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Expression and Polymorphism Analysis of *CFL2* Gene in Chinese Dabieshan Cattle

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Authors' Contribution
YJ conceived and designed the entire experimental plan. SZ performed the experiment, did the statistical analysis and drafted the manuscript. LX and HJ participated in sample collection and growth trait measurements.

Key words
***CFL2* gene, Dabieshan (DBS) cattle, Polymorphism detection, Expression, Association analysis**

ABSTRACT

Cofilin 2 (*CFL2*) is essential for skeletal muscle development and maintenance through regulating the length of actin filaments. In this study, we aimed to identify common variations in *CFL2* gene and investigate their effects on growth traits in Chinese Dabieshan (DBS) cattle. By DNA sequencing and (forced) PCR-RFLP methods, three polymorphisms (g.1500G>A, g.1694T>A and g.2213C>G) were identified and genotyped in our population (n=298). Genetic diversity analysis showed that g.1500G>A and g.1694T>A belonged to an intermediate level of genetic diversity (0.25<PIC<0.5), and SNP g.2213C>G belonged to a low polymorphism level (PIC<0.25). LD (Linkage disequilibrium) analysis showed that SNP g.1694T>A and g.2213C>G had a strong linkage (r^2>0.33), a total of four different haplotypes were constructed and the frequency of the main haplotypes AAG accounted for over 61.2 % of the total individuals. Association analysis indicated that all of the three SNPs were significantly associated with growth traits in the detected population. Furthermore, real-time PCR indicated that *CFL2* mRNA was varied expressed in all studied tissues. The results of our study provide evidence that polymorphisms in *CFL2* gene are associated with growth traits, and *CFL2* gene could be utilized as molecular markers for future assisted selection in cattle breeding program.

INTRODUCTION

Dabieshan cattle are a precious local breed belonging to the central plains group and found in Anhui Province. They are usually red in hair, small in physique, strong in bones, well proportioned in development, and have the superiority such as crude feed tolerance, higher disease resistance and adaptation to environment.

Cofilins are part of the minimum set of proteins that are essential in embryonic development, health and disease. While it was first discovered in porcine brain (Nishida *et al*., 1984), many cofilin homologues have been characterized genetically and biochemically in various organisms including vertebrate, plant and protozoan system (Abe *et al*., 1990; McKim *et al*., 1994; Ono *et al*., 1994). With the role in remodelling the actin cytoskeleton, *CFL1* is enriched in sub-cellular locations that are associated with high actin turnover, specifically in neuronal axons and the contractile rings formed during the final stages of mitosis (Bamburg, 1999; Maciver *et al*., 2002; Vartiainen *et al*., 2002). *CFL2*, by contrast, is predominantly localized between the Z-discs in muscle sarcomeres, where it regulates the length of actin filaments (Kremneva *et al*., 2014).

Three isoforms: actin depolymerizing factor (ADF, also known as destrin), cofilin-1 (*CFL1*) and cofilin-2 (*CFL2*), were expressed in human and mice (Maciver *et al*., 2002). Further study indicated that *CFL1* was predominantly expressed in embryonic mouse striated muscle, while during subsequent muscle development, *CFL2* expression increases to become the predominant isoforms (Mohri *et al*., 2000). In human, the mutations of *CFL2* gene causing nemaline myopathy, indicating that it play a critical role in skeletal muscle function, including development and maintenance (Agrawal *et al*., 2007; Ong *et al*., 2014). Another study indicated that *CFL2* gene, although not critical for muscle development, is essential for muscle maintenance (Gurniak *et al*., 2014).

As the research moves along, more potential characteristics of *CFL2* gene were discovered. Recent research found that *CFL2* gene may be a key candidate in growth traits of domesticated animal. In chicken, g. 2545G>A polymorphism was significantly associated with shank girth and shank length at 4 weeks (P<0.05) (Zhao, 2011). In Chinese QC (Qinchuan) cattle, three SNPs of *CFL2* gene were significantly associated with growth

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traits, including rump length, chest girth, chest breadth, chest depth, hip width and body mass (P<0.01) (Sun *et al*., 2015). The studies indicated that *CFL2* gene may involve in animal growth traits through exerting its effect on actin. However, more studies should be carried out to validate the function of *CFL2* gene in animal growth and development.

Current studies indicated that *CFL2* gene is an attractive candidate gene for pathology, such as myopathies (Fattori *et al*., 2018), cardiomyopathy (Rangrez *et al*., 2017) and Gastric Cancer (Bian *et al*., 2018); however, little is known in animal growing development. To explore the functional implication of *CFL2* gene in Chinese DBS cattle, multiple approaches were undertaken in our study. The structure of bovine *CFL2* gene was characterized and three SNPs were genotyped in studied cattle. Sequently, genetic diversity was analyzed and statistical analysis were undertaken to discover the relationship between the genetic variation and the growth traits of cattle. Finally, quantitative real-time PCR was employed to analyze the spatial expression of *CFL2* mRNA in different tissues.

MATERIALS AND METHODS

Sample collection and growth trait measurements

This study was conducted on a total of 298 healthy and unrelated female DBS cattle (36±6 months old), and all these animals were reared in the County of Yingshang and Taihu, Anhui province. Both maintenance and feeding for all animals were similar according to the obligatory standards. Ear marginal tissues were collected from the aforementioned cattle, and genomic DNA were extracted using the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China) and estimated by spectrophotometer, and then, the genomic DNA was diluted to 50ng/μL for PCR amplication. Data of growth traits, including withers height, body length, height at hip cross, chest girth, abdominal girth, hip width, hucklebone width and shin circumferencec were recorded and used for subsequent analysis.

The tissue samples, such as heart, liver, spleen, lung, kidney, stomach, longissimus dorsi muscle, crureus and subcutaneous adipose tissue, with 2mm cube in diameter, were collected from adult healthy female DBS cattle for spatial expression analysis. All tissue samples were harvested, immediately frozen in liquid nitrogen, and stored at -80^{0}C until analysis.

Spatial expression of CFL2 mRNA in DBS cattle

Quantitative real-time PCR was employed to detect spatial expression of *CFL2* mRNA in DBS cattle. Total RNA were extracted from tissue samples using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) and cDNA was prepared using the reverse transcription kit (Takara, Dalian, China) according to the manufacturer's procedures. Gene-specific primers (*EXP-F: 5'-ATATGCTTTG-TACGATGCCAC-3', EXP-R: 5'-AGCCATTTACTTGC-CACTCAT-3'*) were designed for quantitative PCR, and *GAPDH* (F: 5'-*AACCACGAGAAGTATAACAA-CACCC*-3', R: 5'-*TGGTCATAAGTCCCTCCACGAT*-3') was amplified as an internal control. Each real-time PCR reaction was performed in a final volume of 20μL reaction mixture containing SYBR Premix Ex Taq (2×), Rox Reference Dye II (50×) (TaKaRa, Dalian, China), gene-specific primers, template cDNA and sterile water. The PCR amplification was performed on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) under the following cycling conditions: 30s at 95°C, followed by 40 cycles at 95°C for 5s, 60°C for 30s and 72°C for 34s. RT-PCRs were performed in triplicate and the results were analyzed by the $2^{-\Delta\Delta Ct}$ method (Livak *et al*., 2001).

SNP detection

Based on the sequence of the bovine *CFL2* gene (GenBank accession number: NM_001076154.2), primer pairs were designed for SNP scanning and three SNP sites were identified by sequencing. A total of four cattle breed: Dabieshan cattle (DBS), Jiaxian red cattle (JX), Wannan cattle (WN) and Luxi cattle (LX), were involved to explore the SNP sites, and three SNPs g.1500G>A (*HinfI*) in intron 2, g.1694T>A (*AseI*) in exon 4 and g.2213C>G (*HaeIII*) in 3'UTR were discovered.

The three SNPs were genotyped by (forced) PCR-RFLP technique in 298 cattle in our experimental population. The PCR reactions were performed in a volume of 25 μL containing 20 ng DNA template, 12.5uL 2*Taq Mix, 1.0 μL each of primer (100 ng/μL) and sterile water. The PCR amplification comprised of the initial denaturation at 95 °C for 5 min, 32 cycles of 30 sec at 94 °C, 30 sec at Tm specific for primer, and 30 sec at 72°C, followed by a final extension at 72°C for 5 min. The RFLP reaction mixture consisted of 1 uL 10*buffer, 10 U restriction enzyme (NEB, Ipswich, MA, USA), 5 uL PCR products and sterile water. Samples were incubated at 37°C for restriction enzyme overnight. The digested products were analyzed by 3% agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV illumination. The primers, fragment sizes and restriction enzymes selected were according to the study of Sun *et al*. (2015).

Statistical analysis

The allele and genotype frequencies were estimated by direct counting, Hardy–Weinberg equilibrium (HWE) was tested by POPGENE software (Version 3.2) based

on the likelihood ratio for different locus-population combinations (Yeh *et al*., 1997). Population genetic indexes, including gene homozygosity (Ho), gene heterozygosity (He, Ho + He =1), effective allele numbers (Ne) and polymorphism information content (PIC), were calculated according to Nei's methods (Nei *et al*., 1974; Botstein *et al*., 1980).

Linkage disequilibrium (LD) of the SNPs was analyzed by Haploview software according to the expectation maximization (EM) algorithm (Barrett *et al*., 2005). Haplotypes constructed was performed with the online SHEsis software (http://analysis2.bio-x.cn/myAnalysis.php) (Shi *et al*., 2005). The association between SNPs and growth traits in DBS cattle was analyzed using general linear models (GLM) with SAS software.

$$Yijk = \mu + Bi + Gj + \varepsilon ijk$$ (Wang *et al*., 2006)

Where Yijk is the phenotypic value of a target trait; μ represents the population mean, Bi is the combination effect, Gj is the genotype effect and εijk represents the random error.

RESULTS

Sequence analysis of bovine CFL2 gene

Sequence analysis revealed that bovine *CFL2* cDNA (NM_001076154.2) comprised a 501-bp open reading frame (ORF) flanked by a 22-bp 5′UTR and 851-bp 3′UTR sequences. Bovine *CFL2* gene shows 94% and 86% identity with human (NM_001243645.1) and mouse (NM_007688.2) counterparts, respectively. Further comparison revealed that each of the 5′-donor and 3′-acceptor splice sites conformed to the GT-AG rule (Table I). Bovine *CFL2* gene encodes a polypeptide of 166 amino acid residues with a calculated molecular mass of 18.7 kD and an isolectric point of 8.1714 (http://weblab.cbi.pku.edu.cn/program.inputForm.do?program=pepstats (v6.0.1)). The amino acid sequence shares 89% and 99% sequence similarity with human (NP_001230574) and mouse (NP_031714.1) homologues, respectively. However, the bovine and mouse *CFL2* has additional 17 amino acids more than human, respectively (shown in Fig. 1). Amino acid sequence analysis of bovine CFL2 revealed that the precursor protein contains actin depolymerisation factor (ADF), similar to the domains of the human (Q9Y281) CFL2 proteins.

Spatial expression of CFL2 mRNA in DBS cattle

Quantitative real-time PCR analysis showed that bovine *CFL2* mRNA expression varied greatly in diverse tissues, it is highly expressed in heart and stomach, moderately expressed in subcutaneous adipose tissue and crureus, and weakly expressed in other tissues.

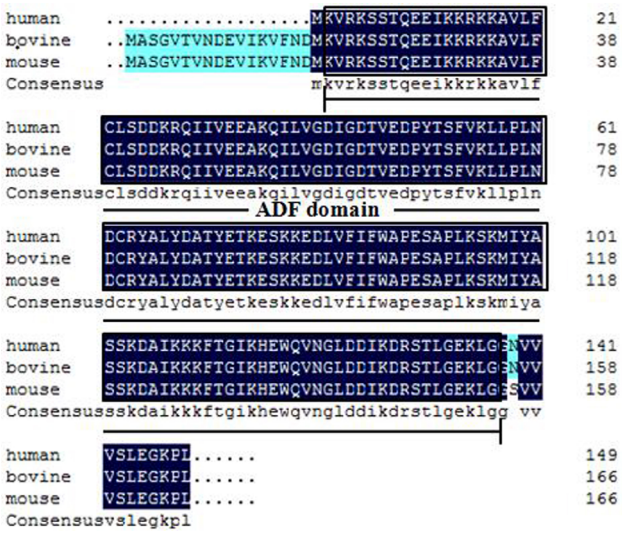

Fig. 1. Deduced amino acid sequence of bovine CFL2 compared with human CFL2 (NP_001230574) and mouse CFL2 (NP_001830.1). Shading shows identical amino acid residues among the three species. Common structural domains are indicated by boxes including ADF domain.

Table I. The exon/intron organizations of bovine *CFL2* gene.

Gene	Number	Exon size(bp)	Intron size (bp)	5' splice donor	3' splice acceptor
CFL2	1	3	1076		ATGgtaagg
	2	308	118	ttcagGCTTC	TTCTGgtgtg
	3	76	86	tgtagGGCTC	TACAGgtaca
	4	113		tttagGTAAT	

SNP detection and genetic diversity analyses

Three SNP sites were discovered through scanning DNA sequence of bovine *CFL2* gene. The three SNPs, g.1500G>A in intron 2 with a *HinfI* site, g.1694T>A in exon 4 with a *VspI* site and g. 2213C>G in 3'UTR with a *HaeIII* site were genotyped by (forced) PCR-RFLP technique in 298 cattle in our experimental population. After digestion by *HinfI*, the 389 bp PCR products were digested into 279 bp and 110 bp fragments (allele A) (Fig. 2A). At the g.1694T>A locus, the 197 bp PCR amplicon can be digested by *VspI*, producing 172 bp and 25 bp fragments for allele T (Fig. 2B). At the g.2213C>G locus, the 276 bp PCR fragment can be digested by *HaeIII* in fragment lengths of 252 bp and 24 bp for allele C (Fig. 2C). The 25 bp and 24 bp fragments were too small to stay in gel.

The genotype and allele frequencies are summarized in Table II. The results showed that the minor allele frequencies (MAF) ranged from 0.12-0.38.

Table II. Allele frequency of *CFL2* gene in DBS cattle.

Loci	Genotypic frequencies			Allelic frequencies		WE	Diversity parameter			
						χ2	Ho	He	Ne	PIC
g.1500G>A	GG	GA	AA	G	A					
	0.16	0.45	0.39	0.38	0.62	0.27	0.528	0.472	1.893	0.361
g.1694T>A	TT	TA	AA	T	A					
	0.06	0.46	0.48	0.29	0.71	3.46	0.588	0.412	1.701	0.327
g.2213C>G	CC	CG	GG	C	G					
	0.01	0.22	0.77	0.12	0.88	1.56	0.793	0.207	1.261	0.186

Table III. Association analysis of *CFL2* gene different genotypes with growth traits in Dabieshan cattle.

genotypes	body length (cm)	withers height (cm)	height at hip cross(cm)	chest girth (cm)	abdominal girth (cm)	shin circumference (cm)	hip width (cm)	hucklebone width (cm)
g.1500G>A								
AA	126.04±0.90	110.21±0.54a	109.50±0.57	147.99±1.47	169.37±1.47	16.79±0.14	31.66±0.44	17.41±0.16
AG	125.55±0.81	109.89±0.55a	109.89±0.43	148.98±0.89	170,19±1.27	16.94±0.16	32.11±0.37	17.40±0.24
GG	125.76±1.73	107.46±1.10b	109.36±0.94	148.49±1.82	168.00±2.37	16.52±0.25	31.12±0.78	16.85±0.41
P-value	0.925	0.040	0.805	0.838	0.701	0.361	0.415	0.429
g.1694T>A								
TT	124.83±3.06	107.39±2.07	109.50±1.53	146.89±3.98a	169.33±4.21	16.44±0.42	30.02±1.22	17.00±0.66
TA	126.01±0.82	109.83±0.56	109.72±0.46	149.70±0.89b	169.80±1.26	16.93±0.15	32.11±0.37	17.15±0.24
AA	125.68±0.81	109.75±0.50	109.60±0.49	146.29±1.24a	169.29±1.32	16.76±0.14	32.04±0.40	17.52±0.23
P-value	0.880	0.299	0.976	0.048	0.960	0.441	0.053	0.475
g.2213C>G								
CC	126.00±0.60	109.00±0.91	111.50±0.35	140.00±0.40a	161.00±1.19a	17.00±0.05	27.50±0.55a	17.00±0.20
CG	124.51±1.17	110.58±0.79	109.96±0.72	148.03±1.21b	167.36±1.48b	17.00±0.20	31.64±0.50b	17.22±0.32
GG	125.85±0.64	109.14±0.41	109.34±0.35	146.69±0.88b	170.24±1.01b	16.71±0.10	31.69±0.31b	17.20±0.15
P-value	0.573	0.253	0.606	0.036	0.042	0.272	0.046	0.992

Note: 1). The values is marked with mean ± SE; 2). Values with different superscripts within the same row differ significantly at $p<0.05$ (a, b).

SNP g.2213C>G was almost completely monomorphic (MAF=0.01) in our population and other two SNPs were polymorphic. A chi-square test showed that the genotypic distributions within all individuals were in agreement with the Hardy–Weinberg equilibrium ($p>0.05$). Genetic indices, including *Ho*, *He*, *Ne* and *PIC*, were measured to detect the informativeness of the identified SNPs. PIC values ranged from 0.186 to 0.361, and SNP g.1500G>A revealed the highest PIC value (0.361), which corresponds to the highest He (0.472).

Linkage disequilibrium, haplotypes and association analysis

The linkage disequilibrium was evaluated for all pairs of SNPs using r^2, and the values of $r^2>0.33$ might indicate a sufficiently strong linkage disequilibrium (LD) to be useful for mapping (Ardlie *et al*., 2002). The LD between the three SNPs in the population indicated that the D′ values ranged from 0.886 to 0.938, and the r^2 values ranged from 0.170 to 0.653. SNP g.1500G>A and g.1694T>A had a strong LD and there was a low LD between the rests of any two sites (Table IV).

Haplotypes in 298 individuals were analyzed using the online SHEsis software, and four major haplotypes accounting for 99.1% of the alleles were observed (Table V), excepting for 3 haplotypes with a frequency of <0.03. The haplotype 'AAG' was the most common haplotype and has a great frequency of 61.2%, next coming the haplotype GTG, GAG and GTC were quite rare, at a frequency of 0.090 and 0.101, respectively.

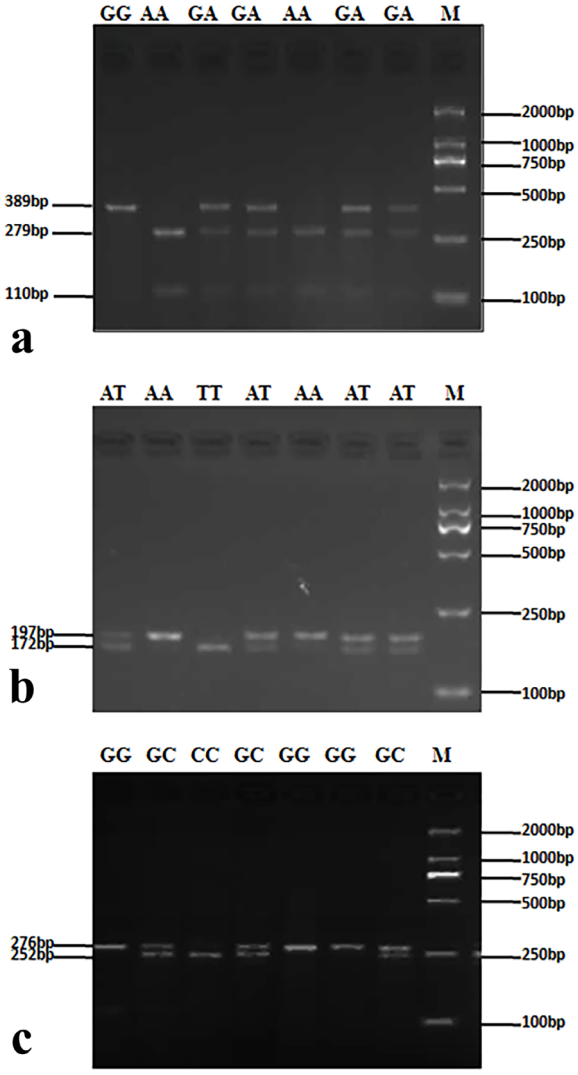

Fig. 2. Electrophoretic patterns of SNP genotyping by PCR-RFLP method; (a) is the PCR-*HinfI*-RFLP analysis result of SNP at g.1500G>A in intron 2 of the *CFL2* gene, genotype GA (389 + 279 + 110 bp), genotype AA (279 + 110 bp) and genotype GG (389 bp); (b) is the PCR-*VspI*-RFLP analysis result of SNP at g.1694T>A in exon 4 of the *CFL2* gene, genotype AT (197 + 172 + 25 bp, 25 bp fragment was too small to stay in gel), genotype TT (172 + 25 bp) and genotype AA (197 bp); (c) is the PCR- *HaeI-II*-RFLP analysis result of SNP at g.2213C>G in 3'UTR of the *CFL2* gene, genotype CG (276 + 252 + 24 bp; 24 bp fragment was too small to stay in gel), genotype CC (252 + 24 bp) and genotype GG (276 bp); M refers to the DNA molecular weight marker.

The associations between *CFL2* genotypes and growth traits were analyzed in our population. As shown in Table III, SNP g.1500G>A was associated with withers height (p=0.040), SNP g.1694T>A was associated with chest girth (p=0.048), and g.2213C>G site has a significant association with chest girth (p=0.036), abdominal girth (p=0.042) and hip width (p=0.046). Furthermore, based on the haplotypes analysis, nine combined genotypes were found in the animal DNA samples of this study. However, the associated analysis suggested that no significant differences were detected between the combined genotypes of the three SNPs and eight growth traits in DBS cattle (p>0.05) (Table VI).

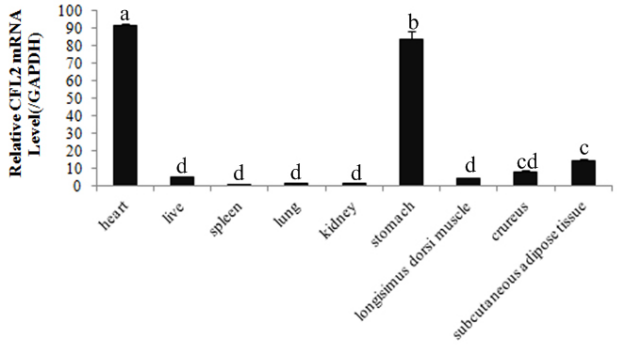

Fig. 3. Expression pattern analysis of CFL2 mRNA in DBS cattle by real-time PCR method. The value of CLF2 in spleen was arbitrarily set to 1.0. The different letters (a, b, c and d) indicate a p-value of less than 0.05 in a Student's t-test.

Table IV. The estimated values of linkage disequilibrium analysis between three mutation sites within the bovine *CFL2* gene.

Loci	g.1500G>A	g.1694T>A	g.2213C>G
g.1500G>A		D′=0.938	D′=0.924
g.1694T>A	r^2= 0.653		D′=0.886
g.2213C>G	r^2=0.170	r^2=0.239	

Table V. Haplotypes of the *CFL2* gene and their frequencies in the DBS cattle breed.

Haplotype	g.1500G>A	g.1694T>A	g.2213C>G	Frequency
1	A	A	G	0.612
2	G	A	G	0.090
3	G	T	C	0.101
4	G	T	G	0.188

Note: frequency>0.03 has been ignored in the analysis.

Table VI. Associations of combined genotypes with growth traits in Dabieshan cattle (mean ± SE).

Genotype of combination	Number of combinations	Body length (cm)	Body height (cm)	Height at hip cross (cm)	Chest girth (cm)	abdominal girth(cm)	shank circumference (cm)	Hucklebone width (cm)	Hip width (cm)
AAAAGG	112	125.93±0.98	110.12±0.58	109.54±0.55	148.04±1.59	169.41±1.59	16.79±1.56	31.60±2.19	17.48±0.43
AGATGG	63	126.62±0.88	109.33±0.65	109.60±1.43	150.91±0.99	172.60±1.52	16.81±0.80	32.62±0.63	17.29±0.32
AGAAGG	21	123.24±0.96	108.33±0.59	110.34±0.45	149.91±0.86	169.65±1.43	16.90±0.87	31.38±1.34	18.00±0.56
AGATGC	47	125.15±0.95	111.32±0.60	110.09±1.52	145.98±1.08	167.19±1.30	17.13±0.69	31.74±0.71	17.29±1.59
GGATGG	16	126.63±1.25	106.93±0.58	108.31±0.79	148.75±1.88	167.69±1.64	16.75±1.57	30.69±1.83	15.85±0.47
GGATGC	7	124.86±0.89	111.00±0.83	111.28±1.89	147.00±1.26	167.00±1.06	17.17±1.32	31.00±0.51	17.86±0.85
GGAAGG	4	129.25+0.78	107.75±0.53	108.67±1.72	149.50±1.30	165.50±1.77	15.50±1.00	34.33±1.08	17.67±0.52
GGTTGG	10	125.10±1.03	106.20±1.99	108.30±1.32	148.30±2.16	171.30±0.69	16.10±0.52	29.90±1.19	16.50±0.88
GGTTGC	7	123.14±1.12	109.00±0.59	110.58±0.67	149.57±1.09	165.00±0.90	16.86±1.26	31.00±0.51	17.67±0.80
P-value		0.598	0.303	0.118	0.389	0.564	0.591	0.206	0.864

Note: Number >3 has been ignored in the analysis.

DISCUSSION

The results of our study provide the comparison of *CFL2* genome structure with that human and mouse, demonstrated remarkably high similarity among the three species; they all contained four exons and three introns. The ADF (Actin depolymerisation factor) domain, may be severing actin filaments and binding to actin monomers, is highly conserved among human, mouse and cattle. Comparing our results with human *CFL2* microarray expression data (http://genome.ucsc.edu/), the expression pattern of bovine *CFL2* gene was partial coincided with the expression of the corresponding gene in human, which it was expressed at considerable levels in heart, and this could be linked to their tissue-specific function. Furthermore, the expression analysis indicated that *CFL2* gene was actively transcribed in heart and stomach, subcutaneous adipose tissue, crureus, longissimus dorsi muscle and live tissues, which were rich in muscle fiber, indicated that *CFL2* gene play a vital role in muscle tissues, and may further affect the phenotype of domesticated animal.

CFL2 gene was reported involving in the muscle development and play a critical role in muscle maintenance (Gurniak *et al*., 2014). *CFL2* mutations may affect F-actin accumulation and trigger congenital myopathy with protein aggregates and nemaline bodies in human (Fagerberg *et al*., 2014; Yue *et al*., 2014). In chicken and Chinese QC cattle, SNPs of *CFL2* gene were associated with the growth traits and may affect the phenotypes of the individuals. Our study documented three SNPs of *CFL2* gene were associated with growth traits of DBS cattle. SNP g.1500G>A was associated with withers height, and the cattle with genotypes AA and AG had significantly greater withers height than those with genotypes *HinfI*-GG ($p<0.05$), demonstrating that the allele *HinfI*-A might be correlated with an increase in withers height in the DBS population. SNP g.2213C>G site has a significant association with chest girth, abdominal girth and hip width, and the animals with genotypes GG and CG had significantly greater chest girth, abdominal girth and hip width than those with genotypes CC ($p<0.05$), indicating that the allele *HaeIII*-G might be correlated with an increase in chest girth, abdominal girth and hip width in the DBS population. The SNPs g.1500G>A in intron 2 and g.2213C>G in 3' UTR may exert specific biological functions of genes by influencing transcriptional efficiency (Greenwood *et al*., 2003; Le Hir *et al*., 2003). In exon 4, SNP g.1694T>A has a significant association with chest girth, the animals with genotypes TA had significantly greater chest girth than those with genotypes TT and AA ($P<0.05$). Moreover, SNP g.1694T>A was a synonymous mutation (Ile 131 Ile), and may alter the function of proteins and change cellular response to specific targets by affecting messenger RNA splicing, stability and protein structure (Hunt *et al*., 2009).

Haplotypes, which can be regarded as a collection of ordered markers, are specifically physical arrangements of SNP alleles on the same chromosome (Olivier *et al*., 2003). In general, because of the higher heterozygosity and multiallelic nature, haplotypes may provide greater power than individual marker analysis for genetic disease and trait associations (Akey *et al*., 2001). With the integration of haplotypes, the molecular markers could be accurately identified and the single SNP could be precisely

associated with character information (Scheike *et al*., 2010). Especially when the marker alleles are not in strong LD with each other, the haplotypes analysis has a greatest power advantage (Morris *et al*., 2002). Unfortunately, the association analysis of combined genotypes of *CFL2* gene among our detected individuals indicated no convincing associations with any of the studied traits; this may be due to the limited population, and more samples and more traits should be considered in the future.

CONCLUSIONS

In conclusion, three polymorphisms of *CFL2* gene in DBS cattle were observed in this study. We have also defined the LD and haplotypes in the DBS cattle and identified the preponderant haplotype AAG, which would provide a background for more extensive characterization of the bovine *CFL2* gene. Although the combined genotypes of *CFL2* gene have no convincing associations with the studied traits, association analyses indicated that its polymorphisms were associated with growth traits of DBS cattle. In addition, the spatial expression indicated that *CFL2* gene was varied expression in adult DBS cattle tissues. Our study suggests *CFL2* gene may affect the phenotype of domesticated animal, and its polymorphisms might be used as a genetic marker and for the breeding of new beef cattle.

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Statement of conflict of interest

The authors declare that they have no conflict of interest.

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