

# Genetic Polymorphisms of Myogenin Gene and Their Associations with Growth Traits in the Chinese Tibetan Sheep

Yonggang SUN<sup>1</sup> Yincang HAN<sup>1</sup> 

<sup>1</sup>Qinghai Academy of Animal Science and Veterinary Medicine, Qinghai University, Xining, CHINA

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## Abstract

Myogenin gene encodes for skeletal muscle-specific transcription factors with highly conserved basic helix-loop-helix domain and play key role in growth and muscle development. In this study, the polymorphisms of myogenin gene were investigated to check whether they are associated with growth traits in Chinese-Tibetan sheep. In three sheep breeds with a total number of 632 individuals, three SNPs, including two in exon 1 (C109A and C183T) and one in intron 2 (A1403C) were found by DNA sequencing. Among those, C109A is a missense mutation (Gly37Arg). The single marker association analysis showed that the three mutations were significantly associated with growth traits (at  $P<0.01$  or  $P<0.05$ ). In conclusion, our results suggest that myogenin gene variation may be considered molecular markers for growth traits in Chinese-Tibetansheep.

**Keywords:** Chinese Tibetan sheep, Myogenin gene, SNP, Growth traits

## Çin Tibet Koyununda Myogenin Geninin Genetik Polimorfizmi ve Büyüme Özellikleri ile Olan İlişkisi

### Özet

Myogenin geni oldukça korunaklı olan temel heliks-ilmek-heliks domain yapısında olup iskelet kas-spesifik transkripsiyon faktörlerini kodlar ve büyüme ile kas gelişimde önemli rol oynar. Bu çalışmada, myogenin geninin polimorfizmi ve Çin-Tibet koyununda büyüme özellikleri ile ilişkisinin olup olmadığı araştırıldı. Üç koyun ırkından oluşan toplam 632 hayvanda, DNA sekansı ile ikisi ekzon 1 (C109A ve C183T) ve biri intron 2 (A1403C)'de olmak üzere üç SNP bulundu. Bunlar arasında C109A missen mutasyonuydu (Gly37Arg). Tek markır asosiyasyon analizi, üç mutasyonun anlamlı derecede büyüme özellikleri ile ilişkili olduğunu gösterdi ( $P<0.01$  veya  $P<0.05$ ). Sonuç olarak, elde edilen sonuçlar ışığında myogenin gen varyasyonunun Çin-Tibet koyununda büyüme özellikleri için moleküler belirteç olabileceği saptandı.

**Anahtar sözcükler:** Çin-Tiber koyunu, Myogenin geni, SNP, Büyüme özellikleri

## INTRODUCTION

Myogenic regulatory factors (MRFs) belong to the family of conserved basic helix-loop-helix (bHLH) transcription factors<sup>[1]</sup>, including *Mfy5*, *Mfy6*, *MyoD* and myogenin<sup>[2,3]</sup>. They are well known to control the determination of the myogenesis, from commitment and proliferation, through muscle fiber formation, to postnatal maturation and muscle function<sup>[3]</sup>.

Myogenin gene plays a role during the terminal transformation of myoblasts into myofibres. Specifically, myogenin gene expression abrogates myoblast proliferation potential and regulates the differentiation of mono-

nucleated myoblasts into multinucleated myofibers<sup>[4]</sup>. The expression of myogenin gene was continuous in all myogenic cell lines and associated with the number of muscle fibres during myogenesis<sup>[5]</sup>. Mice that lack myogenin shows no muscle fiber development, leading to striking phenotypes<sup>[6]</sup>. Additionally, the myogenin gene regulates the expression of muscle-specific genes, which encode several proteins that control the formation and apoptosis (or necrosis) of muscle fibers<sup>[7]</sup>. Taken together, these findings lend credence to the hypothesis that myogenin gene is considered excellent candidate gene for growth-related traits in livestock due to its potential roles in the development of skeletal muscles.



### İletişim (Correspondence)



+86 971 5315935



qhanyincang@126.com

At present, studies on mutation of myogenin gene associated with growth traits were mainly focused on swine, chicken, and cattle. Few studies were carried out on sheep. Therefore, the objective of this study was to detect ovine myogenin gene and to explore their possible association with growth traits in native Chinese breeds.

## MATERIAL and METHODS

### Experimental Population

A total of 632 ewes were collected from Chinese Tibetan sheep including Black Tibetan sheep (BT, N = 226), Gaoyuan Tibetan sheep (GT, N = 191), and Oula Tibetan sheep (OT, N = 215). All the sheep were in the artificial insemination system that were raised in provinces of Qinghai, Gansu, and Henan, respectively. Their growth traits (body weight, height, length and chest circumference were recorded at 3 years of age.

### Genomic DNA Isolation and Genotyping

Genomic DNA was extracted from sheep blood (jugular vein samples) by the standard phenol-chloroform extraction procedure. DNA quantity and purity ( $A_{260}/A_{280}$  ratio) for each sample was assessed using a Nano-Drop™1000 Spectrometer (Thermo Scientific, Waltham, MA, USA), then stored at -20°C.

Primers to amplify of the ovine Myogenin gene were designed based on sequences in the NCBI database sequences (GenBank accession No. NC\_019469.1) using Primer v5.0 software (PREMIER Biosoft, Palo Alto, CA, USA). The information of the primers of Myogenin gene was shown in Table 1. A PCR was conducted in 20- $\mu$ L reactions containing 50 ng DNA, 10 pM each primer, 0.20 mM dNTPs, 2.5 mM  $MgCl_2$ , and 0.5 U Taq DNA polymerase (TaKaRa,

Shiga, Japan). The following PCR reaction conditions were used: 5 min at 95°C; 35 cycles of 30 s at 94°C, annealing for 35 s at optimum temperature, 40 s at 72°C, and final extension at 72°C for 10 min. Digested products were detected by electrophoresis on 1.0% agarose gels.

Thirty random DNA samples were mixed to form a single DNA pools, from which mutation of Myogenin gene were detected. Then, the PCR products were amplified from the 632 Chinese Tibetan sheep directly sequenced in both directions (Sangon, Shanghai, China).

### Statistical Analysis

Genotype and allele frequencies, gene heterozygosity ( $H_e$ ), effective allele numbers ( $N_e$ ), polymorphism information content (PIC), and tests for deviation from Hardy-Weinberg equilibrium (HWE) were calculated by POPGENE v. 1.32<sup>[8]</sup>.

The association analysis between single marker and growth traits were analyzed by general linear model (GLM) procedure of SPSS 21 (IBM, Armonk, NY, USA). The following statistical linear model is used:  $Y_{ij} = u + G_i + S_j + E_{ij}$  where  $Y_{ij}$  is the traits measured on each of the individual cattle,  $u$  is the overall population mean for the traits,  $G_i$  is the fixed effect associated with the genotype,  $S_j$  is the fixed effect with season, and  $E_{ij}$  was the standard error.

## RESULTS

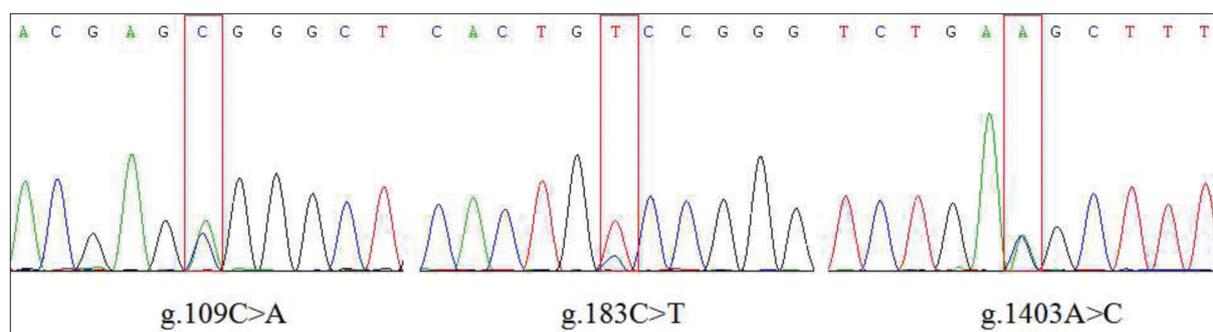
### Identification of SNPs

Amplification and sequencing of Myogenin gene among three different sheep breeds revealed three variations, named C109A, C183T, and A1403C, respectively (Fig. 1). Of these, C109A and C183T were identified in exon 1 and A1403C was found in intron 2. Sequence analysis showed that C109A was a missense mutation (Gly37Arg),

**Table 1.** Genotype frequencies (%) of the myogenin for the SNPs in Chinese Tibetan sheep

Site	Breed	Genotypic Frequency			Allele Frequency		$\chi^2$ (HWE*)	$H_e$	$N_e$	PIC
g.109C>A		CC	CA	AA	C	A				
	BT	0.7611	0.1416	0.0973	0.8319	0.1681	55.1166	0.2406	0.2797	1.3884
	GT	0.5759	0.3194	0.1047	0.7356	0.2644	6.1169	0.3133	0.3890	1.6366
	OT	0.6279	0.2977	0.0744	0.7767	0.2233	4.3180	0.2867	0.3468	1.5310
g.183C>T		CC	CT	TT	C	T				
	BT	0.2080	0.3230	0.4690	0.3695	0.6305	21.2634	0.3574	0.4659	1.8724
	GT	0.2723	0.3037	0.4241	0.4241	0.5759	27.3398	0.3692	0.4885	1.9549
	OT	0.1953	0.2651	0.5395	0.3279	0.6721	34.1447	0.3436	0.4408	1.7882
g.1403A>C		AA	AC	CC	A	C				
	BT	0.0605	0.2186	0.7209	0.1698	0.8302	10.8372	0.2422	0.2819	1.3925
	GT	0.0628	0.3560	0.5812	0.2408	0.7592	0.1330	0.2988	0.3657	1.5765
	OT	0.0605	0.2186	0.7209	0.1698	0.8302	10.8372	0.2422	0.2819	1.3925

HWE, Hardy-Weinberg equilibrium;  $\chi_{0.05}^2 = 5.991$ ,  $\chi_{0.01}^2 = 9.210$



**Fig 1.** Sequencing map of the three identified SNPs in myogenin gene

**Table 2.** Association of different genotypes of SNPs in myogenin with growth traits in BT sheep

Site	Genotypes	Body Weight (cm)	Body Height (cm)	Body Length (cm)	Chest Circumference (cm)
g.109C>A	CC	39.81±3.05 <sup>b</sup>	59.87±5.38 <sup>bb</sup>	63.68±5.36 <sup>B</sup>	87.84±8.14
	CA	41.78±4.52	63.07±6.28 <sup>a</sup>	68.15±3.25 <sup>A</sup>	87.38±8.61
	AA	42.99±3.77 <sup>a</sup>	65.91±5.42 <sup>A</sup>	68.77±3.70 <sup>A</sup>	89.42±7.33
g.183C>T	CC	38.88±3.69 <sup>bb</sup>	62.77±5.69 <sup>b</sup>	65.45±3.68 <sup>b</sup>	85.45±7.21 <sup>B</sup>
	CT	41.56±3.44 <sup>a</sup>	63.19±6.39 <sup>b</sup>	68.33±3.72	87.08±7.55
	TT	43.52±4.85 <sup>A</sup>	66.22±6.17 <sup>a</sup>	68.62±3.58 <sup>a</sup>	90.36±8.19 <sup>A</sup>
g.1403A>C	AA	41.73±4.63	63.84±5.00	68.26±5.77	87.90±7.02
	AC	41.86±3.69	63.59±4.66	68.13±6.21	87.92±8.19
	CC	43.00±3.26	65.75±4.17	68.42±6.34	89.52±8.32

Means with different superscripted lower and upper case letters are significantly different at  $P<0.05$  and  $P<0.01$ , respectively

while C183T resulted in a synonymous mutation (Cys61Cys). The detailed information about all SNPs are presented in Table 1.

### Genotyping and Allele Frequencies

Genotyping was performed by DNA sequencing method. Allele frequencies of the SNP were investigated and performed by the  $\chi^2$  test in three different sheep breeds (Table 1). The data shown here demonstrates that C (C109A), T (C183T) and C (A1403C) were the most prevalent alleles. By chi-square test, C109A in OT sheep and A1403C in GT sheep were in HWE ( $\chi^2 < \chi_{0.05}^2$ ). In this study, PIC values ranged from 0.2406 to 0.3692, according to the conventions for PIC classification (PIC < 0.2500 is considered low polymorphism, 0.2500-0.5000 is intermediate polymorphism, and > 0.5000 is high polymorphism), our data showed that C109A in BT sheep and A1403C in OT sheep has low genetic diversity, while others possessed an intermediate genetic diversity.

### Effect of The Polymorphism Locus on Growth Traits in BT Sheep

Table 2 showed the effects of the SNPs on growth traits in GT sheep. At the C109A locus, individuals with genotype AA had higher values than those with CC for body height

and body length ( $P<0.01$ ). Additionally, the body weight of individuals with genotype AA was higher than those with genotype CC ( $P<0.05$ ). At the C183T locus, individuals with genotype TT had higher values than those with CC on body weight and chest circumference ( $P<0.01$ ), while genotype TT had higher mean values for body length and body height than those with genotype CC ( $P<0.05$ ). As with similar the BT sheep, there were no significant correlation between A1403C and growth traits.

### Effect of The Polymorphism Locus on Growth Traits in GT Sheep

Association results of single markers with four growth traits in the GT sheep population are shown in Table 3. At the C109A locus, individuals with genotype CC had higher values than those with AA for body weight ( $P<0.05$ ). At the C183T locus, significant differences in body weight and height were observed between the CC and TT genotypes ( $P<0.05$ ). Compared with TT, individuals with the CC genotype showed better performance for body length and chest circumference ( $P<0.01$ ). At the A1403C locus, individuals with genotype AA had higher values than those with CC for body weight ( $P<0.05$ ). In addition, the body length of individuals with genotype AA was higher than those with genotype CC ( $P<0.01$ ).

**Table 3.** Association of different genotypes of SNPs in myogenin with growth traits in GT sheep

Site	Genotypes	Body Weight (cm)	Body Height (cm)	Body Length (cm)	Chest Circumference (cm)
g.109C>A	CC	47.79±3.36 <sup>a</sup>	66.54±5.89	70.35±5.02	92.41±7.95
	CA	46.05±3.22	65.38±5.64	69.33±5.11	91.33±7.77
	AA	43.49±4.49 <sup>b</sup>	66.37±6.54	68.92±3.90	91.22±8.21
g.183C>T	CC	47.98±3.69 <sup>a</sup>	69.94±7.25 <sup>a</sup>	74.41±4.29 <sup>Aa</sup>	95.43±8.33 <sup>Aa</sup>
	CT	44.93±4.20 <sup>b</sup>	65.88±7.02	68.21±3.82 <sup>b</sup>	91.75±8.02 <sup>b</sup>
	TT	43.15±4.65 <sup>b</sup>	63.88±6.32 <sup>b</sup>	66.82±3.24 <sup>B</sup>	88.75±6.58 <sup>B</sup>
g.1403A>C	AA	48.55±4.42 <sup>a</sup>	67.11±4.39	73.32±6.26 <sup>A</sup>	94.54±8.41
	AC	47.32±3.36 <sup>a</sup>	66.71±4.92	70.01±6.34	91.88±8.81
	CC	43.20±5.36 <sup>b</sup>	65.68±5.39	68.44±6.10 <sup>B</sup>	90.91±7.27

Means with different superscripted lower and upper case letters are significantly different at  $P<0.05$  and  $P<0.01$ , respectively

**Table 4.** Association of different genotypes of SNPs in myogenin with growth traits in OT sheep

Site	Genotypes	Body Weight (cm)	Body Height (cm)	Body Length (cm)	Chest Circumference (cm)
g.109C>A	CC	59.01±3.69 <sup>A</sup>	74.21±7.32 <sup>A</sup>	76.01±4.24 <sup>a</sup>	93.83±8.25
	CA	56.96±4.35	72.50±6.21	73.82±3.59	90.72±8.46
	AA	53.61±5.11 <sup>B</sup>	67.10±6.03 <sup>B</sup>	72.29±4.22 <sup>b</sup>	90.37±7.62
g.183C>T	CC	59.42±4.28 <sup>a</sup>	73.64±7.21	75.88±3.52	93.83±7.56 <sup>a</sup>
	CT	56.36±4.19	72.95±6.50	74.00±3.89	91.40±7.31
	TT	55.17±4.89 <sup>b</sup>	71.28±6.24	73.98±5.21	89.94±8.26 <sup>b</sup>
g.1403A>C	AA	61.74±5.11 <sup>a</sup>	75.32±5.69 <sup>a</sup>	76.32±8.36	94.36±8.96
	AC	57.36±5.37 <sup>b</sup>	73.95±6.28	75.57±7.25	92.89±9.22
	CC	57.89±3.56 <sup>b</sup>	72.73±5.87 <sup>b</sup>	74.81±7.11	92.42±7.85

Means with different superscripted lower and upper case letters are significantly different at  $P<0.05$  and  $P<0.01$ , respectively

### Effect of The Polymorphism Locus on Growth Traits in OT Sheep

As shown in Table 4, the association analysis between each marker and the growth traits in OT sheep. At the C109A locus, individuals with genotype CC had increased body weight and height compared with the AA genotype ( $P<0.01$ ). At the C183T locus, individuals with genotype CC had higher values than those with TT on body weight and chest circumference ( $P<0.05$ ). At the A1403C locus, individuals with genotype AA had higher values than those with CC on body weight and body height ( $P<0.05$ ).

## DISCUSSION

Chinese Tibetan sheep were the first artificially bred sheep in the natural ecosystem of the Qinghai-Tibetan plateau and has adapted well to these conditions [9]. Undoubtedly, they are an important species of grazing livestock with great economic value and highly tolerant to unfavorable weather conditions, such as extreme cold, lower atmospheric oxygen and air pressure [10]. However, slower growth rate has hampered the commercialization of Tibetan sheep production. Therefore, this study aimed to

identify potential polymorphisms of the ovine myogenin genes and to explore their relationships with growth traits in Chinese-Tibetan sheep.

In the present study, we detected the two SNPs (C109A and C183T) in exon and other one SNP (A1403C) mapping to intron to reveal their associations with growth traits in Chinese -Tibetan sheep. Specifically, At the C109A locus, individuals with genotype CC has significantly greater body weight in all breeds compared with genotype AA, meaning that C allele might be associated with an increase in body weight. At the C183T locus, individuals with genotype CC had significantly greater body weight and chest circumference in all breeds compared with genotype TT, C allele appeared to be the beneficial genotype for body weight and chest circumference. At the A1403C locus, individuals with genotype AA had significantly greater body weight in all breeds compared with genotype CC, A allele appeared to be the beneficial genotype for body weight.

Recent evidence suggests that myogenin gene plays an essential role in skeletal muscle development and adult homeostasis [11]. Different myogenin gene function or timing of expression could have a major influence

on the number of muscle fibers that develop during myogenesis<sup>[12]</sup>. Growing observations indicate that genetic polymorphisms in myogenin gene are associated with growth traits in livestock. Xue et al.<sup>[13]</sup> identified a novel polymorphism (T314C) in the myogenin gene that was associated with growth traits in native Chinese breeds. Anton et al.<sup>[14]</sup> showed that one SNP in myogenin gene had a strong effect on growth rate in Hungarian large pig. One SNP (T36C in exon 3) detected in myogenin gene was associated with an alteration in body weight in Jinghai yellow chicken<sup>[15]</sup>. Combined with the results of our study, it was suggested that myogenin gene may mediate, directly or indirectly, growth traits in animals.

We noted that C183T was the synonymous mutation, and did not change the structure of the encoded proteins, but our results demonstrated that it was still associated with some of the growth traits. Growing evidence suggests that synonymous mutation could affect both the splice donor site or nearby regions and regulatory motifs<sup>[16-19]</sup>. In this study, we deduce that such associations may be the result of linkage disequilibrium between this SNP and other genes on the same chromosome that has a significant effect on the growth traits studied here. Importantly, further verifications are needed to understand the underlying mechanisms.

In summary, three mutations were identified in the ovine myogenin gene in this study. Substantial differences in allele frequencies were observed among three different breeds. The results presented here show significant associations between the myogenin gene SNPs with growth and in Chinese Tibetan sheep. The obtained results of this study will contribute to the understanding of the regulatory mechanisms of the myogenin gene, and may be used in molecular marker-assisted selection for excellent growth traits.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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