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Original Article

Relationship of Polymorphisms of the Mutation (225131) of the *pou1f1* Gene with some Productive Traits in Iraqi Camel Females (*Camelus dromedarius*)

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Abstract

Camels are semi-ruminant placental mammals, classified as two-toed, padded-footed mammals, and belong to the family Camelidae, which includes the one-humped camels *(Camelus dromedaries*), and the two-humped camels (*Camelus bactrianus*), llama, Alpaca, Vicuna, and Guanaco. The study used 50 Iraqi single-humped camel females who belonged to private fields in the AL-Furat AL-Awsat region, which involved three Iraqi cities (Babylon, Diwaniyah, and Muthanna). All the Biotechnological and Molecular Genetics analyses were performed in the Altakadum Laboratory, Baghdad, in order to determine the genotypes and their distribution ratios for the POU1F1 gene and the relationship of the Polymorphism of the gene with some productive traits, growth characteristics (weight and body dimensions) and blood biochemical parameters of animals. The mutation 225131 was in the second exon region, in which there was a change in the amino acid C.49 CAA>CAC Gln>His. Three genotypes were discovered in this mutation in the second studied piece, which included the region of the first intron and the second exon, with a length of 777 base pairs using DNA sequencing technology. The results indicated that there were highly significant differences ($P \leq 0.01$) in the distribution ratios of the genotypes resulting from the mutation. The results also showed a significant relationship between these genotypes with somebody dimensions, as there was a significant superiority ($P \leq 0.05$) for individuals with mutant CC genotype over the wild AA and hetero AC genotypes in each of the traits, body height from the front (220.66±1.76, 215.12±0.92, 212.80 ±2.33) cm and body length (186.66±1.20, 179.47±1.10, 170.00±4.96) cm and head length (55.00±2.08, 50.78±0.46, 51.20 ±1.31) cm for the mutant, wild and hetero, genotypes respectively. Concerning the characteristics of the length of the milking season, daily and total milk production, and its chemical components, there was no significant relationship between the genotypes resulting from the studied mutation.

Keywords: Genotypes, Body dimensions, Exon, Milk production

1. Introduction

Camels are semi-ruminant placental mammals, and they are classified as two-toed, padded-footed mammals and belong to the family Camelidae, which includes the one-humped camels *(Camelus dromedaries*), and the two-humped camels (*Camelus bactrianus*), llama, Alpaca, Vicuna and Guanaco (1). The number of chromosomes in camels is 74, nearly identical, with only slight differences in the amount and patterns of heterochromatin distribution (2, 3). Camels constitute approximately 20%, estimated around 16 million heads of the total livestock in the Arab world (4).

Determining the genotypes of any gene and studying the quantitative trait loci (QTL), and detecting the presence of mutations are of great importance in the

application of selection programs and increase the genetic yield, and the application of molecular technologies in evaluating the performance of ruminants through the study of genetic homogeneity within the breed and the relationship between genes with multiple effects to predict productivity as well as study its genetic diversity to preserve it as a genetic source (5, 6). Quantitative traits are affected by many factors, including hereditary and non-hereditary, and the composition of the herd and the location of the study had a significant effect on some economic traits of ruminants, especially milk production and composition (7).

The genetic and phenotypic characterization of camels to evaluate the biological diversity and differences between the current Iraqi breeds is a prerequisite for facilitating their conservation and use in breeding and improvement programs, as Iraqi camels are not classified, and the information available about them is minimal at the local, national, regional and global levels (8). The genetic formations of these genes are revealed using modern techniques, as their discovery led to a qualitative shift that changed the course of biological sciences. Recently, the technique of sequencing the nitrogenous bases analysis of genes or parts of them was used to determine the genotypes and discover mutations and use them as markers, and their impact on different traits (9, 10) and among the genes with multiple genotypes is the POU1F1 gene, which is one of the transcription factors of the POU-family. POU1F1 gene in camels is located on the first chromosome, consists of 9 exons, and has a size of 237287 PB (11). The POU1F1 gene is associated with reproduction and many economic and productive traits, including animal weight, milk production, and its components because it is responsible for the gene expression of growth hormone (GH), prolactin (PRL), and thyroid-stimulating hormone (TSH-ß) (12-14). It was found that mutations that occur in the POU1F1 gene lead to a different expression of the genes GH, PRL, TSH, and POU1F1 itself and then affect the level of these hormones, which in turn leads to a change in the body's ability to perform vital functions such as growth, development of mammary glands, production and secretion of milk (15, 16).

Despite the growing interest in the Polymorphism of the POU1F1 gene and its association with the productive traits of different agricultural animals during the past years, and since there are no studies on the Polymorphism of this gene in camels, the study aimed to the following determining the genetic phenotypes of the POU1F1 gene in Iraqi camel females, determining the relationship between the genetic manifestations of the POU1F1 gene, total and daily milk production and the length of the milking season, chemical components of milk and biochemical blood parameters.

2. Materials and Methods

2.1. Experimental Animals and Study Site

50 Iraqi camel females were used in this study. These animals single-humped belonged to private fields in the AL-Furat AL-Awsat region and were randomly selected from Babylon city (n=20), 15 females from Diwaniyah city (156 km south of Baghdad city), and 15 females from Muthanna city (270 km south of Baghdad city), whose ages range from 8-15 years.

2.2. Blood Samples Collection

Blood samples were drawn and collected from the jugular vein and the abdominal milk vein, with one sample for each camel and an amount of 10 ml for each sample, using a medical syringe with a capacity of 20 ml.

2.3. DNA Extraction

DNA was extracted from blood samples drawn using the kit according to the manufacturer's instructions attached to this kit Presto™ Mini gDNA Kit, Geneaid, Taiwan.

2.4. Primers Designed

The primers (Forward and Reverse) were supplied by the Macrogen company in a lyophilized powder. Lyophilized primers were dissolved in nuclease-free water to give a final concentration as a stock solution, which is 100 pmol/µl. A working solution of these primers was prepared by adding 10 µl of stock solution

(stored at -20 °C) to 90 µl of nuclease-free water to obtain the final concentration of the working solution of 10 picomols/µl (Table 1).

2.5. Reaction Setup and Thermal Cycling Protocol

Materials and PCR reaction conditions showed in tables 2 and 3.

2.6. DNA Sequencing

PCR products were sent to the Microgene Corporation - Korea to read the nitrogenous base sequences and detect their genetic mutations. The results were obtained by email, then analyzed using generous software.

2.7. Daily Milk Yield (DMY)

The females were milked twice a day, in the morning and the evening, and the milk production was recorded per day by weighing the milk using a scale. The total milk production was also calculated according to the following equation **:**

Total milk production = daily milk production rate **x** number of milking days

2.8. Animal body dimensions and weight

The dimensions of the body were taken by measuring tape in centimeters and for each animal in a state of natural standing as much as possible on a flat floor (17). The body weight was also estimated according to the method Yāgîl (18) through the following equation:

Live weight (kg) = shoulder height (m) x heart girth (m) x hump girth (m) x 50

2.9. Statistical ANALYSIS

The data were analyzed using Statistical Analysis System SAS (19) to study the effect of the genetic phenotypes of the POU1F1 genes on the studied traits according to the mathematical model below, and significant differences were compared using the least square means method.

$Yijk = \mu + Pi + Aj + eijk$

Since: Yijk: Viewed value k.

µ: the general average of the adjective.

Pi: influence of genotypes for the POU1F1 gene and each SNP.

Aj: the effect of age (8-15 years). eijk: the naturally distributed random error with a mean equal to zero and a variance of $\sigma 2e$.

The Chi-square-$\chi 2$ test was also used to compare the percentages of the genotype distribution in the POU1F1 gene.

Table 1. Primer sequence

Primers	Start	Stop	Length	Tm	GC%
Forward 5`-CTTCAACCTACGTCCATCATAC-3`	243	265	22	60	45.5
Reverse 5`-CTAGTCAAGATTCCCTCAAACC-3`	998	1020	22	60	45.5

Table 2. Master mix components and volumes

No.	Components of Master Mix	Volume	
		1 Sample	50 Sample
1	Master Mix	12.5 µl	625 µl
2	Forward primer	1 µl	50 µl
3	Reverse primer	1 µl	50 µl
4	Nuclease Free Water	7.5 µl	375 µl
5	DNA	3 µl	µl 150
6	Total volume	25 µl	µl1250

Table 3. Thermal cycling protocol

Stages	Temperature	Time	Number of courses
Initial Denaturation	95	5 Minute	1
Denaturation	95	30 Second	30
Annealing	60	30 Second	
Extension	72	Minute 1	
Final Extension	72	Minute 7	1
Hold	10	Minute 10	

3. Results and Discussion

3.1. Identification of *POU1F1* Gene Polymorphisms

In the current study, three SNPs of the *POU1F1* gene were detected in fragment 909 bp of the *POU1F1* gene in Iraqi she-camels by direct DNA sequencing (Figures 1 and 2).

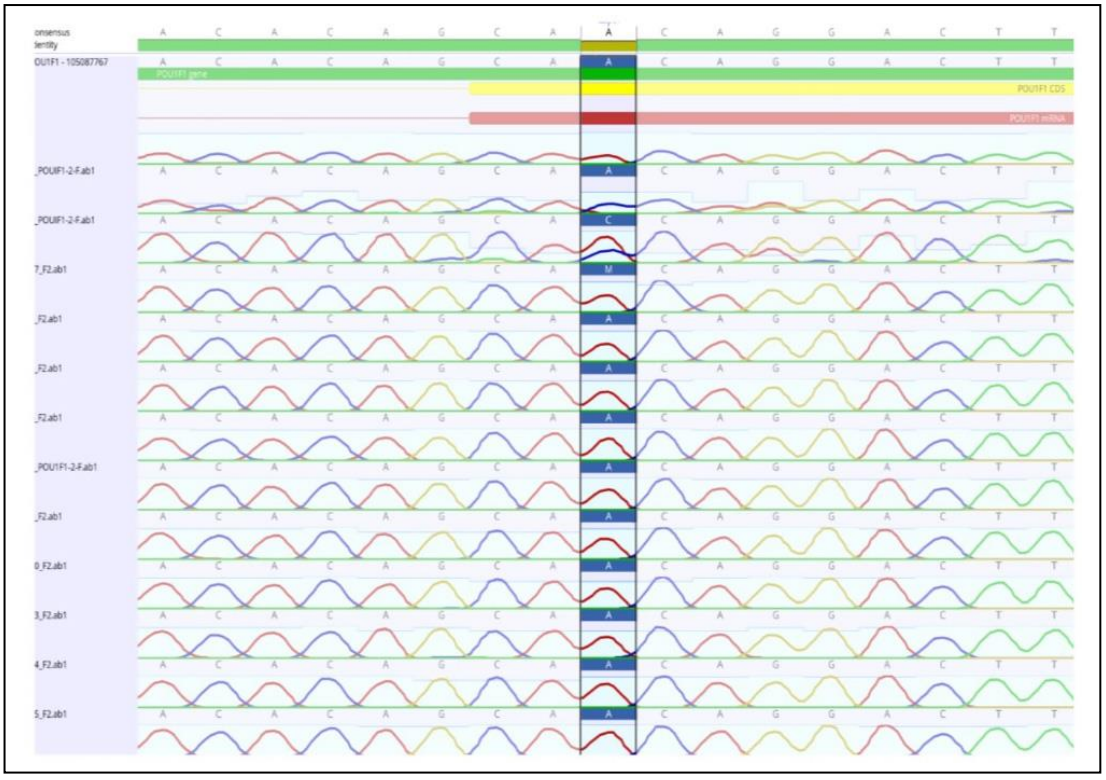

Figure 1. The site of the mutation (225131) in the second studied segment of the POU1F1 gene

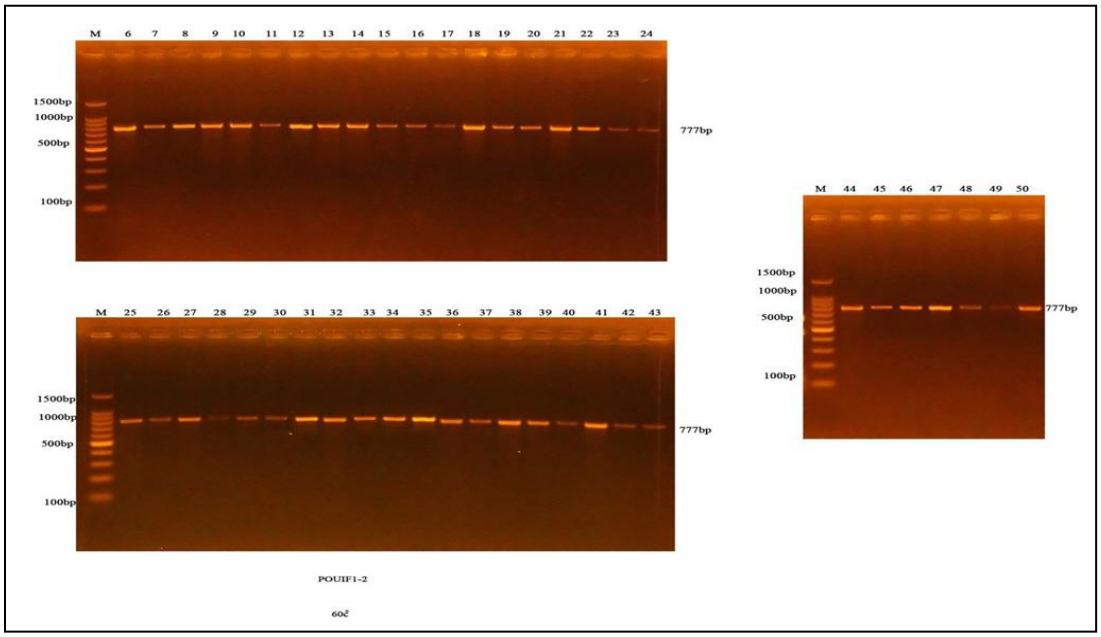

Figure 2. Result of the amplification of POU1F1 gene of camel female's samples

3.2. Percentages, Number, and Allelic Frequency of the Mutation (225131)

It is evident from table 4 that there are highly significant differences ($P \leq 0.01$) in the distribution ratios of the three genotypes of the mutation (225131) in Iraqi camel females, as we find that the percentages of these structures amounted to 84, 10 and 6% for each of the wild AA, hetero AC, and mutant CC was sequential, the allelic frequency of wild A was 0.92, and the mutant C was 0.08.

Table 4. Number, percentages of genotypes, and allelic frequency of POU1F1 gene (225131/A>C)

Genotype	No.	Percentage (%)
Wild: AA	42	84.00
Hetero: AC	5	10.00
Mutant: CC	3	6.00
Total	50	% 100
Chi-square value (χ^2)	----	** 92.840
Allele	**Frequency**	
A	0.92	
C	0.08	

**($P \leq 0.01$)

3.3. The Relationship of the Mutation (225131) with Milk Production and Its Chemical Components

The results in table 5 show no significant effects between the three genotypes resulting from this mutation with each of the length of the milking season, daily and total milk production, and its chemical components.

3.4. The Relationship of the Mutation (225131) with Body Dimensions and Animal Weight of Iraqi Camel Females

The results of table 6 showed that there were no significant differences between the three genotypes resulting from this mutation with both body weight and body dimensions except for body height from the front, body length, and head length in Iraqi camel females. The results showed that the mutant CC was significant ($P \leq 0.05$) on both the wild AA and hetero AC, the results were (220.66±1.76, 186.66±1.20, and 55±2.08) in favor of the CC mutant, respectively.

3.5. Relationship of Mutation (225131) with Biochemical Blood Parameters

Table 7 showed no significant differences between the three genotypes resulting from this mutation with all blood biochemical parameters.

Table 5. Relationship of the genotypes of the POU1F1 gene (225131/A>T) with milk production and its chemical components

Traits	(Mean±Standard error) Genotypes			Significant level
	AA	AC	CC	
Length of the milk (season day)	247.28±3.93	258.80±13.59	255.00±12.05	NS.
Milk production for the third month (kg/day)	4.09±0.16	3.88±0.52	4.75±0.52	NS.
Milk production for the fourth month (kg/day)	4.33±0.14	3.84±0.55	4.90±0.21	NS.
Milk production for the fifth month (kg/day)	4.74±0.14	4.30±0.62	5.50±0.28	NS.
Total Milk Production (kg)	1101.44±34.86	1048.66±141.82	1302.61±128.08	NS.
Lactose in milk (%)	4.75±0.15	5.16±0.38	5.56±0.96	NS.
Milk Triglycerides (g/l)	33.63±0.65	37.16±0.83	34.02±1.72	NS.
Milk Cholesterol (mg/l)	42.16±2.68	45.04±2.10	36.63±12.68	N.S.
Milk protein (%)	3.33±0.14	3.67±0.11	3.02±0.42	N.S.
Total Solids in Milk (%)	12.89±0.34	13.34±0.34	12.37±0.77	NS.
Fatty acids in milk (%)	0.940±0.85	0.098±0.01	0.093±0.04	NS.

NS: Not significant

Table 6. Relationship of the genotypes of the POU1F1 gene (225131/A>T) with body dimensions and animal weight

Traits	(Mean±Standard error) Genotypes			Significant level
	AA	AC	CC	
Front Body Height (cm)	215.12±0.92[ab]	212.80±2.33[b]	220.66±1.76[a]	*
shoulder height (cm)	193.69±1.29	192.80±3.99	195.00±1.52	NS.
body length (cm)	179.47±1.10[ab]	170.00±4.96[b]	186.66±1.20[a]	*
head length (cm)	50.78±0.46[b]	51.20±1.31[ab]	55.00±2.08a	*
neck length (cm)	111.88±0.96	109.00±0.89	115.33±3.75	NS.
tail length (cm)	52.28±0.57	50.60±1.63	51.66±2.40	NS.
Heart girth (cm)	206.69±1.53	212.00±3.80	204.00±9.01	NS.
hump girth (cm)	278.57±2.70	290.80±7.53	287.66±16.69	NS.
animal weight (kg)	559.85±11.28	595.53±30.25	573.44±50.05	NS.

The averages with different letters within the same row differ significantly between them
* ($P \leq 0.05$), NS: Not significant

Table 7. Relationship of the genotypes of the POU1F1 gene (225131/A>T) with biochemical blood parameters

Traits	(Mean±Standard error) Genotypes			Significant level
	AA	AC	CC	
Glucose (mg/dL)	100.43±2.57	104.75±1.74	102.88±2.98	NS.
Total Protein (g/dL)	7.40±2.02	6.77±0.38	5.94±0.15	N.S.
Cholesterol (mg/dL)	87.88±2.09	93.36±1.79	88.82±1.93	N.S.
Triglycerides (mg/dL)	56.12±1.08	54.79±1.23	57.90±2.15	N.S.

N.S. : Not significant

The study's results showed that the mutation (225131) was in the second exon region of the second studied piece, in which there was a change in the amino acid C.49 CAA>CAC Gln>His. Regarding the length of the milking season and the total and daily milk production, the study's results showed the absence of a clear significant relationship between it and all the genotypes resulting from these mutations. The results of the current study were different Zaky, Abdel-Khalek (20) in total milk production reached 1559 kg. While it was very close in the daily milk production, which amounted to 4.69 kg/day, it also differed from Nagy, Fábri (21) in the daily milk production, reaching 4 kg. It also differed Chamekh, Khorchani (22) in the daily milk production of 4.21 kg/day and the total milk production of 1388.41 kg. This result can be explained by the fact that the effect of the multiple manifestations of this gene in milk production is due to the role of the proteins of this gene in regulating the gene expression of the prolactin gene, in addition to the fact that the gene expression of the POU1F1 gene is confined to the anterior lobe of the pituitary gland, in which the lactotroph cells responsible for secreting the hormone prolactin are located, as well as the presence of expression of this gene in the mammary gland cells in lactoblasts (23).

Concerning the components of milk, the results of our study were in agreement with what was found by Shamsia (24), who found that the percentages of lactose, protein, fat and non-fat solids amounted to 4.86, 3.46, 4, and 12.2%, respectively. Also, our results were close to Nagy, Fábri (21) in each the percentage of protein and lactose amounted to 2.95 and 4.19%, respectively, while it was higher in the percentage of total solids, which amounted to 10.46%. However, our results differed Chamekh, Khorchani (22) concerning protein and total solid percentage, which amounted to 2.67 and 10.75%, respectively. Our results coincided with Babiker and El-Zubeir (25) in the percentage of protein and lactose, which were 3.32 and 4.59%, respectively. However, it differed Yadav, Kumar (26), who found that the percentages of fat, protein, lactose and non-fat solids were 2.20, 2.80, 2.86, and 8.58%, respectively. This difference is also due to the

difference in the animal's breed, the herd's health status, the type of feeding, and the geographical location.

The results of the current study differed from the findings of Tandoh and Gwaza (27) regarding body weight when they found that the average weight of Nigerian camels was 754.8 kg. Our results also differed from those of Yosef, Kefelegn (28), who found that the average weights of camels for some Ethiopian breeds were 439.76, 533.95, 567.00, and 375.14 kg in each of the Jijiga, Hoor, and Shanili breeds, respectively. Also, our results differed from those of Zaalan (29), who found that the Judi breed was superior to the Khawar and Hurra breeds. The average weights of the camels for the Judi, Khawar, and Hurra breeds were 660.78, 449.08, and 425.32 kg, respectively.

With regard to shoulder height, heart girth, and hump girth, our results differed from what was found by Belkhir, Chehma (30), who concluded that the shoulder height was 192.2 and 178.1 cm, heart girth was 181.5 and 190.1 cm, and hump girth was 220 and 163.8 cm for both the Tarji and the Saharan breeds respectively. Also, our results in shoulder height were very close to the results of Zaalan (29), who registered values of 200.29, 190.06, and 194.80 cm for the Judi, Khawar, and Hurra breeds, respectively.

The studied camel females were distinguished by recording high values for both the height and length of the animal compared to Chniter, Hammadi (31) found, who recorded the animal's heights 194, 193, 190, 186, and 192 cm, and the animal's length 147, 145, 142, 136 and 138 cm in both the Gueoudi, Guiloufi, Merzougui, Tataouine, Medenine, and Tunisia breeds respectively. Our results also outperformed and were higher than was found by Tandoh and Gwaza (27), which recorded the animal's height as 204.2 cm and the animal length as 159.8 cm in Nigerian camels. Part of the variation in the height of the animal may be due to the difference in the size of the hump according to the degree of the animal's body condition (32); the hump is the main store of fat in camels, which represents on average 85% of adipose tissue (33).

In terms of head and neck lengths, our results were similar to what was found by Belkhir, Chehma (30), who registered head lengths of about 51 and 52 cm, while it differed in neck lengths which amounted to 109 and 102 cm for the Targi and Saharawi Algerian breeds respectively. While it was very close to what was found by Shah, Sarwar (34) with respect to the head length, which reordered 50 cm and higher neck length of 87.6 cm in the Pakistani Kohi breed.

In terms of tail length, our results were higher than those obtained by Chniter, Hammadi (31), who found that the tail length was 38 and 41 cm in both the Gueoudi and Guiloufi breeds of Tunisia, respectively, while the results of our study were less compared to 55.80 cm Nigerian camels Tandoh and Gwaza (27). Our results were also less than those recorded by Yosef, Kefelegn (28), who observe that tail length was 59.55 and 63.17 cm in both Jijiga and Hoor breeds, respectively. This may be due to their adaptation to protect themselves from flies.

The differences between the breeds of Arab camels in the expression of anthropometric measurements may be due to the difference in each breed's genotype and the geographical location (35, 36). According to González, Luque (37), it was demonstrated that phenotypic diversity is a good reflector of ecological selection systems and breed history. The phenotype is the genetic performance of the traits and adjusted according to the environmental conditions, and the sum of genetic and environmental variance affects the phenotypic variance (38). As for the geographical location, this may be due to the nature of the agricultural environment and the availability of feed and water, as the quality of feed in terms of phosphorous and calcium available in a given area may have an impact on most body measurements due to its direct role in the development of bones and skeleton (28).

Authors' Contribution

Study concept and design: T. A. H. A. and W. I. I.
Acquisition of data: T. A. H. A. and W. I. I.

Analysis and interpretation of data: T. A. H. A.
Drafting of the manuscript: W. I. I.
Critical revision of the manuscript for important intellectual content: T. A. H. A. and W. I. I.
Statistical analysis: T. A. H. A.
Administrative, technical, and material support: T. A. H. A. and W. I. I.

Ethics

The study design was approved by the ethics committee of the Al-Furat AL-Awsat Technical University, Najaf, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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