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Asian Journal of Animal and Veterinary Advances

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Asian Journal of Animal and Veterinary Advances 7 (10): 1014-1020, 2012
ISSN 1683-9919 / DOI: 10.3923/ajava.2012.1014.1020

cDNA Cloning and Analysis of Polymorphism of RERG Gene in QianBei Ma Goat

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ABSTRACT

The aim of this study was to obtain CDS sequences of RERG (ras-related and estrogen-regulated growth inhibitor) and reveal the polymorphism of exon3, exon4 and part of exon5 of RERG in QianBei Ma goat and evaluate its relationship with growth traits. The cow RERG genome sequences which own the high identity at nucleotide to the goat were selected to design the specific primers. The cDNA encoding RERG was obtained by the reverse transcription PCR (RT-PCR). The purified RT-PCR product was cloned into T vector and then the sequence was analyzed. The SNPs was detected by directional sequencing. One new mutation sites of C264A were identified in the sequence that submitted it to GenBank and get registration number JQ818422. Associations between growth traits and RERG gene polymorphism were investigated. Results suggested that genotype AB were superior to genotype AA and BB in many growth traits which indicated that the genotype AB can be treated as a candidate gene type to facilitate QianBei Ma goat breeding work.

Key words: QianBei Ma goat, RERG gene, cDNA cloning, analysis of polymorphism

INTRODUCTION

RERG (ras-related and estrogen-regulated growth inhibitor) gene belongs to the ras super gene family. Ras super gene family could encode important function protein which can mediate pathways of growth factors, cell factors and various extracellular signals and plays an important roles in adjusting the cell growth, differentiation, survival and appreciation (Habashym *et al*., 2011). It worked as a signal converter or molecular switch in signal transduction (Bourne *et al*., 1991; Yu *et al*., 2003). Finhn *et al*. (2001) used the statistical analysis of microarray to identify and name a new gene--- the ras-related and estrogen-regulated growth inhibitor (RERG). RERG owns 40-50% the identity with some ras super gene family members by using homologous comparison and it also has a GTP conservative combining domain. But it lack the sequence that is participation and lipid modification at C- terminal (Xu *et al*., 2000; Yu *et al*., 1999; Zhi *et al*., 2012).

QianBei Ma goat is one of three excellent goat breeds in Guizhou province, China and is fed widely by local people. It has a strong adaptive capacity, crude feed tolerance, high reproductive rate and meat productivity, good taste and excellent skin quality (Zhi *et al*., 2012; Fen *et al*., 2011). The study (Xing *et al*., 2010) showed that the meat of QianBei Ma goat was excellent and possesses high values of exploration and ultilization. Research should that about RERG gene of QianBei Ma

goat has not been found. For this reason, We took QianBei Ma goat as the research object, the cDNA sequences of RERG gene were cloned by molecular cloning technology, submitted to the GenBank and analysed sequence subsequently. It would provide some theoretical foundation for the further study on the expression and regulation of RERG gene in QianBei Ma goat.

This study also aimed to detect SNP sites in RERG gene of QianBei Ma goat by using the method of double directional sequencing. We taking the detected SNP sites correlation analysis about growth traits of QianBei Ma goat, It provided new theory method and technical route to improve the growing performance of QianBei Ma goat.

MATERIALS AND METHODS

Animals: All procedures involved in animals were approved by the breeding center of QianBei Ma goat in XiShui county, Guizhou province, China. Spleen were collected from adult QianBei Ma goat with ewes and ram, respectively in 2011, then saved in liquid nitrogen. Venous jugular blood samples (8 mL per ewe) were collected from QianBei Ma (322) of 36-48 months that fed under same feeding conditions in 2010.

Packing the blood samples into the plain tube and saving in -40°C. The seven traits (body weight, body length, chest circumference, body high, chest deep, chest wide and tube circumference) were measured.

RNA and DNA extraction: According to procedures of kit of Trizol RNA extracted the total RNA in QianBei Ma goat spleen and kept at -80°C. Genomic DNA was extracted from whole blood by phenol-chloroform method and then dissolved in TE buffer [10 mmol L^{-1} tris-HCl (pH 8.0), 1 mmol L^{-1} EDTA (pH 8.0)] and kept at -40°C (Chu *et al*., 2011).

Primer design and PCR amplification: The cow RERG genome sequence (NM_001076198) which is high identity with goat was selected to design a specific primer by using the Prime5 software. The RERG CDS sequences of QianBei Ma goat which were expected as 629 bp in length were amplified by the specific primer that had been synthesised in ShengGong biotechnology company Shanghai china. Primer GFI-CDS sequence:

- **Forward :** 5'-ATTGTCTACCAGCACCCAGCAT-3'
- **Reverse :** 5'-TCAGCAGTTAGGCAACTTCG-3'

RT-PCR amplification and cloning in CDS of RERG of QianBei Ma goat: According to the procedures of Revertaid™ Fist strand cDNA synthesis kit obtain the transcription product (cDNA). PCR amplification of Primer GFI-CDS: the cDNA 2.5 UL, 10 by PCR Buffer (Mg^{2+}) 2 μL, dNTPs (2.5 mmol L^{-1}) 2.0 UL, up and down primer each 1 μL, Taq enzyme (5 U μL^{-1}) 0.4 UL, Plus ultra pure water to 20 μL^{-1} of total product. Recycling program: 95°C the degeneration 5 min; 94°C degeneration 30 sec, 55°C annealing 40 sec, 72°C extensions 60 sec, 35 circulation; 72°C extensions 10 min, 4°C save, 1% agarose gel electrophoresis. Then cloning sequencing, three times independent sequencing.

The detection of single nucleotide polymorphisms: According to the sequences of REGE gene of cow (NC_007303), Three specific primer had been designed by Prime5 which amplified the third exon, fourth exon and part of fifth exon in REGE gene, respectively. (Table 1) PCR amplification of Primer GFI-1, GFI-2 and GFI-3 2.5 UL, 10 by PCR Buffer (Mg^{2+}) 2 μL, dNTPs (2.5 mmol L^{-1})

Table 1: Primer sequences of PCR amplification

Primers	Sequences of primers	Amplified length (bp)	Annealing temperature (°C)
GFI-1	F: 5'- CATTATACCAATTTTTAGGC-3'	317	55.9
	R: 5'-ATGTCATTCTTTCTTTCAGG-3'		
GFI-2	F: 5'-CCATTTTACCTTCGTTTTGCTC-3'	298	62.1
	R: 5'-CCCTTGGAGCACTGAGTAATTT-3'		
GFI-3	F: 5'-TGCTGAATGTAAGGAATGGTTG-3'	247	62.1
	R: 5'- CATTCTTGGGCTTTTTGATCTC-3'		

2.0 UL, up and down primer each 1 μL, Taq enzyme (5 U μL^{-1}) 0.4 UL, Plus ultra pure water to 20 μL^{-1} of total product. Recycling program: 95°C the degeneration 5 min; 94°C degeneration 30 sec, 55.9/62.1/62.1°C annealing 45 sec, 72°C extensions 60 sec, 35 circulation; 72°C extensions 10 min, 4°C save, 1% agarose gel electrophoresis. PCR products of difference individuals were sequenced by method of two directional sequencing.

Sequences and data analysis: Sequences of REGE gene cDNA had been analysed by DNAStar program. Homologous comparison was done between QianBei Ma goat and cows, rats, chicken and toad by DNAMAN software. Sequence alignment by ClustalX1.81 software was built phylogenetic trees by software MegAlign program (1000 times repeat).

The following fixed effects model was employed for the analysis of growth traits in QianBei Ma goat and least squares mean was used to multiple comparison in growth traits among different genotypes. Age and sex are ignored in the statistical analysis since the testing ewes are at the same age. Yijk = u+markerk+eijk; where Yijk is a record of individual phenotype value; U is a group average; Markerk is a marker gene type effect; Eijk is a random error (Jin *et al*., 2010; Liu *et al*., 2011; Yuan *et al*., 2010).

RESULTS

RERG gene CDS cloning of QianBei Ma goat spleen: According to the predicted sequence, we designed specific primers and amplified the complete CDS. A single PCR amplification product was obtained and inspection the products of RT-PCR amplification with agarose gel electrophoresis (629 bp). It can visible amplification segment Fig. 1. PCR product recovery, cloning, picking to take three positive, cloning, results of three sequencing are consistent.

Analysis of RERG gene cDNA sequence: The sequencing results analysed by DNAMANN 4.0, the size turned out to be same as we expected (629 bp). It contains all coding sequences (CDS) including ATG and TGA. Then submit it to GenBank and get registration the number JN672576. RERG gene cDNA of QianBei Ma goat encoded 199 amino acids. Though genetic analysis by DNAMAN 4.0, we can conclude that its base composition of each base. A = 29.67%; G = 28.00%; T = 21.00%; C = 21.33%, among them A+T = 50.67%; C+G = 49.33%.

Analysis of the homology: The RERG gene sequences of other animals in GenBank database were searched for the analysis of the homology. RERG gene CDS of QianBei Ma goat were induced amino acids by DNAStar software program and constructed the molecular evolution tree compared with other species (Fig. 2). Homologous comparison was done between QianBei Ma goat and cows, rats, chicken and toad. The identify are 98.5, 87.0, 82.7 and 78.0%, respectively.

Detection of RERG gene polymorphism by the method of two directional sequencing: Three primer of GFI-1, GFI-2, GFI-3 were designed to amplified genomic NA. The directional

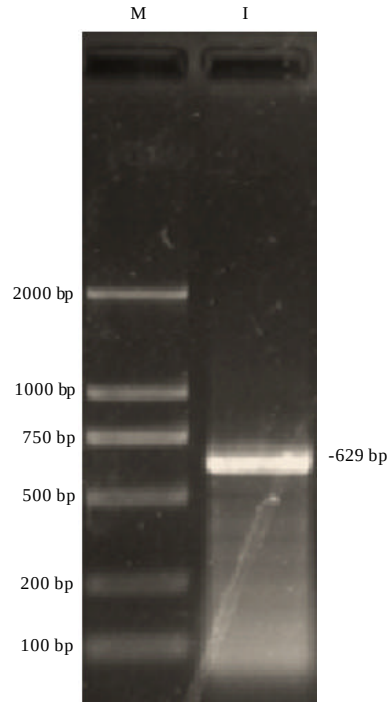

Fig. 1: M DL2000 marker; the products of RT-PCR

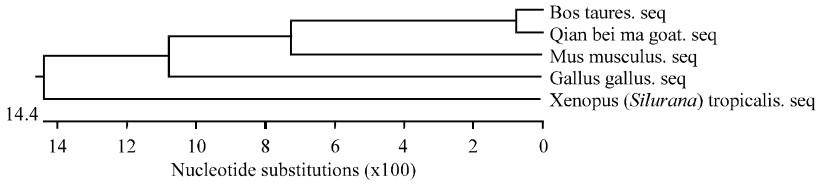

Fig. 2: The phylogenetic trees of RERG molecule of QianBei Ma goat and other species

sequencing was performed after PCR products detection (Fig. 3). Results showed that only the PCR products amplified by primers GFI-1 displayed polymorphisms. Three genotypes (AA, AB and BB) were detected by primer GFI-1 (Fig. 4).

Sequencing of different genotypes and nucleotide mutations: For primer GFI-1, sequencing revealed a nucleotide mutation (264 bp C→A) between genotype AA and genotype BB. The sequence submitted it to GenBank and get registration number JQ818422 (Fig. 4).

Distribution of 3 genotypes in QianBei Ma goat: For RERG gene, this position showed that homozygous type were more than miscellaneous type. Allele frequency results showed that was advantage alleles, AA proved to be advantage alleles type. It belong to moderate polymorphism (Table 2).

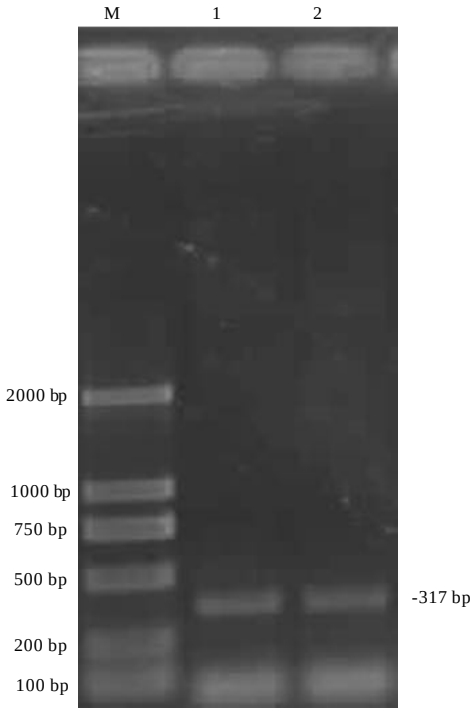

Fig. 3: M DL2000 marker; the products of PCR

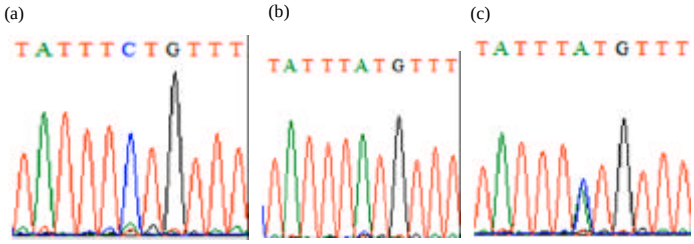

Fig. 4: The sequence comparison of, (a) AA, (b) BB and (c) AB genotype of 264 bp

Table 2: The analysis of genetic diversity of RERG gene

Genotype	No.	Genotype frequency	Allele	Allele frequency	He	Ho	Ne	PIC
AA	192	0.596	A	0.736	0.388	0.612	1.634	0.307
BB	40	0.124	B	0.264				
AB	90	0.280						

PIC>0.5 high diversity; 0.25<PIC<0.5 moderate: PIC<0.25 low diversity

Influence of fixed effects on growth trait in QianBei Ma goat: Body weight, body height and chest circumference index were significantly higher in individuals with genotype AB than AA and BB in ($p<0.01$). The body length difference between genotype AA and BB was significant ($p<0.05$) and the body length the between genotype AA and AB was highly significant ($p<0.01$) (Table 3).

Table 3: Effect of different genotypes on growth trait

	Genotype		
Trait	AA (n = 192)	BB (n = 40)	AB (n = 90)
Weight (kg)	43.700±19.471^{A}	43.525±3.743^{A}	46.433±3.666^{B}
Height (cm)	60.965±3.718^{A}	61.755±3.324^{A}	64.174±5.220^{B}
Length (cm)	67.832±4.351^{Aa}	70.558±2.470^{b}	71.436±3.022^{B}
Chest circumference (cm)	80.781±4.862^{A}	81.148±3.820^{A}	85.380±4.817^{B}
Chest deep (cm)	30.403±1.910	31.113±1.586	32.068±1.671
Chest broad (cm)	18.409±2.102	19.078±1.634	19.259±1.861
Tube circumference (cm)	8.024±0.582	7.958±0.522	8.693±0.860

Data marked with different superscripts differ significantly at $p<0.01$ and $p<0.05$ for capital and small letters, respectively

DISCUSSION

RERG gene was first reported in breast cancer. The functions of RERG are largely unknown (Wang *et al*., 2006). A lot of research is about the cancer but the study of RERG Gene in goat had not been reported.

In the study, a specific primer (GFI-CDS) had been designed by ruminant animals with the cow RERG gene sequences. And the RERG gene in the QianBei Ma goat were cloned successfully. It explained that the sequence of highly identify design primer for PCR gene cloning was feasible. It Contains all coding sequence (CDS) included ATG and TGA. Then submitted to GenBank and the registration number was JN672576. The sequence analysis was conducted subsequently. It provided basic information for further researches including express, carrier construction and transgenic works in RERG gene of goats. But the theory needs experiment to be done. It could be a good functional reference and good enlightenment on correct understanding structure and function of protein if relevant no information.

The phylogenetic tree was divided into three branches: mammals, birds and amphibians. The results were consistent with the rule of species evolution. This suggested that RERG genes can be used as structural gene to distinguish different.

This study reveal the polymorphism of exon3, exon4 and part of exon5 of REGE gene in QianBei Ma goat and evaluated its relationship with growth traits. Only the PCR products amplified by primers GFI-1 displayed polymorphisms. The change at 264 position in the sequence which submitted to and got registration number of JQ818422 detected mutation in QianBei Ma goat. Three genotypes (AA, AB and BB) were detected by primer GFI-1. Genotype AB were better than the genotype AA and BB in many growth traits. The genotype AB of RERG gene could be regarded as marker for growth traits in QianBei Ma goat. It will provide study basis between RERG genes and growth traits as well as certain theoretical foundation of better feeding and genetic resources of QianBei Ma goat.

CONCLUSION

In the present study, the CDS sequences of RERG were cloned and sequenced. Subsequently submitted it on the NCBI and the GenBank registration number was JN672576. Genotype AB were superior to genotype AA and BB in many growth traits, it can be treated as a candidate gene type to facilitate QianBei Ma goat breeding work. It also showed RERG gene can be treated as a candidate gene to facilitate goat breeding work. Therefore, the results in the present study were preliminary.

ACKNOWLEDGMENT

This study was supported by Agricultural science research project of Guizhou [NO. QKH-NY (2008) 3044].

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