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Correlation between sheep *YAP1* temporal and spatial expression trends and *MSTN* and *MyoG* gene expression

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ABSTRACT. The aim of the current study was to investigate the effects of Yes-associated protein 1 (*YAP1*) gene expression after birth on the development of muscle and the relationship between *YAP1* and myostatin (*MSTN*) and myogenin (*MyoG*). Reverse transcription polymerase chain reaction was used to analyze the trends in *YAP1*, *MSTN*, and *MyoG* temporal and spatial expression levels in various skeletal muscles (i.e., longissimus dorsi muscle, soleus muscle, gastrocnemius muscle, and extensor digitorum longus) and across 3 different growth stages (i.e., 2 days old, 2 and 6 months old) of Hu Sheep. The results showed that *YAP1* expression was significantly different in the skeletal muscles of sheep; the expression level gradually increased with age; it was highly expressed in the gastrocnemius muscle and minimally expressed in the longissimus dorsi muscle. *MSTN*, a negative regulator

of skeletal muscle development, was minimally expressed in the soleus muscle and might be related to the enlargement of muscle fiber diameter. *MyoG*, an important factor in regulating skeletal muscle development, was minimally expressed in the longissimus dorsi muscle and extensor digitorum longus, and highly expressed in the gastrocnemius and soleus muscles; it might inhibit the enlargement of muscle fiber diameter after birth. *YAP1* expression was significantly ($P < 0.05$) or extremely significantly ($P < 0.01$) and positively correlated with *MSTN* and *MyoG* at 2 days old, 2 and 6 months old. *YAP1* expression was related to muscle fiber development after birth and might be a candidate gene for the regulation of muscle growth.

Key words: Hu-sheep; *YAP1*; *MSTN*; *MyoG*; Gene expression; Muscle fiber development

INTRODUCTION

Yes-associated protein 1 (*YAP1*), known as YAP, YAP2, YAP65, and YKI, is a major downstream effector of the Hippo pathway, which is used as a coactivator or corepressor within the Hippo pathway. *YAP1* is directly phosphorylated by Lats1/2 on 5 HXRXXS consensus motifs to inhibit *YAP1* from entering the nucleus. *YAP1* plays important roles in cell proliferation and apoptosis, regulation of organ size, cell contact inhibition, and tumorigenesis (Zhao et al., 2008; Liu et al., 2010; Li et al., 2011; Zhang and Zhu, 2011; Zhao and Wang, 2011; Liu et al., 2011). In addition, it is a novel regulator of C2C12 myogenesis (Watt et al., 2010).

Myostatin (*MSTN*) and myogenin (*MyoG*) are the 2 most important regulatory factors related to myoblast differentiation. *MSTN* is a negative regulator and *MyoG* is a positive regulator during myoblast differentiation into myotubes. Smad3 is a key mediator inhibiting myogenesis in TGF-β. Liu et al. (2001) found that inhibition, which separates Smad3 (not Smad2) on *MyoD*, is a key step in TGF-β myoblast signal transduction. Smad3 inhibits the transcription of *MyoD* by closely interacting with it. *MSTN* can induce the phosphorylation of Smad3 to enhance the correlation between Smad3 and *MyoD* (Langley et al., 2002); thus, Smad3 might influence the activity of *MyoD* and mediate the signal transduction of *MSTN*. Further experimentation has shown that *MSTN* inhibits the expression levels of Myf5, *MyoG*, and p21 (Langley et al., 2002). *MyoG* is expressed downstream of *MyoD*, and *MyoD* plays a role in the activation on *MyoG*. *MyoG* may play an important role in the differentiation and integration of myotubes and muscle fibers. Following *MyoG* knockout, myogenesis begins but myoblasts do not differentiate into muscle fibers (Li, 2007). The research indicates that *MSTN* inhibits the activity and expression of *MyoG* by inhibiting the activity of *MyoD*; in addition, it inhibits the differentiation of myoblasts into myotubes by Smad3 expression.

TGF-β is the pathway that is associated with myogenesis; Smad7 plays an antagonistic role in the TGF-β pathway, and *YAP1* may be a novel protein that binds with Smad7 (Ferrigno et al., 2002). Ferrigno et al. (2002) found that *YAP1* potentiated the inhibitory activity of Smad7 against TGF-β-induced, Smad3/4-dependent gene transactivation. Furthermore, *YAP1* augmented the association of Smad7 to activate TGF-β receptor type I (TbRI), and *YAP1* enhanced the inhibitory activity of Smad7 against TGF-β signaling. The phosphorylation of *YAP1* at Ser127 led to changes in the expression levels of MRFs and cell cycle regulators,

which are required for C2C12 myoblast differentiation into myotubes (Watt et al., 2010). However, the mechanistic effect of *YAP1* is involved with muscle fiber development after birth in sheep, and the relationships among *YAP1*, *MSTN*, and *MyoG* are still unknown.

In the present study, *YAP1*, *MSTN*, and *MyoG* spatial and temporal expression trends and the correlations of these gene expressions in sheep skeletal muscles were investigated. The results showed that *YAP1* expression affected sheep muscle fiber development after birth and provided important genetic information for the selection of sheep muscle growth candidate genes.

MATERIAL AND METHODS

Experimental animals

Thirty-six experimental Hu sheep were purchased from Suzhou Sheep Breeding Farm. The experimental sheep were divided into 3 growth stages (i.e., 2 days old, and 2 and 6 months old), including 3 rams and 3 ewes for each stage. Animals were raised under the same conditions. All animals were slaughtered; the longissimus dorsi muscle, soleus muscle, gastrocnemius muscle, and extensor digitorum longus were rapidly collected and preserved in liquid nitrogen.

Reagents and kits rTaq, dNTP, PrimerScript RT reagent Kit, SYBR® Premix Ex TaqTM II (Tli RNaseH Plus), and TRIzol were purchased from (TaKaRa Biotechnology Dalian, Co., Ltd.). Primers were designed via Oligo 7.0 and synthesized by Shanghai Sangon Biological Engineering Co., Ltd. DEPC was purchased from Beijing BioTeke Corporation. Goldview was purchased from SBS Genetech Co., Ltd. Other reagents were purchased from China National Medicines Corporation Ltd.

RNA isolation and complementary DNA (cDNA) synthesis

Total RNA was extracted in the presence of a buffer containing β-mercaptoethanol and guanidine using the RNAiso Plus Kit (TaKaRa Biotechnology Dalian, Co., Ltd.) following manufacturer instructions and eluted with 40 μL RNase-free water. The concentration of RNA was measured using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and a purity (A260/A280) of >1.8 was used. A total of 250 ng RNA from each sample was transcribed into cDNA using the TaKaRa reverse transcription kit (TaKaRa Biotechnology Dalian, Co., Ltd.) according to manufacturer instructions. Primers listed in Table 1 were used for reverse transcription polymerase chain reaction (RT-PCR).

Table 1. Primer sequences for *YAP1*, *MSTN*, *MyoG*, and 18S *rRNA* gene expression assay.

Gene	Reference sequence	Primer sequence	Product (bp)
YAP1	JQ714252	SF: GACAGCGGACTGAGCATGAG SR: CAGGGTGCTTTGGTTGATAGTG	108
MSTN	AF019622	SF: CGCCTGGAAACAGCTCCTAAC SR: CCGTCGCTGCTGTCATCTCT	119
MyoG	AF433651	SF: AATGAAGCCTTCGAGGCCC SR: CGCTCTATGTACTGGATGGCG	101
18S	AY753190	SF: CGGCTACCACATCCAAGGAA SR: GCTGGAATTACCGCGGCT	187

Quantitative PCR detection

The cDNA products were diluted and tested by fluorescent quantitative PCR (FQ-PCR) with ABI 7900; the *18s* ribosomal RNA gene (eukaryon) was used as a reference gene to analyze *YAP1*, *MSTN*, and *MyoG* gene expression levels. Under the same conditions, annealing temperature (5°-63°C) and primer concentrations were optimized for use in the experiment according to the system of the SYBR Green I Kit (TaKaRa Biotechnology Dalian, Co., Ltd.). The optimal reaction system was a 20-μL reaction containing 0.8 μL 10 μM PCR Forward Primer, 0.8 μL 10 μM PCR Reverse Primer, 0.4 μL ROX Reference Dye II, 7 μL H_2O, 10 μL SYBR Green real-time PCR master mix, and 1 μL template. The PCR condition was 40 cycles of 30 s at 95°C, 5 s at 95°C, and 34 s at 60°C. Meanwhile, 1 μL sterile water (instead of the template) was set as the negative control, and 3 parallel experiments were conducted for each sample. The fluorescence signals of the computer automatic analysis were converted into Ct values of *YAP1*, *MSTN*, and *MyoG*, which were used to calculate the initial template copies.

Statistical analysis

SPSS 16.0 was used to calculate the Ct values and standard errors among repeat samples, and the differences in relative gene expression levels were analyzed by the $2^{-\Delta\Delta Ct}$ method. The ΔCt was calculated as the Ct of the target gene minus the Ct of the reference gene. In the comparison for Hu sheep of similar age and skeletal muscle but different gender, the ΔΔCt was calculated as the ΔCt for males minus the ΔCt for females. The ΔΔCt was calculated as the ΔCt for other age groups minus the ΔCt for the 2-day-old group when individuals of the Hu sheep were of the same gender and had similar skeletal muscle but belonged to different age groups. The ΔΔCt was calculated as the ΔCt for other skeletal muscles minus the ΔCt of the longissimus dorsi muscle when individuals of Hu sheep were of the same gender and age group but different skeletal muscles. The $2^{-\Delta\Delta Ct}$ represented the differential expression of the target gene between the experimental and control groups. The value of Hu sheep of similar age groups and skeletal muscles but different genders was compared via *t*-test, while the data for individuals of the same gender and skeletal muscle but different age groups; and the same gender and age groups but different skeletal muscle were compared by ANOVA. Meanwhile, a variation trend histogram of ΔCt was used to verify the conclusion that the value of ΔCt showed a negative relationship with transcriptional quantity.

RESULTS

Specificity of the amplification products

The dissolution curve analysis showed a sharp single peak in each of the *YAP1*, *MSTN*, *MyoG*, and 18S PCR products (Figure 1), indicating that the primers had good specificity and the PCR was well optimized. The dissolution temperatures of *YAP1*, *MSTN*, *MyoG*, and 18S were 84.9, 83.0, 86.3, and 84.8°C, respectively, and there was no amplification product in the negative control. The amplification efficiencies of *YAP1*, *MSTN*, *MyoG*, and 18S were 99.6, 99.4, 99.8, and 99.7%, respectively.

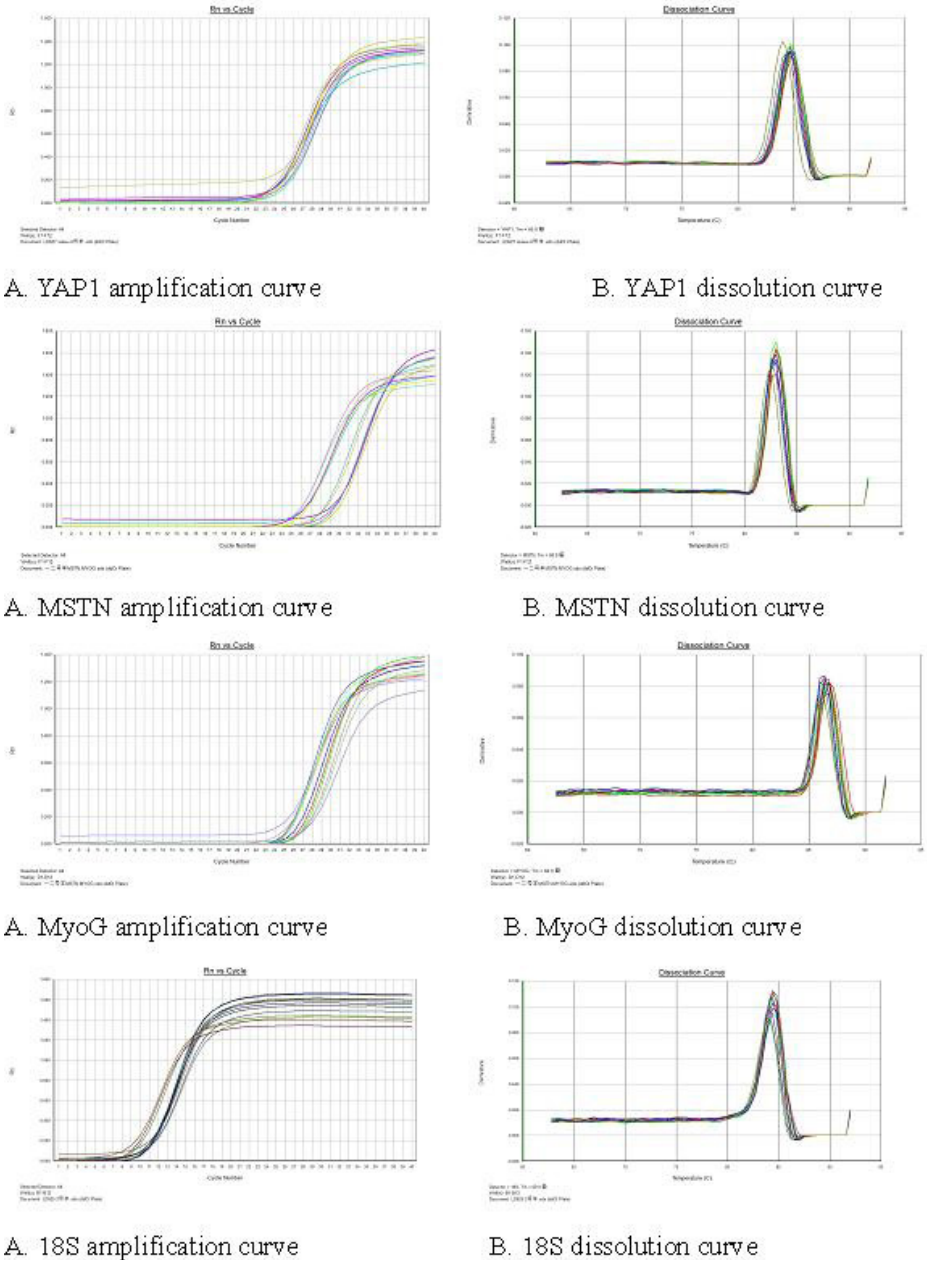

Figure 1. Amplifications and dissociation curves of *YAP1*, *MSTN*, *MyoG*, and 18S.

YAP1 spatial and temporal expression analysis in sheep muscle

At 2 months old, *YAP1* sheep exhibited the highest and lowest expression levels in

the gastrocnemius and longissimus dorsi muscles, respectively, which were shown to be significant or extremely significant ($P < 0.05$). At 6 months of age, *YAP1* was highly and significantly ($P < 0.05$) expressed in the gastrocnemius muscle and extensor digitorum longus; lower expression was observed in the longissimus dorsi muscle (Figure 2). Expression increased gradually with age; however, in the soleus muscle and gastrocnemius of rams, *YAP1* exhibited a nonsignificant ($P > 0.05$) downward trend from 2 to 6 months of age (Figure 3). At 2 months of age, *YAP1* in ewes had a higher expression levels than those in rams, especially in the gastrocnemius and extensor digitorum longus ($P < 0.01$). At 6 months of age, sheep *YAP1* expression levels were significantly higher in rams when compared to those in ewes ($P < 0.05$) in the extensor digitorum longus muscle (Figure 4).

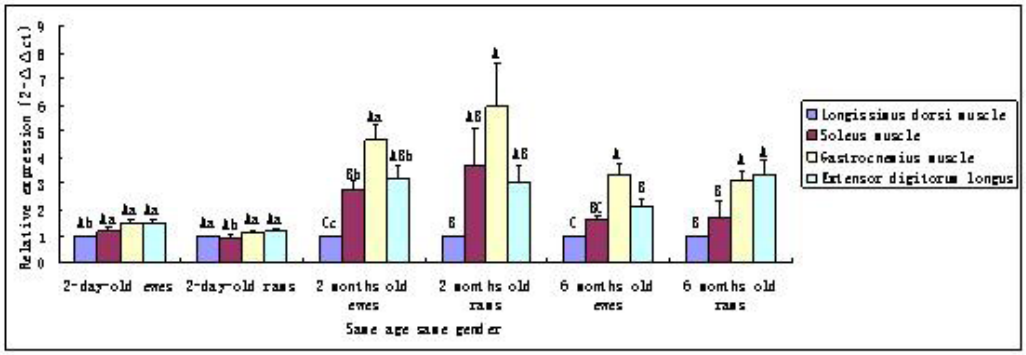

Figure 2. Expression of sheep *YAP1* in the different muscles. A, B, C, a, b, and c show the results of multiple comparisons of similar genders and stages of growth but different muscles. The values with the same letters are not significantly different ($P > 0.05$), and those with different letters are significantly different ($P < 0.05$); different capital letters indicate extremely significantly different ($P < 0.01$) results.

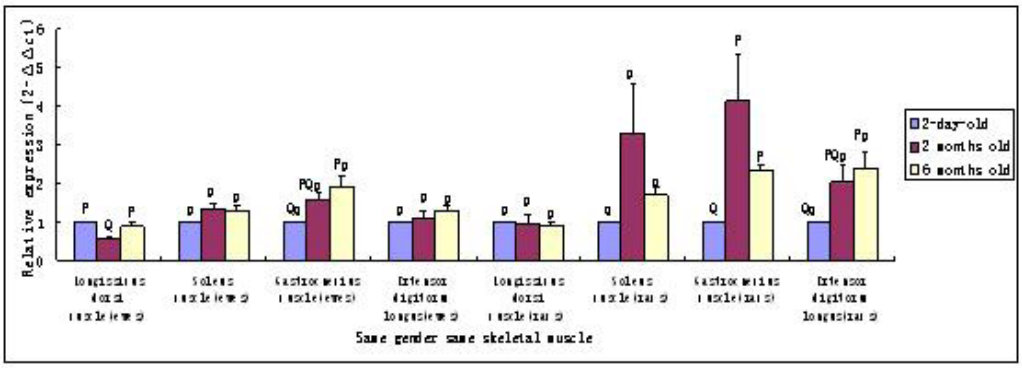

Figure 3. Expression of sheep *YAP1* in different growth stages. P, Q, R, p, q, and r show the results of multiple comparisons of similar genders and muscles but different stages of growth. The values with the same letters are not significantly different ($P > 0.05$), and those with different letters are significantly different ($P < 0.05$); different capital letters indicate extremely significantly different ($P < 0.01$) results.

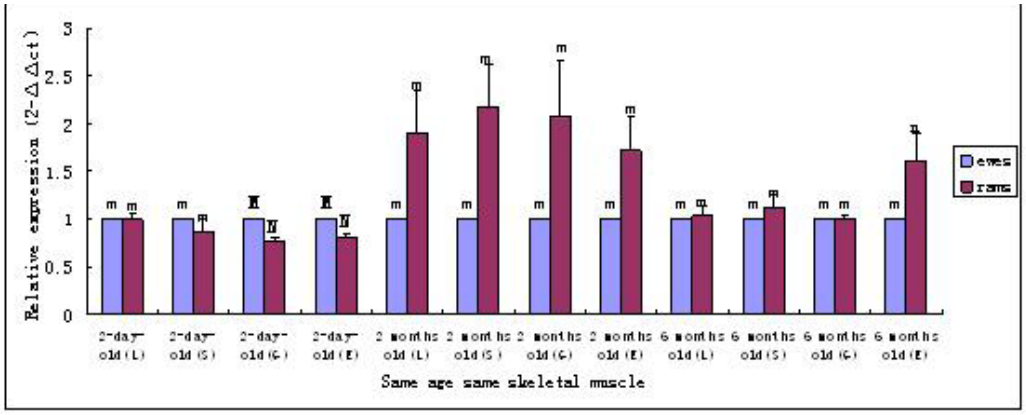

Figure 4. Gender effect on the expression of sheep *YAP1*. M, N, m, and n show the results of multiple comparisons of similar stages of growth and muscles but different genders. The values with the same letters are not significantly different ($P > 0.05$), and those with different letters are significantly different ($P < 0.05$); different capital letters indicate extremely significantly different ($P < 0.01$) results. L, longissimus dorsi muscle; S, soleus muscle; G, gastrocnemius muscle; E, extensor digitorum longus.

MSTN and *MyoG* spatial and temporal expression levels in the sheep muscle

In Figure 5, for all growth stages, *MSTN* had the lowest expression levels in the soleus muscle; at 2-days-old stage, *MSTN* was highly expressed in the longissimus dorsi muscle; at 2 months old, *MSTN* was highly expressed in the gastrocnemius muscle and minimally expressed in the soleus, and the difference between the gastrocnemius muscle and other skeletal muscles was significant ($P < 0.05$). At 6 months old, *MSTN* expression was lowest in the soleus muscle and did not exhibit significant differences in the longissimus dorsi muscle, gastrocnemius muscle, or extensor digitorum longus. Only *MSTN* expression in ram gastrocnemius and longissimus dorsi muscles were highly and significantly different ($P < 0.01$). Across all growth stages, *MyoG* had higher expression levels in the soleus and gastrocnemius muscles, and lower expression levels in the longissimus dorsi muscle and extensor digitorum longus; there were significant differences among the different skeletal muscles ($P < 0.05$). In Figure 6, in the longissimus dorsi muscle and soleus muscle (except in ewes), there were no significant differences between the different growth stages ($P < 0.05$). In the extensor digitorum longus, *MSTN* expression increased with increasing age and reached a highly significant level ($P < 0.01$). In the gastrocnemius muscle, *MSTN* expression increased from 2 days to 2 months of age ($P < 0.01$) and then significantly decreased (gastrocnemius muscle in ewes) or did not significantly change (gastrocnemius muscle in rams) in the different skeletal muscles of ewes. Overall, *MyoG* expression decreased with increasing age in the different skeletal muscles of ewes; moreover, *MyoG* expression initially increased and then decreased with increasing age, except in the extensor digitorum longus (i.e., gradual increase with increasing age). In Figure 7, *MSTN* expression did not exhibit significant differences between the different genders ($P > 0.05$); *MyoG* expression was highly and significantly different between the different genders in the 2-day-old ($P < 0.01$) age group; in the other age groups, there were no significant differences ($P > 0.05$).

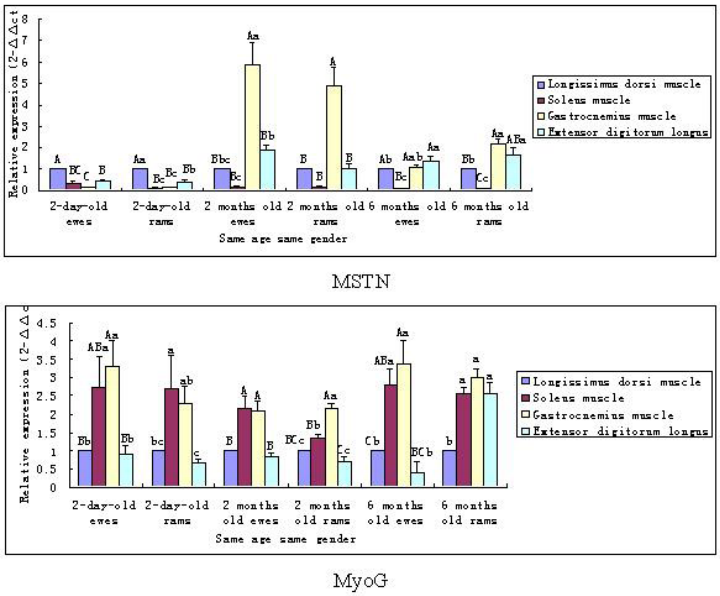

Figure 5. Expression of sheep *MSTN* and *MyoG* in the different muscles. A, B, C, a, b, and c show the results of multiple comparisons of similar genders and stages of growth but different muscles. The values with the same letters are not significantly different ($P > 0.05$), and those with different letters are significantly different ($P < 0.05$); different capital letters indicate extremely significantly different ($P < 0.01$) results.

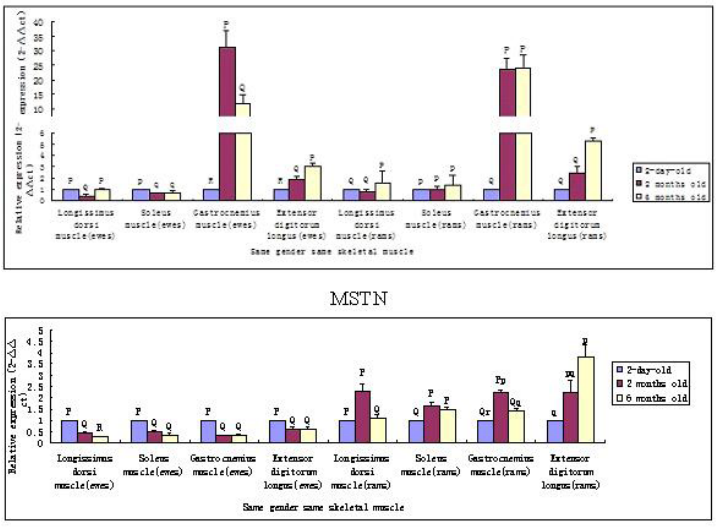

Figure 6. Expression of sheep *MSTN* and *MyoG* in different growth stages. P, Q, R, p, q, and r show the results of multiple comparisons of similar genders and muscles but different stages of growth. The values with the same letters are not significantly different ($P > 0.05$), and those with different letters are significantly different ($P < 0.05$); different capital letters indicate extremely significantly different ($P < 0.01$) results.

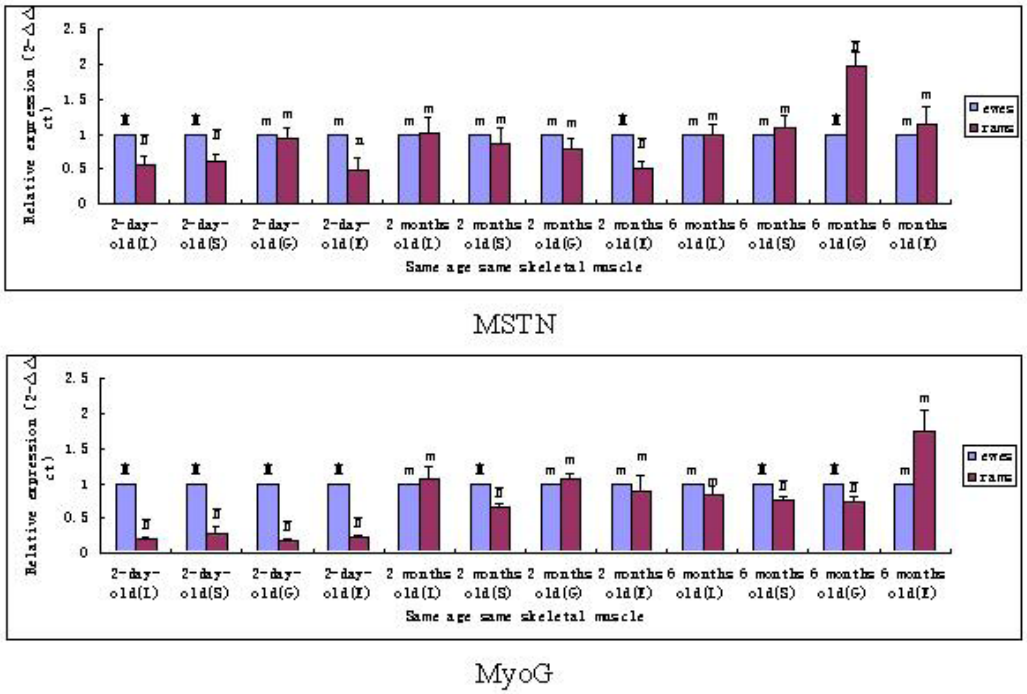

Figure 7. Gender effect on the expression of the sheep *MSTN* and *MyoG* genes. M, N, m, and n show the results of multiple comparisons of similar stages of growth and muscles but different genders. The values with the same letters are not significantly different ($P > 0.05$), and those with different letters are significantly different ($P < 0.05$); different capital letters indicate extremely significantly different ($P < 0.01$) results. L, longissimus dorsi muscle; S, soleus muscle; G, gastrocnemius muscle; E, extensor digitorum longus.

Association of the *YAP1*, *MSTN*, and *MyoG* genes with growth and muscles

At 2 days old, *YAP1* expression was significantly and positively correlated with *MSTN* and *MyoG* ($P < 0.05$; Table 2A). At 2 months old, *YAP1* expression was positively correlated (highly significant at $P < 0.01$) with *MSTN* (Table 2B). At 6 months old, *YAP1* expression was positively correlated (highly significant at $P < 0.01$) with *MSTN* and *MyoG* (Table 2C). For the different growth stages of the longissimus dorsi muscle, *YAP1* expression was positively correlated (highly significant $P < 0.01$) with *MSTN* and *MyoG* (Table 3A). In the soleus muscle, *YAP1* expression was significantly and positively correlated with *MSTN* and *MyoG* ($P < 0.05$; Table 3B). In the gastrocnemius muscle, *YAP1* expression positively correlated (highly significant $P < 0.01$) with *MSTN* (Table 3C). In the extensor digitorum longus, *YAP1* expression was significantly and positively correlated with *MSTN* and *MyoG* ($P < 0.05$; Table 3D). *YAP1* expression for the 3 age groups and 4 skeletal muscles showed that *YAP1* expression was significantly and positively correlated with *MSTN* and *MyoG* ($P < 0.05$; Table 3E).

Table 2. Association of *YAP1*, *MSTN* and *MyoG* gene expression with muscles.

A			
Index	YAP1	MSTN	MyoG
YAP1	1	0.259*	0.441**
MSTN	0.259*	1	0.163
MyoG	0.441**	0.163	1
B			
Index	YAP1	MSTN	MyoG
YAP1	1	0.362**	0.035
MSTN	0.362**	1	0.531**
MyoG	0.035	0.531**	1
C			
Index	YAP1	MSTN	MyoG
YAP1	1	0.894**	0.425**
MSTN	0.894**	1	0.417**
MyoG	0.425**	0.417**	1

A. Two-day-old; **B.** 2-month-old; and **C.** 6-month-old animals.

Table 3. Association of *YAP1*, *MSTN* and *MyoG* gene expression with growth.

A			
Index	YAP1	MSTN	MyoG
YAP1	1	0.506**	0.323*
MSTN	0.506**	1	0.246
MyoG	0.323*	0.246	1
B			
Index	YAP1	MSTN	MyoG
YAP1	1	0.402**	0.322*
MSTN	0.402**	1	0.622**
MyoG	0.322*	0.622**	1
C			
Index	YAP1	MSTN	MyoG
YAP1	1	0.652**	-0.050
MSTN	0.652**	1	0.094
MyoG	-0.050	-0.221	1
D			
Index	YAP1	MSTN	MyoG
YAP1	1	0.535**	0.312*
MSTN	0.535**	1	0.298*
MyoG	0.312*	0.298*	1
E			
Index	YAP1	MSTN	MyoG
YAP1	1	0.370**	0.650**
MSTN	0.370**	1	0.770**
MyoG	0.650**	0.770**	1

A. longissimus dorsi muscle; **B.** soleus muscle; **C.** gastrocnemius muscle; **D.** extensor digitorum longus; and **E.** muscles.

DISCUSSION

Spatial and temporal expression pattern of *YAP1*, *MSTN*, and *MyoG*

Hippo-*YAP1* is a novel pathway that was first discovered in *Drosophila*; it acts as a switch that controls cell division and death and may regulate organ size by inducing apoptosis and inhibiting cell proliferation. The hippo-*YAP1* pathway has similar functions in the developmental processes of mammals, and its signaling pathway is closely related to human tumors (Zhao et al., 2008; Liu et al., 2010; Li et al., 2011; Zhang and Zhu, 2011; Zhao and Wang, 2011; Liu et al., 2011). Downregulation of *YAP1* promotes the vascular smooth muscle cell (VSMC) contractile phenotype by upregulating myocardin expression and promoting association of the serum response factor-myocardin complex with VSMC contractile gene promoters, suggesting that the *YAP1* signaling pathway is a central regulator of the phenotypic switch of VSMCs (Xie et al., 2013). In the present study, *YAP1* expression was significantly different in the 4 skeletal muscles of the 2- and 6-month-old age groups; it was highly expressed in the gastrocnemius muscle and minimally expressed in the longissimus dorsi muscle. *YAP1* expression increased gradually with age in the different skeletal muscles of sheep. Sun et al. (2011) indicated that the muscle fiber diameter of rams was thicker than that of ewes, while the muscle fiber tenderness of rams was poorer than that of ewes. Thus, *YAP1* expression might be associated with the enlargement of muscle fiber diameter. However, there was no significant difference between ram and ewe *YAP1* expression levels.

MSTN is a negative regulator of skeletal muscle development and a member of the TGF-β super family. Currently, there are no reports on *MSTN* expression among the different skeletal muscles. In the present study, the expression of *MSTN* was significantly different in the different skeletal muscles and minimally expressed in the soleus muscle (the tenderness of the soleus was greater than that of the other 3 skeletal muscles), which indicates that *MSTN* may be related to the enlargement of muscle fiber diameter. In Erhualian boar, *MSTN* expression was highest at 45 days of age and then decreased as age increased (Yang et al., 2006). In our previous study, we used semi-RT-PCR to detect *MSTN* gene expression in the longissimus dorsi muscle and found that *MSTN* gene expression in Hu sheep initially increased with increasing age up to 60 days of age; however, after 60 days of age, expression decreased with increasing age (Sun et al., 2010). In the present study, we used FQ-PCR to detect *MSTN* gene expression in the longissimus dorsi muscle and found that, with the exception of an individual muscle at 2 months of age, *MSTN* expression decreased. Overall, *MSTN* expression increased as age increased. In our previous study, we showed that the high level of *MSTN* expression might play a role in regulating the metabolic and contractile maturation of myofibers during early postnatal growth; however, it did not appear to increase or decrease with increasing age. The results of the present study are similar to our previous study. *MSTN* expression was significantly different ($P < 0.05$) between boars and sows (Yang et al., 2006). In our previous study, we found that, with the exception of the 2-day-old age group, there was no significant difference between rams and ewes. Overall, with regard to the other age groups, there were significant differences between the two genders, and the expression in rams was higher than that in ewes. Thus, during the initial period after birth, there was a significant difference between genders for *MSTN* expression (Sun et al., 2010). In this study, with regard to the different age groups, there were significant differences between genders; thus, the results of the present study are in accordance with those of previous studies.

MyoG is a member of the *MyoD* family and an important factor in regulating skeletal muscle development. *MyoG* is a unique gene that can be expressed in all skeletal cell lines in *MyoD*. With the exception of regulating its own expression, *MyoG* could also interact with other members of the *MyoD* family to regulate the expression of each *MyoD* family member. For instance, *MyoG* can regulate MRF4 expression; thus, *MyoG* genetic variants may be associated with myogenesis, eventually leading to variation in meat quality. In the present study, *MyoG* expression was significantly different in the different skeletal muscles. Its expression was lower in the longissimus dorsi muscle and extensor digitorum longus, and much higher in the gastrocnemius and soleus muscle. Therefore, *MyoG* may inhibit the enlargement of muscle fiber diameter after birth. *MyoG* was expressed at low levels at 3 days of age in Erhualian and Large White boars; it peaked at 20 days of age and then decreased with increasing age (Yang et al., 2006). On the other hand, *MyoG* expression significantly increased from 20 to 45 days of age in Erhualian boars ($P < 0.05$) and then decreased, while *MyoG* expression decreased in 90-day-old boars and was significantly lower than that of the other age groups ($P < 0.05$). *MyoG* gene expression in the muscle of rams was lowest at 2 days of age and appeared to increase with the increasing age up to 30 days of age; after 30 days of age, expression initially decreased prior to 120 days of age and then increased. In ewes, the level of gene expression appeared to increase with increasing age up to 30 days of age; after 30 days of age, the expression level initially decreased prior to 90 days of age and then increased with the increasing age (Sun et al., 2010). Therefore, high levels of *MyoG* expression might play a role in regulating the metabolic and contractile maturation of myofibers during early postnatal growth; however, it did not appear to increase or decrease with increasing age. In the present study, *MyoG* expression in ewes decreased with the increasing age; however, in rams, it initially increased and then decreased. These data indicate that *MyoG* expression is associated with gender when its expression is analyzed in the different skeletal muscles.

Correlation analysis between *YAP1* and *MSTN* and *MyoG*

There is an association between *MSTN* and *MyoD*; Smad3 induced phosphorylation after *MSTN* bound with its receptor and enhanced the interactions between Smad3 and *MyoD*. *MSTN* can inhibit the activation and expression of *MyoD* factors by Smad3, and the resulting myoblasts do not differentiate into myotubes (Rebbapragada et al., 2003). In addition, Smad3 may be regulated by transcription factors and receptors to bind with *MSTN* on specific sites upstream of the promoter, thus contributing to the expression of *MSTN* and inhibiting myoblast differentiation (Salehian et al., 2006). In the present study, *YAP1*, *MSTN*, and *MyoG* expression were significantly and positively correlated ($P < 0.01$). The results indicate that *YAP1* may exert an antagonistic effect on *MSTN* before birth and inhibit *MSTN* expression; after birth, *YAP1* promotes *MSTN* expression. Watt et al. (2010) showed that a *YAP1* mutant expression vector could inhibit *MyoG* expression, while Sun et al. (2010) reported that *MSTN* and *MyoG* synergistically promoted the development of muscle in the early developmental stages after birth. The results in the present study showed that *YAP1* expression had an extremely significant positive correlation with *MSTN* and *MyoG* during the early developmental stages after birth, and it revealed that *YAP1* might play an important role in promoting the enlargement of muscle fibers after birth.

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