Protective role of Lipoprotein-Associated Phospholipase A2 Gene (A379V) Polymorphism against Myocardial Infarction among Egyptians

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Introduction

The recognition that atherosclerosis has a strong inflammatory component has stimulated a great deal of research on the role of inflammatory mediators in the atherosclerotic disease process. Oxidation of low density lipoproteins (LDLs) is an initial step in atherogenesis, that generates a myriad of pro-inflammatory phospholipids, including platelet-activating factor (PAF) and its analogs. Platelet-activating factor is degraded by lipoprotein-associated phospholipase A2 (Lp-PLA2), also known as platelet-activating factor-acetylhydrolase (PAF-AH), a circulating enzyme having both pro and anti-inflammatory activities. Lipoprotein-associated phospholipase A2 activity has been postulated to be a risk factor for acute coronary syndrome (ACS); however, whether Lp-PLA2 has a causal or protective role is still unclear. A large number of single nucleotide polymorphisms (SNPs) that affect Lp-PLA2 mass and activity in plasma have been described.

Abstract

**Background:** Oxidation of low density lipoproteins is an initial step of atherogenesis that generates pro-inflammatory phospholipids, including platelet-activating factor (PAF) and its analogs. Platelet-activating factor is degraded by lipoprotein associated phospholipase A2 (Lp-PLA2), also known as platelet-activating factor-acetylhydrolase (PAF-AH), a circulating enzyme having both pro and anti-inflammatory activities. Lipoprotein associated phospholipase A2 activity has been postulated to be a risk factor for acute coronary syndrome (ACS); however, whether Lp-PLA2 has a causal or protective role is still unclear. A large number of single nucleotide polymorphisms (SNPs) that affect Lp-PLA2 mass and activity in plasma have been described.

**Aim:** The aim of the present work is to determine the prevalence of Lp-PLA2 gene A379V single nucleotide polymorphism (SNP) in Egyptians suffering from myocardial infarction (MI) in comparison to healthy controls and to correlate this genetic variant with different cardiovascular risk factors.

**Methods:** Lp-PLA2 gene A379V polymorphism (rs1051931) was investigated in fifty patients having MI and fifty age and sex matched healthy controls using real-time PCR.

**Results:** The homozygous CC genotype, coding for alanine at position 379 of Lp-PLA2 protein, had the highest frequency among patients (72%) compared with controls (46%) while heterozygous CT genotype had the highest frequency among controls (46%) compared with patients (24%) with a significant difference (p=0.033). The major “C” allele had the highest frequency among patients (84%) compared with controls (69%) while the minor “T” allele, coding for valine at the same position, had the highest frequency among controls (31%) compared with patients (16%) with a significant difference (p=0.012).

**Conclusion:** The Lp-PLA2 A379V gene polymorphism was found to be less frequent in MI patients presented with ACS than in healthy controls, suggesting that this SNP might be protective against the development of MI.

**Key words:** lipoprotein associated phospholipase A2, Myocardial infarction, platelet-activating factor-acetylhydrolase, single nucleotide polymorphism.
in certain ethnic groups. The most frequently studied SNPs are R92H (rs1051931), I198T (rs1805018), V279P and A379V (rs 1051931) (46672943 C > T), in alanine (ACG) to valine (ATG), with the C allele being the major allele (coding for alanine) and the T allele being the minor one (coding for valine). This polymorphism is thought to decrease the substrate affinity of Lp-PLA2, possibly prolonging the activity of pAF, which in turn is associated with many inflammatory diseases.12-14

The missense mutation of the PLA2G7 gene, which results in alanine (AGC) to valine (ATG) transition at position 379 of Lp-PLA2 protein, A379V (rs 1051931) (46672943 C > T), has been observed in Caucasians, Chinese, Taiwanese and South Koreans.15-18 This polymorphism is thought to decrease the substrate affinity of Lp-PLA2, possibly prolonging the activity of pAF, which in turn is associated with many inflammatory diseases.13

Materials and Methods

Study Population:

This study was conducted on fifty Egyptian patients; 23 males (46%) and 27 females (54%), all suffering from MI which was confirmed by ECG changes (ST segment elevation) and elevation of cardiac enzymes (CK-MB and troponin). All patients were recruited from the Cardiology Department at Alexandria Main University Hospital and their ages ranged between 32-65 years with a mean of 48 years. Patients with inflammatory or liver diseases were excluded to eliminate the relationship between this gene polymorphism and diseases other than MI.

Fifty healthy individuals, 25 males (50%) and 25 females (50%), whose ages ranged between 30-70 years with a mean of 45 years, were included as a control group. They had no history of hypertension, DM, atherosclerosis or cancer.

Full history was taken from all participants; including smoking habits, physical activity, alcohol consumption, drug history and medical history for hypertension and DM. Also, supine blood pressure was measured for all participants. All subjects signed a written informed consent before enrollment in the study.

Table 1. Clinical Characteristics of the two studied groups.

<table>
<thead>
<tr>
<th>Parameter (mean±SD)</th>
<th>Patients (n=50)</th>
<th>Controls (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.34 ± 7.67</td>
<td>45.16 ± 8.73</td>
</tr>
<tr>
<td>Male</td>
<td>23 (46%)</td>
<td>25 (50%)</td>
</tr>
<tr>
<td>Female</td>
<td>27 (54%)</td>
<td>25 (50%)</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>187.82 ± 92.50*</td>
<td>112.82 ± 29.73</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>228.26 ± 71.98*</td>
<td>165.88 ± 30.11</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>152.32 ± 62.08*</td>
<td>85.80 ± 25.36</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>38.06 ± 13.77*</td>
<td>56.18 ± 12.64</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>1654.46 ± 1390.38*</td>
<td>80.04 ± 32.39</td>
</tr>
<tr>
<td>CK-MB (ng/ml)</td>
<td>163.06 ± 185.24*</td>
<td>0.47 ± 0.32</td>
</tr>
<tr>
<td>Troponin I (ng/ml)</td>
<td>61.92 ± 74.46*</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>324.42 ± 266.37*</td>
<td>23.22 ± 10.09</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>1261.64 ± 965.39*</td>
<td>151.16 ± 30.61</td>
</tr>
<tr>
<td>Hs-CRP (mg/L)</td>
<td>138.48 ± 145.80*</td>
<td>8.54 ± 10.17</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>110.88 ± 33.02*</td>
<td>97.74 ± 16.41</td>
</tr>
</tbody>
</table>

*p value < 0.05 compared with controls.

Table 2. Clinical Characteristics of the two studied groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Genotype</th>
<th>Test of sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid Profile</td>
<td>CC (n=36)</td>
<td>CT (n=12)</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>191.44 ± 93.18</td>
<td>188.92 ± 97.42</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>224.92 ± 77.55</td>
<td>240.0 ± 57.18</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>149.31 ± 63.09</td>
<td>163.25 ± 61.73</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>37.75 ± 15.13</td>
<td>37.17 ± 9.59</td>
</tr>
</tbody>
</table>


The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and has obtained the approval of the Medical Ethics Committee of the Faculty of Medicine, Alexandria University

Routine Laboratory Investigations:

Three milliliters of whole blood were collected from every subject by aseptic veni-puncture in a plain red-topped vacutainer, left to clot slowly at room temperature for 15-30 minutes. The clot was removed by centrifugation at 1000-1200 g for 10 minutes, then the serum was used for measurement of lipid profile (triglycerides, total cholesterol, LDL and HDL), cardiac enzymes (CK-total, CK-MB, troponin, LDH, AST), hs-CRP and fasting blood glucose. All parameters were measured by chemistry auto-analyzer Dimension RxL Max (Siemens Health Care diagnostics, USA).

Genomic Analysis for Detection of Lp-PLA2 A379V (rs 1051931) Gene Polymorphism by 5’ Nucleace Allele Discrimination Assay using Real-Time PCR:

1- DNA Extraction:

Another 2 milliliters of whole blood were aseptically drawn into lavender-topped EDTA vacutainer. Genomic DNA was extracted from EDTA whole blood samples, using QIAGEN total DNA purification kit (QIAMP DNA blood mini kit, QIAGEN, Germany, cat. no. 51104) according to the manufacturer’s instructions. The DNA samples were stored at -20°C until use.

2- 5’ Nuclease Allele Discrimination Assay using Real-Time PCR:

Ready-made “TaqMan SNP Genotyping Assay” (Assay ID C_2032800_20, catalog # 4351379, Applied Biosystems, USA) was used to detect Lp-PLA2 A379V SNP (rs1051931). In Lp-PLA2 A379V polymorphism (46672943 C > T), alanine (ACG) is replaced with valine (ATG), with the C allele being the major allele (coding for alanine) and the T allele being the minor one (coding for valine). This assay kit contains primer/probe mixes (40X); 2 unlabeled sequence-specific forward and reverse primers to amplify the sequence of interest harboring the polymorphism and 2 labeled taqMan minor groove binder (MGB) probes for detecting both the major C and the minor T alleles.

The first probe, labeled with FAM (green fluorescence) as the
Table 3. Relation between the different genotypes and patients’ cardiac enzyme levels.

<table>
<thead>
<tr>
<th>Cardiac Enzymes</th>
<th>Genotype</th>
<th>CC (n=36)</th>
<th>CT (n=12)</th>
<th>TT (n=2)</th>
<th>Test of sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK (U/L) Mean ±SD.</td>
<td>1629.94 ± 1435.48</td>
<td>1929.67 ± 1296.0</td>
<td>444.50 ± 518.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK-MB (ng/ml) Mean ±SD.</td>
<td>149.95 ± 130.17</td>
<td>222.49 ± 302.60</td>
<td>42.40 ± 57.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tnl (ng/ml) Mean ±SD.</td>
<td>63.03 ± 70.99</td>
<td>68.12 ± 89.58</td>
<td>4.86 ± 0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (U/L) Mean ±SD.</td>
<td>323.81 ± 255.50</td>
<td>353.0 ± 315.25</td>
<td>164.0 ± 193.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH (U/L) Mean ±SD.</td>
<td>1213.61 ± 818.83</td>
<td>1555.08 ± 1330.47</td>
<td>365.50 ± 74.25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p: p value for comparing between the three genotypes. KW: Kruskal Wallis test
*
: Statistically significant at p ≤ 0.05

Table 4. Relation between the different genotypes and patients’ glucose and hs-CRP levels.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Genotype</th>
<th>CC (n=36)</th>
<th>CT (n=12)</th>
<th>TT (n=2)</th>
<th>Test of sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP (mg/l) Mean ±SD.</td>
<td>161.93 ± 144.53</td>
<td>90.02 ± 143.96</td>
<td>4.86 ± 0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mwp1</td>
<td>112.72 ± 37.58</td>
<td>108.83 ± 15.99</td>
<td>444.50 ± 518.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mwp2</td>
<td>0.100</td>
<td>0.049*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p: p value for comparing between the three genotypes
p1: p value for comparing between CC with each of CT and TT
p2: p value for comparing between AG and AA
MC: Monte Carlo test
FE: Fisher Exact test
KW: Kruskal Wallis test
MW: Mann Whitney test
*
: Statistically significant at p ≤ 0.05

Results

Patients of both sexes were more often smokers, had a higher prevalence of hypertension, diabetes and a more unfavorable lipid profile compared with controls. The inflammatory marker, hs-CRP, was markedly increased in patients compared with controls. Also, CK-total, CK-MB, troponin, AST and LDH were markedly increased in patients compared with controls. (Table 1)

Regarding the different PLA2G7 A379V genotype distributions between the 2 studied groups, we found that homozygous CC genotype had the highest frequency among patients (72%) compared with controls (46%), while we found that heterozygous CT genotype had the highest frequency among controls (46%) compared with patients (24%) with a statistically significant difference (p=0.033). (Figure 1)
According to different genotype distributions (p=0.033) and allele frequencies (p=0.012), there was no statistically significant difference between the patients’ sex. Also, our results showed no difference in genotype distributions or allele frequencies among patients regarding their sex. However, a statistically significant difference (p=0.043) was found between different genotypes regarding hs-CRP, (Table 3).

Validity of Hardy-Weinberg equilibrium regarding the 3 genotypes of the Lp-PLA2 A379V in all the studied population: As shown in table 5, the incidence of the C allele (p) = [(2X59) + 35]/ 200= 0.765 and the incidence of the T allele (q) = [35 + (2X6)]/ 200 =0.235. The observed and expected values were found nearly identical. This means that the Egyptian population is in Hardy-Weinberg equilibrium for the Lp-PLA2 A379V gene variant.

Validity of Hardy-Weinberg equilibrium regarding the 3 genotypes of the Lp-PLA2 A379V gene among patients: As shown in table 6, the incidence of the C allele (p) = [(2X36) + 12]/ 100 = 0.69 and the incidence of the T allele (q) = [12 + (2X23)]/ 100 = 0.31. The observed and expected values were found to be quite similar denoting that Egyptian patients having MI are in Hardy-Weinberg equilibrium for the Lp-PLA2 A379V gene variant.

Validity of Hardy-Weinberg equilibrium regarding the 3 genotypes of the Lp-PLA2 A379V gene among healthy subjects: As shown in table 7, the incidence of the C allele (p) = [(2X23) + 24]/ 100 = 0.69 and the incidence of the T allele (q) = [24 + (2X22)]/ 100 =0.31. The observed and expected values were found to be quite similar denoting that the controls are also in Hardy-Weinberg equilibrium for the Lp-PLA2 A379V gene variant.

**Discussion**

In our study, we found that homozygous CC genotype, coding for alanine at position 379 of Lp-PLA2 protein, had the highest frequency among patients compared with controls and was associated with increased incidence of MI, while we found that heterozygous CT genotype had the highest frequency among controls compared with patients and was associated with decreased incidence of MI, with a statistically significant difference (p=0.033) between patients and controls. The allelic frequencies for Lp-PLA2 A379V (46672943 C > T) SNP in our studied population did not show any deviation from Hardy-Weinberg equilibrium.

Also, we found that the major “C” allele, coding for alanine, had the highest frequency among patients compared with controls and was associated with increased incidence of MI, while we found that the minor “T” allele, coding for valine, had the highest frequency among controls compared with patients and was associated with decreased incidence of MI. So, there is a significant difference (p=0.012) between patients and controls with predominance of C allele in patients and T allele in controls.

In agreement with our study, Ninio et al., 22 Abuzeid AM et al., 23 and Ling LC et al., 24 reported that the homozygous (TT) and heterozygous (CT) forms of 379V polymorphism were less frequent in MI patients than in controls, suggesting that this allele might be protective against the development of CAD while A379 variant was more prevalent among patients.

In contrast to our study, Liu PY et al.,25 and Casas JP et al.,26 reported that 379V gene variant was more prevalent in Taiwanese patients who presented with acute coronary syndrome (ACS) than in controls. Also, Sutton et al.,27 reported that 379V polymorphism was more prevalent among MI patients.
than controls with a significant difference (p=0.002) which was against our results. This dissimilarity in results may be due to differences in ethnic groups, sample size and selection criteria of patients and controls. However, Wotton P et al., reported absence of any significant association between this polymorphism and coronary heart disease complications. In a Chinese study, the risk of MI was found to be higher among cardiovascular patients harboring the minor T allele compared with the major C allele. In a Taiwanese study, the T allele (379V polymorphism) was associated with lower Lp-PLA2 activity and increased risk of MI. In contrast, a study of European Caucasians revealed that T allele was associated with reduced risk of MI. But other studies on European Caucasians reported no association with CHD risk. In South Koreans, a similar lack of association between A379V and CVD was reported.

Personalized medicine is of growing interest, with a number of pharmacogenetic drug examples, like clopedogril and warfarin, where genetic variants influence the rate of drug metabolism and efficacy. Among the limitations of our study are the relatively small sample size and the inability to correlate the studied polymorphism with enzyme activity or mass.

It could be concluded from this study that the Lp-PLA2 A379V polymorphism was less frequent in Egyptians having MI than in healthy controls and was associated with a lower risk of cardiovascular events, suggesting that the minor T allele, coding for valine, might be protective against the development of MI while A379 variant was more prevalent among patients than controls, suggesting that the major “C” allele, coding for alanine could be used a risk factor for the development of MI. Moreover, there was no significant correlation between A379 and lipid profile, suggesting that the action of this enzyme is independent of other traditional risk factors. So, patients harboring the Lp-PLA2 A379 gene variant, or the C allele, might be candidates for specific Lp-PLA2 enzyme inhibitors, as darapladib.

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References