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The association between the D166E polymorphism of the lipoprotein associated phospholipase A2 and risk of myocardial infarction

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Abstract. – OBJECTIVE: Although numerous studies have evaluated the association between lipoprotein associated phospholipase A2 (Lp-PLA2) gene polymorphisms and coronary heart disease, the conclusions are still inconsistency. Here we detected the correlation between D166E polymorphism of Lp-PLA2 and myocardial infarction (MI). Further, its clinical value as biomarker was assessed.

PATIENTS AND METHODS: A total of 297 patients were enrolled, all diagnosed as MI at the Hebei General Hospital between May 2017 and May 2018, with 262 healthy subjects recruited as controls. Blood specimens of all participants were collected for testing serum lipid, blood glucose, Lp-PLA2, HsCRP, IL-17 and IL-35. The D166E polymorphism was genotyped. The correlation between D166E polymorphism and MI was explored using multiple logistic regression analysis.

RESULTS: We detected higher levels of TC, TG, LDLC, Lp-PLA2, HsCRP and IL-17 but lower levels of HDLC and IL-35 in MI patients, compared with healthy controls ($p<0.05$). Also, the positive ratio of family history is higher in MI patients than that in control. Indexes were collected after one-week and one-month hospitalization, respectively, and levels of Lp-PLA2, HsCRP, IL-17 and IL-35 decreased to the normal levels ($p>0.05$). We also observed positive correlations between Lp-PLA2 with HsCRP and IL-17 ($r=0.6517$, 0.2689), and negative correlations between IL-35 with Lp-PLA2, HsCRP and IL-17 ($r=-0.3142$, -0.3968, -0.2516), respectively. The G allele at D166E accounted for a higher percentage in MI patients than in controls, and so as the GG and GC genotypes ($p<0.05$). Logistic regression analysis showed close associations between MI with Lp-PLA2 and GG genotype at D166E, with odds ratios of 1.239 (1.023-2.017) and 9.863 (4.107-21.331), which suggested they were independent risk factors for the development of coronary heart disease.

CONCLUSIONS: The D166E (C/G) mutation of Lp-PLA2 was a potential risk factor of MI.

Key Words:
Lipoprotein associated phospholipase A2, Gene polymorphism, Myocardial infarction, Inflammatory factors.

Introduction

Myocardial infarction (MI) has already been regarded as one of the top health threats for populations in China due to the rapid increasing morbidity, poor prognosis, and high mortality rate. The development of MI has a complicated progress, with atherosclerosis confirmed as the pathological basis and inflammatory reaction as the incentive for MI[1-6]. Scholars[7-9] reported a variety of susceptible genes participating in the progress, such as angiotensinogen, CCR7 and MMP9. Recently, lipoprotein associated phospholipase A2 (Lp-PLA2), a vascular inflammatory biomarker, was also reported as being associated with MI by promoting the generation of atherosclerosis, especially due to the mutations of A379V, I198T, V279F and R92H sites[10-15]. However, the conclusions of those studies were inconsistent, and no researches have yet reported the association between the mutation of D166E site (Figure 1) and MI.

In this study, we compared serum Lp-PLA2 and D166E polymorphism of Lp-PLA2 as well as inflammatory factors between patients with MI and controls. Then, we evaluated the association between serum Lp-PLA2 and polymorphisms with MI.

Patients and Methods

Patients

A total of 297 patients including 149 male and 148 female aged 48.1±9.8 years, diagnosed with

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MI at the Hebei General Hospital from May 2017 to May 2018, were enrolled in this study. Another 262 healthy subjects matched in age (48.0±10.2 years) and gender (132 male and 130 female) were collected as controls, of whom visited the Hebei General Hospital for physical examination during the same period. Subjects were excluded if diagnosed with any of the following diseases: hepatitis, cirrhosis, nephritis, hydremic nephritis, congenital heart disease, cholecystitis, diabetes mellitus and any other symptoms which might affect the evaluation of the association between Lp-PLA2 and MI. Subjects with a family history of coronary heart disease were also excluded from the control group. Fasting specimens (7 ml) were sampled from all participants within 24 hours of hospitalization or physical examination, with 5 ml used for serum test after centrifugation and another 2 ml for the molecular test. This process was repeated in patients with MI one week and one month after hospitalization. All blood specimens were tested immediately or stored at -80°C. All participants had signed informed consent. This study was approved by the Behavioral and Social Sciences Ethical Review Committee of the Hebei General Hospital (Hebei, China).

Serum Detection

Total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDLC), low-density lipoprotein (LDLC) and glucose were measured by using Beckman AU5821 (Brea, CA, USA). Lp-PLA2 and HsCRP were detected with Abbott ARCHITECT plus i2000 SR (Chicago, IL, USA) and SIEMENS BNII system (München, Germany), respectively. IL-17 and IL-35 levels were measured by an enzyme-linked immunosorbent assay (ELISA). All procedures were conducted according to the manufacturers' instructions.

Polymorphic Analysis of D166E Site of Lp-PLA2

DNA was extracted from blood specimens using TIANamp Genomic DNA kit (Beijing, China). To identify polymorphism of the D166E site of Lp-PLA2, the polymerase chain reaction assay was performed with Mastercycler® gradient PCR analyzer (Eppendorf, Germany) using the following primers: forward primer, 5'-TGGTAGTTGTAATTCTTCCC-3'; reverse primer, 5'- TTGGCCAAAGAGCCCAATTA -3'. Primers were designed using Primer 6.0 software and then optimized by aligning in Genbank. PCR amplification was carried out in a volume of 100 µl containing 1 µg of genomic DNA, 0.2 $mmoll^{-1}$ dNTP, 10×PCR buffer 10 µl, 1.5 $mmoll^{-1}$ $MgCL_2$, 0.5 $\mu moll^{-1}$ of each primer and 2.5 units of KOD plus Pfu DNA polymerases, ddH_2O. The PCR amplification was done with a denaturation step at 95°C for 5 min followed by 35 cycles of PCR amplifications un-

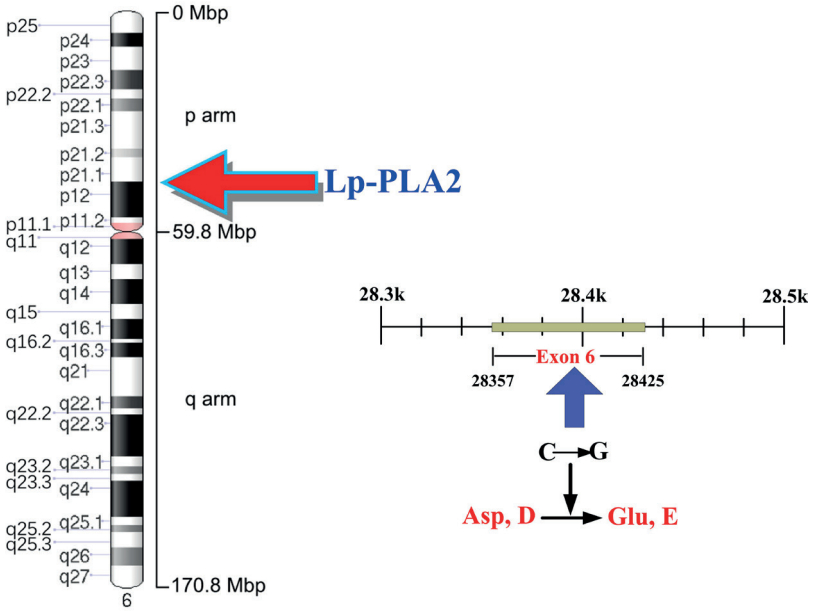

Figure 1. Location of Lp-PLA2 gene and mutation of D166E site. Lp-PLA2 gene is located in 6p21.2-p12 ***(A)***, and mutation of D166E site is located in the 6th exon of Lp-PLA2 gene ***(B)***.

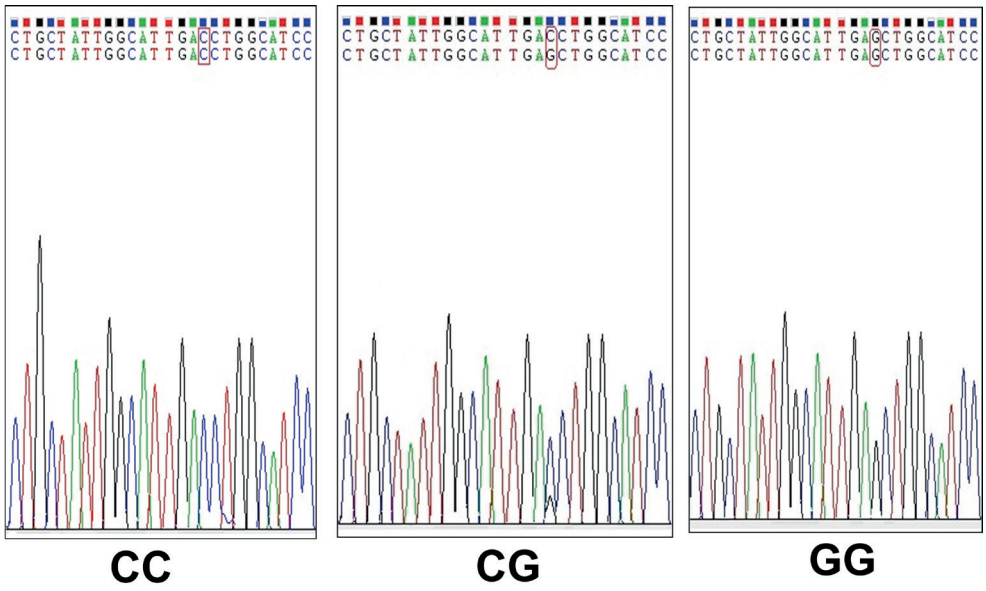

Figure 2. Results of DNA sequencing in D166E site of Lp-PLA2 gene. As shown in Figure 2, 3 genotypes and 2 alleles were verified with DNA sequencing in D166E site of Lp-PLA2 gene. The genotypes were GG (Mutant Homozygote), GC (Mutant Heterozygous), and CC (Wild Type).

der the following conditions: 94°C for 30 s, 60°C for 30 s, and 72°C for 60 s. The last step of PCR was 72°C incubation for 10 min. Amplified fragments were sequenced by ABI3730XL sequencer (Thermo-Fisher Scientific, Waltham, MA, USA) after purification. Sequencing data were aligned with Lp-PLA2 representative sequences downloaded from GenBank, EMBL and DDBJ to identify the mutations.

Statistical Analysis

Measurement data are expressed as mean and SD. Counting data are expressed as a percentage. The results of serum test - levels of TC, TG, LDLC, Lp-PLA2, HsCRP, IL-17 and IL-35 between patients and healthy subjects, were compared using Student's *t*-test, while the results of polymorphic analysis were tested using chi-square test. In this study, logistic regression analysis was applied to assess the association between polymorphism of D166E and MI. Spearman's rank correlation coefficient was applied to describe the correlations between LP-PLA2 with IL-17, IL-35 and HsCRP. All statistical analyses were performed by SPSS 16.0 (SPSS Inc., Chicago, IL, USA) software, with $p<0.05$ regarded as significance. The data were tested for Hardy-Weinberg equilibrium. All the genotypes fitted Hardy-Weinberg equilibrium ($p>0.05$).

Results

Results of Serum and Polymorphic Analysis

Table I indicated higher levels of TC, TG, LDLC, Lp-PLA2, HsCRP and IL-17 but lower levels of HDLC and IL-35 in MI patients, compared with healthy controls ($p<0.05$). No significant difference was observed for Glu between the two groups ($p>0.05$). It was shown that Lp-PLA2, HsCRP, IL-17 and IL-35 returned back to the normal values ($p>0.05$) after one-month hospitalization and treatment ($p>0.05$, Table II). As shown in Figure 2, two different alleles (G/C) were identified at the D166E site of Lp-PLA2, which constituted three genotypes: GG (homozygous mutant), GC (heterozygote mutant) and CC (wild type). As summarized in Table III, the frequency of G allele was significantly higher in MI patients (19.03%) than in healthy subjects (6.11%, $p<0.0001$). In response, GG and CG accounted for 4.72% and 28.62% of all genotypes in MI patients, which were higher than 1.15% and 9.93% in controls ($p=0.0002$). In contrast, the percentage of CC genotype was lower in patients (66.67%) than in controls (88.94%, $p<0.0001$) (Tables I-IV, Figures 2-4).

Results of Spearman Correlation Analysis and Logistic Regression Analysis

Spearman correlation analysis identified that a positive correlation existed between Lp-PLA2

Table I. Comparation of age, serum lipids, Glu, Lp-PLA2, HsCRP, IL-17, IL-35, and family history between MI patients and Control group ($\bar{x}$±s).

	MI group (297)	Control group (262)	t/χ^2	p
Age	48.1±9.8	48.0±10.2	-0.141	0.888
TC (mmol/L)	5.21±1.03	4.17±0.61	19.047	<0.001
TG (mmol/L)	1.83±0.69	1.05±0.27	23.540	<0.001
HDLC (mmol/L)	0.72±0.14	1.29±0.31	-37.506	<0.001
LDLC (mmol/L)	3.65±0.68	2.41±0.39	30.129	<0.001
Glu (mmol/L)	4.92±0.58	4.93±0.57	1.425	0.112
Lp-PLA2 (ng/ml)	375.74±64.33	112.54±27.82	69.462	<0.001
HsCRP (mg/L)	25.41±4.92	4.97±0.83	87.143	<0.001
IL-17 (pg/ml)	179.25±23.66	42.3±11.2	32.458	<0.001
IL-35 (pg/ml)	113.47±22.73	305.7±53.2	-42.159	<0.001
Family history (%)	113 (38.18)	31 (11.84)	83.005	<0.001

and HsCRP, then Lp-PLA2 was correlated positively with IL-17 (r=0.6517 and 0.2689), but negatively with IL-35 (r=-0.3142). Similar negative correlations also were observed between IL-35 with HsCRP and IL-17, with a coefficient value of -0.3968 and -0.2516, respectively. Logistic regression model was fitted by setting MI morbidity as the dependent factor; the levels of TC, TG, LDLC, HDLC, Lp-PLA2, HsCRP, IL-17 and IL-35, and the genotypes of D166E as the independent factors. Dummy variables were used to describe the genotypes of D166E, with (1, 0) representing GG genotype, (0, 1) for GC and (0, 0) for CC. The results indicated close associations between MI with Lp-PLA2 level and GG genotype, with odds ratios of 1.239 (1.023-2.017) and 9.863 (4.107-21.331), respectively (Table V).

Discussion

The development of MI has a complicated progress, with susceptible genes and inflammatory factors participating in such as Lp-PLA2, HsCRP, IL-17 and IL-35[16-18]. Previous studies reported a higher level of Lp-PLA2, an inflammatory factor belonging to phospholipase superfamily, in atherosclerosis plaques, especially in macropha-

Table II. Levels of Lp-PLA2, HsCRP, IL-17, IL-35 in MI patients and Control group ($\bar{x}$±s).

	n	Lp-PLA2 (ng/ml)	HsCRP (μg/ml)	IL-17 (pg/ml)	IL-35 (pg/ml)
MI patients					
Pre- hospitalization and treatment	297	375.74±64.33$^{*\Delta}$	25.41±4.92$^{*\Delta}$	179.25±23.66$^{*\Delta}$	113.47±22.73$^{*\Delta}$
1w after hospitalization and treatment	297	251.94±42.52$^{*\#}$	15.18±2.91$^{*\#}$	101.16±16.42$^{*\#}$	214.68±34.97$^{*\#}$
1m after hospitalization and treatment	297	114.85±24.73$^{\#\Delta}$	5.02±0.56$^{\#\Delta}$	43.71±12.36$^{\#\Delta}$	306.15±52.09$^{\#\Delta}$
Control group	262	112.54±27.82	4.97±0.83	42.3±11.2	305.7±53.2

*Compared with Control group, $p<0.05$; #Compared with MI patients (pre- hospitalization and treatment), $p<0.05$; ΔCompared with MI patients (1 w after hospitalization and treatment), $p<0.05$.

Table III. Genotypes and alleles at the D166E site of Lp-PLA2 gene in MI patients and Control group [n *(%)*.].

		Genotypes			Alleles	
	n	CC	CG	GG	C	G
MI patients	297	198 66.67)	85 (28.62)*	14 (4.72)*	481 (80.98)	113 (19.03)*
Control group	262	233 (88.94)	26 (9.93)	3 (1.15)	492 (93.89)	32 (6.11)$^{\#\Delta}$

*Compared with Control group, $p<0.05$.

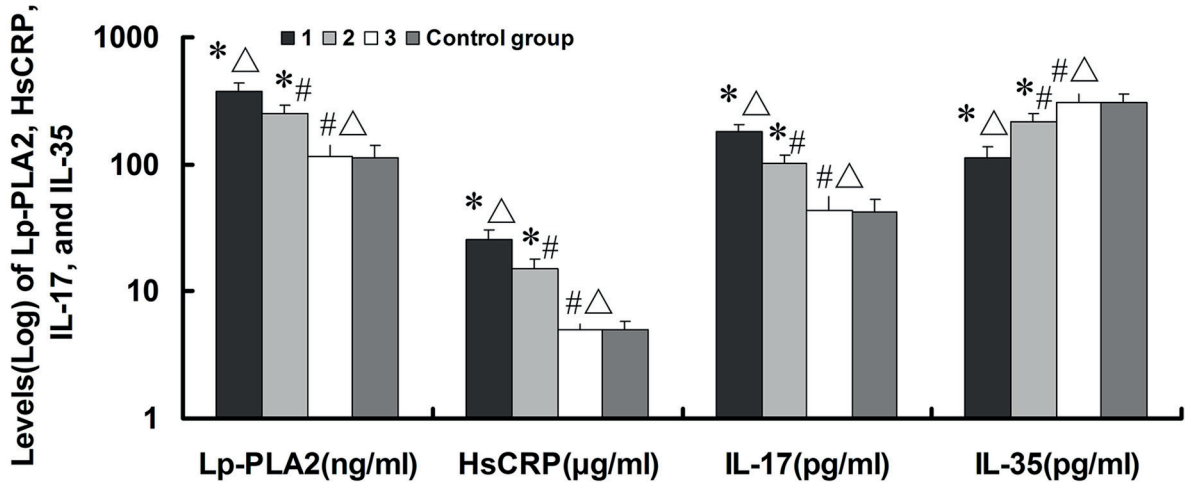

Figure 3. Serum levels of Lp-PLA2, HsCRP, IL-17, IL-35 in MI patients and control group. Serum levels of Lp-PLA2, HsCRP, IL-17 in MI patients before treatment were all higher than those in Control group, whereas IL-35 was lower (p<0.05). After treatment, levels of Lp-PLA2, HsCRP, IL-17 decreased, while IL-35 increased gradually. In Figure 3, 1: MI patients (pre-hospitalization and treatment); 2: MI patients (1 w after hospitalization and treatment); 3: MI patients (1 m after hospitalization and treatment); 4: Control group. *Compared with Control group, p<0.05; #Compared with MI patients (pre- hospitalization and treatment), p<0.05; ΔCompared with MI patients (1 w after hospitalization and treatment), p<0.05.

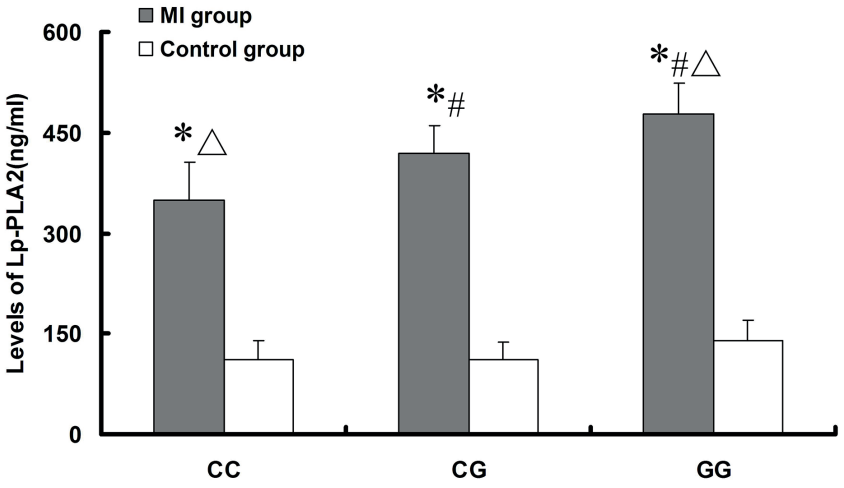

Figure 4. Lp-PLA2 levels in different genotypes. As shown in Figure 4, different levels of Lp-PLA2 were identified in different genotype groups. Levels of Lp-PLA2 were all higher in MI group than in Control group, and levels of Lp-PLA2 in MI group decreased in the order of GG genotype, CG genotype, CC genotype (p<0.05). *Compared with same genotype in Control group, p<0.05; #Compared with CC genotype in same group, p<0.05; ΔCompared with CG genotype in same group, p<0.05.

gocytes of fibrous caps. This indicated a correlation between Lp-PLA2 and coronary heart disease. HsCRP is an acute-phase protein composed of lever cells during inflammatory stimulation[19-21]. IL-17 stimulates the release and gather of inflammatory factors in many diseases, while IL-35 inhibits inflammatory reactions[22-29]. In this study, we observed higher levels of Lp-PLA2, HsCRP and IL-17 but lower levels of IL-35 in MI patients, suggesting the occurrence of inflammatory reaction during the development of MI as well as the association between Lp-PLA2 and MI. We also observed higher levels of TC, TG and LDLC, but a lower level of HDLC, which suggested the correlation between MI and dyslipidemia.

Lp-PLA2 is located in 6p21.2-p12 with 38378bp, which codes a protein chain with 441 amino acid residues[30]. Mutating of the 6th exon nucleotide of

Table IV. Levels of Lp-PLA2 in MI patients and Control group with different genotypes ($\bar{x}$±s). ng/ml).

		CC (198)	CG (85)	GG (14)	F	p
MI patients	(297)	350.10±56.24$^{*\Delta}$	418.61±41.18$^{*\#}$	478.08±45.34$^{*\#\Delta}$	80.322	<0.001
		CC (233)	**CG (26)**	**GG (3)**		
Control group	(262)	112.25±27.95	112.01±25.78	139.95±30.36	1.480	0.2297

*Compared with same genotype in Control group, $p<0.05$; #Compared with CC genotype in same group, $p<0.05$; $^{\Delta}$Compared with CG genotype in same group, $p<0.05$.

Table V. Logistic regression analysis for detection of risk factors of MI.

Variables	B	SE	Wald	df	p	OR	95% CI
TC	0.077	0.295	3.018	1	0.105	1.172	0.579-1.427
TG	0.136	0.118	0.048	1	0.624	0.793	0.402-1.105
HDLC	-0.138	0.264	1.312	1	0.523	1.241	0.767-1.719
LDLC	0.275	0.387	4.026	1	0.079	1.307	0.792-1.899
HsCRP	0.334	0.375	3.197	1	0.367	1.048	0.824-1.238
IL-17	0.644	0.504	1.214	1	0.196	1.483	0.874-2.267
IL-35	1.103	0.447	3.687	1	0.069	0.891	0.613-1.036
Lp-PLA2	0.197	0.354	4.592	1	0.034	1.239	1.023-2.017
Gene				2			
Gene (1)	2.156	0.413	4.238	1	0.029	4.596	2.536-8.173
Gene (2)	3.948	0.526	16.497	1	0.000	9.863	4.107-21.331

the codon coding the 166th amino acid residue, namely D166E, from C to G, will result in Aspartic acid (Asp, D) changing to Glutamic acid (Glu, E), which further changes the spatial structure and biological function of the translated protein chain. This study indicated a higher percentile of G allele, as well as GG and CG genotypes in MI patients, suggesting the mutation of D166E may be associated with MI. Moreover, logistic regression analysis indicated that GG genotype was an independent risk factor of MI. The results also suggested a correlation between serum level of Lp-PLA2 and MI. This implied that Lp-PLA2 affected MI by stimulating the release of IL-17 while inhibiting the activation of IL-35, or the inflammatory reactions during MI caused the increasing level of Lp-PLA2.

Conclusions

We identified a close correlation between D166E mutation of Lp-PLA2 and the development of MI. Therefore, genotype detection of Lp-PLA2 can be a sensitive indicator predicting the risk of MI especially in vulnerable populations. However, the specific mechanism remains unclear and further studies are needed.

Conflict of interest

The authors declare no conflicts of interest.

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