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Tissue expression, association analysis between three novel SNPs of the *RXRα* gene and growth traits in Chinese indigenous cattle

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Retinoid X receptor (RXR) α is a member of the nuclear hormone receptor superfamily that mediates the biological effects on several hormones, vitamins, and regulates lipid, glucose and energy metabolism. In this study, the tissue expression profiles of the bovine *RXRα* gene and association analysis of single nucleotide polymorphisms (SNPs) with growth traits were carried out in 413 Chinese native cattle. The expression profile was analysed in ten Jiaxian cattle tissues by real-time PCR, and the results showed that *RXRα* gene was abundantly expressed in adipose tissue and spleen, moderately expressed in heart, liver, lung, kidney, muscle and testis. Meanwhile, three SNPs (T27919A, T28139C and G28142A) and five haplotypes were identified. Haplotype with TTG was dominant with frequency of 69.1%. *Chi*-square test showed all populations were in Hardy-Weinberg equilibrium ($P>0.05$) at the three sites except Jiaxian cattle at G28142A site and Qinchuan cattle at T27919A site. Statistical analysis of combined sites showed that the individuals with TTGA genotype had significantly higher heart girth than those with TAGG genotype ($P<0.05$) and the animals with AAGA genotype had higher body weight than those with TAGG genotype ($P<0.05$) in T27919A–G28142A site. Heart girth, abdominal circumference and body weight of individuals with TCAG genotype were exceedingly higher than those with TTGG ($P<0.01$), TTGA and TCGG ($P<0.05$) in T28139C–G28142A site. For T27919A–T28139C site, the individuals of TCTA and TCTT genotype had significantly higher heart girth and lower height at hip cross than those with TTTA ($P<0.05$), and the body weight of TCAA and TCTT genotype individuals was higher than those with TTTA ($P<0.05$). In conclusion, these results provided evidence that the polymorphisms of *RXRα* gene were associated with growth traits and might apply to Chinese indigenous yellow cattle breeding program as a possible candidate for marker-assisted selection (MAS).

***RXRα* gene, tissue expression, polymorphisms, association analysis, Chinese indigenous cattle**

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Retinoid X receptor (RXR) α is an indispensable member of the nuclear receptor (NR) superfamily and is considered as a key regulator in differentiation, cellular growth [1]. The same as the other members of ligand-activated transcription factors, RXRα has many protein domains, including a central strong conserved DNA binding domain (DBD), a variable N-terminal domain, a flexible hinge and a C-terminal ligand binding domain (LBD). RXRα functions as a DNA binding partner by forming heterodimers or homodimer with other nuclear receptors, so RXRα occupies a particular position in the NR superfamily, and it also participates in regulating the fatty acid and cholesterol metabolism [2]. All

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RXRα heterodimers preferentially bind response elements composed of direct repeats of two AGGTCA sites with a 1–5 bp spacer. RXRα can play different roles on these heterodimers. RXRα acts either as a structural component of the heterodimer complex, required for DNA binding but not acting as a receptor, or as both a structural and a functional component of the heterodimer, allowing 9-*cis*RA to signal through the corresponding heterodimer. RXRα signaling plays an important role on development, and is able to enhance myogenic differentiation *in vitro* combining with other small molecule inducers [1]. Moreover, RXRα regulates expression of genes encoding of key enzymes of lipid metabolism, metabolism of glucose, bile acids, cholesterol, and it is also a factor for RXRα activators that involved into development of inflammatory response, inducing cell differentiation and apoptosis and inhibiting proliferation [3]. RXRα is also involved into regulation of carcinogenesis and the ligands of the *RXRα* gene may exhibit both stimulating and inhibiting effects on tumor development [4–6]. RXRα was also critical for the mediation of hormones in human health and was a component of disrupting substances that was still harmful to wild animals and humans in source water [7].

In structures, human, mouse and swine *RXRα* gene were located on chromosomes 9, 2 and 17, and their encoding proteins are comprised of 467, 467 and 549 amino acids, respectively. In expression patterns, the mRNA of *RXRα* gene was highly expressed in liver and kidney tissues in human. In mice, it was also detected abundantly in the tissues of lung, spleen and muscles besides liver and kidney, but it had a low expression level in paranephros, brain, heart, intestine and testis [8]. In swine, Ding et al. [9] found the *RXRα* gene abundantly expressed in liver and perirenal adipose tissue. There was only one report about expression profile of the cattle embryo [10], however, the integrated expression profile of bovine *RXRα* gene is still vacant so far. In functions, Carmona et al. [11] reported RXRα by binding 9-*cis*RA induced expression of *UCP2* gene to promote brown fat cell differentiation. The previous research indicated RXRα, together with PPARγ to form PPARγ/RXRα heterodimer in adipocyte, regulated the differentiation of preadipocyte [12]. Transgenic mice of selective knockout the *RXRα* gene in adipose tissue could resist adiposity induced by high fat food [13]. Schoonjans et al. [14] pointed out that RXRα/PPARγ was combined with a specific sequence DR in promoter region of *LPL* gene and could promote the expression of *LPL*. The RXRα ligand LG100268 acted together with PPAR agonists rosiglitazone to promote subcutaneous adipose differentiation of human [15]. In metabolism, it was known that many nuclear receptor-mediated pathways in fatty acid oxidation and lipid metabolism were disordered for the lack of RXRα [16]. For example, the lipid-sensing PPARα-mediated pathway, it was altered for the lack of RXRα, as well as other pathways that include LXR and FXR pathways were also compromised, at least partially, by the absence of RXRα [2].

In animal industry, growth and development traits are one of the most economically important aspects for the livestock production. There are many researches about mutations in candidate genes and their associations with growth and development traits that can be applied to animal breeding programmes [17–19]. The bovine *RXRα* gene was located on chromosome 11 and has 15 exons; it was known that the *RXRα* gene was indirectly associated with enhanced developmental potential of the embryo at the time of superovulation by the expression profiles analysis of the cattle embryo [10]. But there was no more information about the *RXRα* gene on metabolism and development in cattle. In the study, the mRNA expression, polymorphisms, haplotypes construction of the bovine *RXRα* gene and association analysis between the genetic variants and growth traits in Chinese indigenous cattle was performed in order to investigate *RXRα* gene whether can be regarded as the candidate gene for maker assistance selection (MAS) and cattle breeding.

1 Materials and methods

1.1 Animals and samples

Ten tissues (heart, liver, spleen, lung, kidney, rumen, muscle, testis, intestine and adipose) were collected from three mature Jiaxian cattles. All tissues were collected within 30 min after slaughter and frozen immediately in liquid nitrogen and subsequently stored at −80°C.

A total of 413 cattle were randomly selected from commercial farms of Henan, Shanxi, Shandong and Qinghai provinces, China, respectively, and represented five yellow cattle breeds and Gaoyuan yak in China: Luxi cattle (LX, n=123), Jiaxian cattle (JX, n=121), Nanyang cattle (NY, n=43), Qinchuan cattle (QC, n=30), Bohai Black cattle (BBH, n=36), and Gaoyuan yak (GY, n=60). Blood samples were collected from these cattle, and DNA samples were isolated from blood samples in accordance with salt-chloroform extraction protocol. Growth traits and body sizes (height at withers, HAW; body length, BL; heart girth, HG; abdominal circumference, AC; hipbone width, HBW; hip width, HW; height at a hip cross, HHC; body weight, BW) were measured for statistical analysis.

1.2 RNA extraction, cDNA synthesis and real-time PCR

The total RNA of tissue samples were extracted using the RNAisoTM Plus reagent (TaKaRa, Japan). The first-strand cDNA was synthesized from 1 μg of total RNA using cDNA Reverse Transcription Reagent Kit (TaKaRa) according to the manufacture's protocol. Relative *RXRα* expression was quantified by using the $2^{-\Delta\Delta Ct}$ method with an ABI Prism 7300 Sequence Detector (Applied Biosystems, USA). Primers

for *RXRα* gene were designed with Primer Premier 5.0 software according to the sequence (GenBank No. NM_000168). Hypoxanthine phosphoribosyl-transferase 1 (*HPRT1*) (GenBank No. NM_001034035.1) was chosen as the reference gene for internal standardization. PCRs were performed using gene-specific primers pairs for *RXRα* gene (P1, Table 1). Gene expression levels were quantified relatively to the expression of *HPRT1* (P2, Table 1). Each sample was run in triplicate along with the internal control gene by using a standard shuttle PCR protocol (95°C for 5 s, 60°C for 15 s and 72°C for 30 s) for 40 cycles. Data were recorded and analyzed with Sequence Detector software (Applied Biosystems), and means ±SD were calculated for each tissue.

1.3 SNP detection and genotyping

Primers were designed based on the bovine *RXRa* gene (GenBank No. NC_000168) by Primer Premier 5.0 software and synthesized by Nanjing Genscript Biological Engineering Technology Company in China (Table 1). DNA sequencing was used for SNPs scanning by pooled PCR amplification in the bovine *RXRα* gene and the sequences were analyzed by DNAStar soft (V 6.0). The genetic variance sites were genotyped by forced polymerase chain reaction-restriction fragment length polymorphism (forced PCR-RFLP) technology, which introduces a point mutation into one of primers sequences so that the PCR product will contain a restriction endonuclease recognition site. Three pairs of primers (namely as *RXRα-Stu* I, *RXRα-Nru* I and *RXRα-Xho* I) were used to detect these mutations (*RXRα-Stu* I: g.27919 T>A, *RXRα-Nru* I: g.28139 T>C, *RXRα-Xho* I: g.28142 G>A). PCR products (6 μL) were digested with 0.5 U restriction endonuclease for 15 h at 37°C. The digested products were electrophoresed with 12% PAGE in 1×TBE buffer and constant voltage (200 V) for 2 h at room temperature. The gel was stained with 0.1% silver nitrate and the genotypes were estimated based on different electrophoresis patterns.

1.4 Statistical analysis

The genotypic frequencies, allelic frequencies, polymorphism information content (PIC), heterozygosity (He) and effective number of alleles (Ne) and Hardy-Weinberg equilibrium (HWE) were analyzed according to Nei's methods and Botstein's methods [20–22]. The association between the polymorphism of the *RXRa* gene and growth traits was analyzed using the general linear models procedure of SPSS (V17.0). The following model was used:

$$Y_{ijk}=\mu+B_i+G_j+F_p+A_q+e_{ijk},$$

where Y_{ijk} is the phenotype of the animal, μ is the mean of the population, B_i is the effect of breed, G_j is the effect of genotype, F_p is the effect of farm, A_q is the effect of age, e_{ijk} was random error. Significance level of differences between the means for each group was considered at $P<0.05$ and $P<0.01$.

The linkage disequilibrium (LD) construction as measured by D' and r^2 and haplotype frequencies of each population were carried out by the SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) [23,24]. The combined genotypes with the number less than ten were not considered.

2 Results

2.1 Gene expression profiles of the *RXRa* gene

The mRNA expression of the *RXRα* gene in various tissues was determined by real-time PCR normalized against *HPRT1* mRNA levels. A dissociation curve showing a single peak at the melting temperature expected for that amplicon suggests specific amplification. The results showed that the *RXRα* gene was predominantly expressed in adipose and spleen, and moderately expressed in heart, liver, lung, kidney, muscle and testis, with minor expression in rumen. While the mRNA expression of *RXRα* gene was not detected in intestine (Figure 1).

Table 1 Primer pairs designed for the bovine *RXRa* gene

Primer	Primer sequence (5′→3′)	*Tm* (°C)	Length (bp)	Method
P1	Forward: CGGGACAACAACAAGGACTGCC	60	152	qRT-PCR
	Reverse: GCTGGACTCCACCTCGCTCT			
P2	Forward: TGCTGAGGATTTGGAGAAGG	60	154	qRT-PCR
	Reverse: CAACAGGTCGGCAAAGAACT			
RXRα-Stu I	Forward: GACGCGGTTGCTGTGGTA	65	471	PCR-RFLP
	Reverse: GGCTACAGGGAACGGTGAGT			
RXRα-Nru I	Forward: GACGCGGTTGCTGTGGTA	65	471	PCR-RFLP
	Reverse: GGCTACAGGGAACGGTGAGT			
RXRα-Xho I	Forward: CGAGGTGGAGGCGCTTCTCGA	63.2	233	PCR-RFLP
	Reverse: TGGGGCTACAGGGAACGGT			

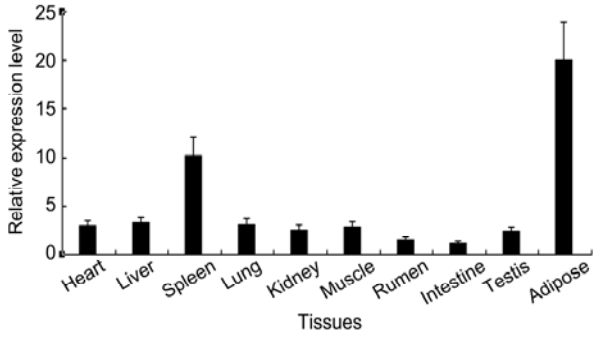

Figure 1 Expression analysis of the *RXRa* gene detected by qRT-PCR in 10 tissues of Jiaxian cattle. Mean±SE were calculated for each tissue type. Tissue distribution of the bovine *RXRa* mRNA assessed by qRT- PCR. The values shown in this figure are the averages of three independent experiments. Error bars represent the SD (n=3) of relative mRNA expression levels of *RXRa* normalized to *HPRT1*.

2.2 Polymorphisms and genetic diversity

In the study, exon 14 and partial sequence in intron 13 of the bovine *RXRα* gene were amplified. Three polymorphism sites (named *RXRα-Stu* I: g.27919 T>A, *RXRα-Nru* I: g.28139 T>C and *RXRα-Xho* I: g.28142 G>A or T27919A, T28139C and G28142A) were identified by DNA pools sequencing. The sequence maps and PCR-RFLP patterns of three SNPs were shown in Figure 2. Two SNPs were missense mutations that introduced amino acid changes, thus likely altered the structure of protein. One SNP (T28139C) resulted in amino acid substitution (Val to Ala). Another SNP (G28142A) caused amino acid substitution (Arg to Lys). The prediction of the secondary structure of RXRa comprised of 28.17% alpha helix, 9.92% extended strand, 5.03% beta-turner, 56.88% random coil. While the secondary structure comprised of 28.57% alpha helix, 12.17% extended strand, 5.29% beta-turner, 53.97% random coil after those two amino acids switches.

The genotypic and allelic frequencies and genetic indexes (Ho, He, Ne and PIC) of three sites were calculated in the six cattle breeds (Table 2). The frequency of allele T demonstrated a high prevalence in the first two sites. For the G28142A locus, the allele G was the predominant allele in Chinese native cattle. The *Chi*-square test showed that the locus of T27919A in the population was in accordance with the HWE (P>0.05) except Qinchuan cattle. All populations were in HEW at T28139C site (P>0.05). However, at G28142A site, the populations also were in HEW except Jiaxian cattle. According to the classification of PIC (low polymorphism if PIC value<0.25, median polymorphism if 0.25<PIC value <0.5 and high polymorphism if PIC value> 0.5) [25], Nanyang, Bohai, and Qinchuan had a moderate genetic diversity at all sites, while Jiaxian were of low level at G28142A site and Luxi had a low polymorphism level at T27919A site (Table 2).

2.3 LD and haplotype analysis of the bovine *RXRα* gene

Five different haplotypes, CAA (24.0%), CAG (2.1%), CTA (1.6%), CTG (3.1%), TTG (69.1%) were identified from the three sites. In the population, the frequency of TGG was the highest, while the frequency of CTA haplotype was the lowest.

All haplotype combinations were found in the cattle breeds. According to the definition of strong linkage (pairwise r^2>0.33) [26], the results of pairwise linkage disequilibrum analysis showed that there was a strong linkage between T27919A and T28139C sites in the studied population (r^2=0.791) as well as T27919A and G28142A (r^2= 0.772), while there was a weak linkage between T28139C and G28142A sites (r^2=0.01).

2.4 Association analysis between polymorphisms and growth traits

The effects of the three SNPs on growth traits were analyzed

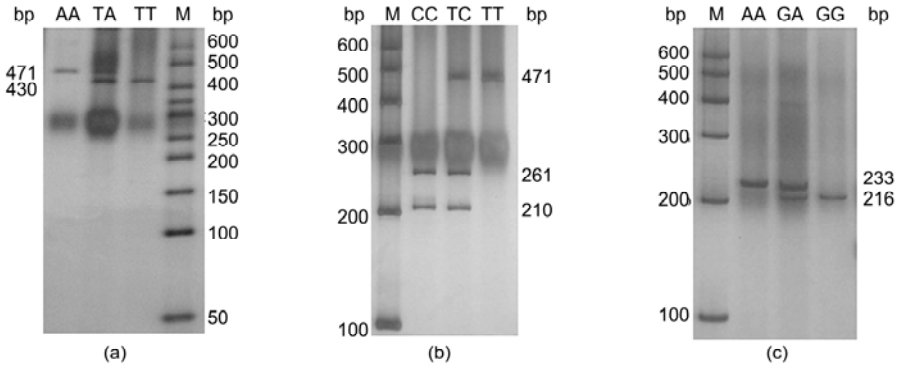

Figure 2 Different genotypes of the *RXRa* gene in Chinese native cattle. (a) Restriction digestion of the 471 bp fragment with the enzyme *Stu* I T27919A locus. Gel=12% PAGE; M: Marker. Genotype: TA=471 bp+431 bp+40 bp; AA=471 bp; TT=431 bp+40 bp. It is difficult to see the 40 bp DNA fragment on 12% PAGE. (b) Restriction digestion of the 471 bp fragment with the enzyme *Nru* I at T28139C locus. Gel=12% PAGE; M: Marker. Genotype: TC=471 bp+261 bp+210 bp; TT=471 bp; CC=261 bp+210 bp. (c) Restriction digestion of the 233bp fragment with the enzyme *Xho* I at G28142A locus. Gel=12% PAGE; M: Marker. Genotype: GA=233 bp+216 bp+17 bp; AA=233 bp; GG=216 bp+17 bp. It is difficult to see the 17 bp DNA fragment on 12% PAGE.

Table 2 Diversity parameters, genotype and allelic frequencies of the bovine *RXRa* gene

SNP	Breeds (number)	Genotype frequencies			Allele frequencies		HWE-*P* value	PIC	He	Ne
		TT	TA	AA	T	A				
T27919A	LX (123)	0.68	0.30	0.02	0.83	0.17	*P*>0.05	0.24	0.29	1.40
	JX(121)	0.55	0.39	0.06	0.75	0.25	*P*>0.05	0.31	0.38	1.60
	BBH (36)	0.51	0.40	0.09	0.73	0.27	*P*>0.05	0.32	0.41	1.69
	QC (30)	0.17	0.80	0.03	0.78	0.22	*P*<0.05	0.37	0.49	1.96
	NY(43)	0.44	0.49	0.07	0.68	0.32	*P*>0.05	0.34	0.43	1.76
	GY (60)	0.45	0.47	0.08	0.68	0.32	*P*>0.05	0.34	0.43	1.76
		TT	TC	CC	T	C				
T28139C	LX (123)	0.50	0.42	0.08	0.71	0.29	*P*>0.05	0.33	0.41	1.70
	JX(121)	0.62	0.33	0.05	0.79	0.21	*P*>0.05	0.28	0.34	1.51
	BBH (36)	0.50	0.41	0.09	0.75	0.25	*P*>0.05	0.31	0.38	1.60
	QC (30)	0.71	0.25	0.03	0.84	0.16	*P*>0.05	0.24	0.27	1.37
	NY(43)	0.55	0.39	0.06	0.85	0.15	*P*>0.05	0.24	0.25	1.34
	GY (60)	0.59	0.36	0.05	0.77	0.23	*P*>0.05	0.29	0.36	1.56
		GG	GA	AA	G	A				
G28142A	LX (123)	0.50	0.46	0.04	0.73	0.24	*P*>0.05	0.32	0.39	1.65
	JX(121)	0.72	0.21	0.07	0.82	0.18	*P*<0.05	0.24	0.29	1.41
	BBH (36)	0.51	0.43	0.06	0.73	0.27	*P*>0.05	0.32	0.40	1.65
	QC (30)	0.70	0.20	0.10	0.80	0.20	*P*>0.05	0.27	0.32	1.47
	NY(43)	0.51	0.41	0.07	0.71	0.29	*P*>0.05	0.32	0.40	1.68
	GY (60)	0.32	0.60	0.08	0.58	0.42	*P*>0.05	0.36	0.47	1.90

statistically (Tables 3–5). At T27919A locus, the results indicated that individuals with AT genotype displayed significantly higher AC than those with AA genotype in Luxi cattle (P<0.05), and individuals with genotype AA displayed significantly higher BL than those with TT (P<0.05) and those with genotype TT had greater BW than those with TT (P<0.01) in Jiaxian cattle. In all cattle concerned, the individuals with genotype TT had higher HG than those with TA (P<0.05) (Table 3); at T28139C locus, the individuals with genotype TC were significantly higher than those with CC at BW and HG for Luxi cattle (P<0.05), and AC of individuals with genotype TT was lower than those with TC in Jiaxian cattle. In all cattle concerned, individuals with CC genotype displayed significantly lower than those with TC at BW (P<0.05), and the HW, HHC of individuals with CC genotype were higher than those with TT (P<0.05) (Table 4); at G28142A locus, the AC of Luxi cattle with genotype AA was significantly higher than those with GG (P<0.05), and for Jiaxian cattle, the HG of individuals with genotype GG was higher than those with AA (P<0.05). In all cattle concerned, the HHC and BW of individuals with GA genotype were higher than those with GG (P<0.05) (Table 5).

The association of the combined genotypes with growth

Table 3 Effect of different genotypes on growth traits at T27919A, T28139C and G28142A locus in yellow cattle[a)]

Loci	Genotypes	HAW (cm)	BL (cm)	HG (cm)	AC (cm)	HBW (cm)	HW (cm)	HHC (cm)	BW (kg)
	AA	132.35±2.99	149.12±3.70	175.41±5.33	203.69±5.91	47.27±1.75	28.68±1.07	133.96±2.41	443.44±31.69
T27919A	TT	128.23±1.05	142.48±1.12	174.06±1.61[a]	201.62±1.79[a]	44.63±0.52	28.04±0.31	131.97±0.72	387.36±9.56
	TA	129.61±0.90	143.40±1.30	171.58±1.87[b]	199.74±2.00[b]	44.31±0.58	27.81±0.36	130.20±0.85	382.33±11.12
	CC	129.17±2.18	143.59±2.71	170.00±3.86	197.34±3.95	44.45±1.17	28.65±0.73[a]	132.55±1.61[a]	369.62±22.97[b]
T28139C	TT	128.27±0.94	143.05±1.16	170.97±1.65	200.27±1.82	44.99±0.54	27.73±0.32[b]	130.27±0.74[b]	373.46±9.85
	TC	130.38±1.06	143.59±1.32	176.63±1.88	202.84±2.12	44.21±0.60	28.13±0.36	132.31±0.83	411.22±11.41[a]
	AA	127.42±2.76	140.90±3.41	171.60±4.88	199.20±5.48	45.03±1,63	28.19±0.94	129.47±2.23	383.81±29.16
G28142A	GG	128.55±0.86	142.25±1.07	171.13±1.53[b]	199.24±1.69	44.42±0.50	27.70±0.29	130.42±0.68[b]	376.34±9.15[b]
	GA	130.58±1.13	145.56±1.40	176.81±2.01[a]	203.97±2.18	44.90±0.63	28.43±0.39	133.04±0.86[a]	408.92±11.95[a]

a) Data with different superscripts within the same column differ significantly at P<0.05 (a, b). HAW, height at withers; BL, body length; HG, heart girth; AC, abdominal circumference; HBW hipbone width; HW, hip width; HHC, height at hip cross. Mean±SE.

Table 4 Effect of different genotypes on growth traits at T27919A, T28139C and G28142A locus in Jiaxian cattle[a)]

Loci	Genotypes	HAW (cm)	BL (cm)	HG (cm)	AC (cm)	HBW (cm)	HW (cm)	HHC (cm)	BW (kg)
	AA	131.29±2.41	150.00±3.61[a]	174.00±5.47	201.00±6.45	47.67±1.90	29.00±1.54	132.25±2.10	461.14±29.77[A]
T27919A	TT	128.00±0.81	143.31±1.21[b]	177.10±1.84	204.35±2.50	45.82±0.74	27.59±0.52	128.21±0.81	381.58±9.92[B]
	TA	126.64±0.97	144.52±1.46	173.04±2.21	200.17±2.67	45.13±0.79	28.33±0.62	128.47±0.87	365.58±12.01
	CC	126.60±2.87	141.80±4.28	174.00±6.53	203.00±7.92	44.75±2.34	30.00±1.82	131.75±2.58	378.20±36.62
T28139C	TT	128.34±0.73	145.22±1.09	175.60±1.67	201.21±2.08[b]	45.54±0.61	27.82±0.46	128.86±0.68	381.47±9.33
	TC	126.18±1.17	141.95±1.75	174.93±2.68	205.47±3.63[a]	46.21±1.08	27.98±0.74	127.24±1.18	378.19±14.71
	AA	126.21±2.43	138.00±3.61[b]	171.71±5.51	198.50±7.90	44.75±2.32	27.71±1.54	126.88±2.59	387.86±30.93
G28142>A	GG	128.18±0.72	144.39±1.06[a]	175.65±1.62	203.68±2.11	46.16±0.62	28.09±0.45	128.23±0.69	378.72±9.09
	GA	126.42±1.31	145.33±1.95	175.38±2.98	199.33±3.45	44.50±1.01	27.60±0.83	130.00±1.13	383.88±16.37

a) Data with different superscripts within the same column differ significantly at $P<0.05$ (a, b), $P<0.01$ (A, B). HAW, height at withers; BL, body length; HG, heart girth; AC, abdominal circumference; HBW hipbone width; HW, hip width; HHC, height at hip cross. Mean±SE.

Table 5 Effect of different genotypes on growth traits at T27919A, T28139C and G28142A locus in Luxi cattle [a)]

Loci	Genotypes	HAW (cm)	BL (cm)	HG (cm)	AC (cm)	HBW (cm)	HW (cm)	HHC (cm)	BW (kg)
	AA	137.00±5.23	147.33±6.30	173.00±9.49	192.33±10.61[b]	46.67±4.18	29.00±2.15	134.67±5.18	387.33±59.81
T27919>A	TT	135.11±0.99	147.84±1.20	178.39±1.80	197.42±2.08	44.12±0.80	28.73±0.41	134.85±0.98	407.93±11.37
	TA	135.35±1.49	147.16±1.79	181.84±2.70	202.06±3.15[a]	45.03±1.19	29.01±0.61	135.04±1.47	435.64±17.03
	CC	132.83±2.12	146.17±2.56	171.50±3.81[b]	194.22±4.34	44.56±1.69	29.11±0.88	134.94±2.11	375.74±24.10[b]
T28139C	TT	135.16±1.46	149.08±1.76	178.40±2.62	199.10±2.99	45.97±1.17	29.08±0.60	134.49±1.45	403.40±16.58
	TC	135.92±1.10	147.19±1.33	181.90±1.97[a]	199.73±2.40	43.57±0.88	28.60±0.45	135.13±1.10	433.52±12.49[a]
	AA	134.50±4.04	147.20±4.83	181.00±7.33	202.40±8.17[a]	46.60±3.24	30.40±1.64	133.70±3.40	424.00±46.50
G28142A	GG	134.70±1.15	146.19±1.37	177.02±2.08	195.64±2.38[b]	43.92±0.92	28.25±0.47	134.27±1.14	405.59±13.20
	GA	135.88±1.21	149.25±1.44	181.66±2.19	201.78±2.56	44.86±0.97	29.31±0.49	135.72±1.19	426.29±13.89

a) Data with different superscripts within the same column differ significantly at $P<0.05$ (a, b). HAW, height at withers; BL, body length; HG, heart girth; AC, abdominal circumference; HBW hipbone width; HW, hip width; HHC, height at hip cross. Mean±SE.

traits was analyzed in five populations. Statistical analysis displayed individuals with TTGA genotype had significantly higher HG than those with TAGG genotype ($P<0.05$) and the AAGA genotype was higher than those with TAGG genotype at BW ($P<0.05$) in T27919A–G28142A site. For the T28139C–G28142A site, BW, HG and AC of individuals with TCAG genotype was exceedingly higher than those with TTGG ($P<0.01$), TTGA and TCGG ($P<0.05$). The individuals with combined genotype TCTA and TCTT had higher HG and lower HHC than those with TTTA ($P<0.05$), and BW of individuals with TCAA and TCTT genotype was higher than those with TTTA ($P<0.05$) in T27919A–T28139C site (Table 6). Other combination genotype had no significance difference with growth traits.

3 Discussion

The RXRα is one of the nuclear hormone receptors which are a large family of transcription factors known to regulate gene transcription and protein expression levels of fatty acid transport and metabolism mediating proteins through the formation of a DNA binding heterodimer complex [27]. There were many reports about expression pofiles and functions for signal transduction of the *RXRα* gene in mice and human. In this study, three SNPs in the *RXRα* gene were firstly detected in cattle populations.

The results of real-time PCR analysis revealed that the mRNA of bovine *RXRα* gene was mainly expressed in adipose tissue, heart, liver, spleen, lung, kidney, muscle and testis, which were inconsistent with the previous observations in mouse [8]. Gene expression in what parts of animals was related to its corresponding gene function. RXRα bound with 9-*cis*RA induced expression of *UCP2* gene to promote brown fat cell differentiation in adipose tissue [11]. It was indicated that RXRα together with PPARγ in adipocyte regulated the differentiation of preadipocyte [12]. Transgenic mice of selective knockout *RXRα* gene in adipose tissue could resist adiposity induced by high fat food [13]. Thus, the high expression level of *RXRα* gene in mature yellow cattle adipose tissue suggested that RXRα may play an important role in lipid and energy metabolism.

In this study, two SNPs were missense mutations that induced amino acid changes, thus likely altered the function

Table 6 Associations of combined genotypes for the three SNPs and growth traits in five populations [a)]

Growth traits	Combined genotypes of T27919A and T28139C		
	TCTA	TCTT	TTTA
HHC(cm)	131.774±1.341[b]	132.423±1.078[a]	129.038±1.070[b]
HG(cm)	176.520±3.070[a]	176.848±2.467[a]	168.449±2.467[b]
BW(kg)	416.135±18.188[a]	403.279±14.522[a]	360.106±14.614[b]
	Combined genotypes of T28139C and G28142A		
	TCAG	TTGG	TCGG
HG (cm)	180.475±2.803[A]	170.203±1.974[B]	173.098±2.695
AC (cm)	208.100±3.374[Aa]	200.144±2.249[B]	199.037±2.904[b]
HAW (cm)	131.344±1.589	127.751±1.119	129.545±1.527
BW (kg)	438.139±16.539[Aa]	372.998±11.742[B]	385.298±16.030[b]
	Combined genotypes of T27919A and G28142A		
	TTGA	TAGG	AAGA
HAW (cm)	133.795±1.119	130.063±1.709	129.156±0.564
HG (cm)	177.493±2.641[a]	168.849±2.365[b]	174.400±9.809
HW (cm)	28.255±0.513	26.341±0.811	27.933±0.267
HHC (cm)	133.262±1.107[A]	129.077±1.026[B]	139.750±6.112
BW (kg)	401.607±15.609	365.696±14.082[b]	488.168±58.404[a]

a) Data with different superscripts within the same column differ significantly at $P<0.05$ (a, b), $P<0.01$(A, B). HAW, height at withers; BL, body length; HG, heart girth; AC, abdominal circumference; HBW hipbone width; HW, hip width; HHC, height at hip cross. Mean±SE.

of protein. The secondary structure prediction of RXRα comprised of 28.17% alpha helix, 9.92% extended strand, 5.03% beta-turner and 56.88% random coil, while the secondary structure comprised of 28.57% alpha helix, 12.17% extended strand, 5.29% beta-turner, 53.97% random coil after those two amino acids switches. One SNP (T28139C) resulted in amino acid substitution (Val to Ala). Val was used to treat hepatic failure disease, while Ala could assist the metabolism of glucose and improve the body energy [28]. In contrast, Ala was more conducive to the cattle, so the missence mutation was important for metabolism in cattle. Another SNP (G28142A) caused amino acid substitution (Arg to Lys). Arg was related to reproduction of male and also was used to treat disease, however, Lysine was an essential alkaline amino acid, it might play important roles in promoting growth and development, reducing the level of triglycerides in blood, helping the body absorbs calcium and also could form collagen [28]. In contrast, Lys was very important for metabolism, growth, development and meat quality in cattle, so this missence mutation was more valuable for our study and may be used in cattle breeding.

Bovine *RXRα* gene was located on chromosome 11 (BTA11) approximately from 105.98 to 106.01 Mb. From the alignment between BTA11 on radiation hybrid (RH) map and Cattle QTL Database (http://www.genome.iastate.edu/cgi-bin/QTLdb/BT/index), we only found milk traits in this region, however, some QTLs about growth and meat quality traits were found on the animals that were usually used to study the growth and meat quality traits, such as the swine and chicken *RXRα* gene. The swine *RXRα* gene located on chromosome 1 about from 288.83 to 288.86 Mb, in this region some QTLs associated with production and meat quality traits was mapped, such as abdominal fat weight [29], backfat [30,31], body weight [32] and average daily gain [32,33]. The chicken *RXRα* gene was located on chromosome 17 about from 7.27 to 7.35 Mb, and we found some QTLs associated with meat quality traits located in this region, such as abdominal fat percentage and drumstick weight [34], bone mineral content and tibia length [35], abdominal fat weight [36], body weight [37]. Thus, the bovine *RXRα* gene, possiblely the same as the swine and chicken *RXRα* gene, may be associated with production traits and be used as a candidate gene for production traits in cattle.

Body measurement is considered as a fitness-related trait in animals, and typically change over the lifetime of an individual, heritable components of phenotypic variance may also show ontogenetic variation. In our study, two SNPs (g.28139 T>C, g.28142 G>A) were found in the bovine *RXRa* gene coding region and one SNP (g.27919 T>A) in the noncoding region. The association analysis showed that the combined genotype TCAG had significantly effect on the growth traits. Consequently, the bovine *RXRa* may be an important candidate gene of growth traits and the SNPs may simply be used as genetic markers on bioeconomic traits.

In conclusion, this study firstly report the expression profiles of the bovine *RXRa* and three novel SNPs in the bovine *RXRa* gene. These could provide a sufficient power for investigating the role of *RXRa* gene for development. The results of association analysis indicated that all three SNPs have significantly associated with growth traits in the populations involved and can be used as genetic markers for cattle breeding.

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