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Original Research

Association of MSTN gene polymorphism with body dimension and physiological performance in original Arabian horses

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ABSTRACT:

This study was under taken at Al- Zawra Park in downtown Baghdad on 50 of the original horses (*Equus caballus*) according to the Mayoralty of Baghdad, as well as the Laboratory of the Scientific Progress of Biotechnology and Molecular Genetics Analysis for the period from 1st July 2017 to 30th December 2017. This study was conducted in order to determine the genotype of myostatin (MSTN) and its relationship with performance (physiological, body dimensions and some of the blood traits), as well as the study of the distribution of their genotype in the sample and the alleles frequency obtained.

The percentages of the distribution of CC, CT and TT were significantly (P<0.01) different for MSTN in the studied sample as their percentages 88.0, 8.0 and 4.0% sequentially, with a recurrence of 0.84 and 0.16% respectively for C and T respectively. There was significant differences (P<0.05) in the breathing depth and the number of breathing times before exercise according to the Genotype of the myostatin gene (MSTN). After morning exercise, the effect was significant (P<0.05) and highly significant in the number of times of breathing.

The length of the body, the height of the back and shoulder length were significantly affected (P<0.05) by the genotype difference of the MSTN gene, while the height of the front and height of the top was not significant for the horses with the genotype CC and CT, and the heart Girth was high significantly affected (P<0.01) with the genotype difference of MSTN gene. Also significant differences (P<0.01) between MSTN genes in the number of red blood cells and the level of hemoglobin, total protein, glucose and cholesterol were found. We can conclude by the study of genotypes of MSTN that could be used to develop horse genetic improvement strategies. The application of the study to a larger sample and multiple sites, and the extraction of overlapping structures between the two genotypes would provide more accurate results for the implementation of the strategy of exclusion, substitution and identification of the best method for horses management.

Keywords:
Horses-MSTN gene-body dimension and physiological performance.

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INTRODUCTION

Scientists continue to study the colour of the cover or the natural appearance and genetic map of horses, and look for more information, especially how the interaction of genes and the presence and effect of mutations, and by understanding the basic information on the inheritance of natural appearance, the breeders are able to obtain more knowledge of genetics and applied to Breeding decisions and the care and management of horses. Horses are one among the most genetically diverse animals as well as the importance of heritage, as represented by the oldest civilizations and the conservation of genetic resources and the production of dowries. In horses, the study of common diseases and the production of several types of medicines and to participate in festivals and exhibitions it is also used in traction vehicles. Also it is the only animal that participates in the olympic games (Rieder *et al*., 2010).

The myostatin gene was identified as one of the genes associated with the candidate growth traits in genetic improvement programs. Its function is to produce and differentiate myoblast. A team of researchers at the University of Dublin studied the genotypes of hundreds of horses and compared them with genetic traits obtained from skeletons of 12 horses with special strength dating from 1764 to 1930, and focused on the study of myostatin, which plays an important role in controlling muscle growth (Dll'olio *et al*., 2010).

Myostatin is a protein known as a negative regulator of muscle mass in mammalian species (Dll'olio *et al*., 2014). Researchers have identified a natural change in the muscles responsible for a gene that works on muscle growth by achieving proteins that regulate cell growth. Hill *et al*. (2010) reported that it is possible to predict the mathematical performance of the horse from the genetic knowledge of the myostatin gene. The present study aimed to determine the genotype of the MISTN gene in the horses sample (extracting the distribution ratios and the first frequency) (RFLP-PCR) and the relationship of these aspects in its performance (physiological qualities, body dimensions and some character traits).

MATERIALS AND METHODS

This study was conducted in the horses division affiliated to the Department of Parks and Afforestation, one of the departments of the Municipality of Baghdad (Zawra park), for the period from 1/7/2017 until 30/12/2017, on a sample of 50 horses. The scientific progress laboratory of Al-Harithiya was utilized for the period of time to separate the genetic material and determine the genotypes of the MISTN gene and its relation with the body and the physiological performance (Katarzyna *et al*., 2015; David and Ngaio, 2017), as well as extracting the distribution ratios of the herd and the frequency of the alleles obtained (Chi-Square test

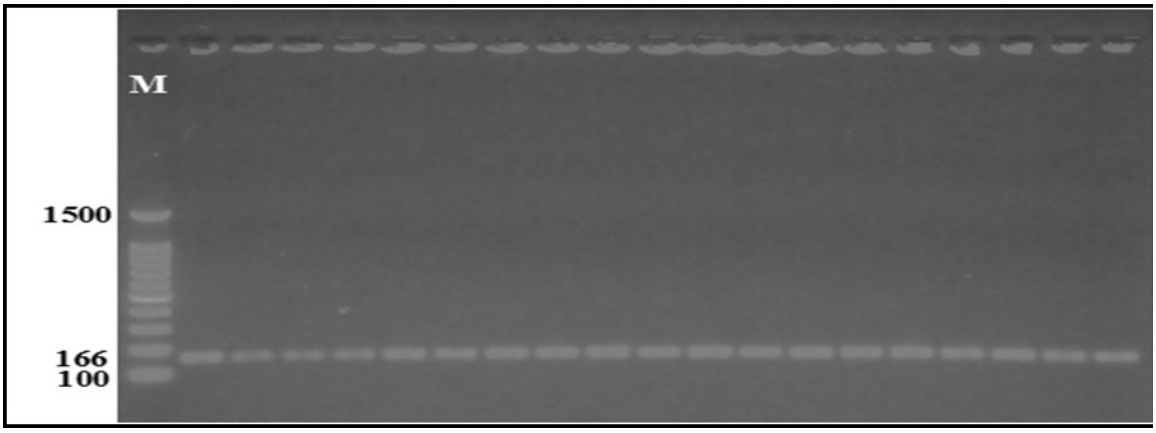

Figure 1. Extraction of the myostatin gene (166 bp) by PCR technology

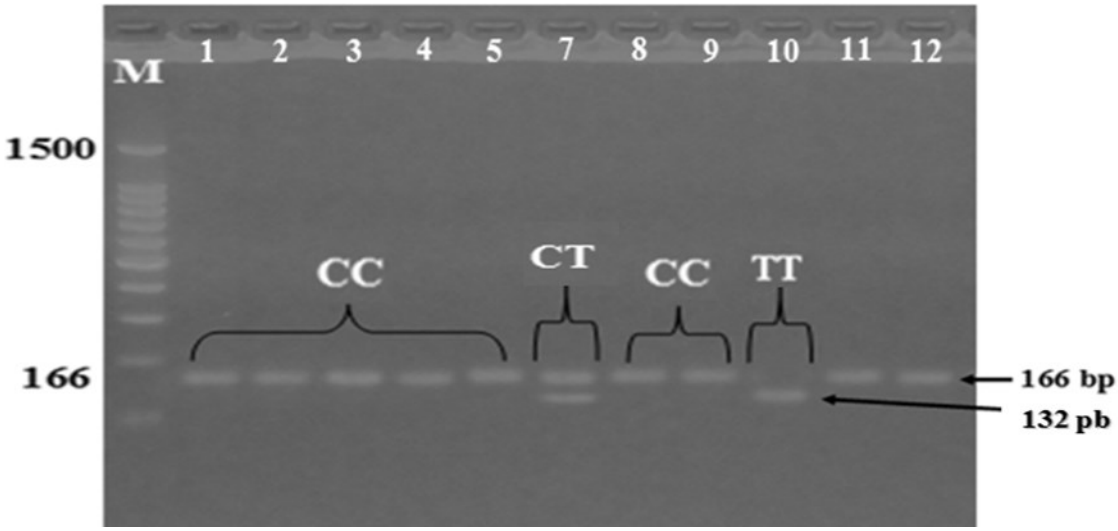

Figure 2. The digestion products of the myostatin gene segment using the *RsaI*-blocking enzyme

within SAS program according to hardy weinberg low).

PCR technology was used to amplify the required segment to complete the molecular detection and polymorphism of the MSTN gene according to the size of the pieces and the type of primers used (Gábor *et al*., 2014). The primers were selected (exon 2, sequence number AY840554) using the knowledge of the phenotypic diversity of the gene resulting from the presence of MSTN mutations (Gábor *et al*., 2014). The gene fragments studied and their location have been confirmed and verified by electronic browsers for vertebrate genome: National Center for Biotechnology Information (NCBI), Ensembl Genome Browser and UCSC (University of California, Santa Cruz) (Kollias and McDermott, 2008).

Table 1. Distribution of genotypes and allele frequency of MSTN gene

(Genotype)	No.	Percentage (%)
CC	44	88.00
CT	4	8.00
TT	2	4.00
Total	50	100%
The value of the square is Kai (χ^2)	----	55.34**
Allele		Repetition
C		0.84
T		0.16
(P<0.01) **		

The following primers were used in this study:

Forward primer = 5'- GAGAAGGCATGACACGGAAG -3';

Reverse primer = 5'- TTGATAGCAGAGTCATAAAGGAAA AGTA-3 ' (Gábor *et al*., 2014).

DNA extraction method

DNA extraction was conducted at the laboratory of Al-Harithiya, from the blood samples using the methodology followed by Gábor *et al*. (2014).

Table 2. PCR program used in this study

Step	Temperature	Time	No of cycle
Initial			
Denaturation	94	5 min	1
First loop			
Denaturation	94	30 sec	
Annealing	62	30 sec	35
Extension	72	30 sec	
Final extension	72	5 min	1

PCR amplification

The MSTN gene was analyzed using the PCR-RFLP techniques, PCR was performed in a final volume of 15µl: 50-90ng/µl genomic DNA, 1µl of each primer, 200µM dNTP, 1.5Nm $MgCl_2$, 10x PCR buffer and 1.0 U Taq DNA for polymorphism. 8µl of PCR product

Table 3. Relationship of the Genotype of MSTN gene with depth of respiration and the number of breathing times

S. No	Genotype	No.	Average ± Standard Error			
			Before exercises		After exercises	
			Number of breath / minute	Breathing depth / sec	Number of breath / minute	Breathing depth / sec
1	CC	44 × 4 = 176	15.91±1.46^{b}	4.18±0.23^{a}	49.18±1.72^{b}	1.25±0.04^{a}
2	CT	4 × 4 = 16	18.00±3.46a^{b}	3.75±0.72^{ab}	49.00±6.35^{b}	1.29±0.16^{a}
3	TT	4 × 2 = 8	22.00±0.47^{a}	2.73±0.07^{b}	64.00±0.73^{a}	0.937±0.02^{b}
4	Level of s.g	The total number 200	*	*	**	*

The averages with different letters within the same column differ significantly at (P<0.05)* and (P<0.01)**

over night was obtained at 37^{0}C, and after digestion, the products were subjected to 1.5% agarose gel-electrophoresis and the genotype were determined with the ultraviolet trans-illuminator by ethidium bromide stain (Gábor *et al*., 2014). The PCR program used in this study is given in Table 2.

***RsaI* restriction enzyme digestion**

Nine µl of amplified products was mixed with 5 Units of *RsaI* enzyme 0.5µl of enzyme buffer and 4ml distilled water, then it was incubated for 3 hours at 37^{0}C (Gábor *et al*., 2014).

Hematological and biochemical analyses

Blood samples (10ml) were collected from each horse at rest in the morning (7:00-8:00) before feeding from the external jugular external vein by the veterinarian, which ensured stress-free course of the treatment. The following hematological parameters were determined in the whole blood (anticoagulant: dipotassium ethylene diamine tetraacetic acid) with the use of the hematology analyser ADVIA 2120 (Siemens AG, Munich, Germany): total protein, hemoglobin, red blood cell, total cholesterol and triglyceride **(**Katarzyna *et al*., 2015).

Physiological traits

Breathing depth and the number of breathing times before and after exercise were measured for each horse (David and Ngaio, 2017).

-Breathing depth: The time required was measure of the process of inspiration and exhalation is one /second.

-Number of breathing times: The number of inhalations and exhalations within one minute.

Statistical analysis

The data were analyzed statistically using the Statistical Analysis System (SAS, 2012) to study the effect of polymorphism of the MSTN gene according to the mathematical model below. Moral differences between the averages were compared with the application of the least squares mean method.

Yijkl = µ + Gi + Aj + Sk + eij; l

Where, Yijkl: the value of the view of the genotype i, age j and sex k, µ: the general mean of the characteristic, Gi: the effect of polymorphism of MSTN genes (TT, CC, CT), Aj: age effect (4-8 years), eijkl is naturally distributed at an average of zero and a variation of σ^{2}e. The chi-square-22 test was used to compare the percentage distribution of genotypes.

RESULTS AND DISCUSSION

Separation of DNA and extraction of myostatin gene

DNA was extracted for MSTN in PCR technology. A 5µl sample was used from each sampling and the results were gel at a concentration of 52% (Gábor *et al*., 2014). The voltages were adjusted to 70 V and 40 amp for an hour and a half and the output of the paging was made to confirm the successful extraction and acquisition of the piece Required in the size of 166 base pairs

Table 4. Relationship of the genotype of the MSTN gene with body measurements

			Mean ± standard error (cm)					
S. No	**(Genotype)**	**No.**	**Height from the top (cm)**	**Height at croup (cm)**	**Height at wither (cm)**	**Body length (cm)**	**Shoulder length (cm)**	**Heart Girth (inches)**
1	CC	44	147.91±5.20^{a}	139.80±5.65^{ab}	147.63±3.31^{a}	147.15±4.69^{b}	67.27±4.69^{a}	174.73±4.23^{a}
2	CT	4	145.50±8.83^{a}	133.50±8.14^{b}	150.50±6.94^{a}	152.50±8.83^{a}	60.00±2.93^{b}	173.00±7.96^{a}
3	TT	2	143.00±3.25^{a}	138.00±3.79^{a}	144.00±3.66^{b}	135.00±4.07^{c}	58.00±0.46^{b}	160.00±5.86^{b}
4	Level of s.g	The total number 50	NS	*	NS	*	*	**

The averages with different alphabets within the same column was significant at '(P<0.01)** and (P<0.05)*; Non-significant: NS

Table 5. Relationship of genotype of the MSTN gene with blood characteristics

			Average ± standard error				
S. No	**(Genotype)**	**No.**	**Total protion (g/L)**	**Hemoglobin (g/dl) Hb**	**Red blood cells (10^{6}X)**	**Cholesterol (mg/dl)**	**Triglyceride (mg/dl)**
1	CC	44 × 2 = 88	70.66±1.30^{a}	12.70±0.12^{b}	7.35±0.07^{b}	107.74±6.79^{b}	23.28±2.63^{a}
2	CT	4 × 2 =8	60.90±0.75^{b}	12.70±0.51^{b}	8.56±0.01^{a}	128.85±14.75^{a}	22.15±1.53^{a}
3	TT	2 × 2 = 4	68.40±0.85^{a}	13.70±0.09^{a}	8.45±0.03^{a}	98.20±1.48^{b}	27.10±0.72^{a}
4	Level of s.g	The total number 50	**	**	**	**	NS

The averages with different letters within the same column was significant at '(P<0.01)**; Non-significant : NS '(P<0.01)**'

(bp) of the myostatin gene, with the use of DNA fragments (Marker 1500-100 bp) (Figure 1) (Gábor *et al*., 2014).

The experimental genotypes of the myostatin gene were determined by applying RFLP-PCR and *RsaI* as described in the materials and methods. The 10µl transfer in the agarose gel was concentrated at 2.5%, the voltages were adjusted at 70V and 40mA for 1.5h. The distribution of the genotypes of the studied animals were according to the number and size of the formed packages. The DNA ladder 100 -1500bp was used in the first well of the gel. The *RsaI* was sliced after identification of the sensitive position within the sequence of the cut-off location of the gene segment, so that one or two packages or three packages of each model were formed from the process of slicing. The genotypes for the MSTN gene in the studied samples are given in Figure 2.

The CC (wild) genotype appears in the size of 166bp. Genetic composition CT (heterozygous) appears in the size of 166bp, 132pb and 34pb. The TT (mutatnt) genotype appears in the size of 132bp and 34pb.

Table 1 shows the number and percentages of genotypes of the myostatin gene, showing significant differences (P<0.01) between the obtained distribution rates of 88.00, 8.00 and 4.00% for CC, CT and TT sequentially. The most common type of wild type (CC) compared with the hybrid genotype was CT. The mutant -TT was the lowest in the samples studied. These results evidenced that the MSTN gene used in our study in the genome of the original breeding horses in Iraq and distribution ratios vary according to the geographical location. The results of previous studies have indicated that there are significant differences (P<0.01) in the gene distribution ratios of MSTN (Hill *et al*., 2011). The prevalence of CC and the lack of genetic structures CT and TT may be due to the adaptation of the first structure to environmental conditions live on the original horses, especially the high temperature for most months of the year or non-validity of the allele T in such circumstances. The C allele recurrence of the MSTN gene in the studied horses sample was 0.84 while the T allele frequency was 0.16, and this result reflected the prevalence of the C allele of the gene in the original horses at central Iraq.

The relationship between the genotype of the MSTN gene physiological traits

Table 5 shows significant differences (P<0.05) in depth of breathing before exercise according to the Genotype of the myostatin gene. The respiratory depth rate was 4.18 ± 0.23 seconds in horses with CC gene, (2.73 ± 0.07 seconds), while the mean difference between the two in terms of morbidity of horses with hybrid genotype CT (3.75 ± 0.72 seconds), and the number of breathing times before exercise was significantly (P<0.05) different Genotype of the gene in this study that has the maximum rate is found in the transient genotype TT (22.00 ± 0.47 / min) and below in the horses with wild genotypes CC (15.91 ± 1.46 / min). Table 5 shows that the depth of respiration after morning exercise varied significantly (P<0.05) according to the Genotype of the casein gene (MSTN). The rates were 1.25 ± 0.04, 1.29 ± 0.16 and 0.937 ± 0.02 seconds for horses with CC and CT and TT (P<0.01) for the number of breathing times. The rates were 49.18 ± 1.72, 49.00 ± 6.35 and 64.00 ± 0.73 / min respectively.

Relationship between the genotype of the MSTN gene to the measured body measurements

The height from top of the horses were not significantly affected by the genotype of MSTN gene, while the difference was significant in body length, width from the posterior and shoulder length, for horses with genetic structure CC (147.63 ± 5.65, 147.91 ± 5.20, 174.73 ± 4.23cm) and genotype CT (150.50 ± 6.94, 145.50 ± 8.83 and 173.00 ± 7.96cm) while the lowest levels were with the horses having transgenic TT structure were in body length, height from the top and shoulder length and according to MSTN (135.00 ± 4.07,

143.00 ± 3.25 and 58.00 ± 0.46cm).

The differences between them were significant (P <0.05), while the variance in the chest circumference was the highest for the horses with the genetic structure CC and CT and lowest in the TT genotype was 174.73 ± 4.23, 173.00 ± 7.96 and 160.00 ± 5.86cm, respectively, and the differences were significant (P<0.01). There are wide genetic differences in the measurements of the body and the difference between the strain and individuals within a single strain, and noted that the genetic factors affecting the dimensions of the body of the mature animal as well as environmental factors, especially age and sex, and that study according to the Mixed model Accuracy (Miserani *et al*., 2002).

Relationship between MSTN gene and blood characteristics

The results of the current study (Table 4 and 5) showed that there were significant differences (P<0.01) between the genotypes of the MSTN gene in the number of red blood cells. The number was high in the blood of horses with hybrid genotype CT (8.56 ± 0.01 X 10^6) Transient TT (8.45 ± 0.03 X 10^6) while the number is less in the wild genotypes CC (7.35 ± 0.07 X 10^6). The coriander is a very important food of the distinctive horses as it carries oxygen and therefore plays an important role in the breeding and performance of this type of animal, and the study of a larger sample may give more accurate results of this characteristic, which is sometimes used in the genetic assessment of horses For selection purposes, (P<0.01) between the genotypes of the myostatin gene in the level of hemoglobin and was the lowest in the blood of horses with transgenic TT structure at a rate of 13.70 ± 0.09 g/dl, and was affected by the concentration of total propane high moral (P<0.01). With different genotypes resulting from the molecular analysis of the myostatin gene and 70.66 ± 1.03 and 68.40 ± 0.85 g/L in the blood of horses with pure CC and TT genotypes, respectively, while the rate was low in horses with hybrid genotype CT (60.90 ± 0.75 g/L). The level of cholesterol significantly differed (P<0.01) with the difference in the genotypes of the myostatin gene, with rates of 107.74 ± 6.79 and 128.85 ± 14.75 and 98.20 ± 1.48 mg/dl for horses with CC, CT and TT respectively. Horses in the level of triglycerides are found to be with the different genotype of MSTN gene.

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