Genomics xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Original Article

Genetic variants in MYF5 affected growth traits and beef quality traits in Chinese Qinchuan cattle

Chunping Zhao[a,*], Sayed Haidar Abbas Raza[a], Rajwali Khan[a], Ahmed Sabek[b,c], Samiullah Khan[d], Irfan Ullah[e], Sameeullah Memon[f], Ayman Hassan Abd El-Aziz[g], Mujahid Ali Shah[h], Li Shijun[a], Liyun Wang[a], Xuchun Liu[a], Yiwei Zhang[a], Linsheng Gui[i], Linsen Zan[a]

[a] College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, China
[b] Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction Ministry of Education, Huazhong Agricultural University, Wuhan 430070, Hubei, China
[c] Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Benha University, Moshtohor 13736, Kalyobiya, Egypt
[d] College of Animal Science and Technology, Yunnan Agricultural University, Kunming, Yunnan 650201, China
[e] Department of Biological Science, Karakoram International University, Ghizer Campus, Gilgit, Baltistan 15200, Pakistan
[f] Yunnan Animal science and Veterinary Institute, Jindian, Kunming 650224, China
[g] Animal Husbandry and Animal Wealth Development Department, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, Egypt
[h] University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zatisi 728/II, 389 25 Vodnany, Czech Republic
[i] College of Agriculture and Animal Husbandry, Qinghai University, Xining, Qinghai 810016, China

ARTICLE INFO

Keywords:
MYF5
SNP
Cattle
Growth trait
Beef quality trait

ABSTRACT

Myogenic factor 5 plays actively roles in the regulation of myogenesis. The aims of this study are to identify the evolution information of MYF5 protein among 10 domestic and mammalian animals, to uncover the expression patterns of MYF5 gene in calves and adults of Qinchuan cattle, and to expose the genetic variants of the MYF5 gene and explore its effect on cattle growth traits and beef quality traits in Qinchuan cattle. The bioinformatics results showed that the MYF5 proteins highly conserved in different mammalian or domestic animals apart from chicken. The expression level of MYF5 gene in the heart, muscle, lung, large intestine and liver was greater than that of other tissues. PCR amplicons sequencing identified four novel SNPs at g.5738A > G, g.5785C > T and g.5816A > G in the 3rd exon region and g.6535A > G in the 3' UTR. Genotypic frequencies of g.5785C > T was harshly deviated from the HWE ($P < .05$). Genetic diversity was low or intermediate for the four SNPs and those SNPs were in the weak linkage disequilibrium. Association analysis results indicated g.5785C > T, g.5816A > G and g.6535A > G significant effect on growth performance and beef quality traits of Qinchuan cattle. H1H3 diplotype had greater body size and better beef quality. All the results implicate that the MYF5 gene might be applied as a promising candidate gene in Qinchuan cattle breeding.

1. Introduction

China has more than 70 native cattle breeds, but most of them were raised as draft cattle, with evident shortcomings for beef production, such as slow growth rate, small body size and low intramuscular fat content [1–4]. Therefore, improvement of Chinese local cattle towards beef production is one of the main objectives of this study. Molecular marker assistant selection is a useful and high-efficient method in modern animal breeding practice. Accordingly, selecting the potential loci for beef quality and quantity is a hotspot in this field [5–13]. Currently, only a small part of functional loci has been identified due to the effect of minor-polygene on these traits. Hence, it is pressing to identify more functional genes and loci to enhance our knowledge of their functions on cattle growth and beef characteristics.

Myogenic factor 5, encoded by the MYF5 gene, plays active roles in the regulation of myogenesis. MYF5 gene pertains to myogenic regulatory factor family, which includes MyoD, myogenin, MYF5, and MYF6, and all the MRF family members contains a helix-loop-helix domain structure, which can identify and bind to *E*-box [14]. Among the MRF family members, MYF5 expressed earliest in the precursor cells

Abbreviations: Body length, (BL); Withers height, (WH); Chest circumference, (CC); Backfat thickness, (BT); Intramuscular fat percentage, (IMF; Hardy-Weinberg equilibrium, (HWE); Ultrasound loin area, (ULA); polymorphism information content, (PIC); linkage disequilibrium, (LD); Single nucleotide polymorphisms, (SNPs)

* Corresponding author at: No.22 Xinong Road, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, China.
E-mail address: zhao.chunping@foxmail.com (C. Zhao).

https://doi.org/10.1016/j.ygeno.2020.03.018
Received 7 February 2020; Received in revised form 7 March 2020; Accepted 22 March 2020

and plays pivotal roles in skeletal muscle differentiation. MYF5 occupies the promoters regions of muscle-specific genes and promote the transformation of fibroblasts into myoblasts. Additionally, MYF5 can stabilize the open state of chromatin, which can promote the transcription and myogenin [15].

Various studies found SNPs in MYF5 are associated with economic traits in domestic animals, for example, meat quality of Ira rabbit [16]; carcass traits of pigeons [17]; intramuscular fat content, water moisture content and pH in the longissimus dorsi of pig [18]. In cattle, polymorphism in the MYF5 gene has been previously described to be associated with growth traits in Canadian cattle [19] and Chinese native cattle [20]; genetic indices, and morphological scores in Marchigiana cattle [21]; growth traits, average daily gain and carcass traits of Korean cattle [22,23]; backfat thickness and meat tenderness in Chinese cattle [24]. All these studies suggested MYF5 plausibly influenced the growth and beef quality of cattle.

In this study, four novel SNPs were found in Chinese native cattle based on the PCR and agarose gel electrophoresis. Furthermore, the SNPs and their haplotypes were found associated with growth traits and beef quality traits. These results may provide new perceptivities into biomarkers of bovine MYF5 gene and its promising utilizations in molecular breeding of beef cattle.

2. Materials and methods

2.1. Bioinformatics analyses

The amino acid sequences of MYF5 gene were acquired from NCBI (www.ncbi.nlm.nih.gov/protein) for *Bos taurus* (NP_776541.1), *Bos indicus* (XP_019815864.1), *Bos mutus* (XP_005888361.1), *Capra hircus* (NP_001273966.1), *Ovis aries* (XP_014950042.1), *Bubalus bubalis* (NP_001277771.1), *Sus scrofa* (NP_001265704.1), *Rattus norvegicus* (NP_001100253.1), *Mus musculus* (NP_032682.1), *Gallus gallus* (NP_001025534.1). MEGA X 10.1.6 (Philadelphia, PA, USA) (www.megasoftware.net) used for both the sequence alignment and construction of phylogenetic tree [25–27]. MUSCLE sequencing alignment, and a neighbor-joining phylogenetic tree were selected for multiple sequence alignment and construction of phylogenetic tree respectively. To reveal the structure characteristics and function of MYF5 proteins in these 10 selected species, the motifs were investigated through MEME suite (http://meme.nbcr.net/) [28,29] and the conserved domains were analyzed through CDD NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) [30,31].

2.2. RNA extraction, cDNA synthesis and MYF5 gene expression pattern analysis

To investigate the expression pattern of MYF5 gene, three 1-day-old Qinchuan Calves and three 24-month-old Qinchuan cow were chosen randomly from National Beef Cattle Improvement Center (Yangling, China). The six subjects were non-relatives for at least three generations. Fourteen tissues including kidney, spleen, liver, heart, lung, longissimus dorsi, subcutaneous fat, perirenal fat, intramuscular fat, rumen, abomasum, omasum, reticulum, large intestine, were collected from six individuals respectively and rapidly stored in liquid nitrogen and then at −80 °C for further analysis. The RNA was extracted using a total RNA kit (Takara, China) and the cDNA was synthesized using PrimeScriptTM RT reagent kit (Takara) based on the recommended procedure. Quantitative Real Time PCR (RT-PCR) was performed using Syber Premix EX Taq Kit (Takara). β-actin was used as a reference gene for normalization. The primers used for RT-PCR were designed with Primer Premier 5.0 (Table 1). The PCR was run by the Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, USA), with the conditions of preheating at 95 °C for 5 min, total 34 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s. The PCR were conducted in triplicate for each sample and the relative expression levels of MYF5 gene were calculated using $2^{-\Delta\Delta Ct}$ method [32].

2.3. Data collection and sampling

414 female Qinchuan cattle with the ages of 24–30 months were fed and managed under the same conditions in the farm of National Beef Cattle Improvement Center (Yangling, China). The phenotype performance for the growth traits including BL (Body length), WH (withers height) and CC (Chest circumference) were measured as previous described [33]. The beef quality traits, including BT (backfat thickness), IMF% (intramuscular fat percentage) and ULA (ultrasound loin area), were evaluated through ultrasound technology as previous standard procedures [34,35]. The blood samples were also collected from these animals and genomic DNAs were extracted and then conserved at −80 °C until genotyping. All the procedures were checked and approved by the Ethical Committee of Animal Care in Northwest A&F University.

2.4. Identifying genetic variants and genotyping

Two pairs of primers (Table 2) were designed using Primer Premier 5 to amplify the 3rd exon region and the 3'UTR of the bovine MYF5 gene. Thirty DNA samples selected at random were amplified to identify if the regions harbor SNPs. The PCR reaction mixture contained 0.6 μL of each primers (10 pM), 1.2 μL of genomic DNA (100 ng), 2 μL of 10× PCR buffer, 2 μL of dNTP (2 mmol/L), 0.8 μL of $MgSO_4$ (25 mol/L), 0.4 μL of KOD with Neo enzyme and dH_2O was added to make the whole volume 20 μL. PCR was performed with Pre-denaturation at 94 °C for 5 min and then followed by 34 cycles of denaturation at 97 °C for 30 s, annealing for 30 s and final extension at 72 °C for 45 Sec. Bi-directional sequencing was operated through Sangon (Shanghai, China). Finally, sequence analysis was conducted by SeqMan software (DNASTAR, USA).

Four SNPs were identified by DNA sequencing, including three mutations in 3rd introns, namely g.5738A > G, g.5785C > T, g.5816A > G, and a SNP in 3'UTR, g.6535A > G. Restriction enzyme analysis showed that the three mutations in 3rd intron contained restriction sites. Therefore, genotyping 414 Qinchuan cattle by PCR-RFLP as the previous protocol [36], using GsaI, *Sex*AI and TaqI (TaKaRa), respectively. To identify the digested products all products were visualized by electrophoresis on 2.5% agarose gels stained with ethidium bromide. The fourth mutation was genotyping by sequencing through Sangon.

2.5. Statistical analysis

The allelic and genotypic frequencies of all four SNPs were calculated directly. The Hardy-Weinberg equilibrium (HWE) was evaluted by a *Chi*-squared test in PopGene 3.2 (Edmonton, Canada). The polymorphism information content (PIC) was calculated following previous instruction [37]. The haplotypes and r^2 linkage disequilibrium (LD) were analyzed through Haploview [38].The general linear model was used for the association analysis between SNPs and phenotypes. The equation was shown as follows: $Yijk = \mu + Gi + Sj + Ak + eijk$, where Y*ijk* was the phenotypic observations; μ was the averaged values; G*i* was the fixed effect of genotype; S*j* was the random effect of sire; A*k* was fixed effect of age; and e*ijk* was the residual effect. All values were presented as the mean ± SE.

The mRNA expression level of MYF5 in tissues were analyzed through GraphPad Prism 6.

Table 1
Primers and amplification conditions for RT-qPCR.

Name	Function	Primer (5′-3′)	Tm (°C)	Production size
MYF5	qPCR	F: CTCTGATGGCATGCCTGAATGT R: GGCAATCCAGGTTGCTCTGA	61	180 bp
β-actin	Reference	F: CACCAACTGGGACGACAT R: ATACAGGGACAGCACAGC	61	202 bp

3. Results

3.1. Biological evolution and conservation of MYF5

The multiple sequence alignments of MYF5 proteins were performed for 10 mammalian or domestic animals, namely mouse (*Mus musculus*), cattle (*Bos Taurus*), zebu cattle (*Bos indicus*), buffalo (*Bubalus bubalis*), yak (*Bos mutus*), goat (*Capra hircus*), sheep (*Ovis aries*), pig (*Sus scrofa*), rat (*Rattus norvegicus*), and chicken (*Gallus gallus*). The protein structure was highly conserved among the 6-ruminants, and somewhat different in pig and rat, but the structure of chicken is more dissimilar to others (Fig. 1). Then phylogenetic tree of MYF5 proteins was depicted through MEGA 7 under the analysis of neighbor-joining, and MEME was used to detect common motifs in the MYF5 proteins of 10 examined species. As shown in Fig. 2, cattle, zebu cattle and yak were more closely related, while pig, rat, mouse and chicken were away from the bovine. Total seven significant motifs were found among 10 species and most of the motifs occupied the same locations except in chicken (Fig. 3). Then MYF5 protein structures were searched for the 10 species through NCBI CDD and three specific conserved domains hits were found in these animals, representing domain super-families, namely Myogenic Basic super family, Helix-loop-helix family and MYF5 super-family (Fig. 4).

3.2. Ontogenic expression analysis

MYF5 mRNA expression was quantitated in 14 tissues from new born calves and adult cows. The results showed that expression patterns were totally different between calves and adult cows. In calves MYF5 only expressed in muscle. Meanwhile it can be detected in all 14 tissues of adult cows and its expression level was high in heart, muscle, lung, large intestine and liver; moderate in abomasum, spleen and subcutaneous fat; low in rumen, perirenal fat, intramuscular fat, kidney, reticulum and omasum (Fig. 5).

3.3. SNP detection and genotype identification

The bovine MYF5 gene is located on chromosome 5 and contains three exons. Four novel SNP loci within the MYF5 gene, including three mutations in the third intron (g.5738A > G, g.5785C > T and g.5816A > G) and one mutation in 3'UTR (g.6535A > G), were detected in Chinese Qinchuan cattle by comparing the sequencing results with the GenBank sequence (NC_037332.1) (Fig. 6). According to the characteristic of mutations, both DNA sequencing and PCR-RFLP were used for genotyping of 414 individuals. The sequencing results and digestion patterns are shown in Table 2. Among these mutations, g.5738A > G locus had two genotypes and the genotype GG was not observed in our samples, while other mutations were detected in all three genotypes.

3.4. Genetic parameters calculation

The genetic parameters of the MFY5 gene in tested population, including genotype and allele frequencies, were directly counted for all 414 cattle. Table 3 showed that AA of g.5738A > G, CC of g.5785C > T, AA of g.5816A > G and AA of g.6535A > G were dominant genotypes compared to corresponding ones in the populations. The *chi*-square test for allelic and genotypic frequencies showed that genotype distribution of g.5785C > T was harshly deviated the HWE ($P < .05$), whereas the rest three SNPs conform to the HWE for ($P > .05$). According to the PIC value of the four mutation sites, the genetic diversity was low (PIC < 0.25) at the g.5738A > G and g.5785C > T loci, whereas two others (g.5816A > G and g.6535A > G) possessed an intermediate genetic diversity (PIC > 0.25).

3.5. Linkage disequilibrium and haplotype analysis

Linkage disequilibrium between polymorphisms of the MYF5 gene were predicted using r^2. As shown in Table 4 the r^2 values ranged from 0.000 to 0.018, indicating that those SNPs were in the weak linkage disequilibrium in Qinchuan populations ($r^2 < 0.33$). Table 5 illustrates the haplotype frequencies at all loci of bovine MYF5 gene. Accordingly, the haplotype analyses results showed that five dominating haplotypes were discovered in the Qinchuan cattle population (above 5.00%), Hap1 (-ACAA-) had the highest frequency, followed by Hap3 (-ACGA-), and Hap2 (-ACAG-). All haplotypes with frequencies less than 0.05 were excluded for further analysis.

3.6. Effects of SNPs and haplotype combinations on growth and beef quality traits

The relationship of the four SNPs and production traits were examined in 414 Qinchuan cattle. As shown in Table 6, except for the g.5738A > G, other three mutations displayed obviously relationships with the economic traits in Qinchuan cattle. For growth traits, the

Table 2
Primers for genotyping in the bovine MYF5 gene.

Locus	Position	Primer (5′-3′)	Tm (°C)	Production size	Restriction enzyme	Genotype pattern (bp)
g.5738A > G	Intron 3	F: ATTTATGGGGGTTTTGGCAG R: CCTGGAGTTGCAGGTTGAGA	61.5	448 bp	GsaI/ CCCAG^C	GG: 136 and 312 GA: 136, 312 and 448 AA: 448
g.5785C > T	Intron 3	F: ATTTATGGGGGTTTTGGCAG R: CCTGGAGTTGCAGGTTGAGA	61.5	448 bp	SexAI/ A^CCWGGT	CC: 448 AG; 178, 270 and 448 TT: 178 and 270
g.5816A > G	Intron 3	F: ATTTATGGGGGTTTTGGCAG R: CCTGGAGTTGCAGGTTGAGA	61.5	448 bp	TaqI/ T^CGA	AA: 448 AG: 212, 236 and 448 GG: 212 and 236
g.6535A > G	3′UTR	F: TTCTTTTCCTTCATCTTTGC R: CTTTTGCTCCACATACACAC	61.0	691 bp	–	–

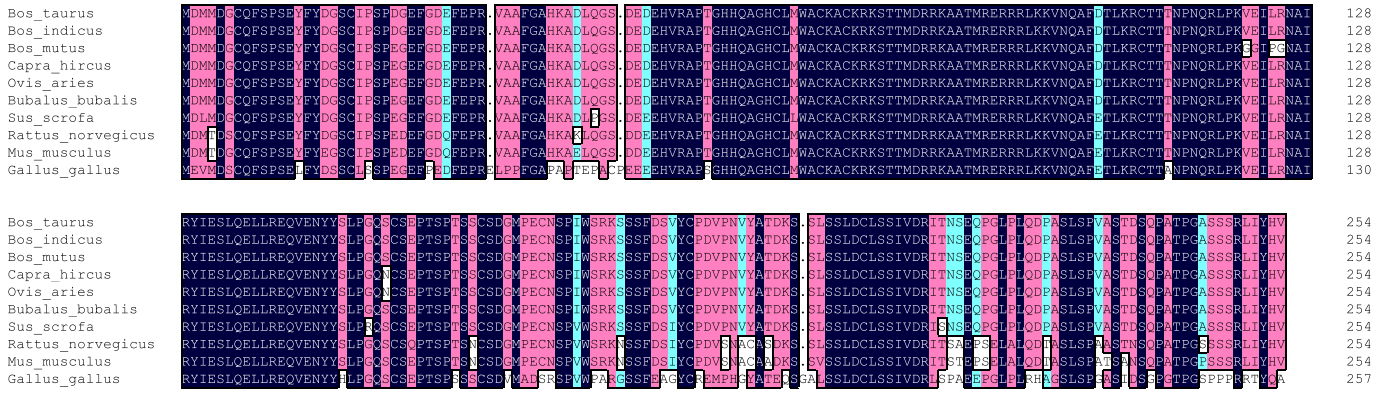

Fig. 1. Multiple sequence alignment of bovine MYF5 for the 10 species. The degree of similarity is delineated using different background shading, with black being 100%; pink 80%; turquoise 60%; yellow 33% and white not conserved. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

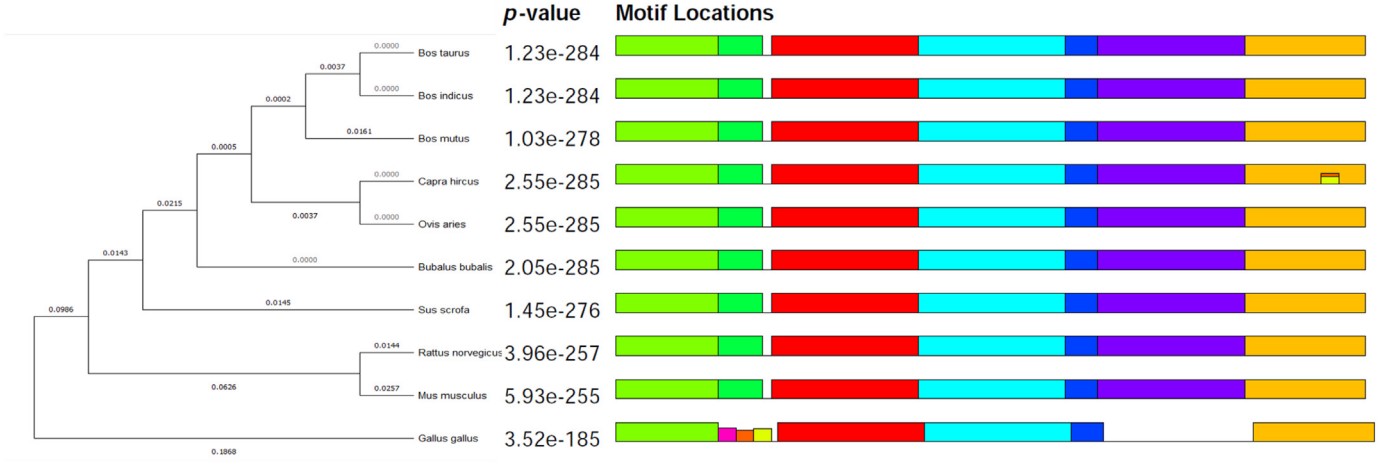

Fig. 2. Phylogenetic tree (Left) and Motif structural analysis (Right) for the 10 species. Seven significant motifs were identified. The length of the color block shows the position, strength and significance of a particular motif site. The length of the motif is proportional to the negative logarithm of the *p*-value of the motif site, truncated at the height for a p-value of 1×10^{-10}. These colors are given through motif analysis performed through MEME suit system.

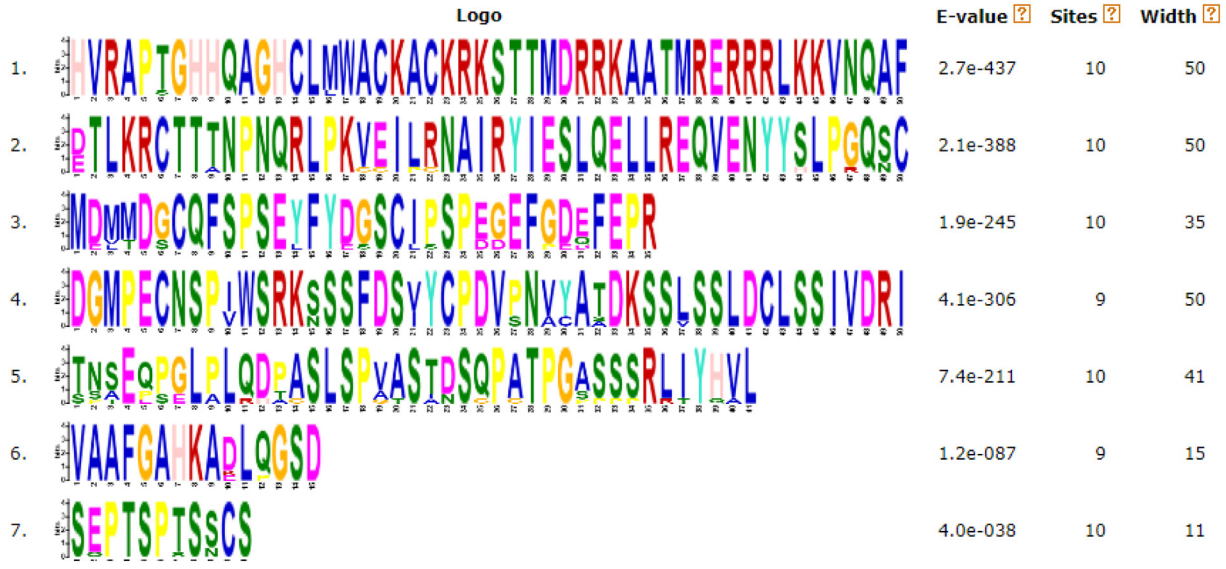

Fig. 3. Significant motifs of MYF5 across the 10 species. Motifs detected using the MEME suite. The different color letters show abbreviation of different amino acids. These colors are given through motif analysis on MEME suit system.

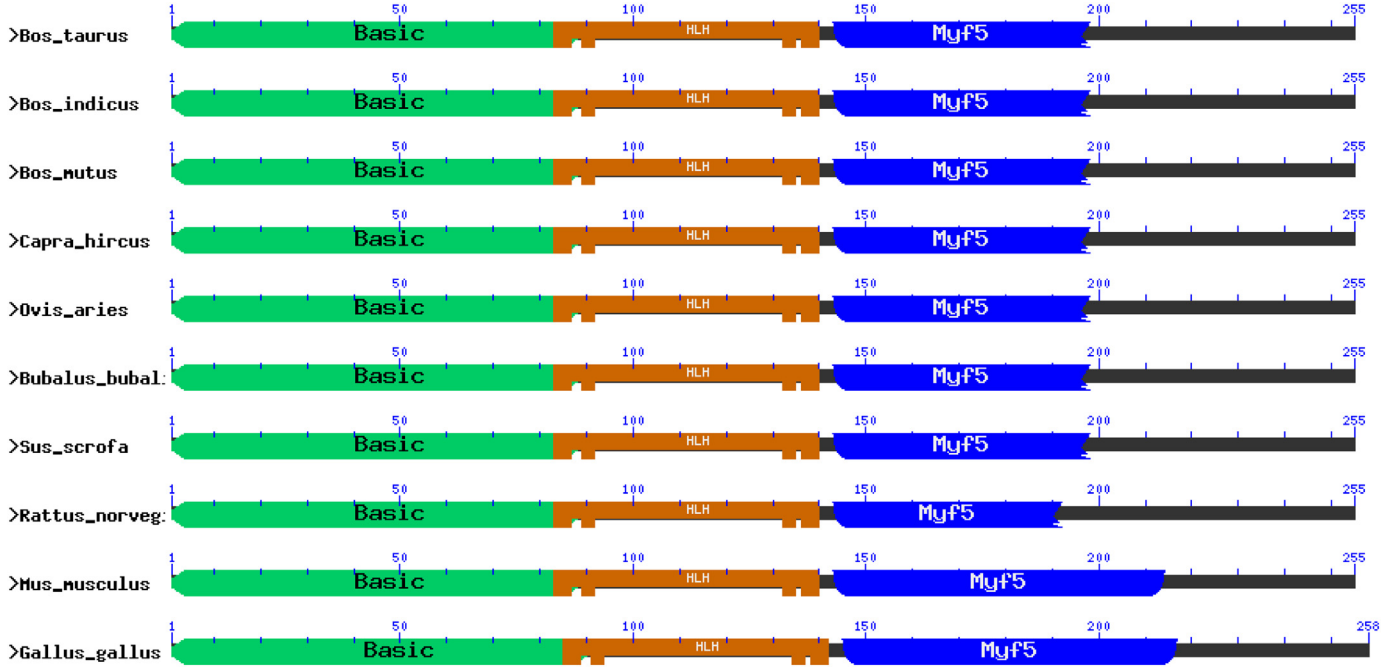

Fig. 4. Structure of MYF5 protein domain families in the 10 species. Each color bar is a specific hit representing a different domain superfamily.

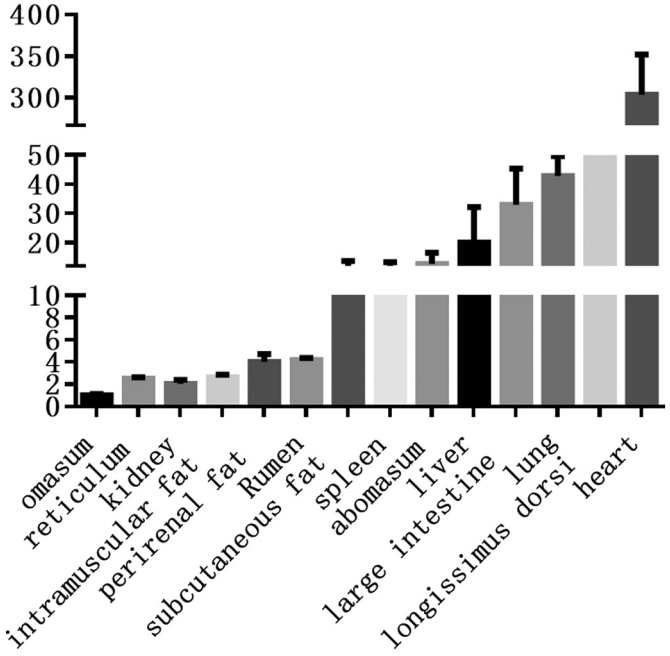

Fig. 5. Sequence variants of the MYF5 gene in Qinchuan cattle.

polymorphisms of g.5785C > T, g.5816A > G and g.6535A > G were significantly associated with body length and chest circumference, and the "CC", "GG" and "AA" were excellent genotypes in those three mutations, respectively. The CC genotype of g.5785C > T was significantly greater than the CT and the TT genotype on withers height ($P < .05$). For ultrasound measured traits, g.5785C > T, g.5816A > G and g.6535A > G polymorphisms affected backfat thickness, and the "CC", "GG" and "AA" genotypes performed better than others. Additionally, g.5816A > G also had effect on loin muscle area. Table 7 listed the association of the haplotype combinations with the growth traits and beef quality traits in examined population. The H1H3 diplotype had significantly greater body length, withers height, chest circumference, back fat thickness and ultrasound loin muscle area than the H1H4 diplotype.

4. Discussion

Studies have shown that effective molecular markers applied in the selection of domestic animals are valuable for improving economical traits and for increasing benefits in the husbandry industry. Of these markers, candidate genes are most investigated by association analysis to link phenotypes and genotypes. In beef cattle, growth traits and beef quality traits are crucial for determining the financial return. These traits directly impact meat yield and quality, and hence, the profit to the farmer. So far the main strength of Chinese beef production system is local yellow cattle, which has obvious weakness for beef production. Improvement of cattle performance is sluggish through traditional breeding methods. So many researches have focused on identifying biomarker for beef quantity and quality traits. Identification of molecular markers related to conformation and carcass characteristics may improve the selection efficiency for economical traits. In our research group, Sirtuin1–7; CRTC2, 3; KLF3, 6; H-FABP; ABHD5; LPL; SIX4; UCPs; ELOVL6, *etc*, genes were found associated with cattle growth and beef quality [5,39].

Previous research found that MYF5 gene played a pivotal role in muscle development and growth. In the meanwhile, it ubiquitously expressed in a variety of tissues in pig, such as the liver, kidneys, heart, spleen, lungs, stomach and intestines *etc* [40]. In this study, MYF5 was detected to be highly expressed in heart, muscle, lung, large intestines, liver, moderate in abomasum, spleen and subcutaneous fat, and also widely expressed in rumen, kidney, *etc*, which is consistent with previous results in pig, implying this gene has extensive regulation roles in the metabolism of these tissues. Interestingly, MYF5 was highly expressed in all fat of three anatomical positions, namely intramuscular fat, subcutaneous fat, perirenal fat, implying this gene plays roles in fat deposition of all position of body.

Previous studies have disagreement reports about the effect of MYF5 mutations on cattle production traits. A research found MYF5 had no influence on growth and conformation traits of Piedmontese cattle [41]. On the contrary, Zhang, RF *et al* found an intron mutation in MYF5 had significant influence on withers height of Qinchuan cattle [20]. They suggested that the intron mutation may be related to other mutations of

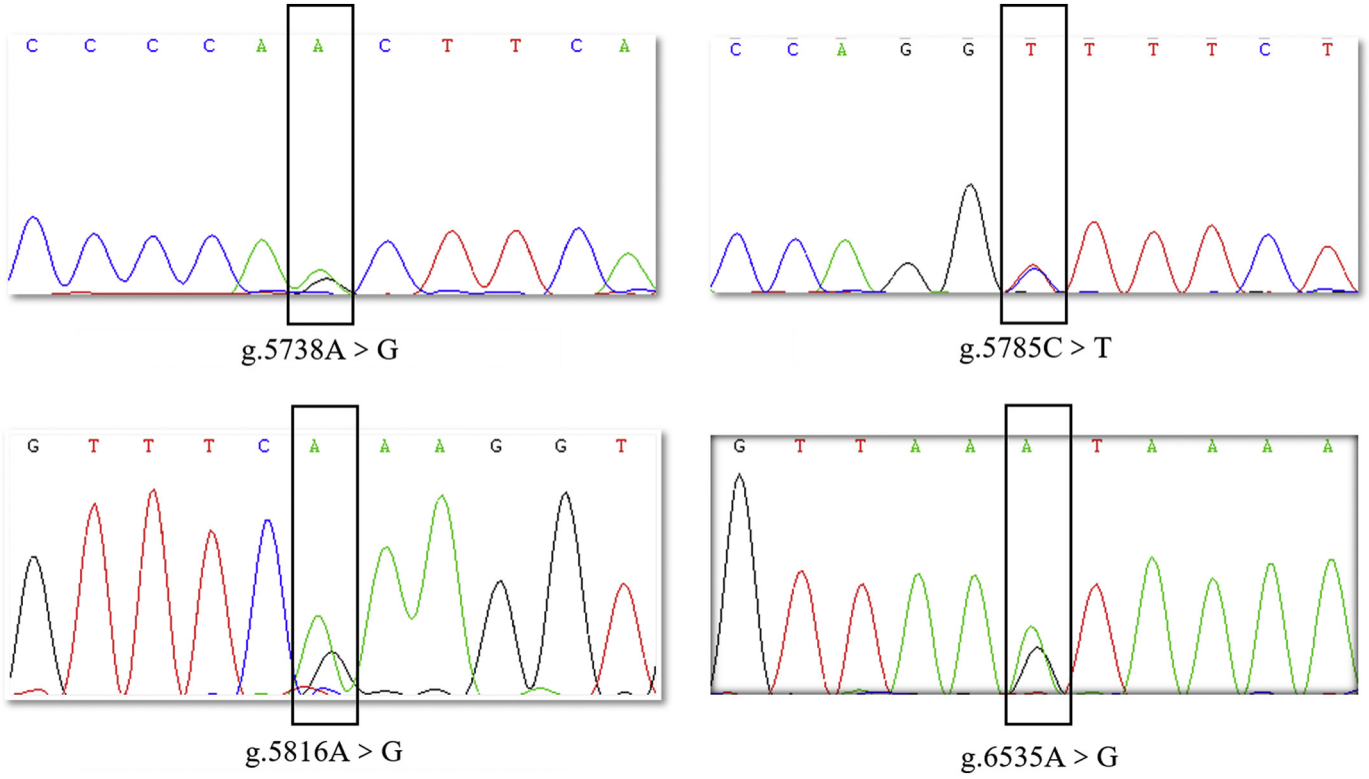

Fig. 6. Sequence variants of the MYF5 gene in Qinchuan cattle.

Table 3
Genotypic and allelic frequencies (%), and related genetic parameters of MYF5.

Locus	Number	Genotype			Minor allele frequency	Heterozygosity	χ^2 (HW)	PIC
g.5738A > G	414	AA (0.792)	AG (0.208)	GG (0.000)	G (0.104)	0.186	P > .05	0.169
g.5785C > T	414	CC (0.742)	CT (0.181)	TT (0.077)	T (0.168)	0.279	P < .05	0.240
g.5816A > G	414	AA (0.580)	AG (0.338)	GG (0.082)	G (0.251)	0.376	P > .05	0.305
g.6535A > G	414	AA (0.681)	AG (0.271)	GG (0.048)	G (0.184)	0.300	P > .05	0.255

Note: HW, Hardy-Weinberg equilibrium.

Table 4
Linkage disequilibrium coefficient among MYF5 SNPs.

r^2	g.5785C > T	g.5816A > G	g.6535A > G
g.5738A > G	0.017	0.002	0.001
g.5785C > T		0.018	0.001
g.5816A > G			0.000

the regulatory regions or coding regions which is crucial for the production traits. They also highlighted previous conclusion that mutations in intron may affect transcriptional efficiency [42]. In our study, we found g.5785C > T and g.6535A > G had a negative effect on body length and chest circumference while g.5816A > G had a positive influence on these two traits. Haplotype combination 1/3 had the most positive influence on the growth traits of Qinchuan cattle. These results further confirmed that MYF5 had effect on growth traits of domestic animals.

Additionally, many previous studies focused on pork quality interfered by mutations on MYF gene family, namely MYOD, MYOG, MYF6 and MYF5. Lee EA *et al* found that two SNPs in porcine MYOD gene were significantly related to the loin eye area and muscle fiber characteristics [43]. Kim JM *et al* reported that the porcine MYOG gene had satisfying effects on the fiber number and loin eye area [44]. Meanwhile, MYF6 was found no influence on carcass meat traits, such as backfat thickness and intramuscular fat content [45,46]. Furthermore, Ryu YC *et al* discovered that the MYF5 significantly affected loin eye

Table 5
Haplotypes of the MYF5 and their frequencies.

Haplotype	Position of sequence variants				Frequency in population
	g.5738A > G	g.5785C > T	g.5816A > G	g.6535A > G	
1	A	C	A	A	0.448
2	A	C	A	G	0.097
3	A	C	G	A	0.173
4	A	T	A	A	0.095
5	G	C	A	A	0.050

Table 6
Association analysis of mutations in MYF5 with growth and ultrasound meat quality traits.

Locus	Genotypes	BL (cm)	WH (cm)	CC (cm)	BT (cm)	IMF (%)	ULA (cm^2)
g.5738A > G	AA (328)	142.73 ± 0.48	126.35 ± 0.36	174.20 ± 0.74	0.92 ± 0.02	7.08 ± 0.04	66.65 ± 1.54
	AG (86)	143.74 ± 0.94	126.35 ± 0.72	175.50 ± 1.45	0.90 ± 0.03	6.90 ± 0.09	63.72 ± 1.69
	P	0.335	0.475	0.217	0.144	0.083	0.328
g.5785C > T	CC (307)	145.07 ± 0.45^{Aa}	127.61 ± 0.36^{a}	176.98 ± 0.73^{A}	0.96 ± 0.02^{a}	7.10 ± 0.05	66.70 ± 1.27
	CT (75)	138.51 ± 0.91^{ABb}	123.39 ± 0.73^{b}	168.52 ± 1.47^{B}	0.79 ± 0.03^{b}	6.89 ± 0.09	63.31 ± 1.73
	TT (32)	132.91 ± 1.39^{BC}	121.20 ± 1.17^{b}	164.38 ± 1.65^{B}	0.88 ± 0.05^{ab}	7.08 ± 0.14	66.02 ± 1.44
	P	0.000	0.019	0.002	0.027	0.234	0.328
g.5816A > G	AA (240)	140.48 ± 0.53^{b}	124.80 ± 0.41	172.05 ± 0.85^{b}	0.87 ± 0.02^{b}	7.07 ± 0.05	64.13 ± 1.34^{b}
	AG (140)	146.31 ± 0.69^{a}	128.47 ± 0.54	177.69 ± 1.11^{a}	0.97 ± 0.02^{a}	7.02 ± 0.07	69.23 ± 1.89^{a}
	GG (34)	146.41 ± 1.18^{a}	128.53 ± 1.10	178.32 ± 1.72^{a}	0.98 ± 0.04^{a}	7.13 ± 0.13	66.10 ± 1.47^{ab}
	P	0.035	0.075	0.017	0.024	0.423	0.028
g.6535A > G	AA (282)	143.92 ± 0.51^{a}	126.73 ± 0.39	175.11 ± 0.79^{A}	0.93 ± 0.02^{a}	7.08 ± 0.05	66.71 ± 1.08
	AG (112)	141.43 ± 0.81^{ab}	125.96 ± 0.63	174.28 ± 1.26^{A}	0.91 ± 0.03^{a}	6.99 ± 0.07	64.53 ± 1.34
	GG (20)	137.63 ± 1.36^{b}	123.13 ± 1.43	166.50 ± 1.66^{B}	0.80 ± 0.06^{b}	7.15 ± 0.13	65.04 ± 1.55
	P	0.019	0.143	0.006	0.0027	0.116	0.335

Note: Values are shown as the least squares means ± standard error. $^{a, b, c}$ Means with different superscripts are significantly different (P < .05). $^{A, B, C}$ Means with different superscripts are significantly different (*P* < .01). BL = Body length, WH = withers height, CC = Chest circumference, BT = backfat thickness, IMF = intramuscular fat percentage, ULA = ultrasound loin area.

Table 7
Associations of combined genotypes with growth and ultrasound meat quality traits.

Combined Genotypes (N)	BL (cm)	WH (cm)	CC (cm)	BT (cm)	IMF (%)	ULA (cm^2)
Hap1/1 (84)	144.16 ± 0.85^{ab}	126.55 ± 0.70^{ab}	175.85 ± 1.34^{A}	0.93 ± 0.03^{A}	7.07 ± 0.08	64.122 ± 0.43^{ab}
Hap1/2 (43)	140.72 ± 1.19^{b}	126.21 ± 0.98^{ab}	175.40 ± 1.87^{A}	0.89 ± 0.04^{b}	7.01 ± 0.12	62.148 ± 0.72^{b}
Hap1/3 (75)	148.65 ± 0.90^{Aa}	129.40 ± 0.74^{a}	178.17 ± 1.42^{A}	0.99 ± 0.03^{Aa}	7.18 ± 0.09	67.188 ± 0.59^{a}
Hap1/4 (21)	134.08 ± 1.46^{Bc}	121.45 ± 1.40^{b}	162.95 ± 1.02^{B}	0.77 ± 0.05^{Bc}	7.08 ± 0.17	61.985 ± 0.77^{b}
P	0.000	0.024	0.000	0.000	0.239	0.027

Note: Values are shown as the least squares means ± standard error. $^{a, b, c}$ Means with different superscripts are significantly different (P < .05). $^{A, B, C}$ Means with different superscripts are significantly different (P < .01).

area and muscle fiber composition in Yorkshire [47] and Cieslak *et al* also detected that polymorphisms for the porcine MYF5 gene were related to the loin eye area and fat deposition [48]. Liu M *et al* found that a mutation in pig MYF5 was associated with intramuscular fat content [18]. But another research found the controverted results that mutations in MYF5 gene did not affect porcine backfat thickness [49]. All these results suggested that porcine MYF5 mutations had significant effect on loin eye area and muscular characteristics, but inconsistent influence on fatty properties. In cattle, Robakowska D *et al* found that a polymorphism in MYF5 influenced its expression in the longissimus dorsi muscle and this SNP was associated with muscle and fat weight in Holstein-Friesian cattle [50]. They suggested that G allele had a favorable effect and T allele had adverse effect on beef yield and quality. They speculated allele G and T modified the activity of the genes by altering its own expression level [50] [51] [52]. In our study, G allele in g.5816A > G had a desirable effect on the backfat thickness and loin eye area, while A allele of g.6535A > G had a favorable effect on the backfat thickness. We speculate the different effect of these two SNP on beef quality may be explained by the position of SNP, namely g.5816A > G in 3rd intron and g.6535A > G in 3'UTR. On the other hand, we used ultrasound measurement, not as accurate as a chloroform–methanol extraction method used in pig's research. Considering the MYF5 is associated with the growth traits and meat quality traits in cattle and other domestic animals in pervious and this studies, further investigation will be essential for exploring the underlying mechanism of MYF5 on growth traits and beef quality of cattle.

5. Conclusion

In summary, we discovered that MYF5 gene was extensively expressed in bovine organs and tissues and four novel SNPs were identified in Chinese indigenous cattle. In addition, g.5785C > T, g.5816A > G and g.6535A > G and diplotype H1H3 might influence growth traits and beef quality traits. These findings may provide deep insights into functional genetic markers for improving economically valuable traits in cattle breeding.

Author Contribution

Chunping Zhao: Sayed Haidar Abbas Raza Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing - original draft, Writing - review & editing. **Rajwali Khan:** Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Writing - review & editing. **Ahmed Sabek: Samiullah Khan: Irfan Ullah:** Data curation, Formal analysis. **Sameeullah Memon: Ayman Hassan Abd El-Aziz** Formal analysis, Writing - review & editing. **Linsheng Gui:** Writing - review & editing. **Mujahid Ali Shah: Li Shijun: Liyun Wang: Xuchun Liu: Yiwei Zhang:** Software, Visualization Software.**Linsen Zan**: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing - review & editing

Ethics approval

All animal experiments were conducted according to the guidelines established by the regulations period. This work was performed at a farm in the Department of Animal Sciences and Northwest A&F University, Yangling, Shaanxi PR China. Ethical approval for this study was obtained from the Ethical Committee of Northwest A&F University. The procedures were approved by the Ethical Committee of China Animal Care Northwest A&F University.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

The work was supported by Shaanxi Key Research and Development Program (2018ZDXM-NY-046), China Postdoctoral Science Foundation (2016M590977), National Key Research and Development Program of China (2018YFD0501700), National Modern Agricultural Industry Technology System (CARS-37), Technology Innovation and Achievement Transformation for the Demonstration Station of Northwest A&F University (TGZX2019-22).

References

[1] S. Wu, Y. Wang, Y. Ning, H. Guo, X. Wang, L. Zhang, R. Khan, G. Cheng, H. Wang, L. Zan, Genetic variants in STAT3 promoter regions and their application in molecular breeding for body size traits in Qinchuan cattle, Int. J. Mol. Sci. 19 (2018) 1035.

[2] C. Mei, H. Wang, Q. Liao, R. Khan, S.H.A. Raza, C. Zhao, H. Wang, G. Cheng, W. Tian, Y. Li, L. Zan, Genome-wide analysis reveals the effects of artificial selection on production and meat quality traits in Qinchuan cattle, Genomics 111 (2019) 1201–1208.

[3] R. Khan, S.H.A. Raza, Z. Junjvlieke, H. Wang, G. Cheng, S.B. Smith, Z. Jiang, A. Li, L. Zan, RNA-seq reveal role of bovine TORC2 in the regulation of adipogenesis, Arch. Biochem. Biophys. 680 (2020) 108236.

[4] J. Hong, C. Mei, S.H.A. Raza, R. Khan, G. Cheng, L. Zan, SIRT5 inhibits bovine preadipocyte differentiation and lipid deposition by activating AMPK and repressing MAPK signal pathways, Genomics 112 (2020) 1065–1076.

[5] S.H.A. Raza, R. Khan, S.A. Abdelnour, M.E. Abd El-Hack, A.F. Khafaga, A. Taha, H. Ohran, C. Mei, N.M. Schreurs, L. Zan, Advances of molecular markers and their application for body variables and carcass traits in Qinchuan cattle, Genes 10 (2019) 717.

[6] R. Khan, S.H.A. Raza, H. Guo, W. Xiaoyu, W. Sen, S.M. Suhail, A. Rahman, I. Ullah, A.H. Abd El-Aziz, Z. Manzari, A. Alshawi, L. Zan, Genetic variants in the TORC2 gene promoter and their association with body measurement and carcass quality traits in Qinchuan cattle, PLoS One 15 (2020) e0227254.

[7] L.S. Gui, S.H.A. Raza, Y.G. Sun, R. Khan, I. Ullah, Y.C. Han, Detection of polymorphisms in the promoter of bovine SIRT1 gene and their effects on intramuscular fat content in Chinese indigenous cattle, Gene 700 (2019) 47–51.

[8] S.H.A. Raza, R. Khan, N.M. Schreurs, H. Guo, L.S. Gui, C. Mei, L. Zan, Expression of the bovine KLF6 gene polymorphisms and their association with carcass and body measures in Qinchuan cattle (Bos Taurus), Genomics 112 (2020) 423–431.

[9] H. Guo, S.H.A. Raza, N.M. Schreurs, R. Khan, D. Wei, L. Wang, S. Zhang, L. Zhang, S. Wu, I. Ullah, S.M. Hosseini, L. Zan, Genetic variants in the promoter region of the KLF3 gene associated with fat deposition in Qinchuan cattle, Gene 672 (2018) 50–55.

[10] S. Wu, Y. Wang, Y. Ning, H. Guo, X. Wang, L. Zhang, R. Khan, G. Cheng, H. Wang, L. Zan, Genetic variants in STAT3 promoter regions and their application in molecular breeding for body size traits in Qinchuan cattle, Int. J. Mol. Sci. 19 (2018).

[11] D. Wei, S.H.A. Raza, J. Zhang, L. Gui, S.U. Rahman, R. Khan, S.M. Hosseini, H.A. Kaleri, L. Zan, Polymorphism in promoter of SIX4 gene shows association with its transcription and body measurement traits in Qinchuan cattle, Gene 656 (2018) 9–16.

[12] S.H.A. Raza, L. Gui, R. Khan, N.M. Schreurs, W. Xiaoyu, S. Wu, C. Mei, L. Wang, X. Ma, D. Wei, H. Guo, S. Zhang, X. Wang, H.A. Kaleri, L. Zan, Association between FASN gene polymorphisms ultrasound carcass traits and intramuscular fat in Qinchuan cattle, Gene 645 (2018) 55–59.

[13] L. Shijun, R. Khan, S.H.A. Raza, H. Jieyun, M. Chugang, N. Kaster, C. Gong, Z. Chunping, N.M. Schreurs, Z. Linsen, Function and characterization of the promoter region of perilipin 1 (PLIN1): roles of E2F1, PLAG1, C/EBPbeta, and SMAD3 in bovine adipocytes, Genomics (2020).

[14] H. Weintraub, R. Davis, S. Tapscott, M. Thayer, M. Krause, R. Benezra, T.K. Blackwell, D. Turner, R. Rupp, S. Hollenberg, The myoD gene family: nodal point during specification of the muscle cell lineage, Sci 251 (1991) 761–766.

[15] K. Singh, F.J. Dilworth, Differential modulation of cell cycle progression distinguishes members of the myogenic regulatory factor family of transcription factors, FEBS J. 280 (2013) 3991–4003.

[16] J. Wang, Y. Hu, M.A. Elzo, Y. Shi, X. Jia, S. Chen, S. Lai, Genetic effect of Myf5 gene in rabbit meat quality traits, Genet 96 (2017) 673–679.

[17] Z.Z. Yin, X.Y. Dong, D.J. Dong, Y.Z. Ma, Association of MYF5 and KLF15 gene polymorphisms with carcass traits in domestic pigeons (Columba livia), Br. Poult. Sci. 57 (2016) 612–618.

[18] M. Liu, J. Peng, D.Q. Xu, R. Zheng, F.E. Li, J.L. Li, B. Zuo, M.G. Lei, Y.Z. Xiong, C.Y. Deng, S.W. Jiang, Association of MYF5 and MYOD1 gene polymorphisms and meat quality traits in large white x Meishan F2 pig populations, Biochem. Genet. 46 (2008) 720–732.

[19] C. Li, J. Basarab, W. Snelling, B. Benkel, B. Murdoch, C. Hansen, S. Moore, Assessment of positional candidate genes myf 5 and igf 1 for growth on bovine chromosome 5 in commercial lines of Bos taurus, J. Anim. Sci. 82 (2004) 1–7.

[20] R. Zhang, H. Chen, C. Lei, C. Zhang, X. Lan, Y. Zhang, H. Zhang, B. Bao, H. Niu, X. Wang, Association between polymorphisms of MSTN and MYF5 genes and growth traits in three Chinese cattle breeds, Asian Aust. J. Anim. Sci. 20 (2007) 1798–1804.

[21] F. Sarti, E. Lasagna, S. Ceccobelli, P. Di Lorenzo, F. Filippini, F. Sbarra, A. Giontella, C. Pieramati, F. Panella, Influence of single nucleotide polymorphisms in the myostatin and myogenic factor 5 muscle growth-related genes on the performance traits of Marchigiana beef cattle, J. Anim. Sci. 92 (2014) 3804–3810.

[22] W. Kim, E. Chung, Association of SNP marker in IGF-I and MYF5 candidate genes with growth traits in Korean cattle, Asian Aust. J. Anim. Sci. 20 (2007) 1061–1065.

[23] M. Bhuiyan, N. Kim, Y. Cho, D. Yoon, K.S. Kim, J. Jeon, J. Lee, Identification of SNPs in MYOD gene family and their associations with carcass traits in cattle, Livest. Sci. 126 (2009) 292–297.

[24] J. Ujan, L. Zan, S. Ujan, C. Adoligbe, H.J.G. Wang, M. Research, Back fat thickness and meat tenderness are associated with a 526 T→ a mutation in the exon 1 promoter region of the MyF-5 gene in Chinese Bos taurus, Genet. Mol. Res. 10 (2011) 3070–3079.

[25] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, MEGA X: molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (2018) 1547–1549.

[26] Z. Junjvlieke, R. Khan, C. Mei, G. Cheng, S. Wang, S.H.A. Raza, J. Hong, X. Wang, W. Yang, L. Zan, Effect of ELOVL6 on the lipid metabolism of bovine adipocytes, Genomics 12 (2019) 981.

[27] C. Liang, A. Li, S.H.A. Raza, R. Khan, X. Wang, S. Wang, G. Wang, Y. Zhang, L. Zan, The molecular characteristics of the FAM13A gene and the role of transcription factors ACSL1 and ASCL2 in its core promoter region, Genes 10 (2019).

[28] T.L. Bailey, M. Boden, F.A. Buske, M. Frith, C.E. Grant, L. Clementi, J. Ren, W.W. Li, W.S. Noble, MEME SUITE: tools for motif discovery and searching, Nucleic Acids Res. 37 (2009) W202–W208.

[29] R. Khan, S.H.A. Raza, N. Schreurs, W. Xiaoyu, W. Hongbao, I. Ullah, A. Rahman, S.M. Suhail, S. Khan, Z. Linsen, Bioinformatics analysis and transcriptional regulation of TORC1 gene through transcription factors NRF1 and Smad3 in bovine preadipocytes, Genomics 112 (2020) 1575–1587.

[30] A. Marchler-Bauer, Y. Bo, L. Han, J. He, C.J. Lanczycki, S. Lu, F. Chitsaz, M.K. Derbyshire, R.C. Geer, N.R. Gonzales, M. Gwadz, D.I. Hurwitz, F. Lu, G.H. Marchler, J.S. Song, N. Thanki, Z. Wang, R.A. Yamashita, D. Zhang, C. Zheng, L.Y. Geer, S.H. Bryant, CDD/SPARCLE: functional classification of proteins via subfamily domain architectures, Nucleic Acids Res. 45 (2017) D200–d203.

[31] X. Wang, R. Khan, S.H.A. Raza, A. Li, Y. Zhang, C. Liang, W. Yang, S. Wu, L. Zan, Molecular characterization of ABHD5 gene promoter in intramuscular preadipocytes of Qinchuan cattle: roles of Evi1 and C/EBPalpha, Gene 690 (2019) 38–47.

[32] L.S. Gui, S.H.A. Raza, M. Garcia, Y.G. Sun, I. Ullah, Y.C. Han, Genetic variants in the SIRT6 transcriptional regulatory region affect gene activity and carcass quality traits in indigenous Chinese beef cattle (Bos taurus), BMC Genomics 19 (2018) 785.

[33] L.S. Gui, Y.R. Zhang, G.Y. Liu, L.S. Zan, Expression of the SIRT2 gene and its relationship with body size traits in Qinchuan cattle (Bos taurus), Int. J. Mol. Sci. 16 (2015) 2458–2471.

[34] G. Rincon, E.A. Farber, C.R. Farber, J.D. Nkrumah, J.F. Medrano, Polymorphisms in the STAT6 gene and their association with carcass traits in feedlot cattle, Anim. Genet. 40 (2009) 878–882.

[35] L. Gui, J. Hong, S.H. Raza, L. Zan, Genetic variants in SIRT3 transcriptional regulatory region affect promoter activity and fat deposition in three cattle breeds, Mol. Cell. Probes 32 (2017) 40–45.

[36] S. Wu, Y. Ning, S.H.A. Raza, C. Zhang, L. Zhang, G. Cheng, H. Wang, N. Schreurs, L. Zan, Genetic variants and haplotype combination in the bovine CRTC3 affected conformation traits in two Chinese native cattle breeds (Bos Taurus), Genomics 111 (2019) 1736–1744.

[37] M. Nei, A.K. Roychoudhury, Sampling variances of heterozygosity and genetic distance, Genet. 76 (1974) 379–390.

[38] P. Sun, R. Zhang, Y. Jiang, X. Wang, J. Li, H. Lv, G. Tang, X. Guo, X. Meng, H. Zhang, R. Zhang, Assessing the patterns of linkage disequilibrium in genic regions of the human genome, FEBS J. 278 (2011) 3748–3755.

[39] M.E. Abd El-Hack, S.A. Abdelnour, A.A. Swelum, M. Arif, The application of gene marker-assisted selection and proteomics for the best meat quality criteria and body measurements in Qinchuan cattle breed, Mol. Biol. Rep. 45 (2018) 1445–1456.

[40] G.Y. Hou, H.L. Zhou, T. Cao, W.J. Xun, D.J. Wang, L.G. Shi, S. Guan, D.F. Wang, M. Li, Expression and variation of Myf5 and MyoD1 genes in different tissues of Wuzhishan pigs, Genet. Mol. Res. 14 (2015) 3729–3735.

[41] C. Lisa, A. Albera, P. Carnier, L.D. Stasio, Variability in candidate genes revealed associations with meat traits in the Piemontese cattle breed, Ital. J. Anim. Sci. 12 (2013) e46.

[42] T.A. Greenwood, J.R. Kelsoe, Promoter and intronic variants affect the transcriptional regulation of the human dopamine transporter gene, Genomics 82 (2003) 511–520.

[43] E.A. Lee, J.M. Kim, K.S. Lim, Y.C. Ryu, W.M. Jeon, K.C. Hong, Effects of variation in porcine MYOD1 gene on muscle fiber characteristics, lean meat production, and meat quality traits, Meat Sci. 92 (2012) 36–43.

[44] J.M. Kim, B.D. Choi, B.C. Kim, S.S. Park, K.C. Hong, Associations of the variation in the porcine myogenin gene with muscle fibre characteristics, lean meat production and meat quality traits, J. Anim. Breed. Genet. 126 (2009) 134–141.

[45] J. Verner, P. Humpolicek, A. Knoll, Impact of MYOD family genes on pork traits in large white and landrace pigs, J. Anim. Breed. Genet. 124 (2007) 81–85.

[46] C. Ernst, M. Davis, Characterization of Regulatory Genes Involved in the Development of Porcine Muscle, from the Research Investment Report, The Ohio State University, Ohio, 1995.

[47] Y.C. Ryu, E.A. Lee, H.H. Chai, J.E. Park, J.M. Kim, Effects of a novel p.A41P mutation in the swine myogenic factor 5(MYF5) gene on protein stabilizing, muscle fiber characteristics and meat quality, Korean J. Food Sci. Anim. Resour. 38 (2018) 711–717.

[48] D. Cieslak, J. Kuryl, W. Kapelanski, M. Pierzchala, S. Grajewska, M. Bocian,

Relationship between Genotypes at at MYOG, MYF3 and MYF5 Loci and Carcass Meat and Fat Deposition Traits in Pigs, Animal Science Reports (2002).
[49] M.F. Te Pas, F.L. Harders, A. Soumillion, L. Born, W. Buist, T.H. Meuwissen, Genetic variation at the porcine MYF-5 gene locus. Lack Of association with meat production traits, Mamm. Genome 10 (1999) 123–127.
[50] D. Robakowska-Hyzorek, J. Oprzadek, B. Zelazowska, R. Olbromski, L. Zwierzchowski, Effect of the g.-723G– > T polymorphism in the bovine myogenic factor 5 (Myf5) gene promoter region on gene transcript level in the longissimus dorsi muscle and on meat traits of polish Holstein-Friesian cattle, Biochem. Genet. 48 (2010) 450–464.
[51] S.H.A. Raza, W. Khan, L. Gui, N.M. Schreurs, X. Wang, C. Mei, X. Yang, C. Gong, L. Zan, Bioinformatics analysis and genetic polymorphisms in genomic region of the bovine SH2B2 gene and their associations with molecular breeding for body size traits in qinchuan beef cattle, Biosci. Rep. 48 (2020) 5–6.
[52] S.H.A. Raza, N. Kaster, R. Khan, S.A. Abdelnour, M.E.A. El-Hack, A.F. Khafaga, A. Taha, H. Ohran, A.A. Swelum, N.M. Schreurs, The Role of MicroRNAs in Muscle Tissue Development in Beef Cattle, Genes (Basel) 11 (2020) 295.