由 ELISA 分析，並同時比較其中 100 位接受冠狀動脈攝影檢察者，評估其冠狀動脈粥樣硬化嚴重度的相關性。

結果 血小板激活素乙醯水解脢 Exon 11 A379V 基因多形性 V 基因在急性冠狀動脈症候群明顯有較高的頻率 (p = 0.02)。這 V 基因多形性同時有較低的血中血小板激活素乙醯水解脢活性 (VV vs. VA vs. AA: 9.8 ± 5.3 vs. 20.5 ± 7.7 vs. 24.8 ± 5.9 nmol/mL; p = 0.03)，並且同時有較複雜的冠狀動脈粥樣硬化程度 (Diffuse score: VV vs. VA vs. AA: 7.3 ± 1.7 vs. 5.2 ± 1.4 vs. 3.4 ± 1.3; p = 0.04)。多變項的回歸分析顯示了三項獨立風險因素：抽煙 (危險度 2.14, 95% CI 1.77 到 8.10, p = 0.02)；糖尿病 (危險度 2.08, 95% CI 1.55 到 5.32, p = 0.007)；與高血壓 (危險度 3.18, 95% CI 1.15 到 7.36, p = 0.002)。但是，血小板激活素乙醯水解脢 Exon 11 A379V 基因多形性變異並非獨立風險因素 (危險度 1.21, 95% CI 0.89 到 5.80, p = 0.18)。

結論 我們認為，在臺灣急性冠狀動脈症候群人口之中，血小板激活素乙醯水解脢 Exon 11 A379V 基因多形性變異，與其發生率並未有顯著相關性；而其與血小板激活素乙醯水解脢活動高低與冠狀動脈粥樣硬化，有顯著相關性。

關鍵詞：血小板激活素乙醯水解脢、基因多形性變異、急性冠狀動脈症候群、動脈粥樣硬化。