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Molecular cloning, sequence analysis and tissue-specific expression of *Akirin2* gene in Tianfu goat

Jisi Ma, Gangyi Xu *, Lu Wan, Nianlu Wang

Institution of Animal Genetics and Breeding, College of Animal Science and Technology, Sichuan Agricultural University, 46# Xinkang Road, Yucity District, Yaan 625014, Sichuan Province, China

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ABSTRACT

The *Akirin2* gene is a nuclear factor and is considered as a potential functional candidate gene for meat quality. To better understand the structures and functions of *Akirin2* gene, the cDNA of the Tianfu goat *Akirin2* gene was cloned. Sequence analysis showed that the Tianfu goat *Akirin2* cDNA full coding sequence (CDS) contains 579 bp nucleotides that encode 192 amino acids. A phylogenic tree of the Akirin2 protein sequence from the Tianfu goat and other species revealed that the Tianfu goat Akirin2 was closely related with cattle and sheep Akirin2. RT-qPCR analysis showed that *Akirin2* was expressed in the myocardium, liver, spleen, lung, kidney, leg muscle, abdominal muscle and the longissimus dorsi muscle. Especially, high expression levels of *Akirin2* were detected in the spleen, lung, and kidney whereas lower expression levels were seen in the liver, myocardium, leg muscle, abdominal muscle and longissimus dorsi muscle. Temporal mRNA expression showed that *Akirin2* expression levels in the longissimus dorsi muscle, first increased then decreased from day 1 to month 12. Western blotting results showed that the Akirin2 protein was only detected in the lung and three skeletal muscle tissues.

1. Introduction

Meat quality is one of the important economic traits in domestic animal production and has become an important research area in recent years. Muscle fiber types and marbling score (MS) are both important influential factors of meat quality (Johnson, 1987; Klont et al., 1998; Karlsson et al., 1999; Sukegawa et al., 2014). We already know that muscle fiber can be divided into four types: slow-oxidative (Type I), fast oxido-glycolytic (Type IIA), and fast glycolytic (Types IIX and IIB) (Brooke and Kaiser, 1970; Lind and K.D., 1991; Argüello et al., 2001). Marbling is defined by the amount and distribution of intramuscular fat in the longissimus dorsi muscle area. The higher MS means better taste, acceptability, palatability and tenderness of meat (Busboom et al., 1993; Sasaki et al., 2009; Kim et al., 2013; Sukegawa et al., 2014). The best quality meat has a higher MS and slow-twitch muscle fiber content (Ryu and Kim, 2005).

The *Akirin* gene is conserved in vertebrates and has two paralogs: *Akirin1* and *Akirin2*. However only one paralog is present in insects and birds in contrast to the eight paralogs that are found in Atlantic salmon (Macqueen and Johnston, 2009). Currently the *Akirin2* gene has been cloned and sequenced in fruitfly, mice, chicken, fish, cattle and pig (Goto et al., 2008; Sasaki et al., 2009; Chen et al., 2012; Kasthuri et al., 2013; Kim et al., 2013). Previous reports showed that the *Akirin2* gene was a nuclear factor involved in the *Drosophila* innate immune response, and that both Japanese black cattle and Korean native cattle contained an *Akirin2* gene single nucleotide polymorphism (SNP) which was significantly associated with MS (Goto et al., 2008; Sasaki et al., 2009; Kim et al., 2013; Sukegawa et al., 2014). Differential-display PCR (ddPCR) showed that the c17–25 expressed sequence tag (EST) exhibited higher expression levels in the high-marbled steer group, compared with low-marbled steer group (Sasaki et al., 2006), where the c17-25 EST corresponds to a segment of the Akirin2 protein. Furthermore, the c17–25 EST was found within in the genomic region of a quantitative trait locus (QTL) of marbling (Takasuga et al., 2007). Thus, the *Akirin2* gene was considered as a functional candidate gene for marbling (Sasaki et al., 2009; Watanabe et al., 2011; Kim et al., 2013; Sukegawa et al., 2014).

Abbreviations: CDS, Coding sequence; RT-qPCR, Reverse-transcriptase quantitative real-time polymerase chain reaction; MS, Marbling score; SNP, Single nucleotide polymorphism; ddPCR, Differential-display polymerase chain reaction; EST, Expressed sequence tag; QTL, Quantitative trait locus; RT-PCR, Real-time reverse transcription polymerase chain reaction; ORF, Open reading frame; AA, Amino acid; PMSF, Phenylmethanesulfonyl fluoride; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; SDS, Sodium dodecyl sulfate, sodium salt; PVDF, Poly(vinylidene fluoride).

* Corresponding author.
E-mail address: goatsxgy@263.net (G. Xu).

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Table 1
Primer pairs used to amplify the *Akirin2* gene.

primer	Sequence (5′-3′)	Fragment length	Application
A1	F: TCCCTTCCCTGACTCCAC R: CTCAACAAGGAACAAGGC	660 bp	cDNA clone
A2	F: CTTATTCACTCTACGGCAGGTT R: TCTCCATATCGTCGCATTATCT	165 bp	Expression
G	F: GCAAGTTCCACGGCACAG R: TCAGCACCAGCATCACCC	118 bp	RT-qPCR

In this report, we isolated the full CDS of the Tianfu goat *Akirin2* gene for the first time, analyzed its nucleotide and protein sequence, and detected its expression levels in different tissues and at different growth stages.

2. Materials and methods

2.1. Animals and sample collection

All animal procedures were performed according to protocols approved by the Biological Studies Animal Care and Use Committee, Sichuan Province, P.R. China. The Goat Breeding Center of Sichuan Agricultural University provided all of the Tianfu goats which were bred under standard conditions. The Tianfu goats were sacrificed on day 1, month 2.5, month 5, month 7.5, and month 12 from the day it was born (n = 6, 3 males and 3 females). We harvested the myocardium, liver, spleen, lung, kidney, leg muscle, abdominal muscle and longissimus dorsi muscle samples, which were first frozen in liquid nitrogen jars and then stored at −80 °C refrigerator for total RNA and total protein extractions.

2.2. Cloning of Tianfu goat Akirin2 gene

Total RNA was extracted as follows: 100 mg Tianfu goat tissue samples were taken out from the −80 °C refrigerator. According to the manufacturer's instructions, total RNA was extracted from the triturated sample using Trizol total RNA extraction kit (TaKaRa, Dalian, China). Then the total RNA was detected using 1.5% agarose gel electrophoresis. First strand cDNA was synthesized from 2 μg of purified total RNA using a real-time reverse transcription PCR (RT-PCR) system (TaKaRa). A pair of primers (Table 1) was designed by Primer Premier 5.0 software from the conserved region of *Bos taurus Akirin2* gene sequences (GenBank: BC104619.1). The PCR reaction system (total volume 10 μl) contained 1 μl cDNA of Tianfu goat lung tissue, 0.5 μl PCR forward primer, 0.5 μl PCR reverse primer, 3 μl RNase-free H_2O (Tiangen, Beijing, China) and 5 μl Taq PCR Mix (TaKaRa). The PCR reactions were run under the following conditions: pre-denaturation at 95 °C for 5 min, followed by 36 cycles (95 °C for 30 s; 61.4 °C for 30 s; 72 °C for 1 min), and ended with a final extension at 72 °C for 10 min. PCR products were detected by 1.5% agarose electrophoresis, and recovered using an E.Z.N.A Gel Extraction Kit (Omega, USA). The ligation reaction system (total volume 10 μl) contained 0.5 μl pMD19-T vector (TaKaRa), 5 μl gel recovered products and 4.5 μl solution one (TaKaRa), which was incubated at 4 °C for more than 12 h. Cloning products were sequenced by LiuHe HuaDa Biotechnology Co, Ltd (Beijing, China).

2.3. Analyzing sequences of Akirin2 gene

The goat *Akirin2* gene open reading frame (ORF) was detected using National Center for Biotechnology Information (NCBI) ORF Finder tool (http://www.ncbi.nlm.nih.gov/projects/gorf/), and sequence analysis of the Tianfu goat *Akirin2* gene was performed by NCBI (http://www.ncbi.nlm.nih.gov) and ExPaSy (http://www.expasy.org) software. Akirin2 protein phosphorylation sites were predicted by the NetPhos 2.0 server (http://www.cbs.dtu.dk/services/NetPhos/). The Akirin2 protein sequences from other related species were aligned with the Tianfu goat Akirin2 sequence by using the DANMAN6.0 software. The phylogenetic tree was constructed by using the MEGA 5.1 software (Tamura et al., 2007). The secondary structures of the deduced amino acid (AA) sequences were predicted by SSpro 4.0 Server (http://download.igb.uci.edu/sspro4.html). The transmembrane domain of the deduced AA sequences was predicted by TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0/). Signal peptides were predicted using the SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP/).

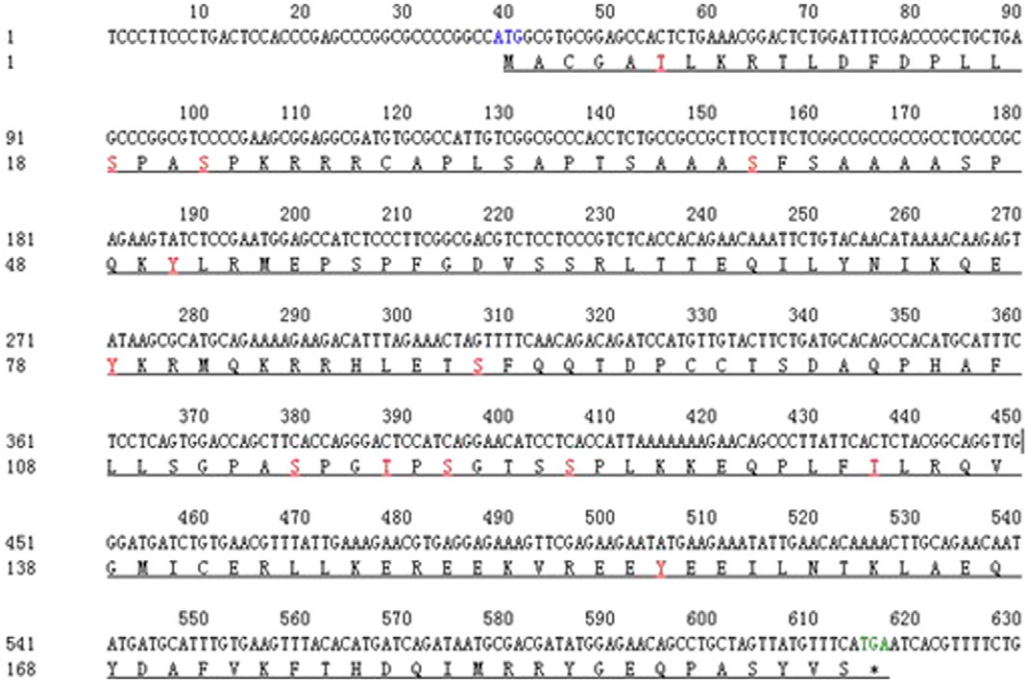

Fig. 1. Nucleotide and deduced amino acid sequences of Tianfu goat *Akirin2* gene. * indicates the stop codon; the blue letters indicate the initiation codon; the green letter indicates the termination codon; the predicted serine, threonine and tyrosine phosphorylation sites are highlighted in red letters.