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Haplotypes of bovine *FoxO1* gene sequence variants and association with growth traits in Qinchuan cattle

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Introduction

The winged helix or forkhead box (Fox) class of transcription factors constitutes a family of structurally related transcriptional activators that have been identified in species ranging from yeast to human. The first member of this transcription factor class was identified as a nuclear homeotic gene involved in embryonic development in *Drosophila melanogaster* (Weigel *et al.* 1989).

The FoxO family of forkhead transcription factors represents a subfamily within the larger group of Fox transcription factors. Mammalian FoxO proteins (FoxO1, FoxO3a, FoxO4 and FoxO6), which are homologous to *Caenorhabditis elegans* protein DAF-16, belong to the O ('other') class of the Fox superfamily (Kaestner *et al.* 2000; Barthel *et al.* 2005). As transcription factors in the nucleus, the primary function of FoxO proteins is to bind to their cognate DNA target sequences as monomers. The cocrystal structure of another Fox protein, HNF-3γ, with DNA shows that there are 14 protein–DNA contacts distributed throughout the forkhead domain, but the third α-helix (H3) plays the most important role in a winged helix/forkhead protein's DNA-binding specificity (Clark *et al.* 1993). In addition, both winged loops also make important interactions with DNA (Clark *et al.* 1993; Boura *et al.* 2007). Although the molecular basis of the DNA-binding specificity of FoxO transcription factors is poorly understood, high-affinity DNA-binding studies have identified a consensus FoxO-recognized element (FRE), (G/C) (T/A)AA(C/T)AA (Biggs Iii *et al.* 1999; Furuyama *et al.* 2000; Gilley *et al.* 2003). FoxO transcription factors appear to be involved in various signalling pathways and control a wide range of biochemical processes including cellular differentiation, tumour suppression, metabolism, cell-cycle arrest, cell death, and protection from stress (Barthel *et al.* 2005; Accili and Arden 2004; Greer and Brunet 2005). Several *in vitro* overexpression studies have suggested that the genes that encode FoxO transcription factors play important roles in several biological processes such as control of the cell cycle, apoptosis and stress response, and some shared downstream transcriptional targets have been identified (Burgering and Medema 2003; Tran *et al.* 2003). The work by Tothova *et al.* (2007) implicates FoxOs as important mediators of the cellular response to oxidative stress, which is involved in the etiology of many human diseases. Recently, it was shown that inhibition of endogenous FoxO proteins attenuated TPA/PDGF-BB-mediated (12-O-tetradecsonyl phorbol-13-acetate / platelet-derived growth factor β-chain dimer) differentiation of neuroblastoma cells (Mei *et al.* 2012). These findings define the FoxO–PDGFRA (PDGF receptor alpha polypeptide) axis as a crucial mechanistic component that governs TPA-induced neuroblastoma differentiation (Mei *et al.* 2012).

FoxO1 is the first identified member of the FoxO family of transcription factors and is involved in transcriptional activity in alveolar rhabdomyosarcomas (Fredericks *et al.* 1995). The *FoxO1* protein shuttles between the nucleus and cytoplasm, and is a widely distributed transcription factor with functional diversity. As a negative regulator, it is involved in differentiation of skeletal muscle and expression of muscle fibre type 1 related gene. Expression of constitutively active *FoxO1* results in decreased myoblast differentiation (Hribal *et al.* 2003) and reduced muscle mass in transgenic mice (Kamei *et al.* 2004). There are reports that FoxO1 is an early

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molecular regulator in differentiation of mesenchymal cells into osteoblasts (Teixeira *et al.* 2010) and plays an important role in integration of hormone-activated signalling pathways with the complex transcriptional cascade that promotes adipocyte differentiation (Nakae *et al.* 2003). A recent study showed that FOXO1 is a direct target of progestin, implicating novel molecular mechanisms of progestin in inhibition of endometrial neoplasia (Kyo *et al.* 2011). In view of these roles, *FoxO1* could be a key candidate in development and metabolism of muscle and adipose tissue.

Bovine *FoxO1* gene is located on chromosome 12, and contains three exons and two introns, and encodes 659 amino acids. However, polymorphisms in and function of bovine *FoxO1* have not been elucidated. The objective of this study was to identify single-nucleotide polymorphisms (SNP) in bovine *FoxO1* using DNA sequencing, PCR-RFLP and PCR - forced RFLP analyses, and to carry out haplotype analysis and identify associations between mutations of bovine *FoxO1* and performance traits that could be useful in cattle breeding.

Qinchuan cattle are a draught breed belonging to the Huanghuai group and found in central Shaanxi in China. They are usually red, but individuals with yellow colouration are also found. They also exhibit a cervical hump. They are one of the most important local breeds in China because of their meat quality and appearance.

Materials and methods

Animal population, DNA extraction and data statistics

Blood samples were obtained from 488 healthy and unrelated female cattle from the breeding centre farm of Qinchuan cattle of Shaanxi province (Wuquan county, Yangling, Shaanxi province, China). Genomic DNA was extracted from 1 mL 2% heparin-treated blood samples and stored at −80°C following standard procedures (Sambrook and Russell 2001). The DNA content was estimated spectrophotometrically, and the genomic DNA was diluted to 50 ng/μL. All DNA samples were stored at −20°C for subsequent analysis. Data on growth traits (wither height, height at hip cross, body length, heart girth, chest breadth, chest depth, rump length, hucklebone width, hip width and body mass) of 197 individuals were recorded at 24 months of age and used for association analysis.

Primer design, PCR amplification, and DNA sequencing

Based on the sequence of bovine *FoxO1* (GenBank accession number: NC007310.5), five pairs of primers were designed (table 1) for PCR amplification of *FoxO1* from cattle genomic DNA. Each amplification reaction was carried out in a 25-μL mixture containing 50 ng genomic DNA, 1 μM of each primer, 1× buffer (including 1.5 mM $MgCl_2$), 200 μM dNTPs (dATP, dTTP, dGTP and dCTP), and 0.6 U of *Taq* DNA polymerase (MBI Fermentas, Vilnius, Lithuania). PCR reactions were carried out using a Touchdown PCR System Thermal Cycler Dice (TaKaRa, Dalian, China). The Touchdown PCR protocol consisted of two phases: phase 1, included an initial step at 95°C for 4 min, followed by 20 cycles of denaturation at 94°C for 30 s, annealing at variable temperatures for 30 s, and extension at 72°C for 1 min. In the first cycle, the annealing temperature was set to 70°C, and at each of the 19 subsequent cycles the annealing temperature was decreased by 1°C (i.e. it varied from 70°C to 50°C at 1°C decrements along the 20 cycles). Phase 2 consist of 20 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min. The final extension was performed at 72°C for 10 min, after the last PCR cycle, the samples were cooled to 4°C. PCR products amplified from genomic DNA were directly sequenced using ABI 3730xl DNA Sequencer (Applied Biosystems, Foster City, USA) and BigDye Terminator Sequencing kit (Nanjing GenScript Biotech, Nanjing, China), and the results were analysed by Dnaman software: (v. 5.2.2) (http://www.lynnon.com/).

PCR-RFLP and PCR - forced RFLP

PCR primers were redesigned to facilitate genotyping of animals for the five SNPs found using PCR, PCR-RFLP, and PCR - forced RFLP, and 3% agarose gel electrophoresis.

Table 1. Primers used for PCR amplification of bovine *FoxO1* gene in Qinchuan cattle.

Primer	Primer sequence	Length, location
P1	1F: 5′-GAGCCGTGAAGTTAAGTTCTG-3′ 1R: 5′-TAGGGCACGCTCTTGACCATC-3′	876 bp/exon 1
P2	2F: 5′-AGTTTAGCCAGTCCAACTCGG-3′ 2R: 5′-CACTTTCAGGCCAAGCGAACT-3′	729 bp/exon 2
P3	3F: 5′-ATCGCAAATCTAAGTGGTT-3′ 3R: 5′-CTCAGGGTTACTGATCTCG-3′	799 bp/exon 3
P4	4F: 5′-GAGGGAGGCAAGAGTGG-3′ 4R: 5′-TGTCGTTGTGCGGAGG-3′	700 bp/exon3
P5	5F: 5′-ATGACGGAGCAGGACG-3′ 5R: 5′-CTGGAAGATACAAGGCAAGT-3′	1076 bp/ exon3

Table 2. Primers used for PCR - forced RFLP analysis of bovine *FoxO1* gene in Qinchuan cattle.

SNP	Amino acid change	Primer sequence	Restriction enzyme and fragment sizes (bp)
A176183G	–	1F: 5′-GACACTGTTTTTAAGATACAGTAGT-3′	*Sca*I
		1R: 5′-GCTTTTTCCAGTTCCTTCATTC-3′	272, 247, 25
C1071T	Ala357Ala	2F: 5′-GGATCGCAGTTTTCCAAGTG-3′	*Pst*I
		2R: 5′-AGACTGGGCAGAGTAGAAGCCATCTCTG-3′	221, 195, 26
G1200A	Pro400Pro	3F: 5′-AACCTTCTCTCGTCACCAAC-3′	*Sma*I
		3R: 5′-TGTCGTTGTGCGGAGG-3′	289, 239, 50
C1245A	Pro415Pro	4F: 5′-AGACGCCCTGCTACTCCTTTGCACGGC-3′	*Bmg*T120I
		4R: 5′-GCCCATCAACACGCTCTGGCCAAG-3′	293, 267, 26
G1732A	Ala578Thr	5F: 5′-GACTCTCCTCCGCACAACGAC-3′	*Hha*I
		5R: 5′-GTCCAAGTCACTGGGGAGCTTC-3′	405, 316, 89

Fragments of bovine *FoxO1* were amplified by the five pairs of primers. SNPs SG1200A and G1732A were genotyped by PCR-RFLP, and SNPs A176183G, C1071T and C1245A were genotyped by the PCR - forced RFLP. Primers, restriction enzymes (TaKaRa, Dalian, China), and fragment sizes are given in table 2. For forced RFLP, specifically modified primers for each of the five SNPs were used so that the PCR product in each case had an internal restriction enzyme site in one allelic sequence and not the other. To detect the five SNPs (A176183G, C1071T, G1200A, C1245A and G1732A) aliquots of 10 μL of PCR products were digested with 10 U of *Sca*I, *Pst*I, *Sma*I, *Bmg*T120I and *Hha*I (TaKaRa, Dalian, China) for 8 h at 37°C, 37°C, 30°C, 37°C and 37°C, respectively. The digested products were detected after electrophoresis at 130 V constant voltage for 0.5–1 h in 3.0% agarose gel by staining with 200 ng/mL ethidium bromide.

Statistical analysis

Gene frequencies were determined by direct counting, and Hardy–Weinberg equilibrium (HWE) was tested for based on the basis of likelihood ratio for different locus–population combinations using POPGENE software (v. 3.2). Gene heterozygosity (H_e), gene homozygosity (H_o), ($H_o + H_e = 1$), effective number of alleles n_e; reciprocal of homozygosity and polymorphism information content (PIC) were computed using POPGENE. Linkage disequilibrium (LD), as measured by $D\prime$ and r^2, and haplotypes were obtained for 488 animals using the online SHEsis software (http://analysis2.bio-x.cn/myAnalysis.php) (Shi and He 2005). Association analysis between single SNP marker genotypes and growth traits was performed by the least squares method as applied in the general linear models procedure of SPSS (v. 17.0) software. The reduced linear model included fixed effects of age and genotype, and was stated as

$$Y_{ijk} = \mu + A_i + G_j + E_{ijk},$$

where Y_{ijk} is the trait measured on each animal k in each combination of age i and genotypes j; μ is the overall population mean, A_i is the fixed effect due to age i, G_j is the fixed effect associated with genotype j, and E_{ijk} is the random error.

Results and discussion

Sequence variants identified in bovine FoxO1

In the present study, genomic DNA of all cattle was successfully amplified using primer pairs for bovine *FoxO1*. We amplified and sequenced all introns and exons and flanking regions of *FoxO1*; DNA samples of individual animals were selected randomly by comparing with a previously reported sequence (GenBank accession number: NC007310.5), five SNPs were located: A176183G (intron 2), C1071T (exon 3), G1200A (exon 3), C1245A (exon 3) and G1732A (exon 3). C1071T, G1200A and C1245A were synonymous mutations, and G1732A resulted in a missense mutation (Ala578Thr).

The five SNPs were genotyped using PCR-RFLP and PCR - forced RFLP. The mutations were detected in bovine *FoxO1* by using *Sca*I, *Pst*I, *Sma*I, *Bmg*T120I and *Hha*I, respectively, in the forced RFLP procedure for A176183G digestion of the 272-bp PCR product with *Sca*I resulted in fragment lengths of 272 bp for genotype GG (homozygous); and 272 bp, 247 bp and 25 bp for genotype AG (heterozygous) (figure 1a). Genotype and allele frequencies were calculated (table 3). The frequency of allele G showed high prevalence in the population (81%) and the GG genotype (63%) was more frequent than the other genotypes (table 3). The χ^2 test showed that the genotypic frequencies were not in agreement with HWE ($P < 0.01$). Homozygous AA was not detected in our sample. This phenomenon showed that the frequency of allele A may present a decreasing trend due to artificial selection, migration, and genetic drift. Perhaps, through natural selection, individuals with genotype AA are eliminated, causing a decline in the number of A alleles. For C1071T, digestion of the 221-bp PCR product with *Pst*I resulted in fragment lengths of 221 bp for genotype CC; 221 bp, 195 bp and 26 bp for genotype CT; and 195 bp and 26 bp for genotype TT (figure 1b). The frequency of allele C showed high prevalence in the population and the CC genotype (69%) was more frequent than the other genotypes (table 3). The χ^2 test showed that the genotypic distribution was not in agreement with HWE ($P < 0.05$). For G1200A, digestion of the 289-bp PCR product with *Sma*I resulted in fragment lengths of 239 bp and 50 bp for genotype GG; 289 bp, 239 bp and

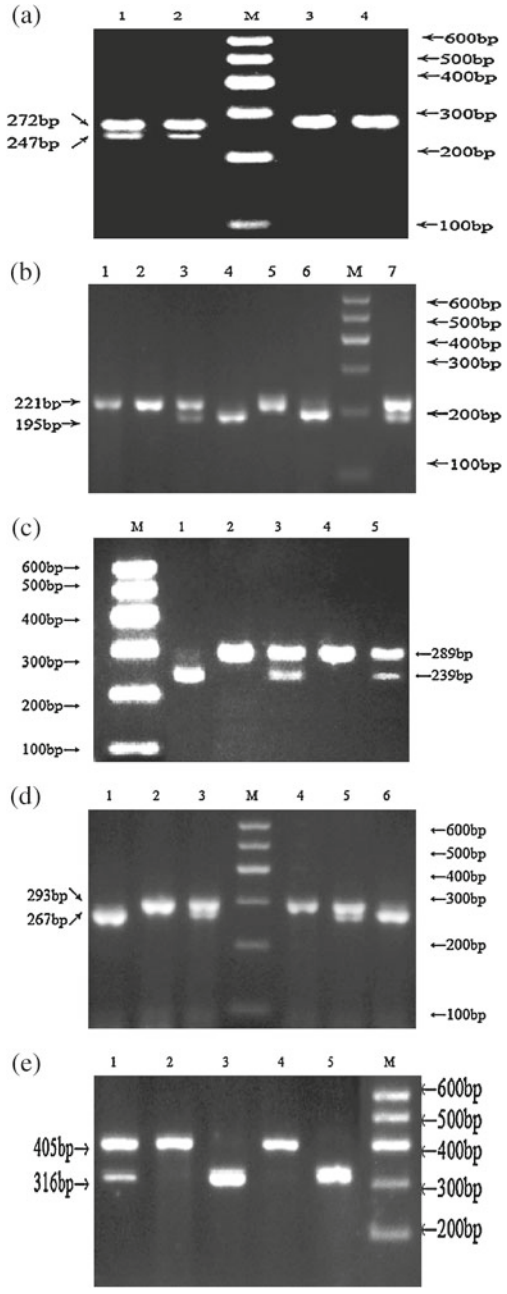

Figure 1. Electrophoretic patterns on 3.0% agarose after digestion with (a) *Sca*I (b) *Pst*I (c) *Sma*I (d) *Bmg*T120I or (e) *Hha*I endonuclease of PCR product containing (a) A176183G, (b) C1071T, (c) G1200A, (d) C1245A or (e) G1732A mutation of bovine *FoxO1* gene, respectively. Lane M, marker. (a) Lanes 1 and 2, genotype AG (272 + 247 + 25 bp; 25 bp fragment was too small to stay in gel); lanes 3 and 4, genotype GG (272 bp). (b) Lanes 3 and 7, genotype CT (221+195+26 bp; 26 bp fragment was too small to stay in gel); lanes 1, 2 and 5, genotype CC (221 bp); lanes 4 and 6, genotype TT (195 + 26 bp). (c) Lanes 3 and 5, genotype GA (289 + 239 + 50 bp; 50 bp fragment was too small to stay in gel); lane 1, genotype GG (239 + 50 bp); lanes 2 and 4, genotype AA (289 bp). (d) Lanes 3 and 5, genotype CA (293 + 267 + 26 bp; 26 bp fragment was too small to stay in gel); lanes 1 and 6, genotype CC (267 + 26 bp); lanes 2 and 4, genotype AA (293 bp). (e) Lane 1, genotype GA (405 + 316 + 89 bp; 89 bp fragment was too small to stay in gel); lanes 3 and 5, genotype GG (316+89 bp); lanes 2 and 4, genotype AA (405 bp).

50 bp for genotype GA; and 289 bp for genotype AA (figure 1c). The frequency of allele A showed high prevalence in the population and the AA genotype (60%) was more frequent than the other genotypes (table 3). The χ^2 test showed that the genotypic distributions was in agreement with HWE ($P > 0.05$). For C1245A, digestion of the 293-bp PCR product with *Bmg*T120I resulted in fragment lengths of 267 bp and 26 bp for genotype CC; 293 bp, 267 bp and 26 bp for genotype CA; and 293 bp for genotype AA (figure 1d). The frequency of allele C showed high prevalence in the population and the CC genotype (44%) was more frequent than the other genotypes (table 3). The χ^2 test showed that the genotypic distribution was in agreement with HWE ($P > 0.05$). For G1732A, digestion of the 405-bp PCR product with *Hha*I resulted in fragment lengths of 316 bp and 89 bp for genotype GG; 405 bp, 316 bp and 89 bp for genotype GA; and 405 bp for genotype AA (figure 1e). The frequency of allele G showed high prevalence in the population and the GG genotype (48%) was more frequent than the other genotypes (table 3). The χ^2 test showed that the genotypic distribution was in agreement with HWE ($P > 0.05$).

Genetic diversity analysis of bovine FoxO1 in Qinchuan cattle

Genetic indices H_o, H_e, N_e, and PIC are also given in table 3. H_o (gene homozygosity) is above 0.5 for all five SNPs. Effective number of alleles approaches 2. The highest and lowest PIC values are 0.355 and 0.235. According to the classification of PIC (PIC value < 0.25, low polymorphism; 0.25 < PIC value < 0.5, intermediate polymorphism; PIC value > 0.5, high polymorphism), the Qinchuan cattle population have intermediate genetic diversity in *FoxO1*. Genetic diversity is essential for species preservation and improvement of production in selected breeds.

Linkage disequilibrium and haplotype analysis of bovine FoxO1 gene in Qinchuan cattle

Table 4 shows the D' and r^2 values. The r^2-values above 0.33 might indicate sufficiently strong LD to be useful for mapping. The estimated D' values among the five SNPs ranged from 0.189 to 0.999; the r^2 values ranged from 0.013 to 0.458; the mean r^2 between any two SNPs was 0.1547. SNP pairs C1071T and G1200A, and G1200A and C1245A show strong LD; LD is low for other pairs (table 4).

Haplotype analysis of the five SNPs using SHEsis showed that 24 different haplotypes could be identified for *FoxO1*. Table 5 shows seven haplotypes whose frequency was >0.03 (haplotypes with frequency <0.03 were ignored). Hap5 (G C A C G) shows the highest haplotype frequency (28.40%). The high-frequency haplotypes have probably been present in the population for a long time. Consequently, most new mutants are derived from common haplotypes, implying that rarer variants represent more recent mutations and are more likely to be related to common haplotypes than to other rare variants (Posada and Crandall 2001).

Table 3. Genotype frequencies and genetic diversity parameters of SNPs in bovine *FoxO1* in Qinchuan cattle.

SNP	Genotype frequencies			Allele frequencies		χ^2 (HWE)	Diversity parameter			
							H_o	H_e	n_e	PIC
A176183G	AA	AG	GG	A	G	18.980	0.695	0.305	1.440	0.259
	0.00	0.37	0.63	0.19	0.81					
C1071T	CC	CT	TT	C	T	6.653	0.729	0.271	1.372	0.235
	0.69	0.30	0.01	0.84	0.16					
G1200A	GG	GA	AA	G	A	3.498	0.66	0.340	1.516	0.282
	0.03	0.37	0.60	0.22	0.78					
C1245A	CC	CA	AA	C	A	5.845	0.539	0.461	1.855	0.355
	0.44	0.41	0.16	0.64	0.36					
G1732A	GG	GA	AA	G	A	0.272	0.575	0.425	1.739	0.335
	0.48	0.44	0.09	0.69	0.31					

χ^2(HWE), Hardy–Weinberg equilibrium χ^2 value; H_o, gene homozygosity; H_e, gene heterozygosity; n_e, effective number of allele; PIC, polymorphism information content.

Association analysis of single SNP markers

The results of the association analysis between *Fox01* SNP and growth traits are shown in table 6. We analysed associations of genotypes of five SNPs in *Fox01* with (W24) growth traits in Qinchuan cattle ($n = 197$). Animals with genotype AA at SNP A176183G had significantly greater body length, chest breadth and chest depth than those with genotypes AG and GG ($P < 0.05$). Genotypes at SNP C1071T did not show significant association with growth traits. Animals with genotypes AA and GA at SNP G1200A had significantly greater rump length than those with genotype GG ($P < 0.05$), suggesting that allele *Sma*I-A at G1200A might be correlated with greater rump length in Qinchuan cattle. Animals with genotypes CA and CC at SNP C1245A had significantly greater chest breadth and chest depth than those with genotype AA ($P < 0.01$ or $P < 0.05$), suggesting that allele C at C1245A might be associated with greater chest breadth and chest depth in Qinchuan cattle. Finally, G1732A is a missense mutation located in exon 3 of bovine *FoxO1*, which may affect translation efficiency, thereby altering the function of FoxO1 protein. Animals with genotypes GA and GG at SNP G1732A had significantly greater withers height, body length, hip width and rump length than those with genotype *Hha*I-AA ($P < 0.01$ or $P < 0.05$), suggesting that allele G at G1732A might be associated with higher measures of these traits in Qinchuan cattle.

Table 4. Estimated values of linkage disequilibrium for SNPs bovine *FoxO1* Qinchuan cattle.

SNP	A176183G	C1071T	G1200A	C1245A	G1732A
A176183G	–	$D' = 0.956$	$D' = 0.865$	$D' = 0.313$	$D' = 0.189$
C1071T	$r^2 = 0.041$	–	$D' = 0.804$	$D' = 0.831$	$D' = 0.999$
G1200A	$r^2 = 0.046$	$r^2 = 0.458$	–	$D' = 0.917$	$D' = 0.999$
C1245A	$r^2 = 0.013$	$r^2 = 0.231$	$r^2 = 0.414$	–	$D' = 0.687$
G1732A	$r^2 = 0.020$	$r^2 = 0.085$	$r^2 = 0.121$	$r^2 = 0.118$	–

Table 5. Haplotypes of *FoxO1* gene and their frequencies in Qinchuan cattle.

Haplotype	A176183G	C1071T	G1200A	C1245A	G1732A	Frequency
1	A	C	A	C	A	0.063
2	A	C	A	C	G	0.071
3	G	C	A	A	G	0.086
4	G	C	A	C	A	0.209
5	G	C	A	C	G	0.284
6	G	C	G	A	G	0.058
7	G	T	G	A	G	0.127

Haplotypes with frequency <0.03 have been ignored.

Table 6. Association of different genotypes of SNPs in *Fox01* with growth traits in Qinchuan cattle.

	Growth trait	Measures (mean±SE)			
SNP	Genotype	AA	AG	GG	*P*
A176183G (*Sca*I)	Withers heights (cm)	130.000 ± 4.065	128.794 ± 0.697	128.488 ± 0.510	0.885
	Height at hip cross (cm)	129.000 ± 5.390	127.000 ± 0.924	125.839 ± 0.676	0.527
	Body length (cm)	150.000^{a} ± 7.228	136.426^{b} ± 1.240	134.012^{b} ± 0.907	0.035
	Heart girth (cm)	180.000 ± 6.966	175.757 ± 1.195	174.622 ± 0.874	0.579
	Chest breadth (cm)	43.000^{a} ± 2.879	36.706^{b} ± 0.494	37.610^{b} ± 0.361	0.050
	Chest depth (cm)	65.000^{a} ± 3.387	60.684^{b} ± 0.581	62.713^{ab} ± 0.425	0.014
	Rump length (cm)	45.000 ± 2.292	44.154 ± 0.393	43.051 ± 0.288	0.063
	Hucklebone width (cm)	27.000 ± 2.604	22.265 ± 0.447	22.882 ± 0.327	0.139
	Hip width (cm)	38.000 ± 2.539	42.103 ± 0.435	41.559 ± 0319	0.207
	Body mass (kg)	450.000 ± 44.619	392.672 ± 7.652	381.542 ± 5.599	0.181
	Genotype	CC	CT	TT	
C1071T (*Pst*I)	Withers heights (cm)	128.937 ± 0.480	127.852 ± 0.778	123.000 ± 5.718	0.307
	Height at hip cross (cm)	126.937 ± 0.634	124.694 ± 1.028	117.000 ± 7.552	0.086
	Body length (cm)	135.335 ± 0.872	134.148 ± 1.413	135.000 ± 10.387	0.775
	Heart girth (cm)	175.518 ± 0.825	174.074 ± 1.338	165.000 ± 9.832	0.389
	Chest breadth (cm)	37.415 ± 0.346	37.269 ± 0.561	33.000 ± 4.122	0.558
	Chest depth (cm)	62.204 ± 0.410	61.667 ± 0.664	58.000 ± 4.881	0.560
	Rump length (cm)	43.514 ± 0.273	43.407 ± 0.443	37.000 ± 3.254	0.139
	Hucklebone width (cm)	22.968 ± 0.309	22.120 ± 0.502	18.000 ± 3.685	0.159
	Hip width (cm)	41.655 ± 0.301	41.981 ± 0.488	35.000 ± 3.585	0.149
	Body mass (kg)	388.877 ± 5.324	379.568 ± 8.633	340.313 ± 63.437	0.506
	Genotype	AA	GA	GG	
G1200A (*Sma*I)	Withers heights (cm)	129.190 ± 0.507	127.701 ± 0.695	125.500 ± 2.846	0.124
	Height at hip cross (cm)	127.175 ± 0.671	124.873 ± 0.920	121.250 ± 3.767	0.055
	Body length (cm)	135.210 ± 0.925	134.896 ± 1.268	130.500 ± 5.189	0.667
	Heart girth (cm)	175.861 ± 0.875	173.687 ± 1.200	173.250 ± 4.911	0.321
	Chest breadth (cm)	37.587 ± 0.367	36.933 ± 0.504	37.000 ± 2.061	0.569
	Chest depth (cm)	62.313 ± 0.435	61.530 ± 0.596	61.750 ± 2.441	0.566
	Rump length (cm)	43.651^{a} ± 0.287	43.328^{a} ± 0.394	39.250^{b} ± 1.613	0.027
	Hucklebone width (cm)	23.083 ± 0.328	22.037 ± 0.450	22.250 ± 1.843	0.169
	Hip width (cm)	41.778 ± 0.322	41.672 ± 0.441	40.250 ± 1.807	0.703
	Body mass (kg)	390.443 ± 5.645	379.096 ± 7.741	365.578 ± 31.681	0.40
	Genotype	AA	CA	CC	
C1245A (*Bmg*T120I)	Withers heights (cm)	128.318 ± 0.995	127.870± 0.688	129.247 ± 0.587	0.300
	Height at hip cross (cm)	125.606 ± 1.324	125.464 ± 0.916	127.089 ± 0.780	0.347
	Body length (cm)	135.545 ± 1.809	134.384 ± 1.251	135.274 ± 1.066	0.819
	Heart girth (cm)	173.015 ± 1.712	175.355 ± 1.184	175.574 ± 1.009	0.419
	Chest breadth (cm)	35.848^{B} ± 0.700	38.514^{A} ± 0.484	37.032^{AB} ± 0.413	0.005
	Chest depth (cm)	60.106^{b} ± 0.836	62.899^{a} ± 0.578	62.079^{a} ± 0.493	0.025
	Rump length (cm)	44.121 ± 0.568	42.949 ± 0.393	43.584 ± 0.335	0.206
	Hucklebone width (cm)	21.333 ± 0.639	23.029 ± 0.442	22.958 ± 0.376	0.063
	Hip width (cm)	42.485 ± 0.626	41.232 ± 0.433	41.789 ± 0.369	0.249
	Body mass (kg)	377.608 ± 11.060	386.199 ± 7.648	388.934 ± 6.518	0.678
	Genotype	AA	GA	GG	
G1732A (*Hha*I)	Withers heights (cm)	125.605^{b} ± 1.293	128.183^{a} ± 0.669	129.425^{a} ± 0.545	0.019
	Height at hip cross (cm)	123.605 ± 1.737	125.739 ± 0.898	127.098 ± 0.732	0.139
	Body length (cm)	129.105^{B} ± 2.324	133.923^{AB} ± 1.202	136.776^{A} ± 0.979	0.006
	Heart girth (cm)	171.368 ± 2.249	175.577 ± 1.164	175.388 ± 0.948	0.224
	Chest breadth (cm)	36.105 ± 0.940	37.937 ± 0.486	37.187 ± 0.396	0.188
	Chest depth (cm)	61.632 ± 1.123	62.134 ± 0.581	62.042 ± 0.473	0.924
	Rump length (cm)	41.632^{B} ± 0.736	43.148^{A} ± 0.381	43.977^{A} ± 0.310	0.009
	Hucklebone width (cm)	23.868 ± 0.843	23.176 ± 0.436	22.196 ± 0.355	0.080
	Hip width (cm)	40.632^{b} ± 0.818	41.155^{ab} ± 0.423	42.271^{a} ± 0.345	0.049
	Body mass (kg)	354.058 ± 14.390	386.057 ± 7.444	391.779 ± 6.064	0.056

Values for the same trait with different superscripts letters (along a line) differ significantly, with $P < 0.01$ (A, B) or $P < 0.05$ (a, b).

Previous studies have shown the structural and functional characterization of the *FoxO1* gene and its genomic localization on bovine chromosome 12 (*Bos Taurus*; BTA 12). The present study has isolated polymorphisms in the *FoxO1* gene that are associated with growth traits in Qinchuan cattle. These data strongly suggest that *FoxO1* polymorphisms might be used as genetic markers in breeding of new beef cattle. However, further research and validation of the various allelic effects, functional mechanisms, and biological activity are needed in an independent sample before the value of the identified *FoxO1* variants or others for marker-assisted selection can be established, especially if the mutations have negative consequences for the breed.

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