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A SNP in *ADIPOQ* Gene can be Used as Molecular Marker for Growth Traits in Goats

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Authors' Contribution
QA, XD and YH conceived the study and designed the work. XD, DW, ZZ, YS, XW, PY and XL analyzed and interpreted the data. QA and XD wrote the manuscript. BR, XW, ZX and HH revised the manuscript critically. All authors read and approved the final manuscript.

Key words
Goat, *ADIPOQ* gene, SNP, Growth traits, Association analysis

ABSTRACT

This study investigated single nucleotide polymorphisms (SNPs) in intronic regions of the *ADIPOQ* gene and estimated their contributions to growth traits of goats. We first found a SNP site at the 14059th base position in the second intron of the *ADIPOQ* gene (NC_030808.1: *g*. 14059 C>T). At the same time, we performed association analysis between this SNP and growth traits in goats. These results showed that the base (C or T) at the SNP was associated with changes in body length with CT and TT genotypes giving greater values than CC genotypes. This site therefore can be used as a molecular breeding marker for early selection of growth traits in goats.

INTRODUCTION

Molecular breeding technology has many advantages over conventional breeding, which can effectively avoid the problems of long generation interval, slow progress, low improvement efficiency, waste of excellent germplasm resources. The key to molecular breeding is molecular marker-assisted selection (MAS). Goat *ADIPOQ* gene is a candidate to target for goat growth traits. It was first found on human chromosome 3, with a total length of 17 kb, including three exons and two introns, encoding 247 amino acids (Wang *et al*., 2007; Pajvani *et al*., 2003). The ADIPOQ protein binds with AdipoR1 or AdipoR2 receptors and can activate adenylate activated protein kinase, p38 mitogen activated protein kinase, Jun amino terminal kinase, nuclear factor kB and other signal pathways and so play a role in the target tissue. Studies have shown that ADIPOQ can directly stimulate adenosine monophosphate-activated protein kinase (AMPK) in skeletal muscle and liver, lead to phosphorylation of acetyl CoA carboxylase, promote fatty acid oxidation, reduce lipid accumulation in skeletal muscle, improve insulin resistance in the liver and reduce the production of liver sugar and the synthesis of very low-density lipoprotein (Yamauchi *et al*., 2002). It was found that the binding of *ADIPOQ* to the AdipoR1/2 receptor increased the expression of the *myhc1* and *myhc2x* genes, decreased the expression of the

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myh2b gene, and changed the composition of muscle fiber via AMPK and peroxisome proliferator-activated receptor alpha (PPARα) signal pathways (Zhang *et al*., 2011). In human, monkey and mouse, it was found that *ADIPOQ* gene is expressed in adipose and the level of *ADIPOQ* in blood reduced when there exists over-deposition of fat, while the expression of the *ADIPOQ* gene and the level of ADIPOQ in blood increased when weight decreased (Zhou *et al*., 2018). Therefore, the *ADIPOQ* gene is related to body weight. Adiponectine (*ADIPOQ*), the product of the *ADIPOQ* gene expression, which belongs to the family of adipocytokines, is a specific hormone secreted by adipocytes and it is mainly related to insulin tolerance regulation, obesity, cardiovascular disease, type II diabetes and other physiological diseases (Yang *et al*., 2006; Liu *et al*., 2018). Therefore, the *ADIPOQ* gene is also related to metabolism.

It was found that the variants of c.– 67G / A and c.– 892C/T in the promoter region of the *ADIPOQ* gene had an effect on the carcass traits and meat quality in pig (Cieslak *et al*., 2013). In the promoter region of the *ADIPOQ* gene in cattle, the variants of c. – 176A/G had an effect on carcass traits; the variants c.- 199 C/T and c.-34 G/A also had some effect on the area of the eye muscle and the thickness of the back fat (Shin and Chung, 2013; Morsei *et al*., 2006). Therefore, there may also be a relationship between the *ADIPOQ* gene and the growth traits in goats. However, molecular markers of goats are rarely reported. The purpose of this paper is to investigate the possibility of a molecular genetic marker related to the growth traits of goats.

MATERIALS AND METHODS

Animals and data collection

In order to explore the genetic variation of the goat *ADIPOQ* gene, 339 individuals representing three goat breeds in China were sampled: Guizhou white goats (N=98), Guizhou black goats (N=155) and Henan hybrid goats (N=86). These breeds are important for goat meat production in China and are reared in the Provinces of Guizhou and Henan. The Guizhou white goats were from three farms (Dejiang County, Guizhou Province); the Guizhou black goats were from three farms (Bijie City, Guizhou Province, China); and the Henan hybrid goats were from four farms (Yongcheng City, Shangqiu City, Henan Province). On each farm, the goats were randomly selected, almost all of them were females, with the age generally between 2-2.5 years old. Basic data (withers height, body length, heart girth, circumference of cannon bone, body weight) for the corresponding individuals were also recorded. The DNA samples were anticoagulated with acid citrin dextrose (ACD) and quickly brought back to the laboratory in an ice box at - 80°C.

Genotyping

The genomic DNA of the samples was extracted using the phenol chloroform method.

The PCR amplification of second intron of the goat *ADIPOQ* gene was conducted using 2×Taq PCR Starmix, with a reaction volume of 10 μL, which contained 1μL of DNA pool, 5 μL of 2×Taq PCR Starmix, 0.5 μL of upstream primers, 0.5 μL of downstream primers and 3μL ddH_2O. F: 5'-GGTTAAGCTTCTTTACCACAGAGTG-3'; R: 5'-CTTCCACACTGACCGAAGTC-3'. Thermal cycling conditions were as follows: pre denaturation at 95 °C for 300 s and one cycle; denaturation at 95 °C for 30 s; annealing at 54.1 °C for 30 s; extension at 72 °C for 30 s and 35 cycles; final extension at 72 °C for 600 s and one cycle; and preservation at 12 °C. The amplified products of PCR were analyzed by 1% agarose gel electrophoresis for 0.5 h. The amplified products were sequenced by Shanghai biotechnology Service Company.

To define genotypes of the variants, 10 μ L PCR products were digested with endonuclease *Ava*II (Takara company). The *Ava*II digestion system was conducted using 1× NE buffer, with a reaction volume of 10 μL, which contained 5μL of PCR product, 0.5μL of 1× NE buffer, 0.1 μL of *Ava*II and 4.4 μL of ddH_2O; digestion took place in a water bath for 40-60 min.

Data analysis

The effective allele number (NE), heterozygosity (He), polymorphism information content (PIC), genotype frequency and allele frequency of goat *ADPIOQ* gene g. 14059 C > T were counted by Microsoft Excel software, and Hardy-Weinberg test was carried out (P<0.01, χ^2-test).

Genotype frequencies were determined for each breed by direct counting using formula

$$P_{BB}, N_{BB}/N$$

where P_{BB} is *the* genotype frequency given a certain nucleotide position, N_{BB} is number of individuals with *BB* genotype in the population, and N is total number of individuals in the population

Gene frequency was determined using formula

$$P_B=(2N_{BB}+N_{Bb1}+N_{Bb2}+N_{Bb3}+N_{Bb4}+\ldots\ldots+N_{Bbn})/2N$$

where P_B is frequency of allele B; N_{BB}, Number of individuals with *BB* genotype in the population; N_{Bbi} is number of individuals with *Bbi* genotype in the population for i = 1,…, n; and b1~bn, n different alleles in a multi-allelic system with allele B.

Population genetic indexes, such as *He*, *Ho*, *Ne*, and *PIC* were calculated according to Nei and Roychoudhurg (1974), respectively. The formulas were as follows:

$$H_0 = \sum_{i=1}^{n} P_i^2 \qquad H_e = 1 - \sum_{i=1}^{n} P_i^2 \qquad N_e = 1/\sum_{i=1}^{n} P_i^2$$

$$PIC = 1 - \sum_{i=1}^{n} P_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2$$

where Pi is the frequency of the i allele and n is the number of alleles.

General linear model (GLM) and one way ANOVA in SPSS 20.0 were used to analyze the correlation among genotypes, combination genotypes and body size traits of SNP loci. The model used in the analysis is $Y_{ij} = \mu + G_i + \varepsilon_{ij}$, where Y_{ij} is the observed value of body size traits, μ is the observed mean value of traits, G_i is the genotype effect, and ε_{ij} is the random error (Boldman *et al*., 1993; Zhao *et al*., 2004; Hendersor, 1986).

RESULTS

Mutation in the intron 2 of ADIPOQ gene

Figure 1 shows A C/T mutation or natural cleavage site in the intron 2 region of the *ADIPOQ* gene in goat by comparing the sequence of the target fragment with the reference sequence (80105499th position of NC_030808.1 exists in the amplification region of the primer pair).

PCR–RFLP analysis of ADIPOQ gene

Figure 2 shows the product after endonuclease AvaII digestion. CC genotype shows two bands of 46bp and 272bp, the CT genotype shows three bands of 272bp, 46bp and 318bp, and the TT genotype shows one band of 318bp. However, the 46bp fragment is too small to be displayed.

Population genetic analysis

There are two alleles (C and T) and three genotypes (CC, CT and TT) at the 14059th position of the *ADIPOQ* gene (Table I). No matter which breed, C gene frequency is higher than T gene frequency, and CT genotype frequency is higher than CC and TT, and TT genotype frequency is the lowest in the three genotypes.

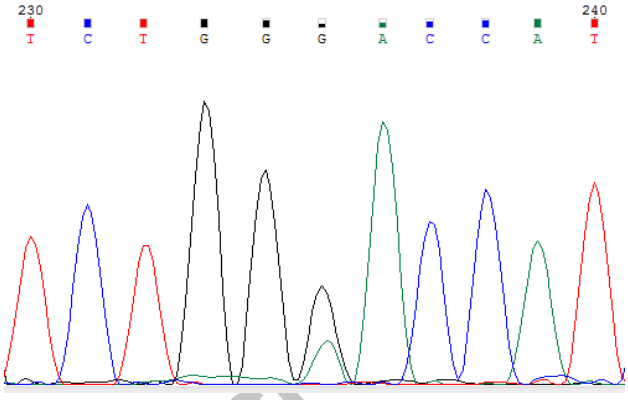

Fig. 1. The sequencing maps of the novel SNP in the goat *ADIPOQ* gene. The SNP site is at the 14059th base position (NC_030808.1: g. 14059 C>T).

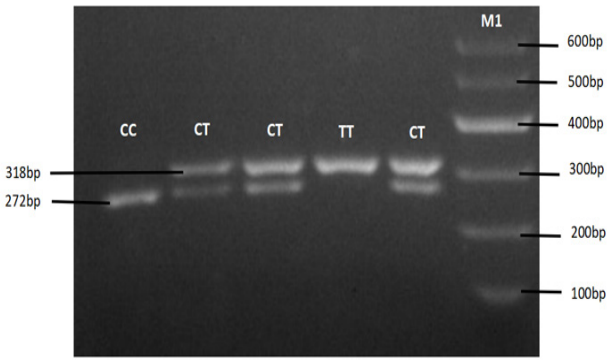

Fig. 2. PCR–RFLP patterns of SNP - AvaII locus in goat the *ADIPOQ* gene. CC = 46 +272 bp. TC = 272 + 46 + 318 bp. TT = 318 bp. M1 = Marker I. The 46 bp fragment was too short to be visible.

Table I. Population genetic analysis of *ADIPOQ* gene in goats.

Breed	Sample size	Genotype frequency			Allele frequency					
		P_{CC}	P_{CT}	P_{TT}	C	T	He	Ne	PIC	$X^2(1)$
Guizhou white goat	98	0.204 (20)	0.724 (71)	0.072 (7)	0.566	0.434	0.491	1.965	0.371	22.097
Guizhou black goat	155	0.200 (31)	0.645 (100)	0.155 (24)	0.523	0.477	0.499	1.996	0.374	13.206
Henan Hybrid goat	86	0.128 (11)	0.814 (70)	0.058 (5)	0.535	0.465	0.498	1.990	0.374	34.775
Total goat	339	0.183 (62)	0.711 (241)	0.106 (36)	0.538	0.462	0.497	1.988	0.374	62.738

Note: $X^2_{0.05}(1) = 6.635$, $X^2(1) > X^2_{0.01}(1)$, $P<0.01$. There was highly significant difference ($P < 0.01$).

Table II. Average growth trait values for three genotypes of goats defined from SNP analysis of the *ADIPOQ* gene.

Growth traits	Genotype (Mean±Standard error)			F-test P-value
	CC	CT	TT	
Withers height (cm)	58.95±1.005	61.04±0.54	62.44±1.03	0.083
Body length (cm)	60.47 ^{b}±1.11	64.31 ^{a}±0.73	65.035 ^{a}±1.50	0.035*
Number	42	169	29	
Heart girth (cm)	72.46±0.99	75.72±0.59	74.23±1.50	0.034
Number	62	240	36	
Circumference of cannon bone (cm)	9.00±0.17	9.37±0.10	9.08±0.26	0.194
Number	28	113	12	
Body weight (kg)	27.11±1.04	27.94±0.632	28.29±0.98	0.791
Number	51	171	31	

Note: For growth traits with F-test *P*-value less than 0.05, there is no significant difference (P>0.05, post hoc t-test) between means with the same superscript letters.

The genetic variation degree of the three populations is similar, and the heterozygosity of Guizhou black goat is highest. In terms of polymorphism information content (PIC), all the goat breeds tested were in moderate polymorphism (PIC > 0.5 was high polymorphism, 0.25 < PIC < 0.5 was moderate polymorphism, PIC < 0.25 was low polymorphism). Among them, Guizhou black goat and Henan hybrid goat gave the highest (0.374). The Chi-squared test for Hardy Weinberg equilibrium showed that the χ^2 value of three goat populations reached a significant level, that is, the gene frequency and genotype frequency of these three goat populations were in the state of Hardy Weinberg disequilibrium ($P < 0.01$).

Association analysis

The results show that different genotypes, which the mutation at the 14059th position of the *ADIPOQ* gene in goats formed, were associated with the growth traits body length and heart girth ($P < 0.05$, F-test). Specifically, mutation of the C/T base at this locus was associated with body length, and mean values for the CT type and TT type were significantly ($P < 0.05$, post-hoc t-test) higher than for the CC type. It is suggested that the allele C of the *ADIPOQ* gene is closely related to the growth traits (body length) of goats (Table II). Therefore, CT and TT can be used as molecular breeding gene markers for early selection of growth traits in goats.

DISCUSSION

It is predicted that the mutation in the *ADIPOQ* gene would affect the metabolism of sugar and lipid, and then affect the growth and development and meat quality in goats. The + 67bp G > C mutation in exon 2 of the *ADIPOQ* gene affects the live weight and carcass weight before slaughter in Tebetan sheep (Yang *et al*., 2014). In Qinchuan cattle, the missense mutation G to C at 64bp of exon 2 of the *ADIPOQ* gene had an effect on live weight, carcass weight and eye muscle area before slaughter; the missense mutation C to T at 50 bp of exon 3 not only affected live weight, carcass weight, leg hip circumference before slaughter, but also back fat thickness and tenderness (Yang, 2009). The *ADIPOQ* gene g.81966377 T > C locus in Hanwoo cattle has an influence on the eye muscle area and the marbling score, on the backfat thickness and carcass weight (MN *et al*., 2019); the dominant allele C of the *ADIPOQ* gene g.8196235 C > T locus is related to low marbling score; the dominant allele D of g.81966364 D > I locus also shows low additive effect on marbling score (Choi *et al*., 2015). Dai *et al*. (2006) showed that the g.81967079 G > A mutation in the *ADIPOQ* gene had an effect on backfat thickness and eye muscle area in pigs. There exists a single base mutation site in the *ADIPOQ* gene related to growth traits in each of these different livestock breeds.

It is very important to study the impact of mutations on the growth traits of livestock and poultry. This paper only studies one mutation site, and more genes and gene mutation sites which could be related to growth traits need further study.

CONCLUSION

In this study, we determined that the single nucleotide mutation in the *ADIPOQ* gene had an effect on growth traits. We conclude that this SNP could be used as a molecular marker in future breeding programs that aim to select for growth traits in goats.

DECLARATIONS

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Ethics approval and IRB approval

A series of animal experiments in our study were followed the relevant laws and policies about animal welfare. Furthermore, all operating procedures involved animals were approved by the Faculty Animal Policy and Welfare Committee of Northwest A and F University (FAPWC-NWAFU, protocol number NWAFAC1008). This article does not contain any studies with human participants or animals performed by any of the authors.

Statement of conflict of interest

The authors have declared no conflict of interest.

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