Relationship of ACTN3-Promoter gene polymorphism and Alpha actin-3 concentration with performance of Original Arabian-WAHO horses

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Abstract

The research was conducted in the Iraqi Equestrian Club (15 km west of Baghdad city center) for the period from 10/1/2021 to 11/30/2022, with the aim of detecting polymorphism in the ACTN3 gene (promoter region) in original Arabian horses (WAHO). There were two variants (2 SNPs) in the Promoter region, which are rs1150531051 with polymorphism of TT, TC and CC, and the frequency of the two alleles T and C amounted to 0.34 and 0.66), and the variant rs1144978872 with polymorphism of GG, GA and AA, and the frequency of the two alleles G and A amounted to 0.68 and 0.32. It was observed that there is a positive and significant regression of the depth of respiration after the exercises on the concentration of alpha-actin 3, and the regression of the number of respiration times after the exercises on alpha-actin-3 and the ratio of alpha-actin-3 to the RNA level is positive and significant and reached 0.905 and 0.569, and there is a positive and significant regression ($P \leq 0.05$) for the speed of horses on the concentration of α-actin 3 (0.552 m/min) as well as on the ratio of α-actin 3 to RNA level (0.617 m/min) in purebred Arabian horses. We can conclude, through studying the genetic features in the ACTN3 gene, the promoter region, that it can be adopted in setting strategies for genetic improvement in horses. The rates of velocity and the number of body dimensions increased with the increase in the concentration of actin, and some values of the coefficient of determination (R^2) were moderate.

Keywords: Original Arabian-WAHO horses- ACTN3/ Promoter - Alpha actin-3 conc. – Regression.

Introduction

The speed of horses is of great economic value, humans invested it to improve the ability to work and the athletic performance of horses, and at the present time endurance is very important in equestrian competitions, as the endurance performance or

endurance of horses has been determined as one of the qualities of low intensity but long-term, Various criteria were used to determine the optimal speed in racing horses by relying on accurate speed data obtained from different races [1 , 2 ,3 , 4], and modeling An athlete providing information about how horses regulate their speed and effort over a given distance [5] Machine learning has changed the horse racing betting market over the past 10 years [6], and endurance performance has also been measured, as horses can achieve an average speed in Endurance races exceeding 25 km/h, especially in the final stage of the race [7].[8] reported that the identification of improvement programs in Arabian horses is most likely related to selection that focuses on improving riding and A race based on genetic variants obtained using Illumina microarray technology, also known as Bead Array or Bead Chip technology, in horses on chromosomes 1, 3, 11, 15, 17 and 22. The identified genes were associated with adaptive Potential such as ATP synthesis coupled to electron transport (COX4I1), vascular smooth muscle contraction (ADCY1), homeostasis and hypopurin metabolism (GAD1), and insulin signaling (CBLB). The sarcomere α-actinin proteins, encoded by the ACTN2 and ACTN3 genes, are major structural components of Z-line proteins and have high sequence similarity: α-actinin2 is found in all skeletal muscle fibers, while α-actinin3 has evolved as a result of specialized expression in type 2 fibers. The second only which is rapidly glycogenolytic [9]. α-actinin-3 is only expressed in type 2 fast glycolytic myofibers, and is essential for rapid muscle contraction (100% for type II myofibers and 50% for type I myo-fibers) [10 , 11]. Functional and structural analysis of the ACTN3 gene has been performed in relation to its association with horse performance characteristics, and so far many researches have focused on identifying SNP differences in the ACTN3 gene for horses in different breeds[12].The study of genetic variation within and between individuals enables researchers to obtain important information about any organism, which may not be available in the light of traditional methods, as well as help in finding and describing levels of genetic variation, and then obtaining basic information about the population structure [13, 14, 15, 16 , 17]. The research aims to determine the genetic polymorphism of the actin gene in a sample of purebred horses, to detect variations in the ACTN3 gene for the promoter region, and the relationship of the multiple polymorphism of the gene in performance (physiological characteristics, body dimensions, rate of speed, and concentration of the actin gene), and to predict traits through the concentration of the actin gene. Actin and its ratio to RNA.

Materials and Methods

The study was conducted in the Iraqi Equestrian Club located in Baghdad / Al-Amriya Governorate, on a sample of purebred Arabian horses participating in the races that take place in the club, from 1/2/2022 to 30/12/2022, with the aim of extracting DNA and estimating the expression level of the actin gene. and measure the level of actin protein in blood serum.

Collecting blood samples

A ten ml of blood was collected from the jugular vein of each animal, and the blood was divided into three tubes. The first tube was added with an EDTA anticoagulant produced by the Jordanian AFCO (Al-Hanoof Factory), and transferred in a refrigerated container to the laboratory for safekeeping. By freezing at - 4 °C until the time of DNA extraction, omeprazole was added to it to benefit from it in knowing gene expression, and the latter was waited 15 minutes until the state of clotting occurred, after which a centrifugation was performed at a speed of 3000 rpm for 5 minutes, and the serum was separated from hemoglobin and transferred The serum is taken with the first tube in a refrigerated box to the laboratory to conduct the necessary analysis to find out the required blood characteristics.

Molecular analysis of the gene

For the purpose of conducting the molecular analysis of the studied gene on the blood samples drawn from the studied horses that were preserved by freezing, they were taken out of the freezer for the purpose of conducting laboratory analyzes to know the genotypes of the genes. According to Temp.C=60 and Product by 870 bp.
F: CGGTCACAGAGCAGTCTAA , **R:** GCTTCTGTAGTGCCCCTTTC
Genomic DNA was isolated from the blood sample according to the ReliaPrep™ Blood gDNA Miniprep System, Promega protocol. A quantitative fluorometer was used to detect the concentration of extracted DNA in order to screen for sample quality for downstream applications. For 1 µl of DNA, 200 µl of diluted Quantifluor dye was mixed. After 5 minutes of incubation at room temperature, DNA concentration values were detected.

PCR reaction conditions

Steps	°C	m: s	Cycle
Initial Denaturation	**95**	**05:00**	**1**
Denaturation	**95**	**00:30**	**30**
Annealing	**60**	**00:30**	
Extension	**72**	**00:30**	
Final extension	**72**	**07:00**	**1**
Hold	**10**	**10:00**	

After PCR amplification, agarose gel electrophoresis was performed to confirm the presence of amplification. The polymerase chain reaction (PCR) was completely based on the standards of the extracted DNA. Shaw in figure 1 and 2 the plot extracted from the F1 promoter region in the ACTN3 gene /and the sample size was (916bp (Figure 1 and 2).

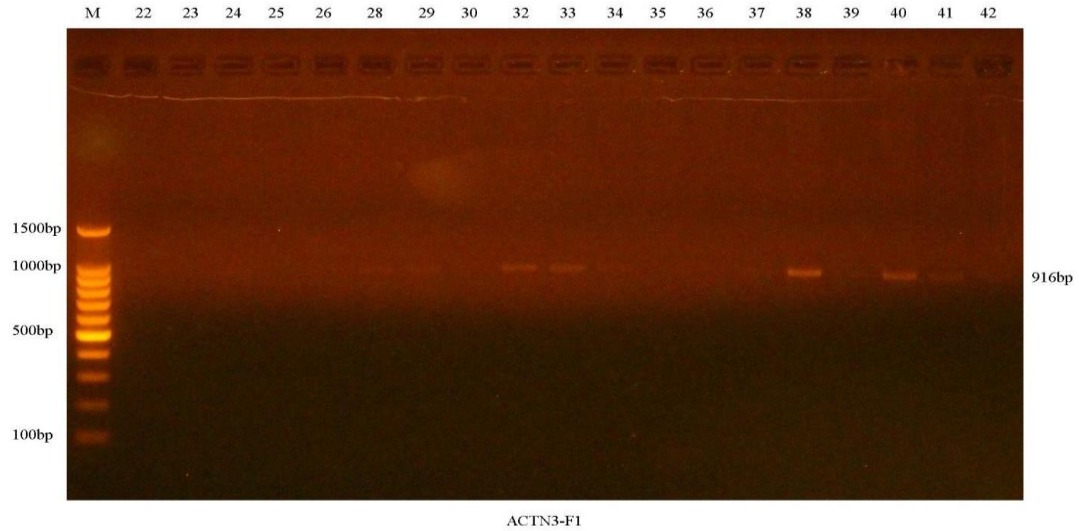

Figure (1): The plot extracted from the F1 promoter region in the ACTN3 gene /and the sample size was (916bp).

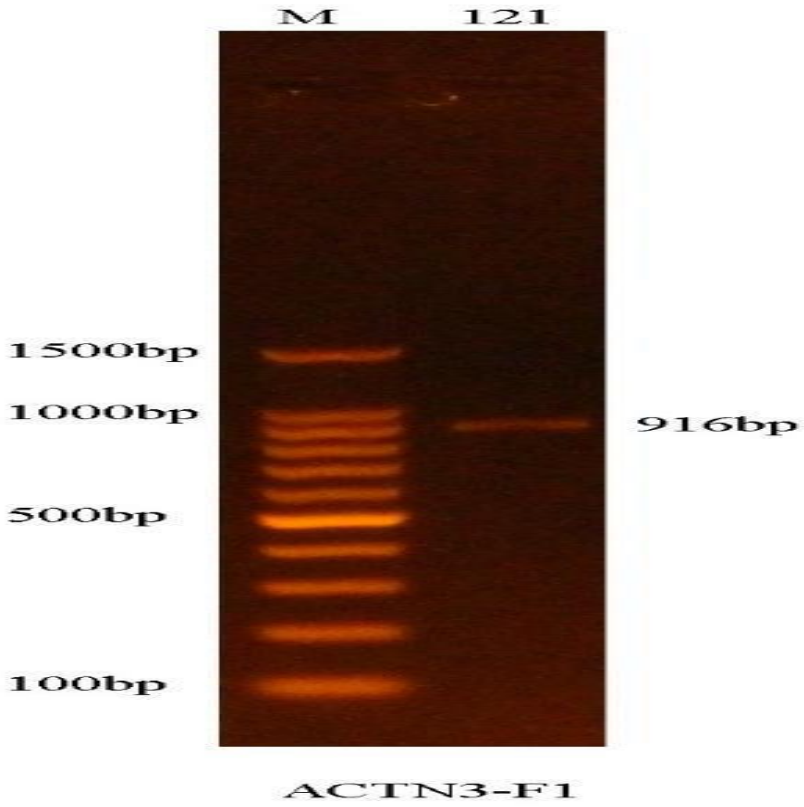

Figure (2): The plot extracted from the results of the amplification of the F1 promoter region in the ACTN3 / Equus caballus gene for sample (121) using BCR technology, and the sample size was (916bp). 1 X TAE buffer, DNA ladder marker, Ethidium bromide (10mg/ml).

Sequence analysis technology was performed to determine genotypes and detect the presence of mutations by sending samples to South Korea, by sending PCR products for Sanger sequencing using the ABI3730XL device, to know the sequences of

automated DNA nitrogenous bases, by Macrogen Corporation - in Korea, and the results were received were emailed and then analyzed using genetic software.

Statistical analysis

The data were analyzed statistically using the program Statistical Analysis System-SAS [18]) to study the effect of the genetic polymorphism of the actin gene (ACTN3) of the Promoter region on the studied traits, and the significant differences between the averages were compared using Duncan's multinomial test by applying the method of least square means and Chi-square- χ^2 test was used to compare the distribution percentages of genotypes for each SNP in the ACTN3 / Promoter region gene.

Results and Discussion

Genotype and allele frequency

There were two variants (2 SNPs) in the Promoter region, which are rs1150531051 with polymorphism of TT, TC and CC, and their distribution ratios were 16.13, 35.48 and 48.39 (P≤0.01), and allele frequency were 0.34 and 0.66 for both alleles T and C respectively, and the variant rs1144978872 with polymorphism of GG, GA and AA, and their distribution ratios were 45.16 45.16 and 9.68 (P≤0.01), and allele frequency were 0.68 and 0.32 for both alleles T and C respectively (Table 1).

Table (1): Genotype distribution and allele frequency of ACTN3 gene/Promoter region in Original Arabian-WAHO horses

SNPs	Genotype	No	Percentage (%)	Allele	Frequency
rs1150531051 /SNP1: T>C	TT	5	16.13	T	0.34
	TC	11	35.48		
	CC	15	48.39	C	0.66
	Total	31	100%		
	Chi-Square (χ^2)		9.064 **	---	
rs1144978872 /SNP2: G>A	GG	14	45.16	G	0.68
	GA	14	45.16		
	AA	3	9.68	A	0.32
	Total	31	100%		
	Chi-Square (χ^2)		9.064 **	---	
**** (P≤0.01).**					

The relationship of genotypes in the gene ACTN3- Promoter region// rs1150531051/ SNP1/ with the studied traits on purebred Arabian horses

It is noted from Table (2) and Figure (3) the relationship of genetic polymorphism in ACTN3- Promoter region / rs1150531051 gene with the studied traits on purebred Arabian horses, as there was a significant variation (P≤0.05) in the rate of speed according to the genotype, and the highest rate of speed was reached for horses with a mutant genotype. CC (1673.02 ± 367.58 m/min) and lower than those with the wild genotype TT (1156.91 ± 12.86 m/min), and this may be attributed to the difference in gene expression depending on the genotype, as the myofibrillar proteins that are active

in muscle contraction and stretch include both the actin protein and myosin, which facilitates the contractile movement of the sarcomere [11]. There were significant differences (P≤0.05) in the body length of purebred Arabian horses according to the genotype of the ACTN3 gene segment, as the TC genotype recorded the highest rate (157.07 ± 1.22 cm), as the expression of α-actinin-3 in muscle fibers is rapidly degraded. Glycogen (Fast Glycolytic), which is necessary for rapid muscle contraction, as well as the variation in muscle contraction, stretching, and contractile sarcomere movement. Therefore, actin has a role in many cellular functions, which may be reflected in some body dimensions, including height, depending on the genetic makeup (Mukund and Subramaniam, 2020). It was observed that the variation in the concentration of alpha-actin 3 according to the genotype in the ACTN3- Promoter region gene of purebred Arabian horses was significant (P≤0.05), with rates of 13.87 ± 1.05, 11.92 ± 0.75, and 10.82 ± 0.88 ng/ml for the genotypes TT, TC, and CC, respectively. This may be due to the difference in gene expression depending on the genotype of the gene.

Relationship of genotypes in ACTN3- Promoter region // rs1144978872/ SNP2 gene with the studied traits of purebred Arabian horses.

The results of the statistical analysis (Table 3) and Figure (4) showed the relationship of the genotypes of the gene ACTN3- Promoter region / rs1144978872 with the studied traits on purebred Arabian horses, as there were significant differences (P≤0.05) in the depth of breathing before exercise, and its rates were 22.07 ± 1.15 and 23.94 ± 0.82 and 27.61 ± 1.27 sec for compositions GG, GA, and AA, respectively. This may be due to the difference in gene expression according to the genotype, as the myofibril proteins that are effective in muscle contraction and stretching include actin and myosin, which facilitate the contractile movement of the sarcomere, and that the expression of α-actinin-3 in muscle fibers is fast glycolytic. They are required for rapid muscle contraction, as well as variability in muscle contraction, stretching, and sarcomere contractile motion, so actin has a role in many cellular functions (Mukund and Subramaniam, 2020). It was found that there were significant differences (P≤0.05) in the body length of purebred Arabian horses according to the genotype of the ACTN3/ Promoter region gene segment, as the mutant genotype AA recorded the highest rate (157.72 ± 1.47 cm) and then the genotype of the hybrid AG (156.26 ± 1.06). cm), while it was lowest in wild horses (GG).

Table (2): Relationship of ACTN3 gene genotype / Promoter region/ rs1150531051/ SNP1 with parameters study in Original Arabian-WAHO horses.

Parameters	Mean ±SE			Level of Sig.
	TT	TC	CC	
Depth of respiration before exercise (sc)	26.51±2.09	24.95±1.62	26.01±2.57	NS
No of respiration before exercise (min.)	16.08±0.71	15.93±0.57	15.17±0.81	NS

Depth of respiration after exercise (sc)	64.37±4.72	59.41±2.88	62.96±5.03	NS
No of respiration after exercise (min.)	81.92±5.27	79.55±1.67	78.27±4.87	NS
Rate of speed (m/min.)	1156.91±12.86 b	1673.02±367.58 a	1287.52±71.06 ab	*
Body length (cm)	142.65±1.47 b	157.07±1.22 a	152.42±3.10 ab	*
Heart girth (cm)	156.89±1.86	161.97±4.15	169.50±12.27	NS
Height from the front (cm)	158.42±1.74	160.74±1.37	164.38±084	
α-ACTN3 conc. (ng/ml)	13.87±1.05	11.92±0.75	10.82±0.88	
Means having with the different letters in same column differed significantly. * (P≤0.05).				

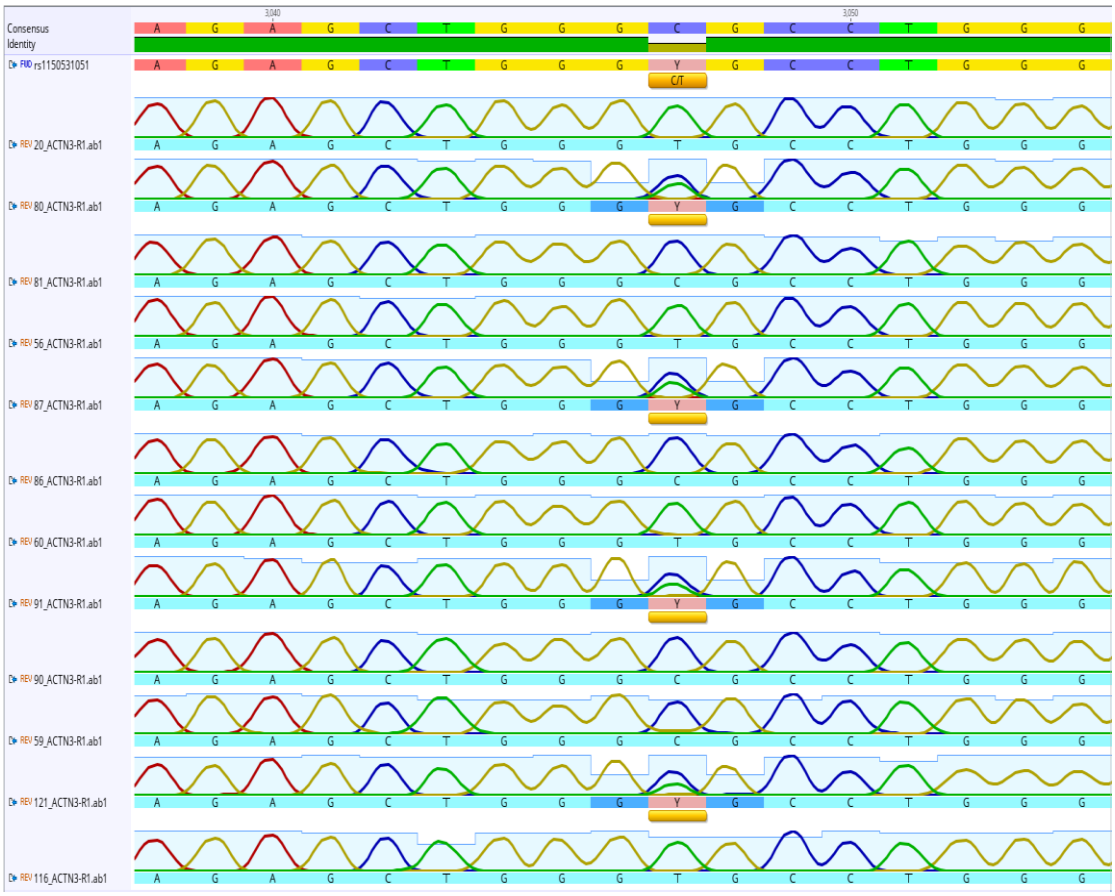

Figure (3): Analysis of rs1150531051/SNP1 of the ACTN3 gene/promoter region using Sanger sequencing

Table (3): Relationship of ACTN3 gene genotype / Promoter region/ rs1144978872/ SNP2 with parameters study in Original Arabian-WAHO horses

Parameters	Means ±SE			Level of Sig.
	GG	GA	AA	
Depth of respiration before exercise (sc)	22.07 ±1.15 b	23.94 ±0.82 ab	27.61 ±1.27 a	*
No of respiration before exercise (min.)	15.93 ±0.58	15.42 ±0.37	14.74 ±0.41	NS
Depth of respiration after exercise (sc)	60.76 ±3.08	57.93 ±3.04	62.79 ±2.76	NS
No of respiration after exercise (min.)	82.27 ±3.92	81.56 ±1.83	79.66 ±2.48	NS
Rate of speed (m/min.)	1652.73 ±137.27	1447.07 ±266.32	1394.85 ±91.52	NS
Body length (cm)	146.59 ±2.18 b	156.26 ±1.06 a	157.72 ±1.47 a	*
Heart girth (cm)	158.37 ±2.75	165.94 ±2.58	161.88 ±3.29	NS
Height from the front (cm)	159.63 ±1.67	163.01 ±1.34	162.58 ±1.25	NS
α-ACTN3 conc. (ng/ml)	11.85 ±0.64	10.94 ±0.78	11.67 ±1.37	NS

Means having with the different letters in same column differed significantly.
* (P≤0.05).

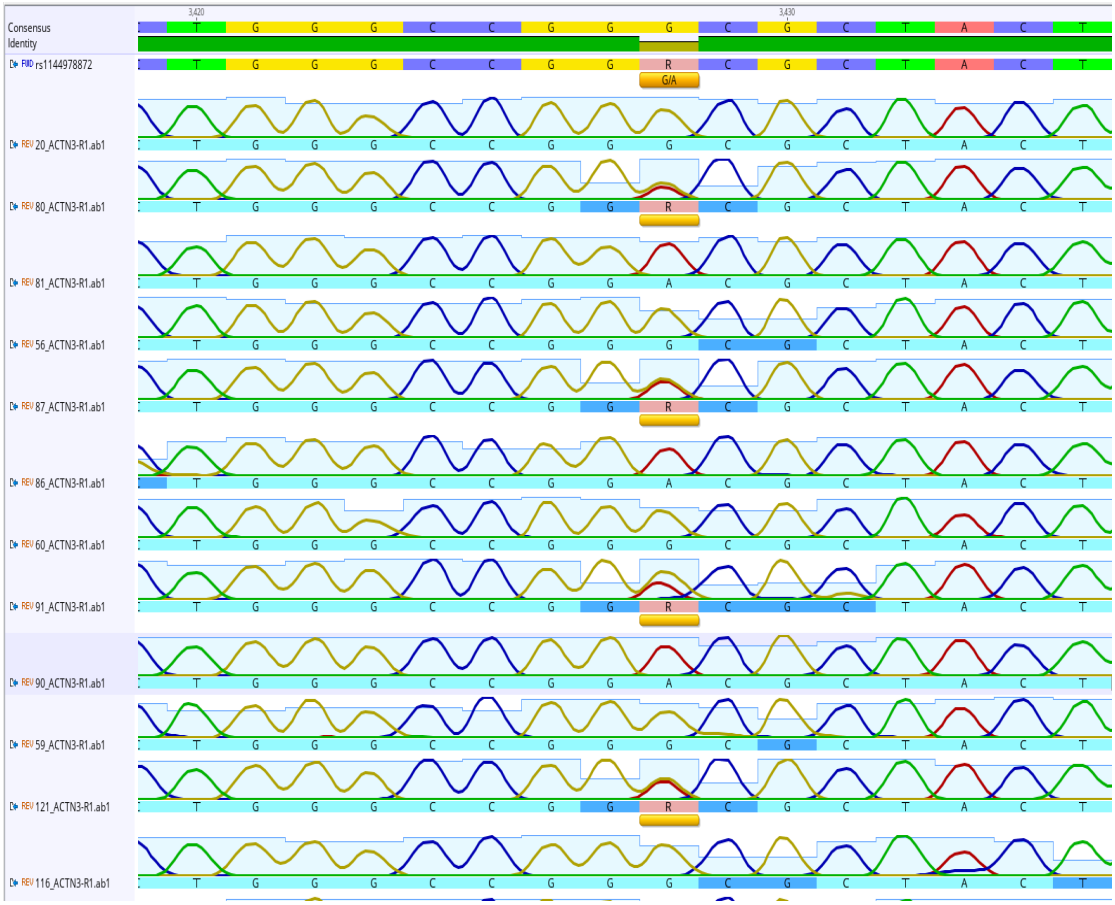

Figure (4): Analysis of rs1144978872/SNP2 of the ACTN3 gene/promoter region using Sanger sequencing.

Regression of some of the studied traits on the concentration of α-actin 3 and the ratio of α-actin 3 to the RNA level

It is clear from Table (4) the prediction equations resulting from the regression of a number of studied traits on the concentration of alpha-actin3, as well as on the ratio of alpha-actin3 to the RNA level in purebred Arabian horses. However, it was not significant on the ratio of alpha-actin 3 to RNA level. The regression of the number of respiration times after exercises on both traits was positive and significant, reaching 0.905 and 0.569, with a determination coefficient (R^2) of 0.22 and 0.14 for each of the concentration of alpha-actin3 and the ratio of alpha-actin3 to the RNA level, respectively, and therefore the concentration of alpha-actin3 explains 22%. The number of times you breathe after exercise. There is a positive and significant ($P \leq 0.05$) regression for the speed of horses on the concentration of α-actin 3 (0.552 m/min) as well as on the ratio of α-actin 3 to RNA level (0.617 m/min) in purebred Arabian horses, with a coefficient of determination of 0.35 and 0.28, and the formula of the expectation equations for these two The two relationships are Y^= 1284.03 + 0.552 X and Y^= 1375.39 + 0.617 X, respectively, which reflects the role of α-actin 3 in horse speed. It appears from Table (4) that the regression of the body length of purebred Arabian horses on alpha-actin3 concentration was not significant, while its regression on the ratio of alpha-actin3 to the RNA level was significant, with a coefficient of 0.498 and a

coefficient of determination of 0.21, while the regression of the chest circumference was significant on alpha-concentration Actin 3 (0.763 cm), while it was not significant on the ratio of α-actin 3 to RNA level (0.406). The results of the current study showed that the regression of height at introduction on the concentration of alpha-actin3 as well as on the ratio of alpha-actin3 to the RNA level in purebred Arabian horses was positive and significant, with a coefficient of 0.851 and 0.682 cm, with a coefficient of determination (R^2) of 0.22 and 0.32. [12] observed that the T>G mutation results in an increase in the free energy of the thermodynamic group ACTN-3 mRNA. [11] reported that the expression of α-actinin-3 in fast glycolytic muscle fibers It is necessary for rapid muscle contraction, as well as the variation in muscle contraction, stretching, and sarcomere contractile motion. Therefore, actin has a role in many cellular functions, which may be reflected in some physiological characteristics and body dimensions in horses [11, 19].

Table (4): Regression of parameters study on α-ACTN3 conc. and α-ACTN3 /RNA conc. ration in Original Arabian-WAHO horses

Parameters	Regression coefficient-b	Prediction equation	Level of Sig.	R^2
	α-ACTN3 conc. (ng/ml)			
Depth of respiration after exercise	0.792	Y^= 57.71+ 0.792 X	*	0.16
No of respiration after exercise	0.905	Y^= 76.63+ 0.905 X	*	0.22
Rate of speed	0.552	Y^= 1284.03+ 0.552 X	**	0.35
Body length	0.319	Y^= 155.47+ 0.319X	NS	0. 07
Heart girth	0.763	Y^= 154.92+ 0.763X	**	0.18
Height from the front	0.851	Y^= 162.28+ 0.851 X	**	0.22
	ACTN3/RNA conc. ration			
Depth of respiration after exercise	0.265	Y^= 56.83+ 0.265 X	NS	0.05
No of respiration after exercise	0.569	Y^= 81.27+ 0.569 X	*	0.14
Rate of speed	0.617	Y^= 1375.39+ 0.617 X	**	0.28
Body length	0.498	Y^= 162.85+ 0.498X	*	0.21
Heart girth	0.406	Y^= 157.03+ 0.406X	NS	0.16
Height from the front	0.682	Y^= 161.75+ 0.682 X	*	0.32

* (P≤0.05), ** (P≤0.01). R^2: Determination coefficient:

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