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Molecular Cloning, Structural Analysis and Tissue-Specific Expression of *Akirin1* Gene in Tianfu Goat

[1]JisiMa, [1]LuWan, [1]Gangyi Xu, [2]Daihua Wang and [1]Nianlu Wang
[1]College of Animal Science and Technology, Institution of Animal Genetics and Breeding, Sichuan Agricultural University, 46# Xinkang Road, Sichuan, Yucity District, 625014 Yaan, China
[2]Agriculture Bureau, Fucheng District, Mianyang, Sichuan, China

Abstract: *Akirin1* gene is a newly discovered nuclear factor which plays an important role in myogenesis and closely contact with meat production. For better understanding of the structure and functions of the *Akirin1* gene and to study its effect in *Capra hircus*, the cDNA of Tianfu goat *Akirin1* gene was cloned and sequenced. The structure of Akirin1 was analyzed using bioinformatics tools. The results showed that the Tianfu goat Akirin1 cDNA full Coding Sequence (CDS) contained 426 bp nucleotides and encoded 141 amino acids protein. Phylogenic tree analysis revealed the goat Akirin1 closely related with cattle Akirin1. Quantitative real-time PCR (qPCR) analyses showed that Akirin1 was expressed in the myocardium, liver, spleen, lung, kidney, leg muscle, abdominal muscle and longissimus dorsi muscle. Especially, high expression levels of Akirin1 were detected in liver, spleen, lung and kidney and low expression levels were seen in myocardium, leg muscle, abdominal muscle and longissimus dorsi muscle. Western blotting results showed Akirin1 protein expression only detected in lung and three skeletal muscle tissues.

Key words: *Akirin1* gene, cDNA clone, complete CDS, sequence analysis, expression analysis

INTRODUCTION

Meat production is one of the most important economic traits in domestic animals industry. Carcass weight and meat percentage both are very important indexes of production performance. Skeletal muscle is an important part of the carcass and closely associated with meat percentage. Skeletal myogenesis is a complicated process and regulated by the muscle-specific transcription factors main including MyoD, myogenin, Myogenic factor 5 (Myf5) and negative regulate factor Myostatin (Weintraub, 1993; Thomas *et al*., 2000; Buckingham, 2006; Le Grand and Rudnicki, 2007). Studying the mechanism of skeletal myogenesis will help to better establish breeding strategies for improving the goat meat production.

Akirin gene is a newly discovered nuclear factor acting on drosophila innate immune responses (Goto *et al*., 2008). The *Akirin* gene is conserved in vertebrates and has two homologues, Akirin1 and Akirin2. In addition, only one copy was present in insects and in birds, eight homologues in Atlantic salmon (Macqueen and Johnston, 2009) and none was found in plants, yeast or bacteria (Goto *et al*., 2008). Up to now, the *Akirin1* gene was cloned from many species such as drosophila, mice, cattle and pig (Goto *et al*., 2008; Marshall *et al*., 2008; Salerno *et al*., 2009; Zhang *et al*., 2009), its expression pattern has been examined in different species (Goto *et al*., 2008; Marshall *et al*., 2008; Macqueen and Johnston, 2009; Macqueen *et al*., 2010b). Except its role in innate immune responses, *Akirin1* gene has recently been shown to play an important role in skeletal myogenesis (Davies, 2006; Kelly, 2006; Marshall *et al*., 2008; Salerno *et al*., 2009; Macqueen *et al*., 2010a, b). In mice, Akirin1 is also named mighty and shown to regulate skeletal myogenesis (Marshall *et al*., 2008). Skeletal myogenesis negative regulation by myostatin, it is through Akirin1 regulates the key steps of muscle regeneration including chemotaxis of inflammatory cells, proliferation and differentiation of myoblasts and Satellite Cell (SC) activation and migration (Thomas *et al*., 2000; Langley *et al*., 2002; Salerno *et al*., 2009). Akirin1 is a downstream target gene of myostatin and negatively regulated by it, Akirin1 expression precedes MyoD in myogenic program (Marshall *et al*., 2008). MyoD is one of the regulators of SC activation and myoblast proliferation, it is possible that Akirin1 expression during SC activation

Corresponding Author: Gangyi Xu, College of Animal Science and Technology, Institution of Animal Genetics and Breeding, Sichuan Agricultural University, 46# Xinkang Road, Sichuan, Yucity District, 625014 Yaan, China

triggers MyoD activity via Insulin-like Growth Factor II (IGF-II) starting further downstream myogenesis (Salerno *et al*., 2009). In the single fiber cultures, Akirin1 protein was only detectable in activated SC and activated SC Akirin1 mRNA expression levels higher than quiescent one, suggesting that Akirin1 may be associated with SC activation (Salerno *et al*., 2009). Furthermore, Akirin1 over-expression in C2C12 lead to myoblasts chemotactic ability and myogenic differentiation were reinforced and *in vivo* increases muscle fiber diameter (Marshall *et al*., 2008; Salerno *et al*., 2009). As researchers all know postnatal animal skeletal myogenesis mainly depend on muscle fiber diameter increased (Gros *et al*., 2005) these indicated that *Akirin1* gene is a novel factor to regulate skeletal myogenesis and a candidate gene of the goat meat production.

In this report, researchers isolated the full coding sequence of the Tianfu goat *Akirin1* gene for the first time analyzed its nucleotide and protein primary structure, detect its expression levels in different tissues on different growth stages. The results of the study will lay the foundations for better understanding the function of *Akirin1* gene.

MATERIALS AND METHODS

Animals and sample collection: All of the Tianfu goats were bred under standard conditions in Goat Breeding Center of Sichuan Agricultural University and slaughtered on day 1, months 2.5, 5, 7.5 and 12 of life (n = 6). The tissue samples including myocardium, liver, spleen, lung, kidney, leg muscle, abdominal muscle and longissimus dorsi muscle were immediately harvested from each goat after slaughtering and frozen in liquid nitrogen jars then store at -80°C refrigerator for total RNA and total protein extraction.

RNA isolation and synthesis of cDNA: About 150 mg of the tissue sample was taken out randomly from the -80°C refrigerator then triturated in liquid nitrogen. According instructions, total RNA was extracted from the triturated sample using Trizol total RNA extraction kit (TaKaRa, Dalian, China). The total RNA was detected by 1% agarose gel electrophoresis. First strand cDNA was synthesized using 2 μg of purified total RNA in a real-time Reverse Transcription (RT-PCR) System (TaKaRa) according to the PrimeScript™ RT reagent kit (TaKaRa) instructions. The first strand cDNA was obtained and preserved at -20°C.

Cloning of the *Akirin1* gene: A pair of primers Table 1 was designed by Primer Premier 5.0 Software in the conserved region of the *Bos taurus* (GenBank: BC151342.1) and human (GenBank: BC119745.1) *Akirin1* gene sequences. The PCR started operation as following conditions pre-denaturation at 95°C for 5 min followed by 32 cycles (95°C for 30 sec; 57.6°C for 30 sec; 72°C for 40 sec) and ended with a final extension at 72°C for 10 min. The PCR products were detected by 1.5% agarose gel electrophoresis and recovered using an E.Z.N.A Gel Extraction kit (Omega, America). The recovered products were cloned by pMD19-T vector (TaKaRa) and sequenced by LiuHe HuaDa Biotechnology (Beijing) Co., Ltd.

Table 1: Primer pairs used to amplify the *Akirin1* gene

Primer	Sequence (5'-3')	Fragment length	Application
A1	F: CCTGGTCTTTCAGCGGCAT R: CTTGAGGTCAAACCTGGTA	482 bp	cDNA clone
A2	F: ATAGTCGTTATCAGAGGTGGAG R: TGTCGGAGGGTAAAGGTG	157 bp	Expression
G	F: GCAAGTTCCACGGCACAG R: TCAGCACCAGCATCACCC	118 bp	qPCR

Bioinformatics sequence analysis: The *Akirin1* gene translation and sequence alignment using National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) and ExPASy (http://www.ExPASY.org) Software. The goat *Akirin1* gene Open Reading Frame (ORF) finding using NCBI ORF Finder tool (http://www.ncbi.nlm.nih.gov/projects/gorf/) and the Akirin1 protein sequences from related species were aligned with the goat sequence using the DANMAN6.0 Software. The phylogenetic tree was constructed by MEGA 4.0 Software (Tamura *et al*., 2007). The secondary structures of the deduced amino acid sequences were predicted by SOPMA (http://npsa-pbil.ibcp.fr/). The transmembrane domain of the deduced amino acid sequences were predicted by TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0/). Signal peptides were predicted using the signal P 3.0 server (http://www.cbs.dtu.dk/services/SignalP/).

Analysis of the tissue-specific expression of the *Akirin1* gene: Total RNA of eight tissues from Tianfu goat were extracted as described earlier. Analysis of tissue-specific expression of *Akirin1* was performed by qPCR and employing the Glyceraldeyhyde-3-Phosphate Dehydrogenase (*GAPDH*) gene as an internal control. Each qPCR reaction (total volume 25 μL) contained 12.5 μL SYBR Premix ExTaq (TaKaRa), 2 μL normalized template cDNA and 10 pm primer pairs. The primers are shown in Table 1 (A2 and G). The qPCR amplification program as follows: 95°C for 10 sec then 40 cycles of 95°C for 5 sec and 60°C for 30 sec, final a temperature increment of 0.5°C/sec from 65-95°C. The $2^{-\Delta\Delta C_T}$ Method was used to analyze the relative expression levels of Akirin1 in each of the tissues (Livak and Schmittgen, 2001).

Western blotting analysis of Akirin1 protein expression was performed as follow: various tissues were lysed in Lysis buffer containing protease inhibitors, phosphatase inhibitors and Phenylmethanesulfonyl Fluoride (PMSF). According to whole protein extraction kit (Sangon Biotech Co., Ltd. Shanghai) instruction complete protein extraction. Protein concentration measure using the BCA Protein Assay kit (BiYoTime, IL, Suzhou, China) with BSA as a standard. The GAPDH protein was selected as internal control. Equal amounts of protein (10 μg/lane) were separated on SDS-polyacrylamide gels and transferred onto PVDF membranes (BIO-RAD Co., USA). After blocking with Tris-Buffered Saline Tween-20 (TBST: 0.14 mol L^{-1} NaCl 0.02 mol L^{-1} Tris-base (pH 7.6) and 0.05% Tween) containing 5% nonfat dried milk for 2 h at 37°C, the membranes were hybridized with 1:1000 primary antibody (Akirin1 (source of rabbit) (Abcam, UK) and GAPDH (source of mouse) (BiYoTime, IL, Suzhou, China)), incubated overnight at 4°C and then washed 3 times with TBST for 15 min. After washing, membranes were incubated with horseradish peroxidase-conjugated secondary antibody for 2 h at 37°C, washed 4 times with TBST for 10 min and 2 times with TBS for 5 min, finally using an chemiluminescence system visualized (Amersham Biosciences, Inc. USA). The protein relative expression levels of Akirin1 protein were analyzed using the Grey value Method (Wang *et al*., 2012).

Statistical analysis: All data are expressed as the mean±SEM. Statistical analysis was performed using one-way ANOVA with the SPSS 13.0 Statistical Software Package (SPSS Inc., Chicago, IL, USA). The threshold of significance was defined as $p<0.05$.

RESULTS AND DISCUSSION

cDNA cloning and sequence analysis of the *Akirin1* gene: A 482 bp fragment of Akirin1 was obtained by homologous cloning using the cDNA from the lung of the Tianfu goat as the template (GenBank: KF515991). The BLAST result of NCBI's nucleotide sequence database revealed that the caprine Akirin1 fragment was significantly similar to the Akirin1 sequence from other mammals. The Tianfu goat Akirin1 nucleotide sequence and deduced amino acid sequence are shown in Fig. 1. An ORF extending from positions 18-443 in the nucleotide sequence encoded a 141 amino acids long protein.

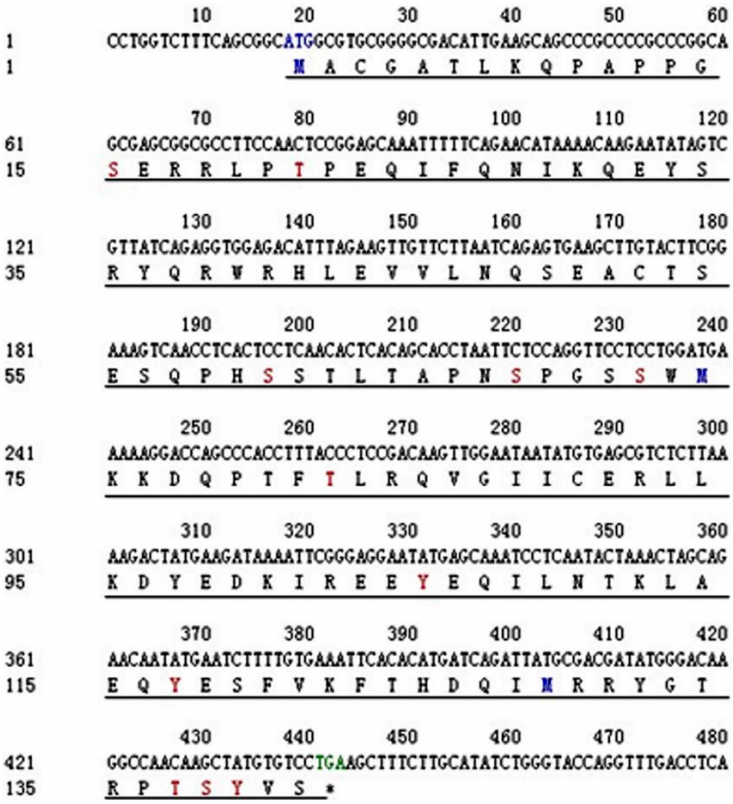

Fig. 1: Nucleotide and deduced amino acid sequences of caprine Akirin1 *indicates the stop codon, the blue letters indicate potential initiation codons, the green letter indicates the termination codon, the predicted serine, threonine and tyrosine phosphorylation sites are highlighted in red

Analysis of the translated amino acid sequence of Akirin1: The deduced amino acid sequence molecular weight is 16.8 kDa and isoelectric point is 8.44. Akirin1 amino acid sequence have 17 negatively charged residues (Asp+Glu) and 19 positively charged residues (Arg+Lys), indicating that the protein should have a positive charge overall. The instability index was computed by SWISS-MODEL (Guex and Peitsch, 1997) to be 74.98 which classified the protein as unstable. Aliphatic index was 61.56 and the grand average of hydropathicity was -0.901. The hydropathicity correlation analysis revealed that the protein was highly hydrophilia. Eleven phosphorylation sites were predicted by network. No signal peptide was identified by the SignalP 3.0 Software. The secondary structure of the protein was predicted to be mainly α-helix and random coil, connected by extended strand (-fold) (Fig. 2). The TMHMM result indicated that the protein had no obvious transmembrane domain, suggesting that Akirin1 is neither an epimembranous accepter nor can be located in the membrane.

Characteristics of the deduced protein and phylogenetic analysis of Akirin1: The deduced amino acid sequence of Tianfu goat Akirin1 was compared with nine Akirin1 sequences from other mammals using ClustalW (Thompson *et al*., 1994). The coding sequence of the goats *Akirin1* gene was 73% identical with *Bos taurus* Akirin1 and 73, 71, 71, 71, 70, 71, 72 and 65% identical with *Ovis aries*, *Sus scrofa*, *Pan troglodytes*, *Macaca mulatta*, *Ochotona princeps*, *Homo sapiens*, *Orcinus orca* and *Mus musculus*, respectively. The amino acid sequences of the Tianfu goat *Akirin1* gene were 73, 73, 72, 72, 72, 72, 60, 72 and 69% identical with *Bos taurus*, *Ovis aries*, *Sus scrofa*, *Pan troglodytes*, *Macaca mulatta*, *Ochotona princeps*, *Homo sapiens*, *Orcinus orca* and *Mus musculus*, respectively. All species GeneBank ID of Akirin1 sequences showed in Table 2. Except for 51 Amino Acids (AA) deletion at the N-terminal end of the sequences, the only difference between the goats and cattle amino acid sequences was at position 66 (Glu in goats and Asp in cattle) and position 117 (Asn in goats and Ser in cattle) (Fig. 3a).

The phylogenetic tree was constructed using the deduced caprine Akirin1 and other species' Akirin1 amino acid sequences (Fig. 3b). The results showed that caprine Akirin1 amino acid sequence compare with other species, the highest homology was with *Bos taurus* and *Ovis aries* and the lowest homology was with danio rerio.

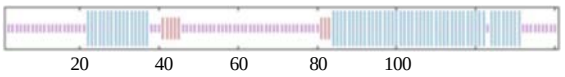

Fig. 2: The predicted secondary structure of the Akirin1 amino acid sequence the HNN secondary structure prediction method was used. The blue line represents α-helix, the red line represents extended strand and the purple line represents random coil

Expression pattern analysis of Akirin1: qPCR was used to analyze the Akirin1 mRNA transcription levels in different tissues on month 12 Tianfu goat. The *Akirin1* gene expression was detected in all the tissues tested. Particularly high expression levels of Akirin1 were found in spleen, lung and kidney ($p<0.01$) while lower levels were seen in liver ($p<0.01$) very little expression was detected in myocardium, leg muscle, abdominal muscle and longissimus dorsi muscle ($p<0.01$) (Fig. 4a). Furthermore, temporal mRNA expression of *Akirin1* gene was found to follow a trend according to the age of Tianfu goat. During longissimus dorsi muscle development, the expression of Akirin1 was first increased from day 1 to months 5 then gradually decreased to month 12, the highest expression was seen on month 5 ($p<0.01$) and the lowest expression on month 12 (Fig. 4b). In leg muscle, the expression of Akirin1 was gradually reduced from day 1 to month 12, the highest expression was detected on day 1 and the lowest on month 12 (Fig. 4c).

The Western blotting results showed different levels of the Akirin1 protein in myocardium, liver, spleen, lung, kidney, leg muscles, abdominal muscle and longissimus dorsi muscle on month 12. No Akirin1 protein was found in the myocardium, liver, spleen and kidney (Fig. 5a). Particularly high expression levels of Akirin1 protein was detected in longissimus dorsi muscle ($p<0.01$) while lower levels were found in leg muscle and abdominal muscle ($p<0.01$) very little expression was detected in lung ($p>0.05$) (Fig. 5b). In addition, the temporal protein expression results showed different levels of Akirin1 protein in longissimus dorsi muscle from day 1 to month 12 (Fig. 6). The protein expression of Akirin1 was first

Table 2: List of the Akirin1 sequences used in the analyses

Organisms	GeneBank ID	Nucleotide identity (%)	Amino acid identity (%)
Capra hircus	KF515991	-	-
Bos taurus	BC151342.1	73	73
Ovis aries	NP_001121144.1	73	73
Sus scrofa	NP_001121927.1	71	72
Pan troglodytes	JAA33906.1	71	72
Ochotona princeps	XP_004591613.1	70	72
Homo sapiens	XP_005271255.1	71	60
Orcinus orca	XP_004266467.1	72	72
Mus musculus	NP_075912.2	65	69
Gorilla	XP_004025555.1	71	72
Condylura cristata	XP_004678882.1	70	71
Ceratotherium	XP_004426049.1	71	71
Tursiops truncatus	XP_004322506.1	72	72
Papio anubis	XP_003919146.1	70	60
Danio rerio	NP_001007187.1	50	55
Macaca mulatta	AFH34017.1	71	72

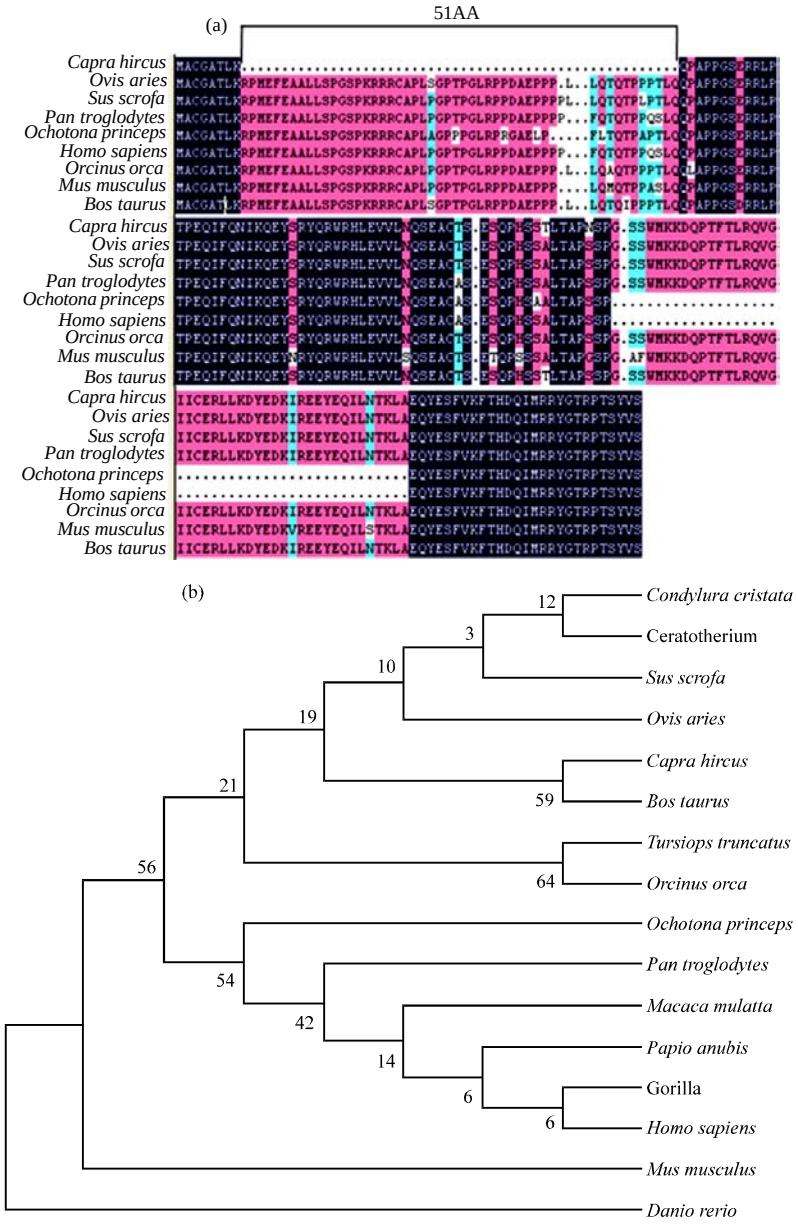

Fig. 3: Phylogenetic tree and alignment of the Akirin1 amino acid sequences from caprine and other species the GenBank accession numbers of the Akirin1 sequences are listed in Table 2; a) Amino acid sequence alignment of caprine Akirin1 with the predicted Akirin1 sequences from eight other mammals; b) Phylogenetic tree of the amino acid sequences of Akirin1 from goat and other mammals

increased then decreased in longissimus dorsi muscle with increasing age of the Tianfu goats: high expression levels were seen on month 5 ($p<0.01$) while lower levels were found on months 2.5 and 7.5 ($p<0.01$) then little expression was detected on day 1 and month 12 (Fig. 6).

Meat production is one of the important economic traits in domestic animals. Skeletal myogenesis is one of the most important elements affecting meat production and is controlled by multiple genes (Weintraub, 1993; Thomas *et al*., 2000; Langley *et al*., 2002; Buckingham, 2006; Le Grand and Rudnicki, 2007). The *Akirin1* gene showed a significant influence on myoblast proliferation and differentiation and increases muscle fiber diameter (Marshall *et al*., 2008; Salerno *et al*., 2009). This implied that the *Akirin1* gene may be is a new factor to regulate skeletal myogenesis and then affect meat production.

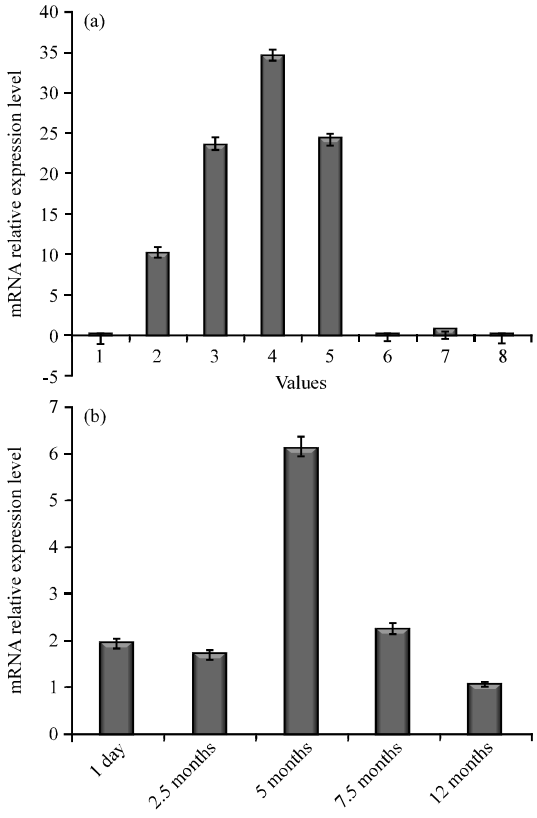

Fig. 4: Relative expression levels of caprine Akirin1 mRNA in different tissues and at different ages. The mRNA was detected by quantitative real-time PCR; a) expression levels of caprine Akirin1 in different tissues. The horizontal axis indicates the various tissues from 12 months old goats. Numbers 1-8 represents myocardium, liver, spleen, lung, kidney, leg muscle, abdominal muscle, longissimus dorsi muscle, respectively; b) expression levels of caprine Akirin1 in five growth stages in longissimus dorsi muscle; c) expression levels of caprine Akirin1 in five growth stages in leg muscle. Mon indicates month. The bars represent the mean±SEM (n = 6)

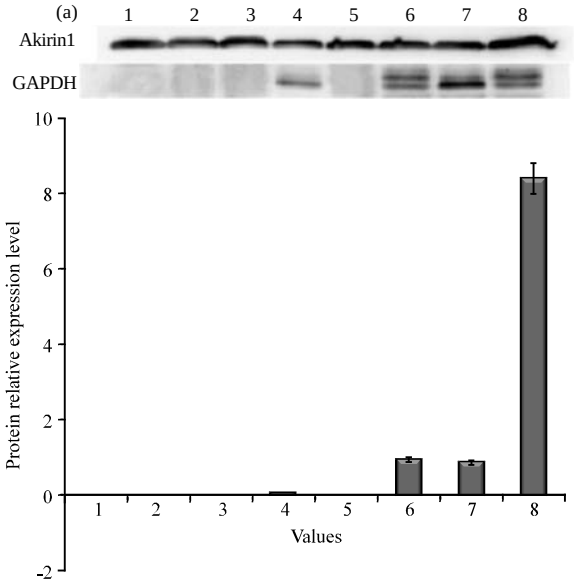

Fig. 5: Western blotting of caprine Akirin1 protein levels in eight tissues Numbers 1-8 represent myocardium, liver, spleen, lungs, kidney, leg muscle, abdominal muscle and longissimus dorsi muscle, respectively; a) Western blotting results for caprine Akirin1; b) relative Akirin1 protein levels in 12 months old goats. Bars represent the mean±SEM (n = 6)

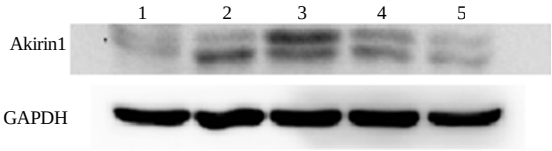

Fig. 6: Western blotting results of caprine longissimus dorsi muscle Akirin1 protein levels in five ages Numbers 1-5 represent day 1, months 2.5, 5, 7.5 and 12, respectively

Recent studies have shown several lines of evidence supporting Akirin1 plays an important role in skeletal myogenesis. First, Akirin1 has been reported to promote skeletal muscle growth and differentiation (Kelly, 2006; Marshall *et al*., 2008). During myoblast growth and differentiation Akirin1 expression peak was observed considerably earlier than MyoD (Marshall *et al*., 2008).

Moreover, over-expression Akirin1 in C2C12 cells make the skeletal muscle growth and differentiation marker genes include MyoD, myogenin and MHC expression levels increased (Davies, 2006; Marshall *et al*., 2008; Salerno *et al*., 2009). Second, the expression of Akirin1 is negatively regulated by Myostatin (Marshall *et al*., 2008; Salerno *et al*., 2009). Myostatin is widely expressed in skeletal muscle tissue and regulates key steps of muscle regeneration (Weintraub, 1993; McPherron *et al*., 1997; Salerno *et al*., 2009; Huang *et al*., 2011). Furthermore, Akirin1 expression level was reportedly only increased in the skeletal muscle which from Myostatin-null mice (Salerno *et al*., 2009). Third, Akirin1 has been shown to induce the quiescent SC activation and migration (Davies, 2006; Marshall *et al*., 2008). Akirin1 over-expression in C2C12 cells significantly reduced the expression levels of quiescent SC marker gene CD34 and Sca-1 (Beauchamp *et al*., 2000; Kelly, 2006; Marshall *et al*., 2008). In addition, in the single fiber cultures, Akirin1 protein was only detectable in activated SC (Salerno *et al*., 2009). These results can further confirm

that Akirin1 may be a good candidate gene for meat production and this should be investigated further.

In the study, researchers obtained the cDNA sequence of Tianfu goat *Akirin1* gene and detected its expression levels on mRNA and protein. The *Akirin1* gene cDNA sequence contains a 426 bp long ORF with coding 141 amino acids and molecular mass of 16.8 kDa. *Akirin1* gene nucleotide sequences alignment result showed that except for 153 bp deletion at the N-terminal end of the sequences, Akirin1 sequence was significant similar with most of mammals. In addition, the phylogenetic tree analysis revealed that except for 51 amino acids deletion at the N-terminal end of the sequences (Fig. 3), the caprine Akirin1 protein sequence had a close genetic relationship with other mammals Akirin1 sequences. This deletion maybe a kind of alternative splicing (Sharp, 1994; Celotto and Graveley, 2001; Kelemen *et al*., 2013).

Next researchers use qPCR detected the mRNA expression of Tianfu goat *Akirin1* gene, the results showed that the high expression levels were detected in liver, spleen, lung and kidney, the comparatively low expression were found in three skeletal muscle tissues and myocardium. This is consistent with previous research, found that mouse Akirin1 was expressed in many tissues such as lung, kidney and liver (Marshall *et al*., 2008). They also found Akirin1 expression level was comparatively lower in skeletal muscle tissues (Marshall *et al*., 2008; Yan *et al*., 2013). In addition, temporal mRNA expression result showed that in longissimus dorsi muscle the expression of Akirin1 was first increased then decreased from day 1 to months 12, the highest expression was seen on month 5 in leg muscle, the expression of Akirin1 was gradually reduced from day 1 to months 12 with highest levels seen on day 1 and lowest on month 12, suggesting that Akirin1 may be a potential indicator to promote animal muscle growth.

Finally, researchers used Western blotting to detect Akirin1 protein expression levels in eight tissues of 12 months old Tianfu goat. Researchers found that Akirin1 protein was only detected in lung, leg muscle, abdominal muscle and longissimus dorsi muscle, no found in myocardium, liver, spleen and kidney. As researchers know, protein is an executor of gene function, the expression of protein is an important index to definition the concrete function of gene. Moreover, the Akirin1 protein major expression at three skeletal muscle tissues. This may imply that Akirin1 is a muscle-specific protein. In addition, researchers detected the Akirin1 protein expression levels of Tianfu goat longissimus dorsi muscle at five growth stages. The results shown that protein expression of Akirin1 was first increased from day 1 to months 5 then gradually decreased to month 12, the highest expression levels were seen on month 5. This is consistent with *Akirin1* gene temporal mRNA expression in longissimus dorsi muscle, further suggesting that *Akirin1* gene may play an important role in muscle development.

Curiously, researchers detected two parallel signals only in three skeletal muscle tissues. Moreover, the signals were similar in leg muscle and longissimus dorsi muscle but in abdominal muscle the above one more strong than another. The reason of this phenomenon perhaps is alternative splicing. Researchers already know that alternative splicing is common phenomenon at eukaryote genome (Sharp, 1994), it is one of the most important mechanisms to generate a large number of mRNA and protein isoforms (Celotto and Graveley, 2001; Kelemen *et al*., 2013). Further, alternative splicing changes the structure of transcripts and their encoded proteins, different tissues specificity expression through different splicing model to accomplish (Smith and Valcarcel, 2000; Johnson *et al*., 2003; Sharp, 2005). This may explain that two parallel signals were detected in three skeletal muscle tissues and Akirin1 protein expression pattern were same at leg muscle and longissimus dorsi muscle but different in abdominal muscle. Alternative splicing maybe also the cause of Tianfu goat Akirin1 amino acid sequence lost 51AA at the N-terminal end of the sequences compared with other mammals. Above results showed that Akirin1 protein in skeletal muscle tissues have relative high expression levels and existed different expression pattern at different skeletal muscle. These suggesting that Akirin1 may be a new factor to regulate skeletal myogenesis and should be further researched.

CONCLUSION

Researchers have isolated Tianfu goat *Akirin1* gene for the first time analyzed the sequence and performed temporal and spatial expression difference using qPCR and Western blotting in Tianfu goat. These results presented here lay the foundations for future research on the functions of *Akirin1* gene in skeletal myogenesis and also provides some theoretical basis for further research in molecular-assisted selection during animal breeding.

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