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Research Article

SNPs Detected in the *SIRT1* and *H-FABP* Genes and Their Association with Growth Traits in Yak

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Abstract

The aims of this study were to investigate whether the Sirtuin 1 *(SIRT1)* and Hear fatty acid binding protein *(H-FABP)* genes could be used as candidate genes in the breeding of yak. In 409 indigenous Chinese yaks, two single nucleotide polymorphisms (SNPs) were identified through DNA sequencing technology, including a SNP within the 5'UTR of *SIRT1* (g.1906A>G), and a SNP in the exon 1 of *H-FABP* (g.6643C>T). The chi-square test suggested that all the variations were in Hardy-Weinberg equilibrium (P>0.05). An association analysis suggested that g.1906A>G of *SIRT1* gene and g.6643C>T of *H-FABP* gene exhibited significant correlation with body weight and body length (P<0.01). These results indicated that these SNPs could be used as meritorious and available genetic markers in yak growth traits breeding.

Keywords: *SIRT1 gene, H-FABP gene, Growth traits, Yak, Single nucleotide polymorphism*

Yak Sığırında SIRT1 ve H-FABP Genlerinde Belirlenen Tek Nükleotid Polimorfizmleri ve Büyüme Özellikleri İle İlişkisi

Öz

Bu çalışmanın amacı Yak sığırı yetiştiriciliğinde Sirtuin 1 *(SIRT1)* ve Kalp tipi yağ asidi bağlayıcı protein *(H-FABP)* genlerinin kullanılabilirliğinin araştırılmasıdır. Yerel 409 Çin yak sığırında *SIRT1* geninin (g.1906A>G) 5'UTR'sinde ve *H-FABP* geninin (g.6643C>T) ekzon 1'inde olmak üzere iki adet tek nükleotid polimorfizmi (SNP) DNA sekanslama teknolojisi ile tespit edildi. Ki kare testi tüm varyasyonların Hardy-Weinberg denklemi içerisinde olduğunu gösterdi (P>0.05). İlişki analizi, *SIRT1* geninin g.1906A>G ve *H-FABP* geninin g.6643C>T'sinin vücut ağırlığı ve uzunluğu ile anlamlı derecede ilişkili olduğunu gösterdi (P<0.01). Bu sonuçlar, SNP'lerin Yak sığırı büyüme özelliklerine bağlı yetiştiricilikte kullanılabilecek önemli genetik belirteçler olabileceğini göstermiştir.

Anahtar sözcükler: *SIRT1 geni, H-FABP geni, Büyüme özellikleri, Yak, Tek nükleotid polimorfizmi*

INTRODUCTION

Yak *(Bos grunniens)* was distributed mainly in the Qinghai-Tibetan Plateau at altitudes from 3000 m to 5000 m above sea level, which could survive in conditions of extreme harshness with extreme cold, poor oxygen concentrations, and low air pressure [1,2]. At present, the total population of yak in China were estimated to be 15 million and accounted for over 90% of those distributed all over the world [3]. This species provided hides, meat, and milk for local Tibetans needs and played a crucial role in contribution to the animal husbandry economy in Qinghai-Tibetan Plateau [4].

Mammalian Sirtuin 1 *(SIRT1)* was localized in the nucleus, wherein it influenced the activity of transcription factors via deacetylate histones [5]. In vivo and vitro, *SIRT1* regulated body growth through modulated insulin resistance and body glucose equilibrium [6,7]. In response to fasting, the secretion of insulin in *SIRT1*$^{-/-}$ mice was significant restrained [8], when compared with wild-type littermates in pancreatic β-cells. In turn, β-cells-specific *SIRT1*-overexpression transgenic

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mice influenced adenosine triphosphate (ATP) production by repressing uncoupling protein 2 *(UCP2)*, consequently contributing to enhanced glucose-stimulated insulin secretion [9]. *SIRT1* deacetylated peroxisome proliferator-activated receptor gamma coactivator 1-alpha *(PGC-1α)* at multiple lysine sites, resulting in alteration of genetic programs for gluconeogenesis and glycolysis in the liver [10]. In addition, *SIRT2* deacetylated forkhead box O 1 *(FoxO1)*, in parallel with influences the activation of NeuroD and MafA, thereby inhibiting the secretion of insulin in β-cells [11].

Hear type fatty acid binding protein *(H-FABP)* was expressed predominantly in hear and skeletal muscle [12], wherein played a crucial role in signal transduction pathways such as the uptake or utilization of long chain fatty acids [13]. The *H-FABP*-nullt mice exhibited improved insulin sensitivity, which was perhaps related to the increased reliance on glucose [14]. The expressions of *UCP2*, *UCP3*, *ACOX1*, and *PGC-1α* involving in lipid and glycolysis metabolism were modified by the knockdown of *H-FABP* gene in brown adipocytes [15]. As a transcriptional factor, *KLF15* gene regulated diverse arrays of biological processes including cell proliferation, differentiation and apoptosis [16]. Previous studies demonstrated that the *KLF15* gene might modify the core promoter of *H-FABP* gene, thereby influencing the growth-related traits in mammal [17].

Based on the functional role in metabolism, polymorphism of *SIRT1* and *H-FABP* genes had been previously demonstrated in pig [18], cattle [19], and human [20]. However, there were no reports on associations between the variations of these two genes and growth traits in yak. The main purpose of this study was to evaluate the genetic association between polymorphism of the *SIRT1* and *H-FABP* genes and growth traits in Chinses domestic yak.

MATERIAL and METHODS

Experimental Animals

In total, 409 female yak aged from 12 to 24 months were randomly collected, which belonged to five farm reared in Qilian county, Qinghai Province of China. They were reared under the same environmental dry-lot nutrition standard conditions. Blood samples were collected from the jugular vein, and stored at -20°C. Genomic DNA was extracted using a DNA extraction Kit (OMGAM Bio-Tek, Doraville, USA) following instructions provided in the attached protocol. The DNA concentration was estimated spectrophotometrically, and then the DNA was diluted to 50 ng/uL. Meanwhile, the phenotypes traits including body weight, body length, withers height and chest circumference were measured according to Gilbert's method [21]. For the accuracy of the results, all individuals were measured once by the same person.

SNP Screening and Genotyping

Available sequence information from yak *SIRT1* gene (Genbank accession no NW_005395486.1) and *H-FABP* (Genbank accession no NW_005395183.1) were used to design PCR primers *(Table 1)*. 5 primer pairs were synthesized by Sangon Biotech (Shanghai, Chian) Co., Ltd. The PCR was carried out in 30 uL mixture containing 50 ng DNA, 10 pM of each primer, 0.2 mM dNTP, 2.5 mM $MgCl_2$, and 0.5 U Taq DNA polymerase. The cycling protocol was as follows: initial denaturation at 94°C for 5 min, with 35 cycles of denaturation at 94°C for 30 s, annealing for 30 s at optimum temperature, and elongation at 72°C for 30 s. The final extension was performed at 72°C for 10 min. All PCR products were sequenced in forward direction by using the ABI 3730 sequencer (Applied Biosystems, Foster City, CA, USA), and the results were analyzed by DNAMAN software version 5.2.2 (Madison, WI, USA). In this study, all 409 samples were genotyped by directly sequencing method of PCR products respectively.

Statistical Analysis

Genotyping and allele frequencies, Hardy-Weinberg equilibrium (HWE) and polymorphism information content (PIC) were calculated by online website *(http://www.msrcall.com/Gdicall.aspx)*. The effect of genotypes of SNPs on the

Table 1. *Primers used for PCR amplification*

Primer Name	Primer Sequence (5' to 3')	Annealing Temperature	Product Length	Amplified Region
SIRT1-L1	CCTGATTTCATTGGGATA	62.5°C	777 bp	5'UTR
	AAGGCTGAGCAAATAACC			
SIRT1-L2	CTTGGACTTGGCATTCTC	60.0°C	351 bp	Eexon 4
	TGGGCTCTTTACCACTCT			
SIRT1-L3	TTTTGGCTTACAGGAACT	58.3°C	749 bp	3'UTR
	AGGCGTTTACTAATCTGC			
H-FABP-L1	CTATGTAACGTCTTTGAAGG	61.0°C	509 bp	Eexon 1
	ACAGGCAACAGGTAGATGCT			
H-FABP-L2	GGCTGGCTGAGCTCTGGCTC	60.5°C	548 bp	Eexon 2
	AGTGAGGCTTTGTGCTCTGC			

growth traits was analyzed by SPSS software (Version 18.0). The model applied was: $Y_{ijk} = \mu + G_i + A_j + F_k + E_{ijk}$, where Y_{ijk} is the trait value for each individual, μ is the overall population mean, G_i is the fixed effect associated with the i^{th} genotype, A_j is the fixed effect of the i^{th} age, F_k is the fixed effect of the i^{th} farm and E_i is the random error. In this model, P-values less than 0.05 were considered to be statistically significant.

RESULTS

SNP Detection and Genetic Diversity Analyses

As is presented in *Fig. 1*, two variations were identified through DNA sequencing analysis, including one SNP within 5'UTR of *SIRT1* gene (g.1906A>G), and one SNP located in exon 1 of *H-FABP* gene (g.6643C>T). The g.1906A>G locus had 3 genotypes (AA, AG and GG, respectively). The g.6643C>T locus had 2 genotypes, the TT genotype was not observed in the sampled animals.

Table 2 illustrates the genotypic and allelic frequencies at locus of *SIRT1* and *H-FABP* genes. The "A" allele of g.1906A>G (*SIRT1*) and the "C" allele of g.6643C>T (H-FABP) were found to be predominant (72.98% and 90.10%, respectively). In addition, the g.1906A>G had moderate polymorphic status (0.25 <PIC<0.05).

Association Analysis

In the present study, one SNP (g.1906A>G) in the 5'UTR of *SIRT1* gene was identified. Statistical results showed that the animals with genotype AA exhibited significantly higher body weight and body length compared to genotype GG (P=0.000, P=0.004, respectively).

For the *H-FABP* gene, statistical analyses indicated animals with genotype CC of g.6643C>T had significantly higher body weight and body length than those with genotype CT (P=0.000 and P=0.031, respectively), demonstrating that allele "C" might be associated with an increase in growth traits in yak *(Table 3)*.

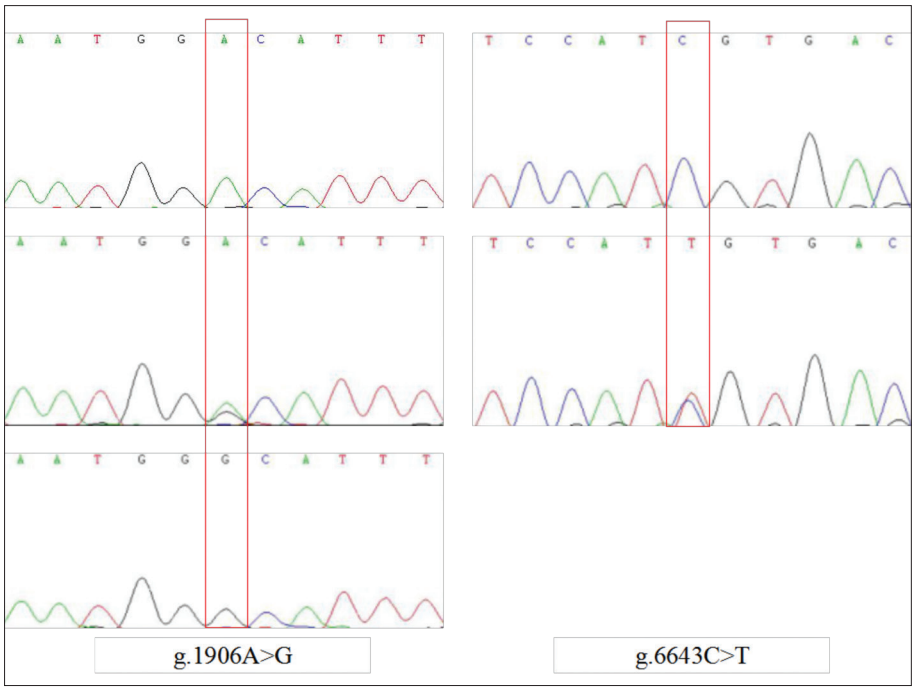

Fig 1. The sequencing map of the SNPs

Table 2. *Distribution of genotype and allele frequencies in yak*

Site	Genotype (N)	Genotypic Frequency (%)	Alleles	Allele Frequency (%)	χ^2 (HWE)	PIC
g.1906A>G (*SIRT1*)	AA (225)	55.01	G	72.98	P>0.05	0.32
	AG (147)	35.94	T	27.02		
	GG (37)	9.05				
g.6643C>T (*H-FABP*)	CC (328)	80.20	C	90.10	*P*>0.05	0.16
	CT (81)	19.80	T	9.90		
	TT (0)	0.00				

χ^2 (HWE): Hardy-Weinberg equilibrium χ^2 value, Hard-Weinberg equilibrium (P>0.05), Hardy-Weinberg disequilibrium (P<0.05)

Table 3. *Association of growth traits with marker genotypes within SIRT1 and H-FABP genes in yak*

Site	Traits	Genotype (Mean±SE)			P Value
		AA	AG	GG	
g.1906A>G (*SIRT1*)	BW (kg)	175.09±0.87^{A}	165.58±1.10^{B}	156.90±2.15^{C}	0.000
	BH (cm)	106.98±0.63	104.64±0.78	105.84±1.55	0.313
	BL (cm)	120.89±0.37^{A}	116.89±0.46	113.27±0.91^{B}	0.004
	CC (cm)	144.91±0.39	142.48±0.49	140.16±0.94	0.147
g.6643C>T (*H-FABP*)		CC	CT		
	BW (kg)	172.41±0.75^{A}	160.38±1.24^{B}		0.000
	BH (cm)	107.62±0.28	106.30±0.56		0.464
	BL (cm)	119.99±0.30^{a}	113.79±0.63^{b}		0.031
	CC (cm)	143.87±0.33	142.52±0.67		0.232

^{A,B,C} *Means with different superscripts are significantly different (P<0.01)*

DISCUSSION

Through deacetylating specific transcription factors (i.e., *PPARγ* and *FOXO1)* [22], the *SIRT1* gene modulated mitochondrial capacity [23], insulin secretion [9] and plasma glucose levels [24]. Previously, a polymorphism (g.-274A>G) within the promoter region of *SIRT1* gene in a Nanyang cattle breed was significantly correlated with body height and body weight [25]. Three SNPs (g.25764G>A, g.25846A>G and g.25868T>C) of *SIRT1* gene were identified in Qinchuan cattle, and were observed to be associated with body length and withers height [26]. Additionally, the novel 12-bp indel of *SIRT1* gene were associated with body weight, chest circumference and height at hip cross Chinese beef cattle [27]. In the present study, the statistical results showed that the individuals with genotype AA of g.4845C>T of *SIRT1* gene were significantly associated with body weight and body length than the other genotype. Those results were consistent with the previous findings of other species [18-20].

The g.1906A>G was located in region of 5'UTR of *SIRT1* gene, without changing the structure of amino acid. Recently, there were some reports about the effects of variants located in region of 5'UTR on the gene expression pattern [28]. A SNP within 5'UTR region of zinc finger, BED-type containing 6 *(ZBED6)* gene resulted in transcription factor change, and then altered the transcription activity and mRNA expression in beef cattle [29]. A 5'UTR-region SNP of growth hormone-releasing hormone receptor *(GHRHR)* gene locus had been found to be associated with body weight and average daily gain in Chinese cattle [30]. Thus, it was an interesting work to find out the mechanism for the association between these 5'UTR-region SNPs and the growth traits in yak.

The *H-FABP* gene contained four exons, and was expressed predominantly in skeletal muscle and subcutaneous fat [12]. Because of its important role in metabolism, association between *H-FABP* polymorphisms and economic characters in livestock was extensively studied. In Berkshire pig breed, one SNP *(H-FABP/Hinf*1) was found to be associated with the live weight and fatty acid composition [31]. Similarly, this SNP had a significant effect on moisture, tenderness and flavor score of Korean native pig [32]. For the g.6643C>T of *H-FABP* locus, only 2 genotypes were detected. The result was due to the lack of genotype in the population or the small size of experimental population. The genotypic frequencies of g.6643C>T of *H-FABP* gene conformed to Hardy-Weinberg equilibrium might be a result of random mating in yak [33]. Additionally, our experimental populations belonged to intermediate genetic diversity. This reflected that this genetic marker could provide more reasonable and effective genetic information [34].

Sequence alignment showed that the g.6643C>T of *H-FABP* gene was synonymous (Thr→Thr), and thus did not change the structure of the encoded protein. It had been demonstrated previously that synonymous SNPs could modify the stability of mRNA [35,36], thereby influencing the phenotypic traits in mammal. A synonymous mutation (g.25557A>G) in the silent information regulator 6 (*SIRT6*) gene was found to be associated with intramuscular fat in Qinchuan cattle [37]. Additionally, synonymous mutation (c.72G>A) of the *UCP2* gene was related to growth performance, carcass characteristics and meat quality in rabbits [38].

Results of the present study suggested that g.1906A>G of *SIRT1* and g.6643C>T of *H-FABP* were significantly correlated with growth traits in yak. It inferred that these SNPs could modify stability/expression of mRNA, therefore influencing the growth-related phenotypes in yak.

In summary, genotypes of *SIRT1* and *H-FABP* were confirmed to be significantly associated with body weight and body length in yak. Our investigation provided evidence that both *SIRT1* and *H-FABP* genes could be used as molecular markers for better growth traits of yak.

Conflicts of Interest

The authors declare no conflict of interest.

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