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Molecular cloning, polymorphisms, and expression analysis of the *RERG* gene in indigenous Chinese goats

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ABSTRACT. The current study aimed to investigate the coding sequence, polymorphisms, and expression of the *RERG* gene in indigenous Chinese goats. cDNA of *RERG*, obtained through reverse transcription PCR was analyzed using bioinformatic techniques. Polymorphisms in the exon regions of the *RERG* gene were identified and their associations with growth traits in three varieties of indigenous Chinese goats were investigated. Expression of the *RERG* gene in three goat breeds of the same age was detected using real-time quantitative PCR. The results revealed that the cDNA of *RERG,* which contained a complete open reading frame of 20-620 bp, was 629 bp in length. The associated accession numbers in GenBank are JN672576, JQ917222, and JN580309 for the QianBei Ma goat, the GuiZhou white goat, and the GuiZhou black goat, respectively. Four consistent SNP sites were found in the exon regions of the *RERG* gene for the three goat breeds. mRNA expression of the *RERG* gene differed between different tissues in adult goats of same age. The highest expression was observed in lung

and spleen tissues, while the lowest expression was recorded in thymus gland tissue. In addition, the expression of the *RERG* gene in the muscle of Guizhou white goat, GuiZhou black goat, and QianBei Ma goat decreased sequentially. Our results lay the foundations for further investigation into the role of the *RERG* gene in goat growth traits.

Key words: *RERG* gene; cDNA clone; Indigenous Chinese goats; qRT-PCR; PCR-SSCP

INTRODUCTION

The *RERG* gene (ras-related and estrogen-regulated growth inhibitor) belongs to the ras supergene family and encodes functional proteins that play important roles in adjusting growth, differentiation, survival, and proliferation of cells (Habashy HO et al., 2011; Habashy et al., 2011). The gene was identified and named in 2001 (Finlin et al., 2001) and has been shown to work as a signal converter in signal transduction pathways (Bourne et al., 1991; Yu et al., 2003). The *RERG* gene shares 40-50% homology with other ras supergene family members and it also has a GTP conservative binding domain (Yu et al., 1999; Xu et al., 2000); however, it lacks the fragment involved in participation and lipid modification at the C-terminal (Chen et al., 2012).

The QianBei Ma goat, one of three high quality goat breeds found in GuiZhou province, China, is fed widely by locals. This goat exhibits traits of high disease resistance, high crude feed tolerance, high reproductive rate, delicious tasting fresh flesh tastes, and outstanding skin quality (Luo et al., 2010; Cai et al., 2011). The GuiZhou white goat also has many excellent properties in terms of body size, high fertility, good taste, and outstanding skin quality, and it is also suitable for grazing in mountainous areas (Ran et al., 2009). The GuiZhou black goat, which has a strong physical build and good taste, has been listed as an excellent local variety since 1984 (Tian et al., 2010).

We selected these three indigenous goat breeds (QianBei ma goat, GuiZhou white goat, and GuiZhou black goat) as the research subjects. The cDNA sequences of the *RERG* gene were cloned and analyzed successfully. Single nucleotide polymorphisms (SNPs) within the *RERG* gene were identified using PCR-single strand conformation polymorphism (SSCP) and *RERG* expression level was evaluated in the three goat breeds using quantitative RT-PCR (qRT-PCR). In the meantime, we expect to provide a further reference to reveal biological function of RERG gene.

MATERIAL AND METHODS

Animals

QianBei Ma goats (322) between 36 and 48 months old were obtained from the QianBei Ma goat breeding center in XiShui county, GuiZhou province, China. GuiZhou white goats (100) between 36 and 48 months old were obtained from the GuiZhou white goat breeding center in YanHe county, GuiZhou province, China. GuiZhou black goats (100) between 36 and 48 months were obtained from the Animal and Veterinary Research Institute of GuiZhou province, China.

All experimental animals were fed and maintained under the same conditions during 2010. Seven growth traits (body weight, withers height, body length, heart girth, chest depth, chest breadth, and cannon circumference) were measured (Table 1).

Eight tissue types (heart, liver, spleen, lung, kidney, muscle, ovarian, and thymus gland)

were collected from three ewes of between 36 and 48 months old of each breed and frozen immediately in liquid nitrogen, and then stored at -80°C.

Table 1. Growth traits recorded for three indigenous Chinese goat varieties.

Breed (n)/trait	Minimum	Maximum	Mean	SE	CV (%)
QianBei ma goats (322)					
Body weight (kg)	32.00	63.00	43.7171	5.55683	12.71
Withers height (cm)	53.00	72.80	61.2295	3.51656	5.740
Body length (cm)	54.80	78.90	68.1357	3.87098	5.680
Heart girth (cm)	72.00	92.50	81.7416	3.76769	4.61
Chest depth (cm)	20.90	35.00	30.6578	1.68149	5.48
Chest breadth (cm)	13.40	22.70	18.7901	1.92322	10.24
Cannon circumference (cm)	7.00	10.50	8.0736	0.56998	7.06
GuiZhou white goats (100)					
Body weight (kg)	21.50	39.00	30.0000	3.71236	12.37
Withers height (cm)	44.50	63.00	53.2750	3.72961	7.00
Body length (cm)	50.00	79.00	61.9460	4.80258	7.75
Heart girth (cm)	55.00	85.00	69.3920	5.78901	8.34
Chest depth (cm)	22.80	30.50	26.7640	1.53731	5.74
Chest breadth (cm)	13.00	21.00	16.5440	1.69153	10.22
Cannon circumference (cm)	6.80	9.00	7.8210	0.53698	6.87
GuiZhou black goats (100)					
Body weight (kg)	22.85	58.00	41.5635	6.01275	14.47
Withers height (cm)	52.50	70.60	60.7090	3.54857	5.85
Body length (cm)	54.80	74.50	67.2980	3.89488	5.79
Heart girth (cm)	65.50	90.00	80.2700	4.38251	5.46
Chest depth (cm)	25.10	34.00	30.2220	1.70066	5.63
Chest breadth (cm)	12.10	22.00	18.3790	2.09474	11.40
Cannon circumference (cm)	7.00	9.50	7.9270	0.51871	6.54

N, total number of goats measured; SE, standard error; CV, coefficient of variation.

Total RNA and genomic DNA extraction

Trizol (Invitrogen, USA) was used to extract total RNA from the heart, liver, spleen, lung, kidney, muscle, ovary, and thymus tissues. RNA quality and concentration were evaluated using 1% agarose gel electrophoresis and a UV spectrophotometer from Beijing labtech Instrument co., ltd. Total RNA samples were stored at -80°C. Genomic DNA was extracted from blood samples using the phenol-chloroform method, and then dissolved in TE buffer (10 mM Tris-HCl, pH 8.0) and stored at -40°C (Chu et al., 2011).

Primer design

The *RERG* gene sequence from cows (accession numbers: NM_001076198 and NW_001495088) was used as a reference, which has high homology with goats. In order to amplify the *RERG* gene in goats, Primer5.0 (Fen, C.H et al., 2012) was used to design specific primers, and β-actin was used as a housekeeping gene (Table 2).

RT-PCR amplification and CDS cloning

The transcription products (cDNA) of the *RERG* gene were obtained according to the protocol of the RevertAid™ First Strand cDNA Synthesis Kit. PCR amplification conditions were as follows: 5 μL RNA template, 2 μL 10X PCR Buffer (Mg^{2+}), 2.0 μL dNTPs (2.5 mM), 1 μL of each primer(10μL), 0.4 μL Taq (5 U/μL) and ultra-pure water to a total volume of 20 μL. The PCR cycling

conditions were as follows: 95°C for 5 min followed by 35 cycles at 94°C for 30 s, 55°C for 40 s (annealing), 72°C for 60 s, and a final extension at 72°C for 10 min. The products were detected using 1% agarose gel electrophoresis and then cloned and sequenced independently three times.

Table 2. Summary of the primers used to amplify the RERG gene in QianBei Ma goats, GuiZhou white goats, and GuiZhou black goats.

Primers	Sequence 5'→3'	Amplified length (bp)	Annealing temperature (°C)
RERG-CDS	F: ATTGTCTACCAGCACCCAGCAT	CDS (629)	55.0
	R: TCAGCAGTTAGGCAACTTCG		
RERG -1	F: ATGGGCTATTCCTCTTTCTGG		
	R: TATCAAATCACGCAAAGAGCC	exon 1 (279)	55.9
RERG -2	F: CCATTTTACCTTCGTTTTGCTC		
	R: AAATTACTCAGTGCTCCAAGGG	exon 2 (298)	62.1
RERG -3	F: TGCTGAATGTAAGGAATGGTTG		
	R: GAGATCAAAAAGCCCAAGAATG	exon 3 (247)	62.1
RERG -4	F: TGAGATCAAAAAGCCCAAGAA		
	R: ACAGAAGAAGGGGAGAAGCTG	exon 3 (100)	60.5
RERG -5	F: ACAGAAGAAGGGGAGAAGCTG		
	R: TCAGCAGTTAGGCAACTTCGT	exon 3 (223)	62.0
RERG -6	F: CAGCAGTTAGGCAACTTCGTC		
	R: ATGTTGGGAAGTTGCAGAGTG	exon 3 (185)	60.0
RERG -7	F: ATGTTGGGAAGTTGCAGAGTG		
	R: TCACCTTGCTTCCATAGGTTG	exon 3 (173)	61.0
RERG -8	F: CACCTTCACCTTGCTTCCATA		
	R: TGGATCAGGAAGATCCCCTAG	exon 3 (202)	60.5
RERG -9	F: CAATGCAAGAGACACATTGGTT		
	R: TGAAGTGAGGCTTTTCTGAATG	exon 3 (275)	61.8
RERG -10	F: TGAAGTGAGGCTTTTCTGAATG		
	R: GTCTTTATTTCACCTGGGCTTG	exon 3 (274)	62.2
RERG -11	F: AAGCCCAGGTGAAATAAAGACA		
	R: CTACTCTTCTGTTGAGGACC	exon 3 (192)	60.3
RERG -12	F: CTGTTGAGGACCCCAGGTT		
	R: TGATAAAGCTTTATGATTCT	exon 3 (217)	60.8
RERG -13	F: TGGTTAGAGTTCACTATGCCTGAA		
	R: AGTTGGAGGCTTTAATTCACCA	exon 3 (231)	62.5
RERG -14	F: CACCAATGCATTTGGACAAC		
	R: TCAGGCAGTCTTTCTTGTGGT	exon 3 (104)	61.5
β-actin	F: CGCAGAAAACGAGATGAGATT		
	R: TGTCACCTTCACCGTTCCAGT	Housekeeping gene (119)	59.0
RERG-qRT	F: GAATCAACCTACCGACACCAAG		
	R: CAGCACTTCCTCAAAACTTCCT	qRT-PCR (171)	59.0

Detection of polymorphisms using PCR-SSCP

Each PCR product (1.5 µL) was transferred to an Eppendorf tube, mixed with 6 µL gel loading solution, containing 98% formamide, 0.025% bromophenol blue, 0.025% xylene cyanol, 20 mM EDTA, pH 8.0, and 10% glycerol. The mixture was centrifuged and denatured at 98°C for 10 min, then chilled on ice for 5 min and loaded on to a neutral polyacrylamide gel (Chu et al., 2011). Following electrophoresis, the DNA fragments in the gels were visualized using silver staining, photographed and analyzed.

Quantitative real-time PCR (qRT-PCR)

After cloning and sequencing, the qRT-PCR assays were analyzed in a PCR Thermal Cycler Dice and Real Time system (Takara, Japan). A 25 µL reaction volume contained 12.5 µL SYBR Premix Ex TaqII (2*), 1 µL upstream primer, 1 µL downstream primer, 2 µL RT product, and 9.5 µL dH_2O. qPCR

amplification conditions were as follows: 95°C predegeneration for 10 s; 95°C denaturation for 5 s; 60°C annealing for 30 s, and 72°C extension for 40 cycles. Tests were first performed in an ordinary qPCR reaction to enable optimization of experimental conditions, including annealing temperature and primer concentration. β-Actin served as a reference gene and each sample was run in triplicate.

Statistical analysis

The following fixed effects model was employed to analyze growth traits in QianBei Ma goats and the least squares mean was used for multiple comparisons of growth traits among different genotypes. Age and sex were ignored in the statistical analysis since the ewes tested were of the same age.

$$Y_{ijk}=u+ \text{marker}_k +e_{ijk}$$

where, Y_{ijk} is a record of individual phenotype value; U is the group average; marker_k is a marker gene type effect; E_{ijk} is a random error (Jin et al., 2010; Yuan et al., 2010; Liu et al., 2011).

RESULTS

Cloning and sequence analysis of the coding sequence (CDS)

The sequencing results were analyzed using DNAMAN v4.0. (Yuan et al., 2010). The sequence for the *RERG* gene is 629 bp long, as expected, and it encodes 199 amino acids. The sequence contained all coding sequences (CDS), including ATG and TGA. The sequences were submitted to GenBank under the following accession numbers: JN672576 (QianBei Ma goat), JQ917222 (GuiZhou white goat), and JN580309 (GuiZhou black goat). Based on the results of the genetic analysis using DNAMAN v4.0, nucleotide composition of the target sequences were as follows: QianBei Ma goat: A = 29.67, G = 28.00, T = 21.00, C = 21.33%, among these A+T = 50.67, C+G = 49.33%; GuiZhou white goats: A = 29.50, G = 28.00, T = 21.00, C = 21.50%, among these A+T = 50.50, C+G = 49.50%; GuiZhou black goat: A = 29.50, G = 28.00, T = 20.50, C = 22.00%, among these A+T = 50.00, C+G = 50.00%. Sequence homology between the QianBei Ma goat and the GuiZhou white goat was 98.5%, between the QianBei Ma goat and the GuiZhou black goat it was 98.8%, and between the GuiZhou white goat and the GuiZhou black goat it was 99.3%.

Sequence analysis of amino acid

The secondary structure of the *RERG* gene was predicted using SOPMA (Geourjon and Deleage, 1995), GOR (Garnier et al., 1996), and HNN (Ou et al., 2007) in ExPASy. The predictions of secondary structure using the methods above were the same for each methodology. For QianBei Ma goats, GuiZhou white goats, and GuiZhou black goats, the Alpha helix, extended strand, and random coil were 56.28, 15.58, and 21.11%; 54.77, 15.58, and 23.12%; 54.27, 16.08, and 25.13%, respectively.

Polymorphisms

The primers RERG-1 to RERG-14 were designed to amplify genomic DNA. PCR-SSCP

was performed to identify polymorphisms within the *RERG* gene. The results showed that primers RERG-3, RERG-8, RERG-11, and RERG-13 amplified regions of genomic DNA with polymorphic sites in all three goat breeds (Figure 1).

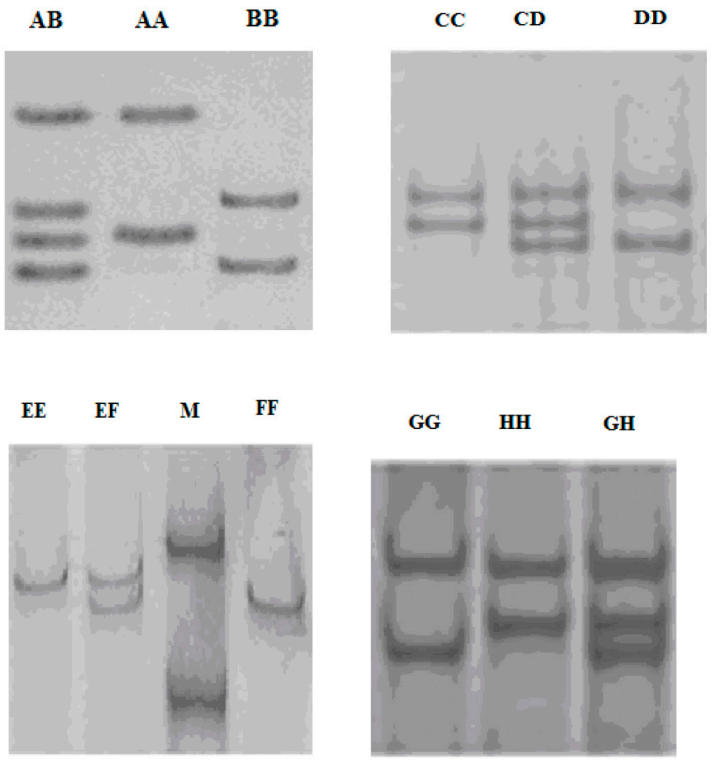

Figure 1. SSCP analysis of PCR amplifications of regions of the RERG gene in QianBei Ma goats, GuiZhou white goats, and GuiZhou black goats.

The PCR products of different genotypes were purified and the DNA directly sequenced. The target sequences had four mutation sites in the third exon at the positions of 56, 826, 1434, and 1798 bp. For primer RERG-3, a nucleotide substitution mutation (56 bp, G→C) existed between genotype AA and genotype BB. A SNP site (826 bp, A→G) was present between genotype CC and genotype DD for primer RERG-8. A nucleotide mutation (1434 bp, T→C and 1798 bp, T→A) was also observed between genotypes EE and FF (primer RERG-11) and genotypes GG and HH (primer RERG-13), respectively (Figure 2).

Genetic characteristics of the SNP sites

The genetic characteristics of the four mutation sites in each of the three goat breeds were also investigated in this study. Alleles A, C, E, and F were observed to be advantageous alleles at the four mutation sites, 56(G/C), 826(A/G), 1434(T/C) and 1798(T/A), in each goat breed. In all cases, the allele frequencies were high, exceeding 0.5 (Table 3).

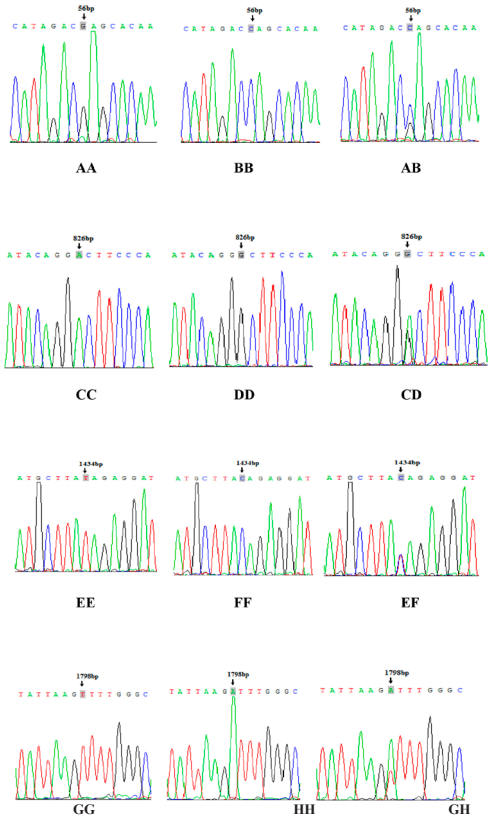

Figure 2. Partial sequencing maps of heterozygotes discriminated by PCR-SSCP in the RERG gene in QianBei Ma goats, GuiZhou white goats, and GuiZhou black goats.

Table 3. Analysis of genetic diversity of regions of the RERG gene in QianBei Ma goats, GuiZhou white goats, and GuiZhou black goats.

Site	Breed	Genotype frequencies			Allele frequencies		H_E	H_O	N_E	PIC
RERG-3		AA	AB	BB	A	B				
56 (G/C)	QianBei Ma	0.54	0.177	0.283	0.682	0.318	0.273	0.627	1.595	0.347
	GuiZhou White	0.56	0.21	0.23	0.675	0.325	0.439	0.561	1.783	0.352
	GuiZhou Black	0.45	0.37	0.18	0.539	0.461	0.497	0.503	1.988	0.462
RERG-8		CC	CD	DD	C	D				
826 (A/G)	QianBei Ma	0.618	0.199	0.183	0.71	0.29	0.412	0.588	1.7	0.326
	GuiZhou White	0.47	0.38	0.15	0.545	0.455	0.496	0.504	1.984	0.457
	GuiZhou Black	0.5	0.3	0.2	0.6	0.4	0.48	0.52	1.923	0.41
RERG-11		EE	EF	FF	E	F				
1434 (T/C)	QianBei Ma	0.553	0.342	0.105	0.606	0.394	0.473	0.522	1.916	0.405
	GuiZhou White	0.38	0.35	0.27	0.515	0.485	0.5	0.5	2	0.485
	GuiZhou Black	0.41	0.48	0.11	0.65	0.35	0.455	0.545	1.835	0.332
RERG-13		GG	GH	HH	G	H				
1798 (T/A)	QianBei Ma	0.652	0.078	0.27	0.787	0.213	0.336	0.664	1.506	0.27
	GuiZhou White	0.58	0.25	0.17	0.665	0.335	0.446	0.554	1.805	0.359
	GuiZhou Black	0.57	0.09	0.34	0.615	0.385	0.474	0.526	1.90	0.362

PIC >0.5 = high diversity.

Influence of fixed effects on growth trait of goats

A fixed effects model was employed to analyze growth traits in QianBei Ma goats. Body weight and body length were found to be significantly greater in individuals with genotypes AB and EF than with genotypes AA, BB, EE, and FF ($P < 0.01$). The differences in body weight between genotypes CD, CC, and DD were highly significant ($P < 0.01$). There were no significant differences in growth traits at the fourth SNP site (Table 4).

Table 4. Association of four loci with growth traits in 322 QianBei Ma goats. n, number of goats with the specified genotype.

Trait	Genotype		
	AA (N = 174)	BB (N = 92)	AB (N = 56)
Body weight (kg)	43.43 ± 5.00^{A}	42.66 ± 6.23^{A}	46.37 ± 5.32^{B}
Withers height (cm)	61.44 ± 3.52^{a}	60.52 ± 3.45^{b}	61.72 ± 3.50^{a}
Body length (cm)	67.90 ± 3.98^{A}	67.56 ± 3.68^{A}	69.84 ± 3.41^{B}
Heart girth (cm)	81.82 ± 3.73^{b}	80.83 ± 3.84^{Aa}	82.99 ± 3.43^{Bc}
Chest depth (cm)	30.71 ± 1.57^{B}	30.11 ± 1.80^{A}	31.41 ± 1.53^{C}
Chest breadth (cm)	18.68 ± 1.89^{A}	18.54 ± 2.00^{A}	19.54 ± 1.70^{B}
Cannon circumference (cm)	8.06 ± 0.57	8.06 ± 0.64	8.11 ± 0.45
Trait			
	CC (N = 199)	DD (N = 59)	CD (N = 64)
Body weight (kg)	42.84 ± 5.28^{Aa}	44.01 ± 6.22^{a}	46.17 ± 5.06^{Bb}
Withers height (cm)	61.25 ± 3.49	60.62 ± 3.79	61.71 ± 3.31
Body length (cm)	67.65 ± 3.91^{A}	67.79 ± 3.57^{A}	70.00 ± 3.53^{B}
Heart girth (cm)	81.52 ± 3.82^{a}	81.43 ± 3.88^{b}	82.74 ± 3.35
Chest depth (cm)	30.61 ± 1.55^{Aa}	30.13 ± 2.06^{Ab}	31.28 ± 1.5^{B}
Chest breadth (cm)	18.56 ± 1.93^{A}	19.01 ± 1.9^{B}	19.31 ± 1.84
Cannon circumference (cm)	8.05 ± 0.55	8.13 ± 0.75	8.10 ± 0.43
Trait			
	EE (N = 178)	FF (N = 34)	EF (N = 110)
Body weight (kg)	42.7 ± 5.43^{A}	41.97 ± 6.56^{A}	45.9 ± 4.75^{B}
Withers height (cm)	61.18 ± 3.37	60.77 ± 4.16	61.45 ± 3.56
Body length (cm)	67.66 ± 4.04^{A}	67.18 ± 3.93^{A}	69.2 ± 3.34^{B}
Heart girth (cm)	81.30 ± 3.75^{A}	80.26 ± 3.95^{A}	82.88 ± 3.46^{B}
Chest depth (cm)	30.52 ± 1.61^{a}	30.2 ± 1.59^{a}	31.02 ± 1.76^{b}
Chest breadth (cm)	18.55 ± 1.89^{A}	18.27 ± 2.46^{A}	19.33 ± 1.66^{B}
Cannon circumference (cm)	7.98 ± 0.52^{A}	8.1 ± 0.60^{B}	8.21 ± 0.61
Trait			
	GG (N = 210)	HH (N = 87)	GH (N = 25)
Body weight (kg)	43.65 ± 4.91	43.94 ± 6.38	43.52 ± 7.53
Withers height (cm)	61.39 ± 3.42	60.86 ± 3.66	61.13 ± 3.86
Body length (cm)	68.08 ± 3.94	68.34 ± 3.6	67.88 ± 4.29
Heart girth (cm)	81.78 ± 3.78	81.77 ± 3.5	81.34 ± 4.61
Chest depth (cm)	30.68 ± 1.55	30.66 ± 1.97	30.48 ± 1.68
Chest breadth (cm)	18.70 ± 1.86	19.12 ± 1.95	18.41 ± 2.25
Cannon circumference (cm)	8.07 ± 0.53	8.11 ± 0.65	7.96 ± 0.58

Data marked with different superscripts within the same line differ significantly, (Uppercase letters, $P < 0.01$, lowercase letters $P < 0.05$). The same as below *ty*; $0.25 < PIC < 0.5$ *moderate*; $PIC < 0.25$ *low diversity*.

Quantitative real-time PCR

RT-PCR amplification and cloning were carried out on total RNA extracted from different tissues. The quantitative results were exported from StepOne software v2.2.2 and analyzed using the $2^{-\Delta\Delta Ct}$ method. Bonnet et al., 2013Relative expression of the *RERG* gene in different tissues of the three goat breeds was calculated and statistical analysis was conducted using SPSS 17 software. The results demonstrate that mRNA expression levels were higher in the lung and spleen than in other tissues. The expression levels in the muscle of Guizhou white goat, GuiZhou black goat and QianBei Ma goat decreased sequentially (Table 5; Figure 3).

Table 5. Expression of the RERG gene in different tissues of QianBei Ma goats, GuiZhou white goats, and GuiZhou black goats.

Breed	Heart	Liver	Spleen	Lung	Kidney	Muscle	Ovarian	Thymus
GuiZhou black goat	1.405 ± 0.161	1.974 ± 0.12	7.318 ± 0.322	10.613 ± 0.334	1.422 ± 0.11	1.838 ± 0.051	2.541 ± 0.22	1.000 ± 0.000
QianBei Ma goat	1.303 ± 0.006	1.906 ± 0.067	7.067 ± 0.082	8.454 ± 0.038	1.431 ± 0.028	1.542 ± 0.008	2.078 ± 0.098	1.296 ± 0.012
GuiZhou white goat	1.508 ± 0.016	1.922 ± 0.05	6.939 ± 0.102	7.627 ± 0.123	1.677 ± 0.022	1.877 ± 0.022	2.295 ± 0.032	1.318 ± 0.04

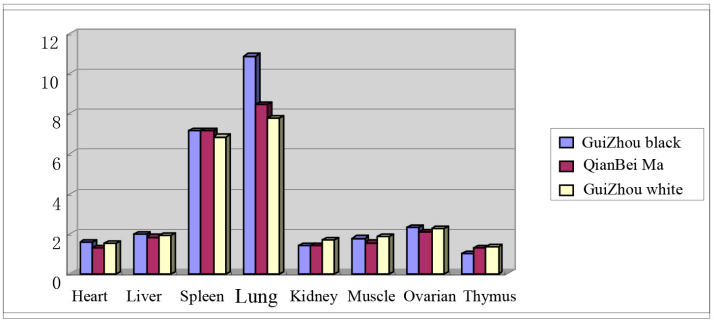

Figure 3. Quantitative expression of the RERG gene in different tissues of the QianBei Ma goat, the GuiZhou white goat, and the GuiZhou black goat.

DISCUSSION

The first report published on the *RERG* gene was a study of breast cancer (Wang et al., 2006). However, a thorough understanding of the functions of the *RERG* gene is still lacking (Wang et al., 2006) because the majority of research is related to cancer (Habashy, 2011). In this study, fourteen primer pairs were designed based on the *RERG* gene sequence of the cow (ruminant). The *RERG* gene in goat was successfully cloned, demonstrating that the method of using a gene with high homology to the target as a reference to design primers for cloning is feasible. The target sequence contained complete CDS, including ATG and TGA (Accession numbers: JN672576, JQ917222, and JN580309, for QianBei Ma goats, GuiZhou white goats, and GuiZhou black goats, respectively). Results of the sequence analysis provided basic information for further research, including vector construction and transgenic work on the *RERG* gene in goats. Sequence homology among the three goat breeds was high: 98.5% between QianBei Ma goats and GuiZhou white goats, 98.8% between QianBei Ma goats and GuiZhou black goats, and 99.3% between GuiZhou white goats and GuiZhou black goats. The results indicate that homology is positively correlated with the distribution of the three goat breeds.

The *RERG* gene plays an important role in adjusting growth, differentiation, survival, and proliferation of cells (Habashym et al., 2011, Habashy et al., 2011). In a previous study, Chen et al. (2012) reported a mutation site in the first intron at 264 bp in the QianBei Ma goat, and results of associations between SNPs and growth traits demonstrated that body weight, body height, and chest breadth were significantly greater in individuals with the AB genotype than those with the AA or BB genotypes ($P < 0.01$). However, Chen et al. (2012) only studied the QianBei Ma goat using direct DNA sequencing. In view of this, our study identified polymorphisms in the *RERG* gene using PCR-SSCP in three indigenous Chinese goats and then evaluated the association of polymorphisms and growth traits in QianBei Ma goats. Four mutation sites (56, 826, 1434,

and 1798 bp) were found in the third exon, while no SNPs were found in the first and second exon regions. In addition, the results of the current study suggest that genotypes AB, CD, EF, and GH are superior to other genotype in many growth traits. The genetic characteristic analysis suggested that alleles of A, C, E, and G were advantageous alleles. The polymorphism information content (PIC) indicated moderate polymorphism, likely due to the input of external blood of the same species with good quality by the local breeding center, which would enrich genetic variation.

Due to the effects of many factors, we only studied SNP correlation analysis with the growth traits of QianBei Ma goats, which suggest that genotypes AB, CD, and EF are superior to other genotypes in many growth traits. These results provide baseline information on the possible effects of the *RERG* gene on growth traits is indigenous Chinese goats, as well as certain theoretical foundations on improved feeding and genetic resources. No significant differences in body weight between genotypes was observed at the 1798(T/A) site, thus, further research is required to confirm whether this site can be used as an advantageous genotype.

The expression of the *RERG* gene was studied using RT-PCR in various tissues of the three goat breeds. The results show that the expression level of the *RERG* gene was relatively consistent across the different breeds. However, mRNA expression levels were higher in lung and spleen tissue than in the other tissues and expression was moderate in muscle tissue. Interestingly, the expression levels differed in muscle for the different goat breeds: the GuiZhou black goats had the highest expression, followed by the QianBei Ma goats and then the GuiZhou white goats. The average weight (Table 1) of the three breeds was the opposite of the expression of the *RERG* gene in muscle, suggesting that expression of the *RERG* gene in muscle may be related to growth and it may play a negative role in goat growth.

In conclusion, in the current study, we cloned the CDS sequences of the *RERG* gene and completed relevant bioinformatics analysis for the first time. The 56(G/C), 826(A/G), and 1434(T/C) polymorphisms were important in improving growth characteristics of indigenous Chinese goats, and the *RERG* gene may be treated as a candidate gene to facilitate breeding of indigenous Chinese goats. Furthermore, we explored the expression of the *RERG* gene in different tissues of different breeds at the same age to lay a solid foundation for future research.

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