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Identification on SNP polymorphisms associated with meat productivity in the local Kazakh horse breed

The aim of our study was to genotype local horses of the Kazakh breed, including the Zhabe u types from the Pavlodar and Zhetysu regions, as well as the Adai type from the Mangystau region, using PCR-RFLP methods for the LCORL and PRKAG3 gene loci. A total of 41 horses were included in the study. The results showed that the frequency of the homozygous genotype TT at the LCORL locus was 81.25% in Zhabe Pavlodar horses, with a heterozygous genotype TC of 18.75%. In Kazakh horses of the Adai type from Mangystau and Zhabe type from Zhetysu, no genetic polymorphism was detected, and all individuals had a homozygous genotype – TT. For the PRKAG3 locus, genetic polymorphism was only found in Adai type horses, with one mare out of 17 tested horses having a heterozygous genotype – CT (5.89%). Horses of the Zhabe type in Pavlodar and Zhetysu regions had a single homozygous CC genotype. The level of homozygosity for the LCORL gene locus in the three studied groups was: 81.25, 100.0 100.0. For the PRKAG3 gene is 100.0, 94.11, 100.0. The level of heterozygosity for the LCORL gene locus in horses of Pavlodar region was 18.75%, for the PRKAG3 gene locus in horses of Mangistau region 5.89%. Thus, LCORL and PRKAG3 gene polymorphisms play a significant role in determining the development of muscle mass, skeletal structure, chest circumference, and growth rate in horses. Therefore, these SNP polymorphisms can potentially serve as DNA markers for evaluating meat production in horses.

Keywords: horse genotyping, Kazakh horses, Zhabe, Adai, LCORL gene, PRKAG3 gene, PCR-RFLP analysis, DNA markers of productivity.

Introduction

Increasing meat production is currently the most relevant task facing livestock breeders in Kazakhstan. In solving this problem, in addition to cattle breeding and sheep farming, there is an opportunity to develop productive horse breeding. In breeding work aimed at improving the productive qualities of stud horses, Kazakh horses of the Zhabe type have exceptional importance. They differ from the main mass of other breeds of herd horses in Kazakhstan by a higher live weight and slightly larger size. An important link in breeding work with Kazakh Zhabe horses is the development of selection methods to improve their breeding and productive qualities. The successful solution of this task largely depends on increasing the efficiency of breeding work through the wide implementation of achievements in population genetics and the maximum realization of the genetic potential of horse productivity [1].

Analysis of foreign literature indicates that the study of polymorphisms in the LCORL and PRKAG3 genes has practical significance, as the alleles of these genes have an associative impact on the development of muscles in horses.

The LCORL (ligand-dependent nuclear receptor corepressor-like) gene has been identified as a candidate gene associated with various traits, including body size, height, and skeletal growth in horses. LCORL is located on chromosome 3 in horses and is highly conserved across species, suggesting a conserved function in growth regulation [2].

One of the earliest studies on LCORL in horses was conducted by Makvandi-Nejad et al. who investigated the association of LCORL with height in Thoroughbred racehorses. The study found that the T allele of LCORL was significantly associated with height in Thoroughbreds, with the T allele carriers being taller than the CC homozygotes [2].

Another study conducted by Tetens et al. identified genetic loci associated with withers height in German Warmblood horses, and the findings indicated that the LCORL/NCAPG region on chromosome 3 plays an important role in determining withers height. The identification of this genetic variant may have important

implications for selective breeding programs aimed at producing horses of specific sizes for different purposes [3].

The results of the study Metzger et al. showed that there was a significant association between LCORL expression and body size in horses. Specifically, larger horses had higher levels of LCORL expression compared to smaller horses. Furthermore, the study identified two single nucleotide polymorphisms (SNPs) in the LCORL gene that were significantly associated with height and weight in horses [4].

Researchers Staiger et al. found a significant association between skeletal variation in the forelimbs and hind limbs of Tennessee Walking Horses and a region on chromosome 3 that includes the LCORL/NCAPG genes. This region had previously been identified as a candidate locus for withers height in horses, and this study provides further evidence of its role in determining skeletal size and morphology [5].

Tozaki et al. (2016) reported that certain sequence variants of the BIEC2-808543 locus, which was located near the LCORL gene, are associated with body composition in Thoroughbred racehorses undergoing training. The study found that specific sequence variants near the LCORL gene, particularly BIEC2-808543, were significantly associated with body composition traits such as body weight, body condition score, and subcutaneous fat thickness. These findings suggest that the LCORL gene may play a role in determining body composition and could potentially be used in breeding programs to select for desired body composition traits in Thoroughbred horses [6].

The PRKAG3 gene, also known as the AMP-activated protein kinase gamma 3 subunits, plays an important role in regulating energy metabolism in skeletal muscle. This gene has been the focus of several studies in horses, particularly in relation to athletic performance and muscle function [7].

A study was conducted by Park et al., to identify genetic variations, specifically for PRKAG3 gene SNPs, which may affect muscle development and intended performance in horses. The study included multiple horse breeds with varying phenotypes. Seven SNPs were identified, five of which resulted in amino acid substitutions. These genetic variations may have an impact on the traits of interest, including muscle development and performance, in horses [7] (The results of the study showed that certain variations in the PRKAG3 gene were associated with differences in muscle glycogen content and exercise performance in horses).

Armeiro et al. investigated the influence of specific genetic variations, or polymorphisms, on muscle performance-related traits associated with the PRKAG3 gene and male fertility-associated traits associated with the SPATA1 gene in Mangalarga horses. The study involved the analysis of DNA samples collected from a group of Mangalarga horses, followed by a series of molecular biology and statistical analyses to examine the relationships between genetic variations and performance traits [8].

In some breeds of livestock, including pigs [9], cattle [10] and goats [11], certain genetic variations in the PRKAG3 gene have been associated with traits related to meat quality, such as tenderness and juiciness.

According to the information provided above the LCORL and PRKAG3 genes has been shown to play a significant role in determining muscle development in horses.

Thus, investigating SNP polymorphisms at the loci of the LCORL and PRKAG3 genes in local horse breeds of the Kazakh population is relevant, as these markers are associated with the meat productivity of horses. The aim of this study was to investigate the distribution of genetic variants at the LCORL and PRKAG3 gene loci, determine the levels of heterozygosity and homozygosity, assess the genetic equilibrium at the studied gene loci, in local horse breeds such as Zhabe and Adai.

This study was conducted to determine the polymorphism of the LCORL and PRKAG3 genes and their association with body size in local Kazakh Zhabe and Adai horses using the PCR-RFLP method.

Experimental

For the study, 41 blood samples from horses with EDTA were used, including 16 samples of the local Zhabe breed (LLP “Akshiman-Agro” of Pavlodar region), 17 samples of the local Adai breed (Peasant farm “Kozhyr-Ata” of Mangystau region) and 8 samples of the local Zhabe breed (Peasant farm “Akimbekov” of Zhetysu region). DNA extraction from blood samples was performed in the laboratory of the Department of Obstetrics, Surgery and Biotechnology of Reproduction of Kazakh National Agrarian Research University using the following method. Firstly, blood samples were thawed at room temperature for an hour. Then, 2.0 ml of thawed blood were placed in numbered tubes, which were centrifuged at 14500 rpm for 8 minutes. The upper phase was removed, leaving the sediment in the tube, to which 500 μl of TES lysis buffer was added and mixed using a vortex. The tube with its contents was centrifuged again at 14500 rpm for 8 minutes. The top part was removed, and 500 μl of TES buffer was

added again to the sediment. After mixing on a vortex until the mixture became homogeneous, the tube was centrifuged at 14500 rpm for 8 minutes. Then, 50 µl of 10% SDS and 5 µl of proteinase K were added to the tube and mixed on a vortex. After that, the tubes were shaken for 15 minutes and left overnight in a thermostat at 37°C. The samples were then removed from the thermostat, and 500 µl of 5M NaCl solution was added to each sample, mixed using a vortex, and centrifuged at 14500 rpm for 5 minutes. Using a pipette, the top layer (supernatant) was transferred to another tube in an amount of 500 µl, and isopropanol was added in a 1:1 ratio. The tube was mixed, centrifuged for 2-3 minutes at 10,000 rpm, and the top part was removed. The remaining DNA in the tube was washed twice with 70% ethanol, and the obtained DNA was dissolved in 1X TE buffer with a volume of 50 µl. The obtained DNA was evaluated using two methods: horizontal electrophoresis in a 0.8% agarose gel and measurement of DNA concentration by microspectrophotometric analysis (NanoDrop™ 2000).

The DNA samples of horses were genotyped for the LCORL gene locus using the following primers: forward F-5'- TGGAGTCAGTTGGGTTTAATG - 3' and reverse R - 5' - GACCGGATAGCATAGAGAGAG - 3'. The resulting amplicon length was 284 bp, and for allele identification, the AluI restriction enzyme with the recognition site AG/CT was used. After restriction, fragments of 284 bp, 169 bp, and 115 bp were formed depending on the animal's genotype. The PCR conditions for genotyping the DNA samples at the LCORL gene locus were as follows: initial denaturation at 94 °C for 5 min, 33 cycles of denaturation at 94 °C for 45 sec, primer annealing at 56.6 °C for 45 sec, extension at 72 °C for 45 sec, and final synthesis at 72 °C for 5 min.

Allele identification in the 8th exon of the PRKAG3 gene was performed using the following primers: forward F-5' - GAGGTGGGACAGTCTGGGGGCT-3' and reverse R - 5' - ACTGAAGGGCTGGGGAAGGGACT -3'. The PCR product length was 182 bp, and to determine the genetic variants, the AluI restriction enzyme was used. After hydrolysis of the amplicon, fragments of 182 bp, 118 bp, and 45 bp were obtained depending on the animal's genotype. The PCR conditions for the PRKAG3 gene were as follows: initial denaturation at 94 °C for 5 min, 34 cycles of denaturation at 94 °C for 30 sec, primer annealing at 66 °C for 30 sec, extension at 72 °C for 30 sec, and final synthesis at 72 °C for 5 min.

Results and Discussion

The work on genotyping DNA samples of horses was carried out at the laboratory of “Green Biotechnology and Cell Engineering” at the Kazakh-Japanese Innovation Center of Kazakh National Agrarian Research University. The average concentration of DNA samples was 289.24 ng/µl, with a minimum value of 1.2 ng/µl and a maximum concentration of 1344.7 ng/µl. Another important quality indicator of isolated DNA is the degree of sample purification, i.e., the ratio of DNA concentration values at A260/A280 wavelength. 85% of DNA samples had values greater than 1.70 and 15% of samples had lower values, less than 1.70. Amplification of the desired fragment of the corresponding genes was carried out according to the temperature regime, and the composition of the reaction mixture was: 2.5 µl of 10X PCR buffer with KCL, 1.0 µl each of forward and reverse primers, 2.0 µl of a mixture of four dNTPs, 0.2 µl of Taq DNA Polymerase (recombinant) 5U/µl, 1.5 µl of 25 mM MgCl2, bidistilled water in the amount of 15.8 µl, and 3.0 µl of DNA samples. The results of the polymerase chain reaction were verified using a 4.0% agarose gel stained with ethidium bromide.

To amplify the necessary regions of the LCORL and PRKAG3 genes, primer sequences and a PCR temperature regime described in the work of foreign scientists were used. The results of the amplification were checked using horizontal electrophoresis on a 4.0% agarose gel (Fig. 1, 2). The identification of alleles of the LCORL and PRKAG3 genes was carried out by hydrolyzing the PCR product with the AluI endonuclease. Thus, for the LCORL gene locus, individuals with the homozygous TT and heterozygous TC genotypes were identified (Fig. 3). However, for the PRKAG3 gene locus, out of the 41 tested samples, only one mare was found to have a heterozygous CT genotype, where fragments of 182 bp, 118 bp, 45 bp, and 19 bp were detected on the electropherogram (Fig. 4). It should be noted that samples 7 and 8 contain an additional fragment with an approximate size of 130 bp, which should not be present on the electropherogram. This may indicate that the observed pattern is the result of an artifact.

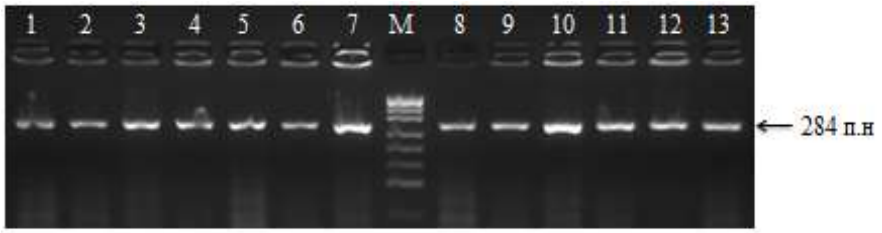

Figure 1. Electropherogram of the PCR product of the LCORL gene, lanes 1-13, with an amplicon size of 284 bp, M - DNA marker pUC19/MspI

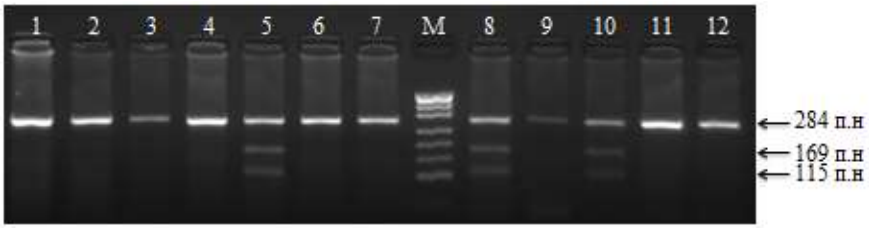

Figure 2. Electropherogram of LCORL gene amplicon after restriction digestion with AluI, lanes 5, 8, 10 - TC heterozygous genotype, fragments of 284 bp, 169 bp, and 115 bp, lanes 1-4, 6-7, 9, 11-12 - TT homozygous individuals with 284 bp fragments, M - DNA marker pUC19/MspI

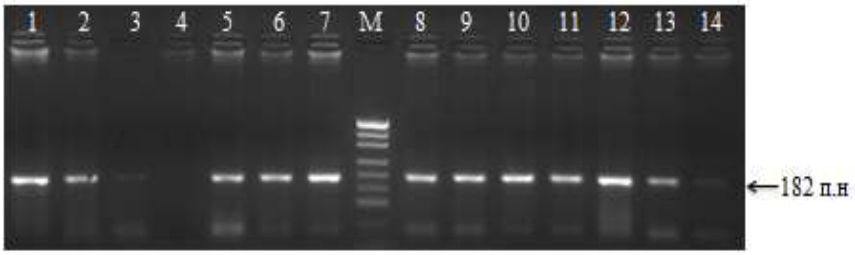

Figure 3. Electropherogram of the PCR product of the PRKAG3 gene. Lane 4 shows negative results, while lanes 1-3, 5-7 and 8-14 show a PCR product with a size of 182 bp., M - DNA marker pUC19/MspI

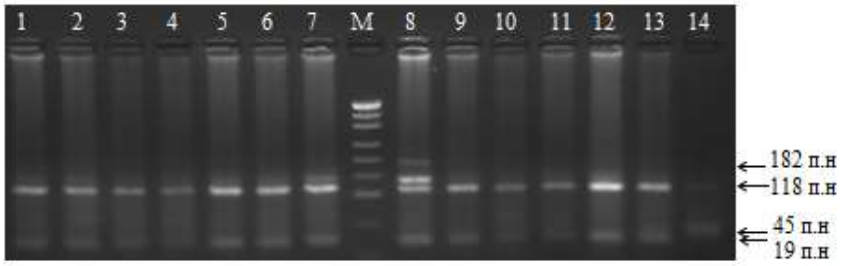

Figure 4. Electropherogram of the amplified product of the PRKAG3 gene after AluI endonuclease restriction, lane 8 - CT heterozygous genotype, fragments 182 bp, 118 bp, 45 bp, lanes 1-7 and 9-14 CChomozygous individuals with 118 bp, 45 bp fragments, M - DNA marker pUC19/MspI

To assess the level of genetic polymorphism, an analysis of the genotyping results was carried out for the LCORL gene locus. The actual occurrence of the homozygous TT genotype in horses of the Zhabe Pavlodar breed was 81.25%, the heterozygous TC genotype was 18.75%, and the other homozygous CC genotype was not detected in the studied animals. For this gene locus, no genetic polymorphism was detected in the DNA of tested horses of the Mangystau Adai type (n=17) and the local Zhabe breed of the Zhetysu region (n=8), with all individuals having a homozygous TT genotype. Genetic polymorphism was detected only in horses of the Mangystau Adai type for the second gene locus, PRKAG3, where one mare out of 17

tested horses had a heterozygous CT genotype (5.89%). According to the genotyping results, horses of the Pavlodar and Zhetysu regions had only one homozygous CC genotype for both gene loci (Table).

T a b l e

Distribution of genetic variants and allele frequencies, level of homozygosity and heterozygosity of individuals for the LCORL and PRKAG3 gene loci in the studied population of horses

Locus name	LLP “Akshiman-Agro” of Pavlodar region				
	Genotype	Genotype frequency	Allele frequency	Ho	He
LCORL	TT (n=13)	81,25%	T=-0,91	81,25	18,75
	TC (n=3)	18,75%	G=0,09		
	CC (n=0)	0,0%			
	Peasant farm “Kozhyr-Ata” of Mangystau region				
LCORL	TT (n=17)	100,0%	T=-1,0	100,0	0,0
	TC (n=0)	0,0%	G=0,0		
	CC (n=0)	0,0%			
	Peasant farm “Akimbekov” of Zhetysu region				
LCORL	TT (n=7)	100,0%	T=-1,0	100,0	0,0
	TC (n=0)	0,0%	G=0,0		
	CC (n=0)	0,0%			
	LLP “Akshiman-Agro” of Pavlodar region				
PRKAG3	CC (n=16)	100,0 %	C=1,0	100,0	0,0
	CT (n=0)	0,0%	T=0,0		
	TT (n=0)	0,0%			
	Peasant farm “Kozhyr-Ata” of Mangystau region				
PRKAG3	CC (n=16)	94,11%	C=0,97	94,11	5,89
	CT (n=1)	5,89%	T=0,03		
	TT (n=0)	0,0%			
	Peasant farm “Akimbekov” of Zhetysu region				
PRKAG3	CC (n=8)	100,0%	C=1,0	100,0	0,0
	CT (n=0)	0,0%	T=0,0		
	TT (n=0)	0,0%			

Gene equilibrium was violated in both gene loci, with a much greater prevalence of the T and C alleles observed, with values of 0.91, 1.0, 1.0, and 1.0, 0.97, and 1.0, respectively, in horses from Pavlodar, Mangystau, and Zhetysu regions. Another criterion characterizing the level of genetic diversity is the determination of the level of homozygosity and heterozygosity in the studied population. For the LCORL gene locus, the level of homozygosity in the three studied groups was 81.25%, 100.0%, and 100.0%, and for the PRKAG3 gene, it was 100.0%, 94.11%, and 100.0%. The level of heterozygosity was 18.75% for the LCORL gene locus in horses from the Pavlodar region and 5.89% for the PRKAG3 gene locus in horses from the Mangystau region. No individuals with homozygous CC and TT genotypes were detected for both studied gene loci.

Conclusion

It is known that the LCORL gene locus in horses is associated with growth parameters, muscle mass formation, chest circumference, and the AY_376689:c.773C>T SNP polymorphism in the PRKAG3 gene influences the growth rate of young animals. The study results indicate a disruption of genetic equilibrium at the LCORL and PRKAG3 gene loci, which is likely due to prolonged selection of the horse population for economically useful traits such as live weight, growth rate, carcass weight, animal endurance, disease resistance, and fertility of stallions. We believe that the preservation of the allele pool of local animal breeds is an important criterion for breeding livestock. The reduction of polymorphism level and genetic diversity in the studied horses is indirectly caused by inbreeding resulting from the use of a single method, natural mating of mares with stud stallions in a particular area for reproductive work. Thus, the LCORL gene determines the formation of muscle mass, skeleton, chest circumference, growth rate, and PRKAG3 gene alleles are associated with the growth rates of young animals. In the future, the indicated SNP polymorphisms can be used as DNA markers of meat productivity in horses.

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Жергілікті қазақ жылқы тұқымындағы ет өнімділігімен байланысты SNP полиморфизмдері бойынша идентификация

Біздің зерттеуіміздің мақсаты — жергілікті қазақ тұқымды жылқылардың генотиптерін анықтау, сонымен қатар Павлодар және Жетісу облыстарындағы Жабы тұқымын, сондай-ақ Маңғыстау облысындағы Адай тұқымын, яғни 41 бас жылқыны LCORL және PRKAG3 гендерінің локустары бойынша ПТР-РФҰП әдістерін қолдана отырып зерттеу. Зерттеу нәтижелері бойынша Павлодар облысының Жабы тұқымының жылқысында LCORL генінің локусы бойынша ТТ гомозиготалы генотипінің кездесу жиілігі — 81,25 %, ТС гетерозиготалы генотипі — 18,75 % құрайтынын көрсетті. Маңғыстау облысының Адай жылқысы мен Жетісу облысының Жабы тұқымындағы қазақ жылқыларында генетикалық полиморфизм анықталмады, барлық гомозиготалы генотип — ТТ болды. PRKAG3 генінің екінші локусында генетикалық полиморфизм тек Адай жылқысында кездесті, сыналған 17 жылқының ішінде бір бие гетерозиготалды генотипке ие болды, яғни СТ (5,89 %). Павлодар және Жетісу облыстарының Жабы тұқымды жылқыларында СС жалғыз гомозиготалы генотипі болған. Зерттелген үш топтағы LCORL генінің локусы бойынша гомозиготалық деңгей: 81,25; 100,0; 100,0. PRKAG3 гені

бойынша — 100,0; 94,11; 100,0. Павлодар облысының жылқыларында LCORL генінің локусы бойынша гетерозиготалық деңгей — 18,75 %, Маңғыстау облысының жылқыларында PRKAG3 генінің локусы бойынша — 5,89 % құрады. Осылайша, LCORL және PRKAG3 генінің полиморфизмдері жылқылардың бұлшықет массасының, қаңқа құрылымының, кеуде шеңберінің және өсу жылдамдығының дамуын анықтауда маңызды рөл атқарады. Сондықтан бұл SNP полиморфизмдері жылқылардағы ет өндірісін бағалау үшін ДНҚ-маркерлері ретінде қызмет ете алатыны зерттелді.

Кілт сөздер: жылқыны генотиптеу, қазақ жылқылары, Жабы, Адай, LCORL гені, PRKAG3 гені, ПТР-РФҰП анализі, өнімділіктің ДНҚ-маркерлері.

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Выявление SNP полиморфизмов, ассоциированных с мясной продуктивностью, у местной казахской породы лошадей

Целью нашего исследования было генотипирование местных лошадей казахской породы: тип Жабе Павлодарской и Жетысуской областей, а также Адайский тип Мангистауской области в количестве 41 голова по локусам генов LCORL и PRKAG3 методом ПЦР–ПДРФ. По результатам исследования встречаемость гомозиготного генотипа ТТ по локусу гена LCORL у лошадей породы Жабе Павлодарской области составила 81,25 %, гетерозиготного генотипа ТС — 18,75 %. У казахских лошадей Адайского типа Мангистауской области и типа Жабе Жетысуской области генетический полиморфизм не выявлен, все особи имели гомозиготный генотип — ТТ. По второму локусу гена PRKAG3 генетический полиморфизм обнаружен только у лошадей Адайского типа, из 17 протестированных лошадей одна кобыла оказалась с гетерозиготным генотипом — СТ (5,89 %). Лошади типа Жабе Павлодарской и Жетысуской областей имели единственный гомозиготный генотип СС. Уровень гомозиготности по локусу гена LCORL у трех исследуемых групп составил: 81,25; 100,0; 100,0. По гену PRKAG3 — 100,0; 94,11; 100,0. Уровень гетерозиготности по локусу гена LCORL у лошадей Павлодарской области составил 18,75 %, по локусу гена PRKAG3 у лошадей Мангистауской области — 5,89 %. Таким образом, полиморфизм генов LCORL и PRKAG3 играют значительную роль в определении развития мышечной массы, строения скелета, окружности грудной клетки, скорости роста лошадей. Следовательно, эти SNP полиморфизмы потенциально могут служить ДНК-маркерами для оценки мясной продуктивности лошадей.

Ключевые слова: генотипирование лошадей, казахские лошади, Жабе, Адай, ген LCORL, ген PRKAG3, ПЦР-ПДРФ-анализ, ДНК-маркеры продуктивности.

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