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Original Research Article

Evaluation of Genetic Polymorphisms in CD36 Gene and Other Co-factors in Al-Baha Population with Myocardial Infarction Disease

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Abstract

To evaluate the role of the genetic mutations of cd36 gene in development of myocardial infarction and to build data for the risk factors for the disease among Saudi population of Al- Baha District. An association case-control study was conducted between May 2014 and April 2015 to investigate the role of CD36 deficiency in the development of MI among Saudi population at Al- Baha District. Cases with MI were enrolled in the study after giving an informed consent to participate. Blood sample was drawn for measurement of serum markers and DNA extraction. Individuals without clinical evidence of (CAD) were enrolled as Controls. Mutations in CD36 gene among patients and controls were screened by polymerase chain reaction (PCR) and restricted fragment length polymorphism (RFLP). SPSS, T-test, Chi-square test and Fisher's exact test were used for statistical analysis. P<0.05 values were considered as significant. Myocardial infarction (MI) is considered as a leading cause of death around the world. An association case-control study of Saudi patients with MI (n=77, M/F=62/15, average age 64.12±13.6) and their normal controls (n=31, M/F=21/10, average age 58±20.3) was conducted between May, 2014 and April, 2015 in Saudi population of Al-Baha area, KSA, to investigate the role of CD36 deficiency and other co-factors in the development of MI. Smoking, high blood pressure, heart failure and diabetes were found to be associated with MI and represent as risk co-factors in predisposition to the disease and their Odd Ratio (*O.R*) were 5.8, 3.91, 3.91 and 1.6 respectively. The single nucleotide polymorphism (478 C›T, Pro90 homozygous) of the gene CD36 was highly prevalent (96%) and its mutant heterozygous (Pro90/Ser90) was not found in the Saudi population in Al-Baha area. The single nucleotide polymorphism (478 C›T, Pro90 homozygous) of the gene CD36 is highly prevalent (96%) and its mutant heterozygous (Pro90/Ser90) was not found in the Saudi population in Al-Baha area. Factors such as smoking, high blood pressure, heart failure and diabetes have strongly associated with MI according to their Odd Ratio (5.8, 3.91, 3.91 and 1.6 respectively) and these factors are represented as co-factors for the disease predisposition. Our study in Al-Baha population represents a preliminary study, so, in order to know the actual and accurate prevalence of the single nucleotide polymorphisms (SNPs) or mutations in the CD36 gene and their association with MI in Saudi population, Further studies are needed for other loci on CD36 gene.

Keywords: CD36 – C478T, Genetic factors, Myocardial infarction, Cholesterol, Polymorphism

INTRODUCTION

Consanguinity refers to the marriage of parents with a recent common ancestor. Consanguineous mating (inbreeding) is an important phenomenon genetically as it brings about an increase in homozygous genotypes and a decrease in the corresponding heterozygous form (Thompson *et al.,* 1991). Saudi Arabia is among the

countries of the world with a high rate of consanguinity (El Hazmi and Swailem, 1995).

Myocardial infarction (MI) is considered as a leading cause of death around the world (Ron *et al*, 2015) and usually caused by occlusion of a coronary artery, which is induced by thrombosis and/or rupture of plaque based on atherosclerosis of the coronary arteries (Libby, 2000). Atherosclerotic coronary diseases (ACD) and MI remain the most significant causes of death in developed countries and by 2020 it is expected to be the first cause of death worldwide (Libby, 2000). Several epidemiologic studies have documented hypertension, hypercholestero lemia, diabetes, obesity, hypertriglyceridemia, sedentary lifestyle and smoking as the most important risk factors for cardiovascular disease (Aboderin *et al*., 2002; Hirano *et al*., 2003; American heart association, 2004). In spite of screening and the treatment for these major risk factors, still small portion of patients with significant cardiovascular disease who do not have any of these risk factors suffer events, such as stroke and MI.

Numerous epidemiologic studies have documented that stroke has a significant genetic component (Paula *et al*., 2015; American Heart Association, 2004; Juan *et al*, 2004). Family studies reported that the incidence of MI in individuals whose first-degree relatives died of MI is 2 to 4 times higher than that in the general population (Shea *et al*., 1984). Twin studies in three populations demonstrated that the concordance of MI was higher among monozygotic twins than among dizygotic twins, especially in females (Marenberg *et al*., 1994).

Based on these observations, it has been suggested that genetic factors may participate in the development of ACD and/or MI, in addition to environmental factors. Several studies have examined the association between the polymorphisms in the candidate genes for ACD or MI. It has been reported that several gene polymorphisms are associated with ACD/MI in certain populations (Ron *et al*., 2014; Ye *et al*., 2006; Libby, 2000).

CD36 is a membrane glycoprotein and has been identified in a wide variety of cell types including platelets, monocytes, erythroblasts, capillary endothelial cells, and mammary epithelial cells (Greenwalt *et al.,* 1992; Tandon *et al.,* 1989; Knowles *et al.,* 1984; Edelman *et al.,*1986; and Greenwalt *et al.,* 1990). Recently, it has been shown that CD36 is one of receptors for oxidized low density lipoproteins and fatty acids, suggesting that CD36 may play a part in atherogenesis and lipid metabolism (Endeman *et al.,* 1993; Abumrad *et al.,* 1993). In humans, CD36 deficiency was first identified in patients with refractoriness to multiple platelet transfusions and is relatively common (2–7%) in persons of Asian and African descent (Hirano *et al*., 2003). CD36 deficiency is divided into two subgroups: type I deficiency, in which neither platelets nor monocytes express CD36, and type II deficiency, in which monocytes express CD36 despite the lack of platelet CD36. Two mutations that are reported to be responsible for CD36 deficiency, a substitution of T for C atnt 478 of CD36 cDNA in codon 90 (proline90→serine) and a dinucleotide deletion at nt 539 in codon 110 (Kajihara *et al*., 2001; Kashiwagi *et al*., 1993). Studies in ^{478}C→T demonstrated that subjects with type I deficiency are homozygous for the Pro90→Ser mutation, whereas subjects with type II deficiency are heterozygous for this mutation (Kajihara et *al*., 2001).

In this study, we investigated the role of the genetic mutations of cd36 gene and the risk factors in development of myocardial infarction among Saudi population of Al- Baha District. We demonstrated that the ^{478}C→T substitution (Pro90, wild homozygote) of the gene CD36 was highly prevalent (96%) and its mutant heterozygote (Pro90/Ser90) was not found in the Saudi population in Al-Baha area. On the other hand, smoking, high blood pressure, heart failure and diabetes were found to be associated with MI and represent as risk co-factors in predisposition to the disease in the Saudi population in Al-Baha area (*O.R*. were 5.8, 3.91, 3.91 and 1.6 respectively).

METHODS

An association case-control study was conducted between May 2014 and April 2015 in Saudi population of Al-Baha area, KSA, to investigate the role of CD36 deficiency in the development of MI.Ethical approval for the study was obtained from the research committee of the academic affairs at King Fahad Hospital, Al-Baha, and from the director of Baljurashi general Hospital, Baljurashi, KSA.

Unrelated patients of MI attending King Fahad and Baljurashi general hospitals and their healthy controls were enrolled in the study.All selected patientshave to give an informed consent to participate in the study. A health questionnaire was answered by the patients and filled by a doctor, and their anthropometric measures were taken. A series of healthy controls from general representing, unrelated subjects from population of Al-Baha and Baljurashi who never have a history of MI or angina, clinical evidence of CAD, stroke or any atherosclerotic disease in the past and are matching in age or older, sex, and tribe were selected as controls after accepting to participate in the study.

Blood samples (5ml) were drawn in a vacutainer tube with EDTA for measurement of serum markers and DNA extraction. Genomic DNA was extracted from peripheral blood leukocytes using the standard extraction protocol (Thermo Fisher Scientific, Gene JET whole blood genomic DNA purification mini kit, K0781, EU, Lithuania). Ethidium bromide (0.5 µg/ml) was incorporated into the agarose gels (1.5 %) and DNA was separated by electrophoresis for 1 hour at (100V), visualized by placing on a UV light source and photographed directly using Micro DOC gel documentation system (Cleaver Scientific, UK.).

Table 1. Selected single nucleotide polymorphism (SNP), chromosomal and structural location.

Polymorphism (SNP)	Gene	Chromosomal location	Structural location
478 C›T	CD36	7q11.12	Exon 4 codon 90

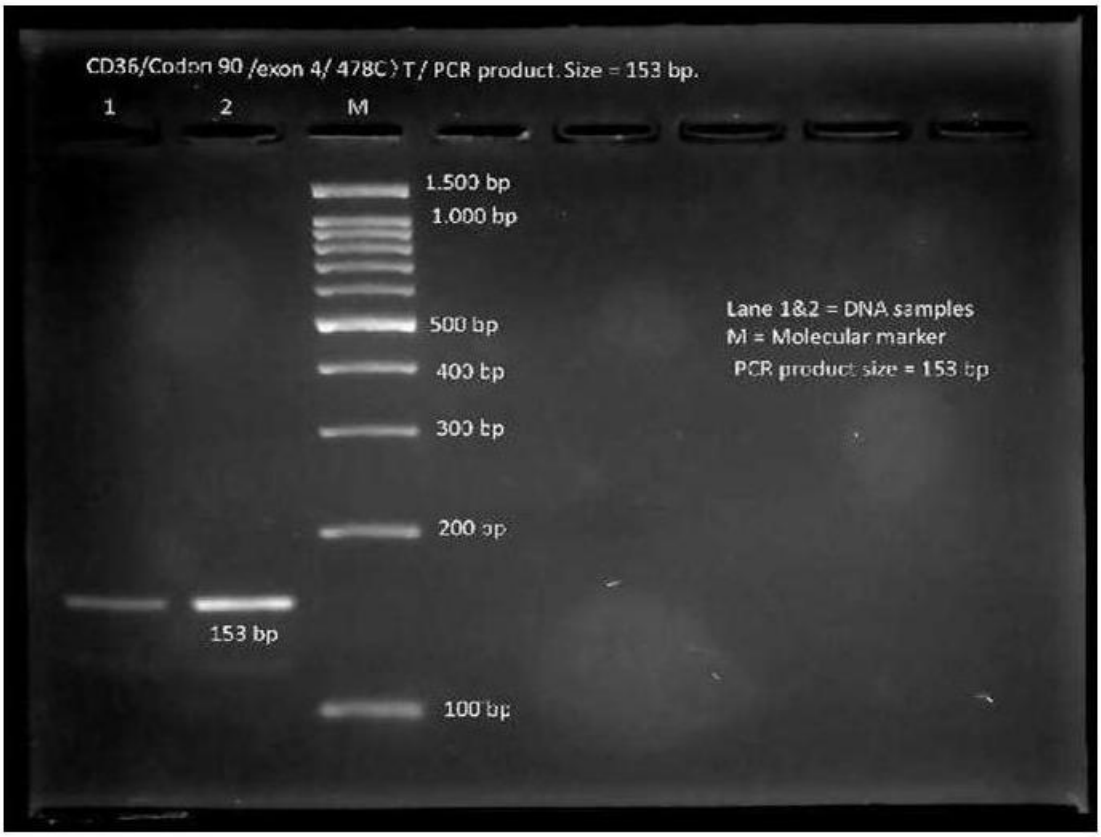

Figure 1. Shows the PCR product of CD36/exon 4/codon 90/ 478 C›T (153 bp, lane 1 and 2), M is a molecular marker (100 bp Ladder); the bands were visualized by UV light (302 nm) after incorporation of EtBr in the agarose gel (2%).

Table 2. Nucleotide sequences of primers used in this study

Primers used in PCR-RFLP		
Mutation	Upstream (Forward) Primer	Downstream (Reverse) Primer
478 C›T	5`-GGCACAGAAGTTTACAGACAG-3`	5`-ATGGTCAAGGTAAGAGTGT

Screening of the genetic variant 478 C›T in codon 90 of the human CD36 gene (*Table 1* and *Figure 2*) among patients and healthy controls was done by PCR-RFLP (Techne, TC-512, Bibby Scientific, UK.) Amplification of the DNA segments of CD36 gene was carried out in a volume of 10 µl containing 100 ng of DNA using primer pairs (*Table 2*, Macrogen, Seoul, Korea).

PCR conditions as follows; denaturation at 98 ˚C for 20 s, followed by annealing at 55 ˚C for 20s and extension at 72 ˚C for 30s for 40 cycles. The expected PCR product size is 153 bp (*Figure 1*). The amplified products (PCR products) were digested at 37 ˚C for 10hrs with 0.4 U of restriction enzyme Sau96I (Thermo Fisher Scientific, EU, Lithuania).

The digested DNA was loaded on agarose gel (2.0%) in TAE buffer and electrophoresed at 100 V for 1 hour. Digestion of the 153-bp PCR product with Sau96I yielded the following three distinct patterns: 94- and 59-bp

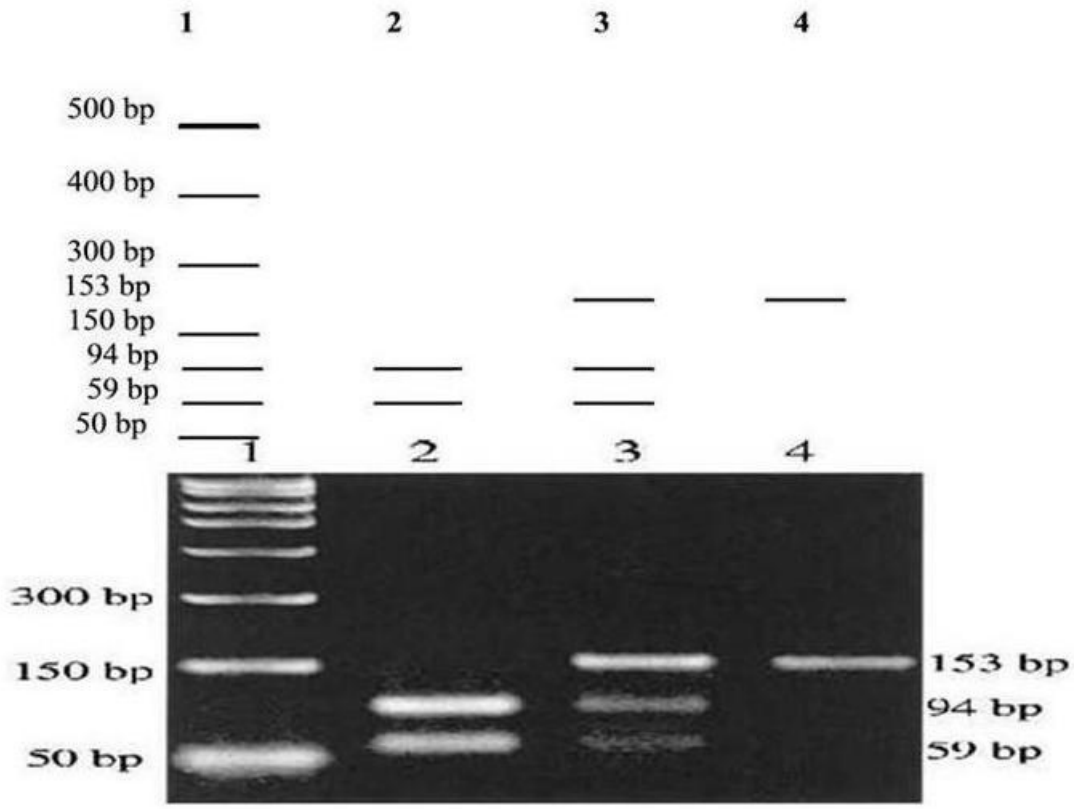

Figure 2. Digestion of 153 bp PCR product of CD36/exon 4/codon 90/ 478 C›T by RFLP. Lane M (1) is a ladder, lane 2 shows 94- and 59-bp products defined the Pro90 homozygote, lane 3 shows 153-, 94 and 59-bp products defined the Pro90/Ser90 heterozygote (mutant type) and lane 4 shows 153-bp product (undigested) defined the Ser90 homozygote (adapted from Kajihara, S. *et al*., 2001).

Table 3. Clinical characteristics of the Saudi MI patients and normal controls in Al-Baha area, KSA

Characteristic	Case (n=77)	Control (n=31)	*P value*	*O.R*
Gender (M/F)	62/15	21/10	0.12	-
Age (years)	64.12 ± 13.6	58 ± 20.2	0.008	-
Smoking (%)	61	32.3	0.27	5.8
High blood pressure (HBP, %)	54	29	0.023	3.91
Diabetes (%)	55	29	0.09	1.6
Total cholesterol (mmol/l)	4.2 ± 1.28	4.29 ± 0.89	0.8	-
LDL cholesterol (mmol/l)	0.52 ± 0.36		0.6	-
Triglyceride (mmol/l)	1.6 ± 0.69	1.6 ± 0.65	0.005	-
TWBC/cell/mm3	8.18 ± 3	7.3 ± 2.5	0.16	-
Hemoglobin (g/dl)	14.28 ± 2.7	14.04 ± 2.6	0.17	-
Fasting blood sugar (FBS, mmol/l)	10.7 ± 5.4	7.91± 3.62	0.7	-
Abnormal lipids	1.47 ± 0.5	1.83 ± 0.37	0.08	-
Raised cardiac enzymes (%)	80.5	3	0.0001	-
Over weight (%)	32.5	20	0.2	-
Peripheral vascular disease (%)	4	3	0.08	-
Stroke (%)	8	0	0.08	-
Cardiac arrest (%)	3	0	0.85	-

Table 3. Continue

Irregular heart beat (%)	10	16	0.04	-
Heart failure (%)	22	0	0.05	3.91
Physical activity (%)	4	10	0.04	0.024
MI (%)	79.2	0	0.0001	-
Abnormal resting echogram (%)	76.6	0	0.0001	-
Abnormal echocardiogram (%)	74	0	0.0001	-
Body weight/kg	74.5 ± 12.6	74.3 ± 16.01	0.48	-
SNP genotype (%)	96	90	0.37	-

Table 4. Cross tabulation of smoking with MI in Al-Baha Saudi population

		MI		Total
		Yes	No	
Smoking	Yes	49	2	51
	No	21	5	26
Total		70	7	77

O.R= 5.8

Table 5. Cross tabulation of high blood pressure with MI in Saudi population from Al-Baha province.

		MI		Total
		Yes	No	
High blood pressure	Yes	47	2	49
	No	24	4	28
Total		71	6	77

O.R= 3.91

products defined the Pro90 homozygote, 153-, 94 and 59-bp products defined the Pro90/Ser90 heterozygote (mutant type) and a 153-bp product defined the Ser90 homozygote (*Figure 2*).

The Statistical Package for the Social Science (SPSS version 21 for Windows 8) was used for statistical analysis. T-test was used for comparing the distribution of a variable between cases and controls groups. The Chi-square test was applied in comparing of categorical data. Genotyping distributions among the subjects and controls were determined using a continuity-adjusted Chi-square or Fisher's exact test for each genotype compared with the homozygous wild-type for 478C→T locus.Odds ratios were calculated with 95% confidence intervals. $P<0.05$ values were considered as significant.

RESULTS

An association case-control study of Saudi patients with MI (n=77, M/F=62/15, average age 64.12±13.6) and their normal controls (n=31, M/F=21/10, average age 58±20.3) was conducted between May, 2014 and April, 2015 in Saudi population of Al-Baha area, KSA, to evaluate the role of known polymorphism at locus (C478T) as a genetic factor leading to CD36 deficiency and to investigate the role of other co-factors in predisposition to MI.

Clinical characteristics of the Saudi MI patients and normal controls

The clinical characteristics of the Saudi MI patients and normal controls in Al-Baha area were represented in table (3). Smoking, high blood pressure, heart failure and diabetes were found to be associated with MI and represented as risk co-factors in predisposition to the disease and their Odds Ratio (*O.R*) were 5.8, 3.91, 3.91 and 1.6 respectively (*Tables*, *4-7*).

Table 6. Cross tabulation of heart failure in Saudi population with MI in Al-Baha district.

		MI		Total
		Yes	No	
Heart failure	Yes	47	2	49
	No	24	4	28
Total		71	6	77

O.R= 3.91

Table 7. Cross tabulation of diabetes with MI in Saudi population at Al-Baha area.

		MI		Total
		Yes	No	
Diabetes	Yes	45	2	47
	No	28	2	30
Total		73	4	77

O.R= 1.6

Table 8. Cross tabulation of physical activity with MI in Saudi population in Al-Baha area.

		MI		Total
		Yes	No	
Physical activity	Yes	2	5	7
	No	66	4	70
Total		68	9	77

O.R=0.024

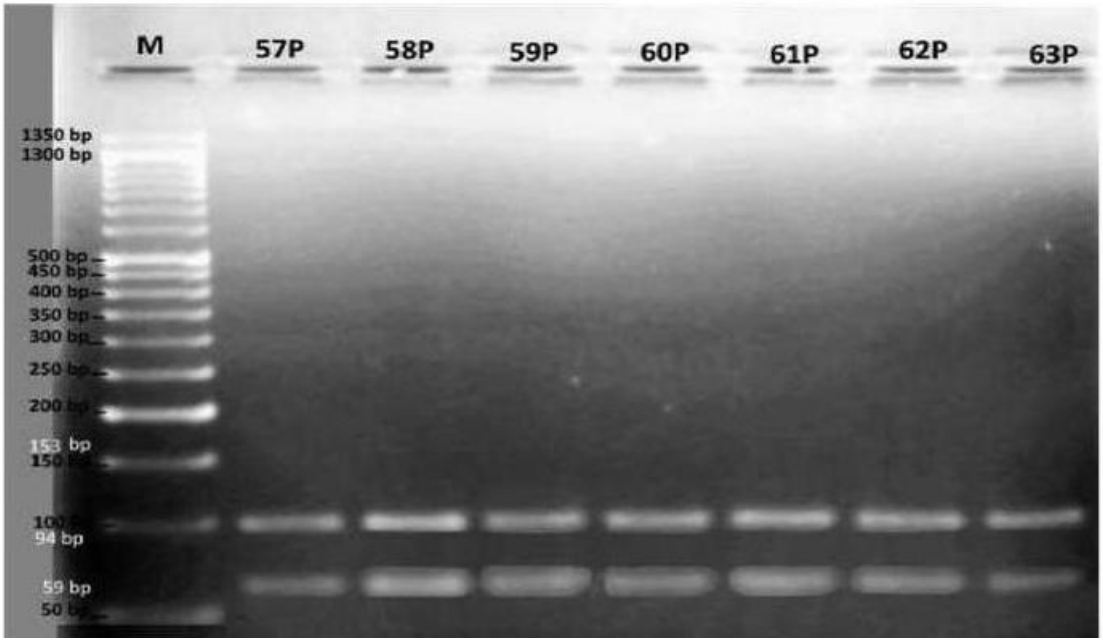

Figure 3. Digestion of 153 bp PCR product of CD36/exon 4/codon 90/ 478 C›T and genotyping by RFLP. Lane M is a ladder; lanes 57P- 63P show 94- and 59-bp products defined the Pro90 homozygote patients.

PCR-RFLP results and genotyping

DNA was extracted from all study subjects (patients and control, n=108) and tested for positivity as described in the methods. The genotyping of CD36/ exon 4/ codon 90/478 C›T of the study subjects was represented in (*Figure 3*).

DISCUSSION

In the present study, the results of controls showed significant clinical measurements when compared to the patients (Table 3). In this regards, the occurrence of the disease in normal individuals depends on the other co-factors (cholesterol level, physical activity, blood pressure, heart failure and diabetes).

Although, 66 (85.7%) of myocardial patients were mainly sedentary (physically not active), the physical activity was found to be not associated with the disease as a risk co-factor for its predisposition (O.R=0.024, Table 8).

Most of MI patients (96%) were genotyped for the above mutation as (Pro90) homozygous. This means that 96% of the study population were not carrying this mutation (478 C›T) because the mutant type of the mutation is (Pro90/Ser90 heterozygous) which was not found in Saudi population of Al-Baha area (Figures 2 and 3).

Our study in Al-Baha population is represented as a preliminary study, so, in order to know the actual and accurate prevalence of the SNPs or mutations in the CD36 gene and their association with MI and other diseases in Saudi population, further studies on other loci of CD36 gene are needed.

CONCLUSION

1. The single nucleotide polymorphism (478 C›T, Pro90 homozygous) of the gene CD36 is highly prevalent (96%) and its mutant heterozygous (Pro90/Ser90) was not found in the Saudi population in Al-Baha area.

2. Factors such as smoking, high blood pressure, heart failure and diabetes have strongly associated with MI according to their Odd Ratio (5.8, 3.91, 3.91 and 1.6 respectively) and these factors are represented as co-factors for the disease predisposition.

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