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Effect of *LCORL* gene polymorphism on body size traits in horse populations

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ABSTRACT. The aim of this study was to determine polymorphism of LCORL gene in horse breeds and its association with body size. PCR-RFLP technique was performed using *Alu*I for genotyping of 306 horses. Results showed that C is the rare allele in Iranian Breeds, because these horses have been used since ancient times as a courier and for war and archery, hence selection has done to benefit of spiky horses with medium body that need less food and are tireless. While, for foreign breeds; frequency of C allele was high that can be concluded these breeds used in fields, forests, and mines. A UPGMA dendrogram based on the Nei's standard genetic distance among studied breeds showed separate clusters for Iranian native and exotic breeds. Statistical association analysis of three observed genotypes with body size showed that there is an association between this polymorphism and body size criteria ($p < 0.01$). Overall, it can be concluded that studied mutation in *LCORL* gene can be used as candidate marker for improving body weight in horse.

Keywords: Association; foreign breeds; Iranian Breeds; PCR-RFLP technique; SNP.

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Introduction

Archaeological land historical evidence demonstrated that domestication of the horse was begun by Aryans (the ancestors of modern Iranians). They employed these animals for various aims like agricultural labors, transportation and as food resources. Aryans created different breeds with discrete characteristics using horse breeding and selection. Unfortunately, only a few Iranian native breeds have remained. Modern Iranian horse breeds are carelessly classified in to five groups including Persian Plateau horses, Turkmen, Karabagh and Karadagh, Persian Arab and Caspian Pony. It is believed that modern horse breeds in the world are taken from four main types (Pony Type 1 or Celtic pony, pony type 2 or Tundra pony, Horse type 1or Plain horse and Horse type 2) migrated from North America to Asia. In addition, it is supposed that, the modern light horse must be originated from either Horse type 1 or 2 or both of them. Among the various modern horse breeds in the world, the Turkmen group (Karabagh) and Caspian pony are very similar to the horse type 1 and 2, respectively, both osteologically and conformationally (Fotovati, 2000).

Another hypothesis has been suggested that shows Caspian pony is the ancestor of a proto-type of Arab. Hence, based on this hypothesis, this horse can be considered as the origin of most modern light horses. The study of native breeds, using molecular techniques is very important and useful for their characterizing (Zamani, Akhondi, & Mohammadabadi, 2015). Conservation of genetic diversity in domestic animal species requires the proper performance of conservation superiorities and sustainable handling plans that should be based on universal information on population structures, including genetic diversity resources among and between breeds (Ruzina et al., 2010; Shamsalddini, Mohammadabadi, & Esmailizadeh, 2016).

Genetic diversity is an essential element for genetic improvement, preserving populations, evolution and adapting to variable environmental situations (Ebrahimi, Mohammadabadi, & Esmailizadeh, 2017; Mousavizadeh et al., 2009). Performing specific gaits is one of the most horse breed's abilities. A gait is a coordination pattern of the limbs identified by timing and sequence of the footfalls. Three factors; speed, genotype and environmental factors play an important role in selecting the gait by horse. Body size is an important characteristic for horses and is crucial for their classification. Ligand-dependent nuclear receptor

corepressor-like (*LCORL*) is located on equine chromosome 3 and was mapped to the QTL region closely located on the most important single-nucleotide polymorphism (SNP). It suggested that the role of *LCORL* was linked to arginine metabolism in growth. This gene has shown interaction with C-terminal binding protein 1 (CTBP1) that is a transcriptional repressor affects the gene expression and cell cycles (Dorman, Shen, Yang, Ezzat, & Asa, 2012; Han, Chen, Liu, & Liu, 2017; Vinayagam et al., 2011). *LCORL* interacts with ubiquity in C through encoding a transcription factor (Kim et al., 2011) and involves in various cellular biological processes (Han et al., 2017; Kimura & Tanaka, 2010).

Association of *LCORL* gene polymorphism with different traits; skeletal growth and skeletal height phenotypes (Carty et al., 2012), withers height (Tetens, Widmann, Kühn, & Thaller, 2013), weight and carcass composition traits (Liu et al., 2015) and performance and conformation traits was indicated using genome wide association study (GWAS). Correlation of body size and *LCORL* polymorphism was reported by some researchers (Boyko et al., 2014; He, Zhang, Li, & Liu, 2015; Signer-Hasler et al., 2012; Tetens et al., 2013) in horse. Schröder (2010) reported valid QTL on chromosome 3 of horse in the region of *LCORL* gene for conformation traits such as head, neck, frame and development. Significant association between QTLs on ECA 3 and 9 of Franches-Montagnes horses and withers height was reported by Signer-Hasler et al. (2012)-. In a study Makvandi-Nejad et al. (2012) showed that LCORL together with three other loci located on ECA 6, 9 and 11 explains approximately 83% of the size variation in different horse populations. Metzger, Schrimpf, Philipp, and Distl (2013) indicated that polymorphism in LCORL gene can be a highly predictive marker of genetic potential for body size. This study was designed to determine polymorphism of LCORL gene in Iranian native horses (Persian Arab, Akhal teke, Yamut, Kurd, Dareshouri, Caspian, Taleshi) and also foreign breeds including Holesteiner, KWPN, Hanoverian, Thoroughbred, Oldenburg, Egyptian Arabian, Selle Français, Zangersheide and Westphalian Iranian horse breeds using of PCR-RFLP technique and its association with body size.

Material and methods

Blood samples were collected from the jugular vein of 306 horses. More details of the animals are presented in Table 1. The samples were kept in EDTA-vacutainer tubes. Samples were kept at -20°C until subsequent use. Genomic DNA was extracted from the blood using an optimized and modified salting-out method. A 284-bp fragment within the horse LCORL gene was amplified using PCR primers 5'-TGGAGTCAGTTGGGTTTAATG-3' and 5'-GACCGGATAGCATAGAGAGAG-3' (He et al., 2015). The PCR amplification was performed in a 25 µl reaction volume, containing negative controls, using CinnaGen PCR Master Kit according to the instructions by the manufacturer (CinnaGen Co., Iran). Initial denaturation for 5 min at 94°C was followed by 33 cycles of 45 s at 94°C, 45 s at 56.6°C, 45 s at 72°C and a 5 min final extension step at 72°C. Amplification products were electrophoresed on 1% agarose gel at constant voltage and 1X TBE for approximately 2 h. The gels were visualized by staining with ethidium bromide and photographed under UV light and then all PCR products were digested with 5 U of AluI restriction enzyme (Fermentas) at 37°C overnight, and the resulting products were separated by the 3% agarose gel and visualized by ethidium bromide staining. Measurement of diversity including gene diversity (H), observed number of alleles (Ne), Shannon's information index etc., were estimated by POPGEN (ver. 3.2) software (McNally, Cotton, Hogg, & Loizou, 2014).

Table 1. Sampling design of the studied animals.

Breed type	Place of sampling within Iran	№ of samples
Pure-bred Arab horse (Persian Arab)	Kerman, Zarand, Rafsanjan	25
Turkmen (Akhal teke)	Kerman, Isfahan	42
Turkmen (Yamut)	Kerman	16
Dareshouri	Kerman, Sirjan, Rafsanjan, Rayen	28
Kurd	Kerman, Rafsanjan, Sirjan, Isfahan	25
Caspian	Rasht, Kerman, Karaj	42
Taleshi	Guilan (Rezvanshahr)	14
Egyptian Arabian	Karaj, Kerman, Sirjan, Zarand, Rafsanjan, Rayen	21
Hanoverian	Tehran, Karaj, Kerman	16
Holesteiner	Tehran, Karaj, Kerman	11
KWPN	Tehran, Karaj, Kerman	20
Thoroughbred	Tehran, Kerman	4
Oldenburg	Tehran, Kerman	4
Westphalian	Tehran, Karaj	2
Selle Francais	Kerman	2
Foreign Arab	Tehran, Kerman	32
Zangersheide	Kerman	2

The association between the *LCORL* gene polymorphism and the body size traits of the 306 horses were studied using the following statistical fixed-model:

$$y_{ijkl} = \mu + B_i + S_j + G(B)_{k(i)} + e_{ijkl}$$

where, y_{ijkl} is the body size traits records; μ is the population mean of the studied trait; B_i is the breed effects, S_j is the effect of sex, $G(B)_{k(i)}$ is the effect of the genotype, and e_{ijkl} is random error. The least square means (LSMs) with standard errors of the body size were calculated for the different genotypes and the means were compared using the adjusted Tukey's procedure via ASReml software (Gogel et al., 2009).

Results and discussion

The extracted DNA had a good quality (Figure 1). The tested DNA of the horses used in the present study was amplified using the specific primers and yielded PCR products at the expected size, 284 bp (Figure 2).

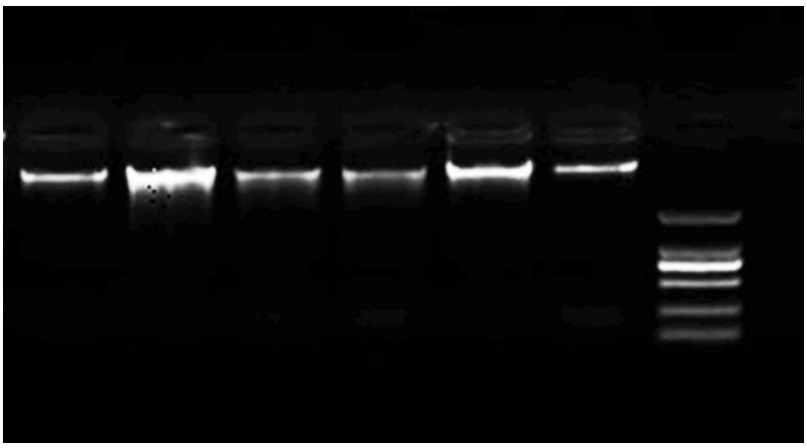

Figure 1. Some samples of the extracted DNA from the studied animals on the 1% agarose gel.

Amplification of the *LCORL* gene produced 284 bp fragments; when these fragments were digested with the restriction enzyme *AluI*, the CC genotype produced two bands: 169 and 115 bp (one restriction site in the C allele), the TT genotype produced one band: 284 bp (no restriction sites in the C allele), and the TC produced three bands: 284, 169 and 115 bp (heterozygote genotype). The different alleles resulted from digestion of the PCR products with the *AluI* restriction enzyme after running on the agarose gel electrophoresis are presented in Figure 3.

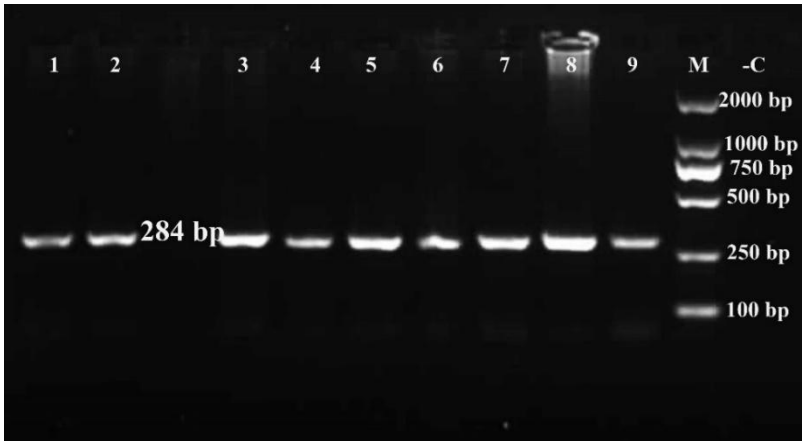

Figure 2. Ethidium bromide-stained agarose gel of amplified PCR products representing amplification of *LCORL* gene in some studied horses. Lines 1-9 are some samples, M is size marker and –C is negative control.

The genotypic and allelic frequencies of *LCORL* gene in studied horses have been shown in Table 2. In this study, the Hardy Weinberg equilibrium was estimated with Chi-square and G-square tests. The studied populations were not found to be in a Hardy-Weinberg equilibrium, as for years they have been under selection. The v*alue*s of the population genetics parameters in studied horses have been shown in Table 3.

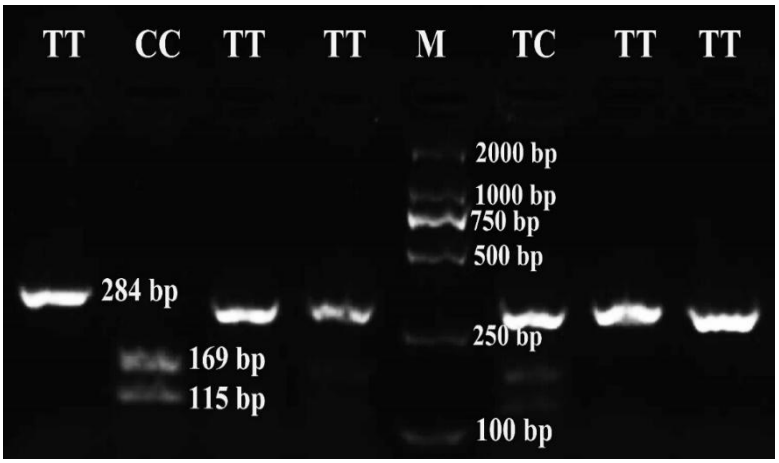

Figure 3. PCR amplified products of *LCORL* gene digested with *AluI* in some studied horses. M is DNA size marker and other lanes are TT, TC and CC genotypes.

Table 2. Genotypic and allelic frequencies of *LCORL* gene in studied horses.

Population	Genotype	N	Genotypic freq.	Allele	Allelic freq.
Pure-bred Arab horse (Persian Arab)	TT	23	0.92	T	0.94
	TC	1	0.04	C	0.06
	CC	1	0.04		
Turkmen (Akhal teke)	TT	42	1.00	T	1.00
				C	0.00
Turkmen (Yamut)	TT	15	0.94	T	0.97
	TC	1	0.06	C	0.03
Dareshouri	TT	26	0.93	T	0.97
	TC	2	0.07	C	0.03
Kurd	TT	25	1.00	T	1.00
				C	0.00
Caspian	TT	42	1.00	T	1.00
				C	0.00
Taleshi	TT	14	1.00	T	1.00
				C	0.00
Egyptian Arabian	TT	21	1.00	T	1.00
				C	0.00
Hanoverian	TT	4	0.25	T	0.56
	TC	10	0.62	C	0.44
	CC	2	0.13		
Holesteiner	TT	5	0.46	T	0.60
	TC	3	0.27	C	0.40
	CC	3	0.27		
KWPN	TT	5	0.25	T	0.52
	TC	11	0.55	C	0.48
	CC	4	0.20		
Thoroughbred	TT	1	0.25	T	0.50
	TC	2	0.50	C	0.50
	CC	1	0.25		
Oldenburg	TT	2	0.50	T	0.62
	TC	1	0.25	C	0.38
	CC	1	0.25		
Westphalian	TC	1	0.50	T	0.25
	CC	1	0.50	C	0.75
Selle Francais	TC	1	0.50	T	0.25
	CC	1	0.50	C	0.75
Foreign Arab	TT	32	100	T	1.00
			0.00	C	0.00
Zangersheide	TC	1	0.50	T	0.25
	CC	1	0.50	C	0.75
All	TT	257	0.84	T	0.90
	TC	34	0.11	C	0.10
	CC	15	0.05		

A UPGMA dendrogram based on the Nei's standard distance matrix showed separate cluster for the Iranian breeds from other commercial horses (Figure 4). Relationship of polymorphism in *LCORL* gene and body size criteria is given in Table 4 as least-squares mean of studied traits, separated by genotypes. Least-square means of the body size traits for the different genotypes of *LCORL* gene are given in Table 5.

Table 3. The v*alue*s of the population genetics parameters in studied horses.

Population	Nº of observed alleles	Nº of effective alleles	Observed heterozygosity	Expected heterozygosity	Shanon's Index
Pure-bred Arab horse (Persian Arab)	2	1.12	0.04	0.12	0.23
Turkmen (Akhal teke)	1	1	0	0	0
Turkmen (Yamut)	2	1.06	0.06	0.06	0.14
Dareshouri	2	1.07	0.07	0.07	0.16
Kurd	1	1	0	0	0
Caspian	1	1	0	0	0
Taleshi	1	1	0	0	0
Egyptian Arabian	1	1	0	0	0
Hanoverian	2	1.96	0.62	0.50	0.68
Holesteiner	2	1.93	0.27	0.50	0.67
KWPN	2	1.99	0.55	0.51	0.69
Thoroughbred	2	2	0.50	0.57	0.69
Oldenburg	2	1.88	0.25	0.54	0.66
Westphalian	2	1.06	0.50	0.50	0.56
Selle Francais	2	1.06	0.50	0.50	0.56
Foreign Arab	1	1	0	0	0
Zangersheide	2	1.06	0.50	0.50	0.56
All	2	1.23	0.11	0.18	0.40

Table 4. Least-square means and standard errors of the studied traits for the different genotypes of *LCORL* gene in all studied horse populations.

Genotype (Nº)	WHT ***	GRC ***	BLG ***	EBW ***	HDL ***	HWD **	OBI N.S.
CC (15)	162.6 ±1.37^{a}	185.9 ±2.23^{a}	166.7 ±2.18^{a}	509.2 ±13.15^{a}	55.6 ±0.59^{a}	19.5 ±0.29^{a}	1.14 ±0.012^{a}
TC (34)	157.4 ±1.07^{b}	178.2 ±1.74^{b}	158.4 ±1.7^{b}	437.2 ±10.27^{b}	53.4 ±0.46^{b}	18.9 ±0.22^{ab}	1.13 ±0.009^{a}
TT (257)	151.4 ±0.64^{c}	172.6 ±1.04^{c}	156.0 ±1.01^{c}	403.2 ±6.12^{c}	52.4 ±0.28^{b}	18.4 ±0.13^{b}	1.14 ±0.006^{a}

EBW: estimated body weight (kg), OBI: obesity index, WHT: withers height (cm), BLG: body length (cm), GRC: girth chest (cm), HDL: head length (cm), HWD: head width. N.S. = not Significant, **: significant effect of genotype on studied traits in 1% ($p < 0.01$), ***: significant effect of genotype on studied traits in 0.1% ($p < 0.001$).

In the studied populations, 3 genotypes including CC, TC and TT were observed. The genotype of the five Iranian studied breeds of Turkmen (Akhal teke), Kurd, Caspian, Taleshi and Foreign Arab horse were TT (monomorph) and then the frequency of C allele was 0 and in the three other Iranian studied breeds consisting of Pure-bred Arab horse (Persian Arab), Dareshouri and Turkmen (Yamut) frequency of C allele was 0.060, 0.035 and 0.030, respectively. It shows that in Iranian Breeds C allele is rare, because these horses have been used since ancient times as a courier and for war and archery, hence selection has done to benefit of spiky horses with medium body that need less food and are tireless. While, for foreign breeds; Selle Francais, Zangersheide, Westphalian, Thoroughbred, KWPN, Hanoverian, Holesteiner and Oldenburg frequency of C allele was 0.75, 0.75, 0.75, 0.50, 0.48, 0.44, 0.40 and 0.38, respectively that can be concluded these breeds used in fields, forests, and mines, therefore, they needed a coarse and strong horses, but after the industrial revolution, the sports aspect of these horses; jumping was considered, and since the height and size of the animal had a high correlation with the jumping power, these breeds have two alleles.

These results are same as the results of He et al. (2015) that studied Yili horse and reported 0.83 and 0.17 for T and C alleles respectively. Phylogenetic tree shows logical results of studied breeds as Iranian populations and European (Selle Français, Zangersheide, Westphalian, Thoroughbred, KWPN, Hanoverian, Holesteiner and Oldenburg) populations are classified in different cluster. From this classification based on *LCORL* gene, it can be concluded that Turkmen (Akhal teke), Kurd, Caspian, Taleshi, Foreign Arab horse, Pure-bred Arab horse (Persian Arab), Dareshouri and Turkmen (Yamut) originated from common ancestor and breed in the same condition and selection criteria, as low genetic distance has also occurred between these populations in their high phenotypic similarity.

Table 5. Least-square means and standard errors of the studied traits for the different genotypes of *LCORL* gene in the studied breeds.

Breed	Genotypes	WHT	GRC	BLG	EBW	HDL	HWD	OBI
Thoroughbred	CC	168.2±4.63	197.7±7.45	183.7±7.19	602.5±43.2	58.4±1.92	18.1±0.93	1.2±0.04
	TC	164.3±3.28	183.8±5.28	168.8±5.09	479.6±30.6	54.1±1.26	18.2±0.66	1.1±0.03
	TT	158.2±4.63	183.7±7.45	186.7±7.19	528.9±43.2	55.9±1.92	20.6±0.93	1.2±0.04
Selle Francais	CC	172.2±4.63	207.7±7.45	192.7±7.19	697.5±43.2	61.9±1.92	22.1±0.093	1.2±0.04
	TC	168.2±4.63	194.7±7.45	183.7±7.19	584.4±43.2	61.4±1.92	21.1±0.93	1.2±0.04
Zangersheide	CC	170.8±4.63	198.3±7.45	166.3±7.19	548.5±43.2	57.6±1.92	20.9±0.93	1.2±0.04
	TC	166.8±4.63	199.3±7.45	166.3±7.19	554±1.92	58.6±1.92	19.9±0.93	1.2±0.04
Oldenburg	CC	173.8±4.63	196.3±7.45	164.3±7.19	531±43.2	56.6±1.92	19.4±0.93	1.1±0.04
	TC	167.8±4.63	190.3±7.45	172.3±7.19	523.1±43.2	57.6±1.92	19.9±0.93	1.1±0.04
	TT	162±3.27	190.5±5.25	171.3±5.07	521.8±30.5	53.5±1.35	19.3±0.66	1.2±0.02
Westphalian	CC	173.8±4.63	195.3±7.45	169.3±7.19	541.5±43.2	58.6±1.92	19.4±0.93	1.1±0.04
	TC	171.8±4.63	207.3±7.45	169.3±7.19	610.3±43.2	58.6±1.92	19.9±0.93	1.2±0.04
Holesteiner	CC	174.8±2.67	202.4±4.29	173.4±4.14	600.6±24.9	63.2±1.11	21±0.54	1.2±0.02
	TC	169.5±2.69	189.6±4.32	161±4.17	486±25.1	57.8±1.11	19.4±0.54	1.1±0.02
	TT	166.8±2.07	186.1±3.32	161.7±3.21	470.7±19.3	56.9±0.86	19.1±0.42	1.1±0.02
Hanoverian	CC	177.3±3.28	205.8±5.28	178.8±5.09	635.9±30.6	59.3±1.36	20.7±0.66	1.2±0.02
	TC	171.5±1.47	195.4±2.26	169.9±2.28	546.3±13.7	57.2±0.61	20.1±0.29	1.1±0.01
	TT	163.4±2.32	187.9±3.72	166.6±3.59	494.4±21.6	54.5±0.96	18.4±0.47	1.2±0.02
KWPN	CC	176±2.32	202.6±3.72	177.9±3.59	614.2±21.6	59.7±0.96	20.7±0.47)	1.2±0.02
	TC	168.8±1.4	194±2.24	168±2.17	530.5±13	58±0.58	19.9±0.28	1.2±0.01
	TT	162.5±2.08	190.7±3.34	161.6±3.22	493.6±19.4	57.6±0.86	19.6±0.42	1.2±0.02
Egyptian Arab	TT	149.8±4.63	163.3±7.45	146.3±7.19	326.9±43.2	50.6±1.92	16.9±0.93	1.1±0.04
Pure-bred Arab horse (Persian Arab)	CC	155.2±4.63	177.7±7.45	170.7±7.19	452.5±43.2	51.4±1.92	18.1±0.93	1.1±0.04
	TC	148.8±4.63	174.3±7.45	157.8±7.19	401.8±43.2	48.6±1.92	19.4±0.93	1.2±0.04
	TT	145.3±0.97	166.1±1.55	154.6±1.5	358.9±9	50.1±0.4	17.9±0.19	1.1±0.01
Foreign Arab	TT	149.9±0.83	171.3±1.33	155.1±1.29	382.6±7.7	49.9±0.34	17.9±0.17	1.1±0.01
Turkmen (Akhal teke)	TT	154.2±0.84	174.3±1.35	163.6±1.3	418.4±7.8	53.2±0.35	18.4±0.17	1.1±0.01
Turkmen (Yamut)	TC	156.8±4.63	180.3±7.45	165.3±7.19	450.4±43.2	52.6±1.92	18.9±0.93	1.2±0.04
	TT	149.1±1.2	168.9±1.93	161.9±1.86	389.4±11.2	52.5±0.51	18.4±0.25	1.1±0.01
Dareshouri	TC	156±3.27	175.5±5.25	156.5±5.07	404.2±30.5	51.5±1.35	17.3±0.66	1.1±0.03
	TT	148.9±0.93	168.8±1.5	154.7±1.45	371.7±8.7	51.1±0.41	17.6±0.2	1.1±0.01
Kurd	TT	146±0.95	169.9±1.53	157.3±1.47	382.3±8.9	51.8±0.39	18.4±0.19	1.2±0.01
Caspian	TT	117±0.77	125.8±1.24	120.5±1.2	162±7.2	43.5±0.32	16.2±0.15	1.1±0.01
Taleshi	TT	125.9±2.08	137.3±3.34	127.3±0.3.22	201.1±19.4	46.5±1.11	17.5±0.54	1.1±0.02

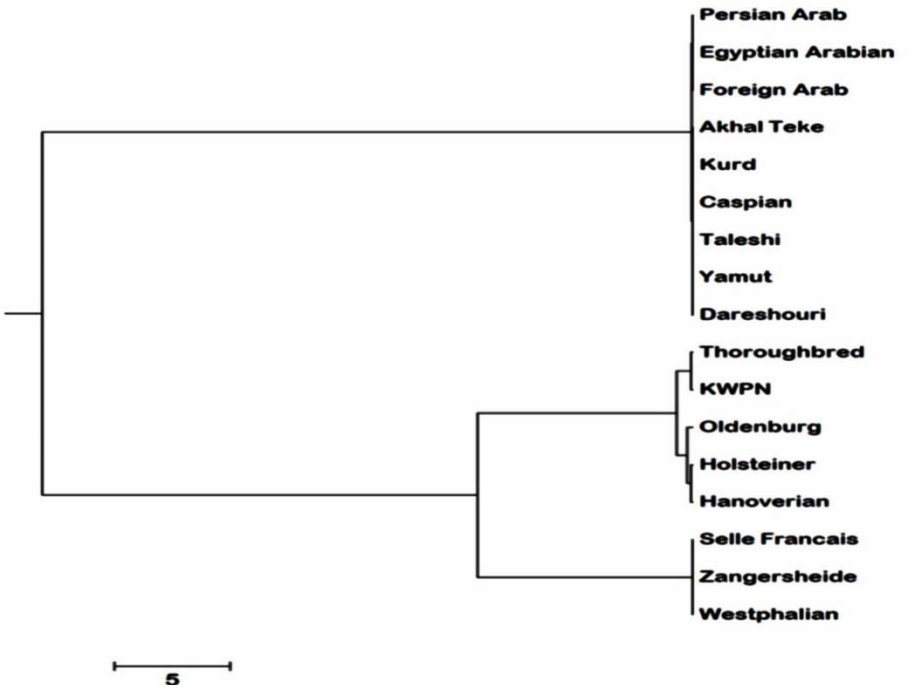

Figure 4. UPGMA phylogenetic tree based on Nei genetic distance.

The studied populations were not found to be in a Hardy-Weinberg equilibrium. The reason for this could be more heterozygotes than homozygotes, mutation, migration, genetic drift and level of crossbreeding in some populations (Khodabakhshzadeh et al., 2016; Mohammadabadi et al., 2010). Although crossing native and commercial breeds can have a faster effect on improving native animal yield than long-term programs as a result, is more beneficial. But this is one of the most important reasons for eliminating the genetic diversity of native breeds.

The excessive use of the crossbreeding and widespread use of commercial breeds can lead to the destruction of the genetic diversity in native breeds, so it is better to use a controlled crossbreeding with a predetermined purpose. Thoroughbred population had the highest Shanon's Index (0.69) and the lowest Shanon's Index (0.14) was belong to Turkmen (Yamut) population. This index for all studied population was 0.34. Heterozygosity v*alu*es were in concordance with those of Yili horse (He et al., 2015). The high average expected heterozygosity within a breed can be due to the high number of identified alleles in the studied loci (Ebrahimi et al., 2017; Mousavizadeh et al., 2009). High heterozygosity may be related to the wider population distribution and larger population size. Results of association analyses between different criteria of body size and *LCORL* gene polymorphism showed effective correlation, as genotype had significant effect on EBW; estimated body weight (kg), WHT; withers height (cm), BLG; body length (cm), GRC; girth chest (cm), HDL; head length (cm) and HWD; head width parameters ($P<0.01$), but there was no correlation with OBI; obesity index. The obesity index is mostly influenced by the treatment and nutrition, and the environment has a great impact on this trait.

The results of comparing the mean of genotypes in the studied breeds showed that the EBW; estimated body weight (kg), WHT; withers height (cm), BLG; body length (cm) and GRC; girth chest (cm) were significantly affected by the *LCORL* polymorphism, so that the individuals with CC genotype had the highest v*alu*e and the lowest v*alu*e for those belonged to TT genotype and animals with TC genotype had the average v*alu*e. For HDL; head length (cm), Tukey's test showed that individuals with CC genotype had a significant difference with TC and TT genotypes, but there was no significant difference between individuals with TC and TT genotypes. Least-squares mean of HWD; head width indicates that the TC genotype is overlapping with the CC and TT genotypes, but the genotypes of CC and TT have a significant difference. These results were the same as results of He et al. (2015) on Yili horse that identified that animals with the CC genotype have significantly larger withers height, body length, chest circumference, and cannon circumference than those with either the TC or TT genotype ($p < 0.01$). However, the TC genotype was larger than the TT genotypes. Other researchers (Boyko et al., 2014; He et al., 2015; Makvandi-Nejad et al., 2012; Metzger et al., 2013; Signer-Hasler et al., 2012; Tetens et al., 2013) reported the association of different alleles of *LCORL* and different criteria affecting body size in horse that confirmed results of this study.

The small differences in the observed results in the present study with the previous study could be due to various breeds and different size of studied populations. Based on reported information by STRING interaction network, LCORL gene has interaction with other genes probably to perform function. On the other hand, it has been shown that the regulated docking of the CtBP proteins on PRDM16 controls the brown and white fat-selective gene programs (Kajimura et al., 2008). It is also reported that inhibition of the KDM1A (LSD1) promotes adipogenic differentiation from hESCs through H3K4 methylation (Xiong et al., 2016). Therefore, it can be concluded that these three genes can interact with each other via their role on adipose tissue metabolism.

Conclusion

Frequencies for the C allele of the *LCORL* gene compared with that of the T allele was low in Iranian native breeds leading to a low to moderate diversity in different breeds indicating that selective breeding has altered the frequency of *LCORL* genotypes with a predicted loss of the C allele. Totally, the results of this study generally indicate a suitable diversity in the studied populations that could be considered as an appropriate genetic reserve for various breeding and breeding purposes. Moreover, based on previous reports and the results of this study, this polymorphism can be used as a guide to select useful markers for genetic variation and horse breeding. Overall, it can be concluded that the mutation studied in this study within the *LCORL* gene is usable as a marker for body size in the horse.

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References

Boyko, A. R., Brooks, S. A., Behan-Braman, A., Castelhano, M., Corey, E., Oliveira, K. C., ... Ainsworth, D. M. (2014). Genomic analysis establishes correlation between growth and laryngeal neuropathy in Thoroughbreds. *BMC genomics, 15*(1), e259. doi: 10.1186/1471-2164-15-259

Carty, C. L., Johnson, N. A., Hutter, C. M., Reiner, A. P., Peters, U., Tang, H., & Kooperberg, C. (2012). Genome-wide association study of body height in African Americans: The women's health initiative SNP health association resource (share). *Human Molecular Genetics, 21*(3), 711-720. doi: 10.1093/hmg/ddr489

Dorman, K., Shen, Z., Yang, C., Ezzat, S., & Asa, S. L. (2012). CtBP1 interacts with Ikaros and modulates pituitary tumor cell survival and response to hypoxia. *Molecular Endocrinology, 26*(3), 447-457. doi: 10.1210/me.2011-1095

Ebrahimi, M. T. V., Mohammadabadi, M., & Esmailizadeh, A. (2017). Using microsatellite markers to analyze genetic diversity in 14 sheep types in Iran. *Archives Animal Breeding, 60*(3), 183-189. doi: doi:10.5194/aab-60-183-2017

Fotovati, A. (2000). Persian horse breeds from ancient time to present and their rules in development of world horse breeds. *Asian-Australas Journal of Animal Science, 13*, 401-401.

Han, Y. J., Chen, Y., Liu, Y., & Liu, X. L. (2017). Sequence variants of the LCORL gene and its association with growth and carcass traits in Qinchuan cattle in China. *Journal of Genetics, 96*(1), 9-17.

He, S., Zhang, L., Li, W., & Liu, M. (2015). BIEC2-808543 SNP in the LCORL gene is associated with body conformation in the Yili horse. *Animal biotechnology, 26*(4), 289-291. doi: 10.1080/10495398.2014.995303

Kajimura, S., Seale, P., Tomaru, T., Erdjument-Bromage, H., Cooper, M. P., Ruas, J. L., ... Spiegelman, B. M. (2008). Regulation of the brown and white fat gene programs through a PRDM16/CtBP transcriptional complex. *Genes & Development, 22*(10), 1397-1409. doi: 10.1101/gad.1666108

Khodabakhshzadeh, R., Mohammadabadi, M. R., Esmailizadeh, A. K., Shahrebabak, H. M., Bordbar, F., & Namin, S. A. (2016). Identification of point mutations in exon 2 of GDF9 gene in Kermani sheep. *Polish Journal of Veterinary Sciences, 19*(2), 281-289. doi: 10.1515/pjvs-2016-0035

Kim, W., Bennett, E. J., Huttlin, E. L., Guo, A., Li, J., Possemato, A., ... Comb, M. J. (2011). Systematic and quantitative assessment of the ubiquitin-modified proteome. *Molecular cell, 44*(2), 325-340. doi: 10.1016/j.molcel.2011.08.025

Kimura, Y., & Tanaka, K. (2010). Regulatory mechanisms involved in the control of ubiquitin homeostasis. *The journal of biochemistry, 147*(6), 793-798. doi: 10.1093/jb/mvq044

Liu, R., Sun, Y., Zhao, G., Wang, H., Zheng, M., Li, P., ... Wen, J. (2015). Identification of loci and genes for growth related traits from a genome-wide association study in a slow-× fast-growing broiler chicken cross. *Genes & Genomics, 37*(10), 829-836. doi: 10.1007/s13258-015-0314-1

Makvandi-Nejad, S., Hoffman, G. E., Allen, J. J., Chu, E., Gu, E., Chandler, A. M., ... Brooks, S. A. (2012). Four loci explain 83% of size variation in the horse. *PloS One, 7*, e39929. doi: 10.1371/journal.pone.0039929

McNally, K., Cotton, R., Hogg, A., & Loizou, G. (2014). PopGen: a virtual human population generator. *Toxicology, 315*, 70-85. doi: 10.1016/j.tox.2013.07.009

Metzger, J., Schrimpf, R., Philipp, U., & Distl, O. (2013). Expression levels of LCORL are associated with body size in horses. *PloS One, 8*(2), e56497. doi: 10.1371/journal.pone.0056497

Mohammadabadi, M. R., Nikbakhti, M., Mirzaee, H. R., Shandi, A., Saghi, D. A., Romanov, M. N., & Moiseyeva, I. G. (2010). Genetic variability in three native Iranian chicken populations of the Khorasan province based on microsatellite markers. *Russian Journal of Genetics, 46*(4), 505-509.

Mousavizadeh, A., Mohammad Abadi, M., Torabi, A., Nassiry, M. R., Ghiasi, H., & Koshkoieh, A. A. (2009). Genetic polymorphism at the growth hormone locus in Iranian Talli goats by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). *Iranian Journal of Biotechnology, 7*(1), 51-53.

Ruzina, M. N., Shtyfurko, T. A., Mohammadabadi, M. R., Gendzhieva, O. B., Tsedev, T., & Sulimova, G. E. (2010). Polymorphism of the BoLA-DRB3 gene in the Mongolian, Kalmyk, and Yakut cattle breeds. *Russian Journal of Genetics, 46*(4), 456-463.

Shamsalddini, S., Mohammadabadi, M. R., & Esmailizadeh, A. K. (2016). Polymorphism of the prolactin gene and its effect on fiber traits in goat. *Russian Journal of Genetics, 52*(4), 405-408.

Signer-Hasler, H., Flury, C., Haase, B., Burger, D., Simianer, H., Leeb, T., & Rieder, S. (2012). A genome-wide association study reveals loci influencing height and other conformation traits in horses. *PloS One, 7*(5), e37282. doi: 10.1371/journal.pone.0037282

Tetens, J., Widmann, P., Kühn, C., & Thaller, G. (2013). A genome- wide association study indicates LCORL/NCAPG as a candidate locus for withers height in G erman W armblood horses. *Animal Genetics, 44*(4), 467-471. doi: 10.1111/age.12031

Vinayagam, A., Stelzl, U., Foulle, R., Plassmann, S., Zenkner, M., Timm, J., ... Wanker, E. E. (2011). A directed protein interaction network for investigating intracellular signal transduction. *Science Signal, 4*(189), rs8-rs8. doi: 10.1126/scisignal.2001699

Xiong, Y., Wang, E., Huang, Y., Guo, X., Yu, Y., Du, Q., ... Sun, Y. (2016). Inhibition of lysine-specific demethylase-1 (LSD1/KDM1A) promotes the adipogenic differentiation of hESCs through H3K4 methylation. *Stem Cell Reviews and Reports, 12*(3), 298-304. doi: 10.1007/s12015-016-9650-z

Zamani, P., Akhondi, M., & Mohammadabadi, M. (2015). Associations of inter-simple sequence repeat loci with predicted breeding values of body weight in sheep. *Small Ruminant Research, 132*, 123-127. doi: 10.1016/j.smallrumres.2015.10.018