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Short Communication

Association Analysis of Growth Hormone (*GH*) Gene and Insulin-Like Growth Factor I (*IGFI*) Gene with Growth Traits in Dazu Black Goat

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ABSTRACT

An association analysis of single-nucleotide polymorphisms (SNPs) in the *GH* gene (*GH-781* and *GH-1148*) and *IGF1* gene (*IGF1-1617*, *IGF1-4700* and *IGF1-5752*) with the growth phenotype was performed in Dazu black goats. The results revealed that *GH-1148* (C/T) was significantly associated with body length, body height and chest circumference ($P<0.05$) at the age of 180 days, while T/T was the dominant genotype. Further, *IGF1-1617* (G/A) was significantly associated with body weight and body height ($P<0.05$) in 60-day-old goats and was the dominant genotype. Therefore, the two markers (*GH-1148* and *IGF1-1617*) identified in the current study may be helpful for the molecular breeding of Dazu black goats in the future.

Goat products have broad market prospects. Goat production is one of the key elements contributing to the economy of farmers living in arid and semi-arid regions (Askari *et al*., 2011). Dazu black goats are an indigenous goat genetic resource in southwest China and have the advantages of a high reproduction rate (the average lambing rate at first birth was 197. 31%, and the average number of lambs produced by female lambs was 272. 32%) (Zhao, 2008), good meat quality, strong adaptability to harsh environments and resistance to disease. However, the goat has not undergone strong artificial selection for production traits and still has the disadvantages of a slow growth rate and low meat yield. Thus, relative growth traits such as weight and meat production still need to be improved in Dazu black goats.

Growth is a complex physiological process that is influenced by various factors, and many molecular studies related to growth traits in domesticated animals have been reported (Cannata *et al*., 2010). It was suggested that the somatotropic axis should be focused upon because growth hormone (GH) and its downstream factor, insulin-like growth factor-1 (IGF-1), play significant roles in growth and metabolism (Yao *et al*., 2007).

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GH mainly participates in stimulating the proliferation, migration, and differentiation of bone and cartilage cells and also regulates the metabolism of proteins, sugars and fat. GH triggers the production of IGFs by binding to the GH receptor (GHR) in target tissue cells. The DNA variations in GH that cause amino acid substitutions have been associated with reproduction and growth traits in cattle, swine, sheep and goats (Ardiyanti *et al*., 2009; Paiva *et al*., 2006; Dettori *et al*., 2019; Zhang *et al*., 2011; Monte *et al*., 2019). The goat *GH* gene consists of five exons, separated by four introns, and polymorphism exists in almost every exon region (El-Halawany *et al*., 2019). Malveriro *et al*. (2001) studied the polymorphism of the *GH* gene in the Angora goat and concluded that different genotypes were significantly associated with milk production (Malveiro *et al*., 2001). Hua *et al*. (2009) assessed the association between different *GH* genotypes and growth traits and reported that goats with the AB genotype at the 781A > G locus had a heavier body weight than did those with the AA genotype at weaning (Hua *et al*., 2009).

Moreover, the other important components of the somatotropic axis, IGFs, are the major mediators of GH-induced growth in vertebrates and can regulate many growth-related processes. IGF1 promotes the differentiation of muscle cells and regulates the growth of skeletal muscle before and after the birth of an animal (Oksbjerg *et al*., 2004). Several studies have demonstrated

that high levels of IGFI in the blood could increase the growth rate (Beckman *et al*., 2004; Buonomo and Baile, 1988). Many genetic polymorphisms in the *IGFI* gene have been reported to be associated with growth traits in bovines (Akis *et al*., 2010; Mullen *et al*., 2011; Abdolmohammadi and Zamani, 2014), swine (Yue *et al*., 2014; Cheng *et al*., 2016; Hao *et al*., 2011), sheep (Meira *et al*., 2019; Dettori *et al*., 2019; Tuersunjiang *et al*., 2017) and goats (Luo *et al*., 2019; Deng *et al*., 2010; Wang *et al*., 2011; Zhang *et al*., 2008). In view of its important roles in animal growth, the *IGF-I* gene has been considered to be a candidate marker associated with growth traits.

The present study was aimed to identify the associations between SNP loci in the *GH* and *IGF1* genes and production traits in the Dazu black goat population to find loci with remarkable genetic effects for marker-assisted selection and to provide potential markers and technical support for molecular breeding of goats in the future.

Materials and methods

A total of 196 blood samples from Dazu black goats were collected at the Chongqing (China) Dazu black goat breeding farm (the parents of the goats were purchased in and around DaZu district in recent years, which means that they are largely genetically unrelated). The data on the birth weight, body weight, body height, body length and chest circumference were obtained from the farm records. The blood samples were stored at -80°C for several weeks, and then genomic DNA was extracted using the standard phenol/chloroform method (Hua *et al*., 2009). The DNA samples were genotyped at 10 SNP sites (see supporting material 1). The PCR mixture contained 0.5 μL of SNaPshot Mix, 3 μL of pooled PCR products, 1 μL of pooled primers, and 0.5 μL of dH_2O for a total volume of 5 μL. All primer and extension primer information is shown in supporting materials 2 and 3. The SNaPshot reaction program consisted of incubation at 95°C for 2 min, followed by 40 cycles of 95°C for 10 s, 52°C for 5 s, and 60°C for 30 s, and incubation at 4°C indefinitely. Finally, the SNaPshot product was purified as follows: 2 μL of the digested SNaPshot reaction product was added to 8 μL of deionized formamide containing 0.4% LIZ120 and denatured at 95°C for 5 min. The product was then quenched at -20°C and sequenced using a 3730XL DNA analyzer (Daniel *et al*., 2015).

Allelic and genotypic frequencies were estimated for each locus by counting alleles and genotypes using the Statistical Analysis System. The Generalized Linear Model (GLM) in SAS (Version 8) was used to analyse the correlations between the genotype and economic traits. The traits corresponding to different genotypes were expressed as LSM± SE.

Results and discussion

By reviewing the literatures (Hua *et al*., 2009; Zhang *et al*., 2008; Seevagan *et al*., 2015; Singh *et al*., 2015) and comparing gene sequences (see support material 4), ten known SNP loci in the *GH* and *IGF-1* genes goat species were identified. Polymorphism of the ten loci were detected in 169 goats and only five loci (*GH-781, GH-1148, IGF1-1617, IGF1-4700* and *IGF1-5752)* showed polymorphism (see supporting material 4), with 2 loci (*GH-1148* and *IGF1-1617*) found to show significant associations with production traits (Table I) in the Dazu Black goat population.

First, two alleles and two genotypes (G/A and G/G) were detected at *GH-781* in exon 2 of the *GH* gene. This variation caused an amino acid change from serine to glycine at residue 35 (Seevagan *et al*., 2015). The results of the correlation analysis showed that there was no significant correlation between the different genotypes and body size and weight traits (P>0.05). The present findings are in agreement with those in Sirohi and Barbari goats (Singh *et al*., 2015). However, in the study by Hua *et al*. (2009), mutation at this site had a significant effect on the chest girth at birth and the weaning weight of Boer goats; goats with the G/A genotype had a body weight that was 2 kg heavier than that of goats with the GG genotype at weaning (Hua *et al*., 2009). Therefore, the association of *GH-781* with growth traits may depend on the variety.

At the other locus of the *GH* gene, *GH-1148*, there were three genotypes (C/C, C/T and T/T). Goats with the T/T and C/T genotypes had a greater body weight, body height and length than did those with the C/C genotype ($P < 0.05$) at the age of 6 months old. Meanwhile, An *et al*. (2011) found that polymorphisms in this locus and in other loci jointly affected the growth traits of the first and second generations after hybridization of Boer goats and Guanzhong milk goats (An *et al*., 2011). These data suggest that this locus can be used as a molecular marker-assisted selection site in a wide range of goat breeds.

Further, in this study, goats with the G/A genotype at the *IGF1-1617* locus of the *IGF-1* gene had a greater body weight and body height than did those with the G/G genotype at the age of 2 months. Although there were no significant differences between the two genotypes in other growth traits, the birth weight, body length and chest girth were higher at 2 months in the G/A genotype than those in the G/G genotype, which indicated that this locus also contributed to the growth of Dazu black goats. The results of this study were consistent with those of previous reports. Thus, Wang *et al*. (2011) showed that the *IGF1-1617* (G/A) and *IGF1*-1620 (C/T) polymorphisms were significantly correlated with villus size and birth weight in Xinjiang, Bogeda Cashmere and Nangjiang Cashmere goats

Table I. Association of the GH and IGF-1 genotypes with body weight and body size indices in Dazu black goats.

locus	Gen-otype	Birth weigh (kg)	60 days				180 days			
			Body weight (kg)	Body length(cm)	Body height (cm)	Chest girth (cm)	Body weight (kg)	Body length (cm)	Body height (cm)	Chest girth (cm)
GH-781	GA	2.25±0.05	7.56±0.17	41.71±0.33	42.56±0.35	46.64±0.38	12.02±0.41	49.47±0.54	47.49±0.52	55.33±0.65
	GG	2.25±0.07	7.63±0.25	42.56±0.48	42.91±0.51	47.02±0.54	12.68±0.71	51.07±0.94	49.33±0.90	56.13±1.12
GH-1148	CC	2.07±0.37	7.20±1.28	40.50±2.51	43.50±2.63	44.75±2.82	7.30±2.70	43.00±3.53a	40.00±3.36a	44.00±4.12A
	CT	2.19±0.06	7.54±0.22	41.77±0.43	42.01±0.45	46.61±0.48	11.98±0.49	49.27±0.65ab	47.33±0.61b	55.50±0.75B
	TT	2.30±0.05	7.63±0.18	42.18±0.36	43.11±0.38	47.05±0.41	12.57±0.50	50.72±0.66b	48.86±0.62b	55.97±0.76B
IGF1-1617	GA	2.44±0.18	9.02±0.63a	44.37±1.24	45.50±1.31a	48.87±1.40				
	GG	2.24±0.04	7.51±0.14b	41.87±0.28	42.53±0.29b	46.66±0.32	12.18±0.35	49.87±0.47	47.95±0.46	55.53±0.56
IGF1-4700	CC	1.90±0.26	7.87±0.90	44.25±1.77	42.25±1.88	48.50±2.00	12.70±2.80	52.00±3.71	45.00±3.55	58.00±4.38
	CT	2.15±0.10	7.31±0.35	41.69±0.68	42.81±0.72	46.63±0.77	12.21±0.84	49.91±1.12	49.09±1.07	56.18±1.32
	TT	2.28±0.04	7.63±0.15	41.99±0.30	42.65±0.32	46.74±0.34	12.17±0.40	49.81±0.54	47.75±0.51	55.33±0.63
IGF1-5752	CC	1.98±0.21	8.62±0.73	43.50±1.44	43.00±1.53	49.33±1.62	11.33±1.61	49.67±2.15	49.33±2.05	55.33±2.53
	CG	2.22±0.07	7.63±0.23	42.33±0.45	43.16±0.48	47.03±0.51	12.11±0.61	49.90±0.81	48.62±0.77	56.19±0.95
	GG	2.29±0.05	7.49±0.18	41.69±0.35	42.34±0.38	46.44±0.40	12.29±0.46	49.89±0.62	47.44±0.59	55.17±0.73

[a,b]Means of a trait under a particular effect with different superscripts differ significantly; figures in parentheses are number of observations.

(Wang *et al*., 2011). In addition, a significant correlation existed between the genotypes and body size in Attappady black goats, and GG was the dominant genotype at the *IGF1-1617* locus (Naicy *et al*., 2017).

In this study, the records of growth traits did not show any significant correlations with the genotypes at the *IGF1-5752* locus of the *IGF-1* gene. However, in Nanjiang yellow goat, studies have reported a significant correlation between the IGF1-5752 (G/C) polymorphism and body size traits, with C/C being the dominant genotype (Zhang *et al*., 2008). Deng *et al*. (2010) also studied the association between the SNP locus IGF1-5752 (G/C) and economic traits, and the results showed significant effects of the polymorphism on milk yield and body size traits in dairy goats; however, the dominant genotype was different for each trait (Deng *et al*., 2010). It was suggested that the sites of variation in the *IGF-1* gene may vary, with different dominant genotypes in different breeds.

In conclusion, the SNPs that we identified (*GH-1148* and *IGF11-1617*) could potentially be used as selection-associated genetic markers for molecular breeding of Dazu black goats. However, because of the small sample size of the population, data for the growth traits of some low-frequency genotypes were not obtained and need to be studied in the future.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2019.51.....

Statement of conflict of interest

The authors have declared no conflict of interest.

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Supplementary Material

Association Analysis of Growth Hormone (*GH*) Gene and Insulin-Like Growth Factor I (*IGFI*) Gene with Growth Traits in Dazu Black Goat

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Supplementary Table I. SNP site information for the *GH* and *IGF-1* genes.

Gene	GenBank no.	Location of mutation site	Variation type	gene	GenBank No.	Location of Mutation site	Variation type
GH	D00476	781	A/G	IGF1	D26119.2	1617	A/G
		1148	C/T			1620	C/T
		1575	A/G			4700	T/C
		2040	C/T			5524	C/T
		2092	A/C			5752	G/C

Supplementary Table II. PCR primer information.

Primer	Primer sequence (5'to3')
GH-781: F	TGAAATGTTCTCAGTCCCTG
GH-781: R	CAGTTGATGCAGGTGCTGAG
GH-1148: F	TTCCGAATAAGGCAGTGAGG
GH-1148: R	TTAAGATGAAGGAGGTCCCC
GH-1575: F	TCGCATCTCACTGCTCCTTA
GH-1575: R	CACGTGTGTTCTCCTGGCTA
GH-2040: F	ACACAAACATGCGGAGTGAC
GH-2040: R	ATTAGGAAAGGACAGTGGGC
GH-2092: F	TGCCAGCCATCTGTTGTTAC
GH-2092: R	CCGGGTCAATTATTCAGCAC
IGF1-1617: F	TTTACCCCAGTCGTTTGAGG
IGF1-1617: R	TAGTGACAAGAGGAGCAGAC
IGF1-1620: F	TTTACCCCAGTCGTTTGAGG
IGF1-1620: R	TAGTGACAAGAGGAGCAGAC
IGF1-4700: F	AAGGACTTCCCCAAGGTTTC
IGF1-4700: R	GCATCTTCACCTGCTGAAAG
IGF1-5524: F	TGGAAAAATCTGGGAGGGTC
IGF1-5524: R	TCATCCACGATTCCTGTCTG
IGF1-5752: F	ACAGGAATCGTGGATGAGTG
IGF1-5752: R	TATTCGGATGCTGCTGCTAC

Supplementary Table III. Primer information for single-base extensions.

Primer	Primer sequence (5'to3')
GH-781(A/G)	GACTGACTGCCTTCCCAGCCAT-GTCCTTGTCC
GH-1148(C/T)	TGACTGACTGACTACACCCAG-GTTGCCTTCTGCTTCTC
GH-1575(A/G)	CTGACTGACTGACTGACTGCTGAAG-GACCTGGAGGAAGGCATCCT
GH-2040(C/T)	TGACTGACTGACTGACTGACTCTGAG-GGTCATGAAGTGTCGCCGCTT
GH-2092(A/C)	GACTGACTTCCAGGGTCTAGGAAGG-CACG
IGF1-1617(A/G)	GACTGACTGACTAAGGAATATTCGTG-GAGGATGGG
IGF1-1620(C/T)	ACTGACTGACTCTCTG-GAATATAAAATTGCTCGCC
IGF1-4700(T/C)	CTGACTGACTGACTAGCCAGGGGAG-CGGGAGCAAGGCA
IGF1-5524(C/T)	GACTCCCACTCTAAAACTAGGCCTCT
IGF1-5752(G/C)	GACTGACTGACTGACTCATAACT-CAGATCTCGCGAAGATGG

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Supplementary Table IV. Genotypic and allelic frequencies of the *GH* and *IGF-1* genes in Dazu black goats.

locus	Geno-type	Num-ber	genetype frequency	Allele	Allele frequency
GH-781	GA	115	0.68	G	0.66
	GG	54	0.32	A	0.34
GH-1148	CC	2	0.01	C	0.14
	CT	70	0.41	T	0.86
	TT	97	0.58		
IGF1-1617	GA	8	0.05	G	0.02
	GG	161	0.95	A	0.98
IGF1-4700	CC	4	0.02	C	0.1
	CT	27	0.16	T	0.9
	TT	138	0.82		
IGF1-5752	CC	6	0.04	C	0.22
	CG	61	0.36	G	0.78
	GG	102	0.60		