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Comparative analysis of myostatin gene and promoter sequences of Qinchuan and Red Angus cattle

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ABSTRACT. To better understand the function of the myostatin gene and its promoter region in bovine, we amplified and sequenced the myostatin gene and promoter from the blood of Qinchuan and Red Angus cattle by using polymerase chain reaction. The sequences of Qinchuan and Red Angus cattle were compared with those of other cattle breeds available in GenBank. Exon splice sites were confirmed by mRNA sequencing. Compared to the published sequence (GenBank accession No. AF320998), 69 single nucleotide polymorphisms (SNPs) were identified in the Qinchuan myostatin gene, only one of which was an insertion mutation in Qinchuan cattle. There was a 16-bp insertion in the first 705-bp intron in 3 Qinchuan cattle. A total of 7 SNPs were identified in exon 3, in which the mutation occurred in the third base of the codon and was synonymous. On comparing the Qinchuan myostatin gene sequence to that of Red Angus cattle, a total of 50 SNPs were identified in the first and third exons. In addition, there were 18 SNPs identified in the Qinchuan cattle promoter region compared with those of other cattle

breeds (GenBank accession No. AF348479), but only 14 SNPs when compared to the Red Angus cattle myostatin promoter region.

Key words: Myostatin; Qinchuan cattle; Red Angus cattle; Promoter; SNPs

INTRODUCTION

Myostatin is a member of the transforming growth factor-beta superfamily of secreted growth and differentiation factors. Myostatin is a negative regulator of skeletal muscle development and growth (McPherron et al., 1997), and is most well known as a potent suppressor of muscle growth, development, and regeneration (Schuelke et al., 2004). Myostatin is therefore essential for the proper regulation of skeletal muscle mass (McPherron and Lee, 1997). Loss and congenital absence of myostatin function has been associated with an increase in muscle mass in mice (McPherron et al., 1997), humans (Schuelke et al., 2004), cattle (McPherron and Lee, 1997; Grobet et al., 1997; Kambadur et al., 1997; Grobet et al., 1998; Karim et al., 2000; Marchitelli et al., 2003; Joulia-Ekaza and Cabello, 2006), sheep (Clop et al., 2006; Boman et al., 2009; Boman and Våge, 2009), and dogs (Mosher et al., 2007). In Belgian Blue cattle, an 11-bp deletion of the myostatin gene results in a truncated protein, as transcription terminates 14 codons downstream of the mutation site (McPherron and Lee, 1997). In Piedmontese cattle, 2 single nucleotide polymorphisms (SNPs) have been identified (McPherron and Lee, 1997). Karim et al. (2000) and Smith et al. (2000) have described additional mutations in the myostatin gene in other cattle breeds. Three, 7, and 4 polymorphisms in exons 1, 2, and 3, respectively, were identified in Nellore cattle (Grisolia et al., 2009). In an analysis of the myostatin genes of *Bos indicus* and *B. taurus*, Tantia et al. (2006) identified SNPs and an insertion mutation in *B. indicus*. Baron et al. (2012) sequenced exon 2 of the myostatin gene in 332 horses of 20 different breeds and compared it against horse myostatin gene sequence deposited in GenBank. The obtained sequences revealed the presence of 11 haplotypes represented by 10 variable nucleotide mutations, 8 of which corresponded to amino acid sequence changes (Baron et al., 2012).

However, no studies on variation in the myostatin gene have yet been conducted on the Qinchuan breed, which is the main cattle breed used in China and has several favorable characteristics, including a tall body, good meat quality, genetic stability, and high adaptability, despite the presence of a congenital defect in which the hind legs are underdeveloped. In order to improve the meat production performance in Qinchuan breeds and to select for improved beef cattle breeds, we here evaluated the role of myostatin in cattle breed quality by comparing myostatin gene sequences in Qinchuan and Red Angus, an internationally famous breed, to those of other cattle breeds that are available in GenBank (accession No. AF320998).

MATERIAL AND METHODS

Animal sources and DNA samples

Samples were obtained from the Yang Ling Ke Yuan Cloning Co. Ltd. Genomic DNA samples were randomly selected from 16 Qinchuan cattle and 4 Red Angus cattle. Samples containing 6 mL whole blood were collected in siliconized vacuum tubes with 1 mL acid citrate dextrose solution. DNA was extracted from 200 μL whole blood samples, using the TIANamp Blood DNA Kit (TIANGEN). The quantity and integrity of DNA were analyzed by electrophoresis on 1% agarose gel with ethidium bromide staining.

Polymerase chain reaction (PCR) conditions

To obtain the myostatin genomic sequence, we designed 3 primer pairs (P1-P6) based on the GenBank myostatin sequence AF320998, using the Primer 5.0 software. To amplify the promoter, we designed primers (P7-P8) based on the GenBank myostatin sequence AF348479 (Spiller et al., 2002). To amplify the mRNA, we designed primers (P9-P10) based on the GenBank myostatin sequence AY160688. All primers used in this study are listed in Table 1.

Table 1. Primers used for PCR amplification.

Name	Position (bp)	Primer sequence	Annealing temperature (°C)	Size (bp)
P1	11→1170	5'-AGTATAAAAGATTCACTGGTGTGGC-3'	58.0	1160
P2	(AF320998)	5'-TGTGTTTACTTCCTTATTGCTCTTACTA-3'		
P3	1054→3588	5'-AGGGTTTTTATGGTCTTCACAGAGTATC-3'	58.0	2535
P4	(AF320998)	5'-AAACAGAAGTAAATACAAGCATAGATAAGCC-3'		
P5	3560→6621	5'-CTTATCTATGCTTGTATTTACTTCTGTTTTC-3'	62.0	3062
P6	(AF320998)	5'-AATTGTTTCTACACATTGGATGTAAGAA-3'		
P7	8721→10312	5'-CAGGGCATCTGGTTTGTGTC-3'	61.7	1592
P8	(AF348479)	5'-TGCCTGCCCAGTCTGAGAGA-3'		
P9	307→1125	5'-GACGGCTCCTTGGAAGACGAT-3'	60.4	819
P10	(AY160688)	5'-TGAACACCCACAGCGATCTA-3'		

Exons 1, 2, and 3 are located at base pairs, 152-524, 2365-2738, 4772-5152, respectively (GenBank accession No. AF320998).

The PCR was performed in a 25-μL reaction volume, containing 100 ng genomic DNA, 1X Taq reaction buffer, 5 nmol dNTPs, 20 pmol each primer, and 0.25 units Taq DNA polymerase (Promega).

Sequencing and analysis

The myostatin gene and promoter were obtained by PCR from Qinchuan and Red Angus cattle genomic DNA. The DNA fragments were cloned into a pMD-18T vector (TaKaRa) and then sequenced with an ABI PRISM 3730 DNA sequencer (Applied Biosystems). Sequence analysis was conducted using the DNAMAN software, version 5.2.2.

mRNA was isolated from the skeletal muscle of Qinchuan and Red Angus cattle with TRIZOL® Reagent (Invitrogen) following manufacturer protocols. cDNA was synthesized with the RevertAid First-Strand cDNA Synthesis Kit (Fermentas). Myostatin-specific cDNA was amplified with the primer P9-P10 and then sequenced to confirm the exon/intron boundaries of the gene.

RESULTS

We first checked the integrity of the genomic DNA on 1% agarose gel electrophoresis. The sequences were also checked manually by splicing and removal of the sequencing primer. The sequences were then compared with those of other bovine breeds that have been deposited to GenBank (*B. indicus* accession No. AY794986; *B. taurus* accession No. AF320998). Observed variations between the sequences of the Qinchuan breeds and *Bos taurus* are shown in Table 2. A total of 69 SNPs were identified, only 1 of which was an insertion mutation in Qinchuan cattle. Furthermore, there was a total of 7 SNPs in exon 3: nt111 (G→C), nt179 (T→C), nt197 (T→A), nt212 (A→G), nt414 (T→C), nt1077 (A→C), and nt1083 (C→T) (Figures 1, 2, and 3, and Table 2).

Table 2. Polymorphism of myostatin gene sequence.

Base position (AF320998)	*Bos taurus* sequence (AF320998)	*Bos indicus* sequence (AY794986)	Qinchuan cattle sequence	Red Angus cattle sequence	Location
262	G	G/C	G/C	G	Exon 1
330	T	T	T/C	T	
348	T	T	T/A	T	
363	A	A	A/G	A	
807	T	T	-	-	Intron 1
870	Insertion	T	T	T	
917	A	A	A/T	A/T	
998	T	T	T/C	T/C	
1020	G	G	G/A	G	
1045	T	-	-	-	
1066	G	T	T/G	G	
1201	G	G	G/A	G	
1229	Insertion	GAGTAGATTATGGCTT	GAGTAGATTATGGCTT	Insertion	
1331	G	T	T/G	T	
1405	A	A	A/G	A	
1423	G	G	G/A	G	
1458	C	C	C/T	C	
1661	C	C	C/T	C	
1664	A	A	A/T	A	
1981	C	A	A	A	
1990	C	T	T	T	
2137	T	G	G/T	G	
2284	Insertion	-	A/-	A	
2311	C	C	T/C	T	
2316	A	A	A/G	A	
2343	Insertion	-	-/T	T	
2405	T	C	C	C	Exon 2
2854	G	T	G/T	G	Intron 2
2855	C	C	T/C	T	
2856	A	A	G/A	G	
2882	T	T	C/T	C	
2974	C	C	C/T	T	
3035	G	A	A/G	A	
3059	A	A	C/A	C	
3096	T	T	T/A	A	
3162	G	A	A	A	
3215	T	A	A	A	
3227	T	G	G	G	
3293	C	C	C/T	C	
3628	G	T	T	T	
3674	A	A	G	G	
3691	A	A	A/T	T	
3768	T	A	A	A	
3838	A	A	A	G	
3908	A	A	A	G	
3919	T	T	T	C	
4112	C	C	T	T	
4204	A	-	-	-	
4290	T	T	T/C	C	
4334	G	C	G/C	G	
4355	G	A	A	A	
4372	A	G	A/G	A	
4421	T	C	C	C	
4432	T	C	T/C	T	
4492	G	G	G/C	C	
4530	G	G	G/C	G	

Continued on next page

Table 2. Continued.

Base position (AF320998)	*Bos taurus* sequence (AF320998)	*Bos indicus* sequence (AY794986)	Qinchuan cattle sequence	Red Angus cattle sequence	Location
4571	G	G	G/A	G	
4691	Insertion	T	-/T	T	
4698	T	T	T	T/C	
5101	A	T	A/C	A	Exon 3
5107	C	T	C/T	C	
5179	C	A	C/A	C	3'-UTR
5253	T	T	T	C	
5303	A	A	A/C	A	
5311	A	T	T/A	T	
5332	C	A	A/C	A	
5353	A	C	C/A	C	
5406	G	C	C	C	
5415	T	C	C/T	C	
5494	C	T	T	T	
5639	G	A	A	A	
5655	A	G	G	G	
5662	G	A	A	A	
5769	A	G	G	G	
5863	T	T	C/T	C	

(-) = Del.

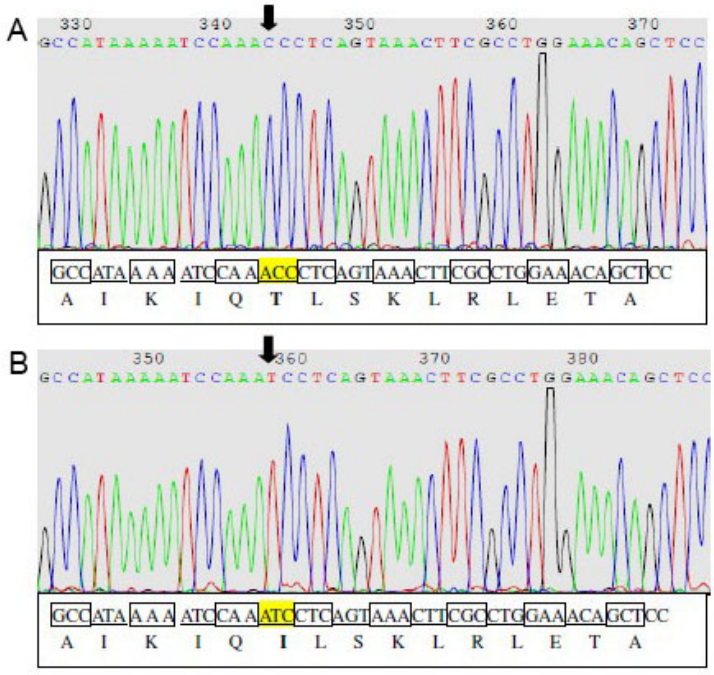

Figure 1. Nucleotide sequence diagram of nt179 (T→C) results in I60T mutant. **A.** Normal cattle; **B.** mutation cattle.

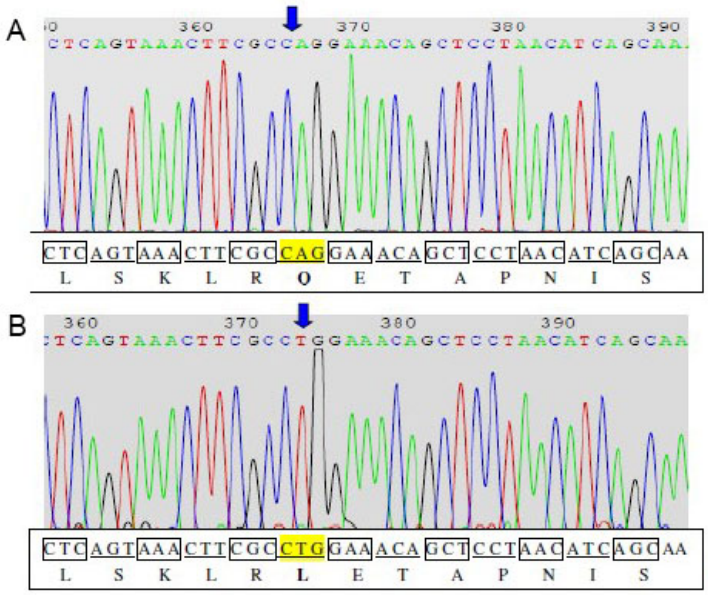

Figure 2. Nucleotide sequence diagram of nt197 (T→A) results in L66Q mutant. **A.** Normal cattle; **B.** mutation cattle.

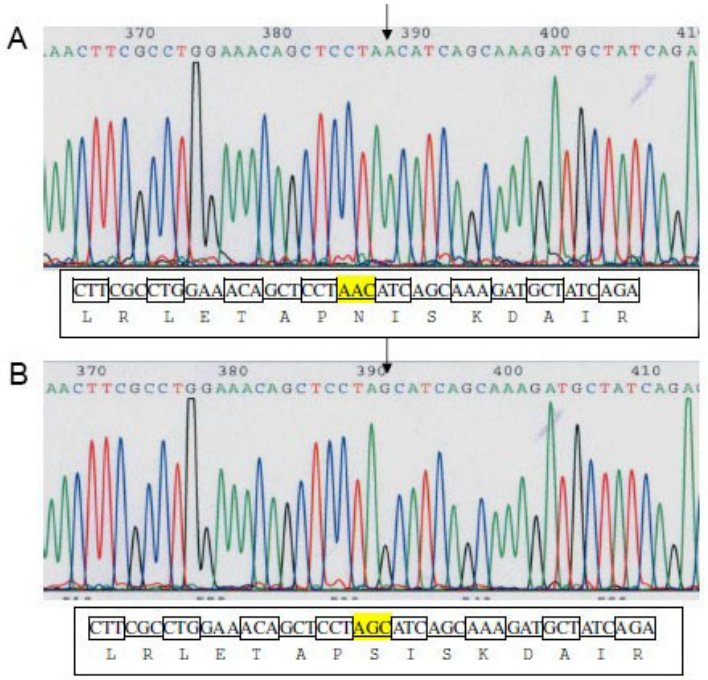

Figure 3. Nucleotide sequence diagram of nt212 (A→G) results in N71S mutant. **A.** Normal cattle; **B.** mutation cattle.

There were 3 base mutations in exon 1 resulting in amino acid changes in the amino acids I60T, L66Q, and N71S. The remaining mutations observed occurred in the first 3 codons and were synonymous; that is, they did not cause changes in amino acids.

One insertion unique to 3 Qinchuan individuals (16-GAGTAGATTATGGCTT) was found in the first 705-bp intron of the myostatin gene. This insertion sequence (GAGTAGATTATGGCTT) (He et al., 2011) is the same as that reported by Tantia et al. (2006).

The results also showed that there were 50 SNPs between Qinchuan and Red Angus cattle breeds, including nt111 (G→C), nt179 (T→C), nt197(T→A), nt212(A→G), nt1077 (A→C), and nt1083 (C→T), in exons 1 and 3. The 3 base mutations in exon 1 caused amino acid changes in amino acids I60T, L66Q, and N71S, whereas the other mutations were synonymous.

There were 18 and 14 SNPs in the Qinchuan myostatin gene promoter compared to the published GenBank sequences (accession No. AF348479) and that of the Red Angus cattle breeds, respectively (Table 3).

Table 3. Polymorphism of myostatin promoter sequence.

Base position (AF348479)	*Bos taurus* sequence (AF348479)	*Bos taurus* sequence (AJ438578)	Qinchuan cattle sequence	Red Angus cattle sequence
9157	T		C	T
9199	C	C	T	C
9213	C	C	-	C
9296	T	T	C	T
9305	A	A	G	A
9308	C	C	G	C
9339	A	A	G	A
9343	T	T	C	T
9348	T	T	C	T
9357	C	T	T	T
9431	G	A	A	A
9442	A	A	G	A
9499	A	A	T	A
9598	C	G	G	G
9607	T	T	C	T
10029	T	T	G	T
10042	C	A	A	A
10047	C	C	A	C

(-) = Del.

DISCUSSION

Qinchuan cattle have been popular farming and meat-utility breeds in China for thousands of years (Pang et al., 2011). This breed is characterized by a number of favorable traits, including a tall body, good meat quality, genetic stability, and adaptability. However, they also have relatively low meat production, and their hindquarters are underdeveloped and grow slowly. Such shortcomings can have serious negative impacts on the economic benefits of Qinchuan when in competition with common foreign commercial beef cattle breeds (Zhang et al., 2007; Han et al., 2008), for example, Red Angus cattle. It has therefore become necessary to improve the meat productivity in Qinchuan cattle breeds while maintaining their other favorable characteristics. As reported previously, the myostatin gene negatively regulates the skeletal muscle, in which the loss or congenital absence of myostatin function is associated

with an increase in muscle mass. In order to improve the meat production performance of Qinchuan as soon as possible and develop a better breed of beef cattle, we investigated muscle production related to single nucleotide polymorphisms in the myostatin gene and its promoter of the Qinchuan and Red Angus cattle breeds. After obtaining the sequences of both breeds, we observed a high degree of conservation in cattle myostatin.

Our results suggest that although the 2 breeds have different growth and development levels, it is unlikely that this is due to nucleotide mutations in the myostatin gene. Some SNPs, nt414 (T→C) and/or nt1083 (C→T), in exon 3 of the myostatin genes of both the Qinchuan and Red Angus breeds have also been observed in European cattle breeds, the Nellore breed, and in Brazilian Murrah buffalo (*Bubalus bubalis*) (Dunner et al., 2003; Mota et al., 2006). This suggests that these 2 sites have a high level of polymorphism, which has previously been generally observed in the myostatin gene of cattle (Grobet et al., 1998).

In the Nellore breed, 11 new polymorphisms were identified in the 3 exons, in which 4 were synonymous mutations, including nt111 (G→T), and 7 were nonsynonymous mutations resulting in amino acid changes (Grisolia et al., 2009). In the present study, we found that polymorphisms at nt111 (G→C) in Qinchuan and Red Angus cattle breeds also resulted in synonymous mutations. The details of SNPs are useful for population diversity and muscle production analysis within cattle breeds. Our results therefore contribute important data to help develop a new Qinchuan breed line through molecular breeding approaches.

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