



ASSOCIATIONS OF SIX SNPS OF *POU1F1-PROPI-PITX1-SIX3* PATHWAY GENES WITH GROWTH TRAITS IN TWO CHINESE INDIGENOUS GOAT BREEDS

Lin Ma^{1*}, Qiaomei Qin^{2*}, Qing Yang¹, Meng Zhang¹, Haiyu Zhao¹, Chuanying Pan¹, Chuzhao Lei¹, Hong Chen^{1*}, Xianyong Lan^{1*}

¹College of Animal Science and Technology, Northwest A&F University, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Yangling, Shaanxi, 712100, China

²Department of Pharmaceutical Science and Technology/Animal Science, Heze University, Heze, Shandong, 274015, China

*These authors equally contributed to this work

*Corresponding authors: lanxianyong79@nwsuaf.edu.cn; chenhong1212@126.com

Abstract

In the process of anterior pituitary gland development and maturation, numerous transcription factors were involved in the pituitary organogenesis. To date, the lack of knowledge on *POU1F1-PROPI-PITX1-SIX3* pathway genes affecting growth traits was reported in Chinese goat breeds. The objective of this work was to identify genetic variants of the *POU1F1*, *PROPI*, *PITX1* and *SIX3* genes, as well as to evaluate their associations with growth traits in goats. The results showed that one novel and five reported single nucleotide polymorphisms (SNP) were confirmed in above four genes. The novel SNP (NC_019460.2:g.1100T>A) was located in intron 1 of *SIX3* gene. Among these loci, DQ826397.1:g.102T>G (SNP1), DQ826397.1:g.279T>C (SNP2) and NC_019460.2:g.1100T>A (SNP6) were associated with growth traits. In details, the SNP1 locus had significant association with hucklebone width ($P<0.05$) and hucklebone width index ($P<0.05$) in the Guanzhong dairy goat. The SNP2 locus was associated with body weight ($P<0.05$), chest circumference ($P<0.01$), chest width ($P<0.05$), hucklebone width ($P<0.05$), cannon circumference ($P<0.01$) and trunk index ($P<0.01$) in the Hainan black goat. The SNP6 locus was significantly associated with hucklebone width index ($P<0.05$) and chest width ($P<0.05$) in the Guanzhong dairy goat and Hainan black goat, respectively. These findings indicated that goat *POU1F1-PROPI-PITX1-SIX3* pathway genes were important genetic factors, which could affect growth traits. Three SNPs detected in this study may be useful for potential marker-assisted selection programs in goat breeding and production.

Keywords: goat, *POU1F1* gene, *PROPI* gene, *PITX1* gene, *SIX3* gene, polymorphism, growth traits

Abbreviations

POU1F1: pituitary specific transcription factor 1; *PROPI*: prophet of PIT1; *PITX1*: paired-like homeodomain transcription factor 1; *SIX3*: SIX homeobox 3; *GH*: growth hormone; *PRL*: prolactin; *TSH*: thyroid-stimulating hormone; *bp*: base

pair; *SNP*: single nucleotide polymorphism; *PCR*: polymerase chain reaction; *RFLP*: restriction fragment length polymorphism; *Ho*: homozygosity; *He*: heterozygosity; *Ne*: effective allele numbers; *PIC*: polymorphism information content; *HWE*: Hardy-Weinberg equilibrium; *GZ*: Guanzhong dairy goat; *HNBG*: Hainan black goat; *BW*: body weight; *BH*: body height; *BL*: body length; *BSL*: body slanting length; *ChC*: chest circumference; *ChD*: chest depth; *ChW*: chest width; *HuW*: hucklebone width; *CaC*: cannon circumference; *TI*: trunk index; *BLI*: body length index; *ChCI*: chest circumference index; *CaCI*: cannon circumference index; *CWI*: chest width index; *HuWI*: hucklebone width index; *CV*: coefficient of variation.

Growth traits are important economic traits in breeding, genetics and reproduction for goat. In goat industry, it is difficult to obtain a big genetic progress using traditional breeding methods, however, application of marker-assisted selection (MAS) to improve these traits is shown to be an efficient strategy (Ahlawat et al., 2015; Pan et al., 2013; Zhao et al., 2013). As we know, goat growth traits are under the control of multiple genes which may be crucial for unraveling the association of genetic variations with growth traits (Jalil-Sarghale et al., 2014; Zhang et al., 2015). But limited studies on pathway genes affecting growth and development are available in goats. Therefore, it is critical to find out numerous candidate genes, which is also the initial and crucial step to quickly and efficiently establish a MAS system in goats breeding (Zhang et al., 2015).

In the process of development and maturation of the anterior pituitary gland, numerous transcription factors are involved in pituitary organogenesis, such as POU homeodomain family (e.g. *POU1F1* and *PROP1*), *PITX* family (e.g. *PITX1* and *PITX2*) and *SIX* family (e.g. *SIX3* and *SIX6*) (Huai et al., 2011; Savage et al., 2003). Currently, *POU1F1*, *PROP1*, *PITX1* and *SIX3* genes belong to the same pathway genes: *POU1F1-PROP1-PITX1-SIX3*.

POU1F1 (also known as *PIT-1* or *GHF-1* previously) is a positive regulator for growth hormone (GH), prolactin (PRL), thyroid stimulating hormone (TSH), and itself in mammals (Cohen et al., 1997; Ribeca et al., 2014). Therefore, mutations on this gene may result in deficiency of GH, PRL, TSH, and *POU1F1* (Lan et al., 2009 b). In our current studies, several polymorphisms were found of *POU1F1* gene, which were related to milk yield and body weight in goat and cattle (Lan et al., 2007 a, b; Zhang et al., 2009; Zhou et al., 2016). Recently, mutations of *POU1F1* gene were reported to be associated with growth and meat quality traits in rabbit (Wang et al., 2015), sheep milk production traits (Mura et al., 2012; Ozmen et al., 2014), sheep weaning weight (Sadeghi et al., 2014), sheep growth and biometric traits (Jalil-Sarghale et al., 2014), milk production traits and litter size in goat (Daga et al., 2013; Feng et al., 2012), milk production in cow (Ahmadi et al., 2015), carcass weight in cattle (Seong et al., 2011), carcass characteristics in crossbred pigs (Kim et al., 2014) and chicken body weight and egg numbers (Yan et al., 2013).

As a paired class homeodomain transcription factor, *PROP1* (also named prophet of *POU1F1*) is specifically expressed in the pituitary gland, and plays a direct or indirect crucial role in the ontogenesis of pituitary gonadotropes, somatotropes, lactotropes and caudomedial thyrotropes (Lan et al., 2009 a). *PROP1* mutations have been found to be associated with litter size and wool traits in sheep (Liu et al., 2015;

Zeng et al., 2011), growth traits in cattle (Pan et al., 2013) and secretion of pituitary hormones in human (Baş et al., 2015; Lazea et al., 2015).

Paired-like homeodomain transcription factor 1 (*PITX1*) plays an important role in pituitary development by indirectly regulating the expression of the *GH* and *PRL* genes (Poulin et al., 2000). Therefore, *PITX1* gene is regarded as a potential candidate gene for building the relationship between the gene polymorphisms and growth traits (Poulin et al., 2000). *PITX1* gene mutations have been found to be associated with milk performance in dairy goats (Lan et al., 2013).

SIX homeobox 3 (*SIX3*) gene is responsible for normal mammalian pituitary development, and its genetic variations or deficiency will cause hypopituitarism, suggesting that this gene is a potential candidate gene for studying association with growth traits in animals (Lan et al., 2011). *SIX3* gene mutations have been found to be associated with growth traits in cattle (Lan et al., 2011).

Considering *POU1F1-PROP1-PITX1-SIX3* pathway genes contribute greatly to growth traits in livestock, it is important to explore the genetic variants of these genes. Our purpose of this study was to identify single nucleotide polymorphisms (SNPs) in above-mentioned genes and carry out their association analysis with growth traits. The results of this study could provide useful genetic markers for animal dairy goats selection and breeding through MAS.

Material and methods

All animal experiments were implemented following relevant laws and institutional guidelines and were approved by Northwest A&F University Institutional Animal Care and Use Committee.

Animals and data collection

In this study, a total of 519 goats from two well-known Chinese native goat breeds (Guanzhong dairy goat (GZ), $n = 235$; Hainan black goat (HNBG), $n = 284$) were used. All selected individuals were healthy and unrelated (Zhao et al., 2013). All GZ individuals were 2 to 4 years old. The GZ goats were reared on the Sanyuan dairy goat breeding farm in Sanyuan county, Shaanxi, P.R. China (Zhao et al., 2013). The HNBG goats were 2 to 3 years old and reared in native breeding farms, in Zanzhou County, Hainan province, China (Zhang et al., 2015). All goats were allowed access to feed *ad libitum* using standard feeding and management protocols.

Body measurement traits for all selected individuals were measured, including body weight (BW), body height (BH), body length (BL), body slanting length (BSL), chest circumference (ChC), chest depth (ChD), chest width (ChW), hucklebone width (HuW), cannon circumference (CaC). Consequently, trunk index (TI), body length index (BLI), chest circumference index (ChCI), cannon circumference index (CaCI), chest width index (CWI) and hucklebone width index (HuWI), were also calculated on the basis of our reported description (Fang et al., 2010; Xu et al., 2010). The number of records and means, maximum, minimum and coefficient of variation (CV) for the analyzed traits are shown in Table 1.

Table 1. Statistics information of the phenotypic traits of GZ and HN BG breeds

Traits	GZ					HN BG				
	mean	SD	max	min	CV	mean	SD	max	min	CV
BW (kg)	-	-	-	-	-	28.28	7.01	51.00	13.50	0.25
BH (cm)	68.48	3.47	75.70	60.00	0.05	52.63	4.15	64.70	40.10	0.08
BL (cm)	76.56	5.23	89.40	62.80	0.07	-	-	-	-	-
BSL (cm)	-	-	-	-	-	55.86	7.01	68.30	13.50	0.25
ChC (cm)	89.02	4.26	98.20	80.20	0.05	71.98	4.15	91.00	40.10	0.08
ChD (cm)	-	-	-	-	-	26.42	4.51	33.00	43.80	0.08
ChW (cm)	28.13	2.72	34.00	22.00	0.10	14.81	6.50	21.60	54.00	0.09
HuW (cm)	23.24	3.33	30.00	16.20	0.14	13.65	2.38	17.20	14.50	0.09
CaC (cm)	10.82	1.30	14.10	6.60	0.12	7.78	1.88	10.00	10.80	0.13
TI (%)	116.62	7.56	137.74	101.74	0.06	129.06	1.37	173.91	9.00	0.10
BLI (%)	111.90	7.03	129.30	96.91	0.06	106.41	0.73	130.42	6.00	0.09
ChCI (%)	130.23	7.97	154.88	117.44	0.06	136.99	9.04	167.08	105.88	0.07
CaCI (%)	15.85	2.15	21.02	9.78	0.14	14.82	8.05	19.27	83.15	0.08
CWI (%)	-	-	-	-	-	56.19	10.14	77.24	105.82	0.07
HuWI (%)	123.54	22.22	177.33	85.71	0.18	108.85	1.32	145.22	11.08	0.09

DNA isolation and DNA pool construction

The genomic DNA samples were isolated from the ear tissues and the leukocytes of the blood sample according to the procedure described by Lan et al. (2007 b). Based on our previous report (Lan et al., 2013), all genomic DNA samples were diluted to the identical concentration (50 ng/ μ L) for the DNA pool construction or the PCR amplification. A total of fifty DNA samples were randomly selected from the GZ and HN BG breeds to construct two different genomic DNA pools, respectively. The genomic DNA pools were used as templates for PCR amplification and exploring genetic variation in *POU1F1*, *PRO1*, *PITX1* and *SIX3* genes.

Primers design, PCR amplification and DNA sequencing

In our previous study (Lan et al., 2007 a, b; Lan et al., 2009 a, b; Lan et al., 2013), we found five SNPs in *POU1F1-PRO1-PITX1-SIX3* pathway genes, namely, DQ826397.1:g.102T>G (SNP1), DQ826397.1:g.279T>C (SNP2), DQ826397.1:g.365T>C (SNP3), AF453512:g.1795C>T (SNP4), NW_00314033:g.201G>A (SNP5). These SNPs could be recognized by the *DdeI*, *AluI*, *PstI*, *Hin6I* and *MspI* (MBI Fermentas, Vilnius, Lithuania) restriction endonuclease, respectively. Based on that, three pairs of primers P1, P2 and P3 were synthesized to test the polymorphisms of the goat *POU1F1* gene, *PRO1* gene and *PITX1* gene, respectively (Table 2).

As the caprine *SIX3* gene sequence was not published currently on NCBI, in this study, a total of four pairs of primers were designed to scan novel genetic variants in the caprine *SIX3* gene based on the ovine *SIX3* gene sequence (GenBank Accession No. NC_019460.2) (Table 2).

The 20 μ L PCR volume included the 40 ng genomic DNA, 0.4 μ M of each primer, 1 \times buffer (including 1.5 mM $MgCl_2$), 160 μ M dNTPs (dATP, dTTP, dGTP and dCTP), and 0.5 units of *Taq* DNA polymerase (MBI Fermentas, Vilnius, Lithuania).

The cycling protocol was 95°C for 5 min, 35 cycles of denaturing at 94°C for 30–50 s, annealing for 30–60 s, extending at 72°C for 35 s, with a final extension at 72°C for 10 min (Table 2).

Table 2. Primers used for detecting SNPs of the goat *POU1F1-PROPI-PITX1-SIX3* pathway genes

Primer name	Primer sequences (5'-3')	Product size (bp)	T_m (°C)	Target region	Note
SNP1, SNP2 and SNP3 (P1) (<i>POU1F1</i>)	F: CCATCATCTCCCTTCTT R: AATGTACAATGTGCCTTCTGAG	450	54.5	Exon 6, partial intron 5, 3' UTR	Cited from Lan et al., 2007 a
SNP4 (P2) (<i>PROPI</i>)	F: ATGTGGTCTGGGATGGATG R: CTGGTGAAGGTTTGGGTTAG	414	54.3	Exon 2, flanking region	Cited from Lan et al., 2009 a
SNP5 (P3) (<i>PITX1</i>)	F: CCGCCTTCCACCTAGCCCGCAC R: TCGTCCATGTCCACGTTTCATCG	230	65.2	Intron 1	Cited from Lan et al., 2013
<i>SIX3</i> -SNPs scanning (P4)	F: CGCCGATTCTCACCACC R: AGGCTCCCAAGGACACGAC	933	58.4	Exon 1 and Intron 1	This study
<i>SIX3</i> -SNPs scanning (P5)	F: AGGCTTGAGCGGACAGGA R: TCCAAGGGCATGGGTGAG	914	59.0	Exon 1 and Intron 1	This study
<i>SIX3</i> -SNPs scanning (P6)	F: CCTGAACCTTTGGCCCTCT R: CTGACTCCGCTGTCTACGC	701	57.0	Intron 1	This study
<i>SIX3</i> -SNPs scanning (P7)	F: GGGATCTGTCTGCTCCAGGGAC R: AGGCTCGACACGCTGGTGGT	520	64.8	Intron 1 and Exon 2	This study
<i>SIX3</i> PCR-RFLP (P8)	F: AGAAGGGCGAGCGTGAAC \boxed{A} T R: TGGATTCTCCCAAGGGTTTT	264	56.0	Intron 1	This study

The framed base \boxed{A} at the P8-forward primer showed a mismatch for creating restriction sites (*NdeI*, CAT-ATG), where the actual nucleotide was "G" at NC_019460.2:g.1100 locus.

To identify mutations within *SIX3* gene of two goat breeds, DNA pool sequencing was carried out using the ABI 3730 sequencer (ABI, Foster City, CA, USA) in the forward and reverse directions.

Genotyping of the SNPs within the goat *POU1F1-PROPI-PITX1-SIX3* pathway genes

Aliquots of 20 μ L PCR products of *POU1F1* gene were digested with 10 U *DdeI*, *AluI*, and *PstI*, for 5 h at 37°C following the supplier's directions, similarly, PCR products of *PROPI* and *PITX1* genes were digested with 10 U *Hin6I* and *MspI*, respectively. The digested products were detected by electrophoresis in 2.0–3.0% agarose gel stained with ethidium bromide (Lan et al., 2007 a, b; Lan et al., 2009 a, b; Lan et al., 2013).

SIX3 gene: Because the NC_019460.2:g.1100T>A (SNP6) could not be recognized by the natural restriction endonuclease, another pair of primer was designed

to establish the *NdeI* Forced-PCR-RFLP, where the actual nucleotide “G” was induced into “A” at NC_019460.2:g.1098 locus. Thus, this introduced that point mutation would create an *NdeI* restriction site (CATATG) in the PCR products from the g.1100A goat carrier, whereas the PCR products from the g.1100T goat carrier lacked this restriction endonuclease site. In the *NdeI* PCR-RFLP analysis, the 20 μ L PCR products were digested with 5 U *NdeI* (MBI Fermentas, Vilnius, Lithuania) for 12 h at 37°C following the manufacturer’s protocol. All digested products were detected by electrophoresis in 3.5% agarose gel stained with ethidium bromide.

Statistical analysis

Genotypic and allelic frequencies were determined through a direct count. Hardy-Weinberg equilibrium (HWE) was analyzed based on a predetermined value of the test statistic (Emigh, 1980). According to Nei’s method (Nei and Roychoudhury, 1974), population parameters, including gene homozygosity (H_o), gene heterozygosity (H_e), effective allele numbers (N_e) and polymorphism information content (PIC), were performed by PopGene version 3.2 (Wang et al., 2013). Distribution differences for genotypic and allelic frequencies between different breeds were analyzed using the χ^2 test. The associations between genotypes and growth traits of goat were analyzed by ANOVA using the statistical software SPSS (Version 22.0).

Results

Genetic variation, genotypic and allelic frequencies and genetic diversity of *POU1F1-PROPI-PITX1-SIX3* pathway genes

The frequencies of genotype and allele of *POU1F1-PROPI-PITX1-SIX3* pathway genes were calculated in the GZ and HNBB breeds. The results were summarized in Table 3.

The present results confirmed the previous findings. For *POU1F1* gene, the 450 bp products were amplified using the specific combination of primers, the digestion with *AluI*, *DdeI* and *PstI*. For *PROPI* gene, the amplified PCR fragments (414 bp) were digested by *Hin6I* endonuclease (Xu et al., 2010). A genetic variant (NW_00314033: g.201G>A, SNP5) was identified at *PITX1* gene. This SNP is located at nt41 position of the first intron, which can be genotyped by the *MspI* PCR-RFLP.

SIX3 gene: The pool sequencing and alignment analysis identified one novel SNP at intron 1 within *SIX3* gene (NC_019460.2:g.1100T>A) in GZ and HNBB breeds (Figure 1 a). The SNP could be genotyped by the *NdeI* PCR-RFLP method. At the *NdeI* locus, the allele “T” demonstrated one band (264 bp), while the allele “A” demonstrated two bands (244 bp and 20 bp). Accordingly, genotype TT showed only one band (264 bp) and genotype CC showed two bands (244 bp and 20 bp); while genotype TC showed three bands (264 bp, 244 bp and 20 bp) (Figure 1 b). It was obvious that 264 bp and 244 bp fragments could clearly classify the genotypes.

Table 3. Genotypes, allele and HWE for the SNPs within the goat *POU1F1-PROPI-PITX1-SIX3* pathway genes in GZ and HNBG breeds

Loci/breed	Sizes	Genotype frequencies			Allele frequencies		HWE
SNP1	N	TT	TG	GG	T	G	P-values
GZ	235	0.022	0.281	0.697	0.163	0.837	P>0.05
HNBG	284	0.294	0.452	0.254	0.520	0.480	P>0.05
SNP2	N	TT	TC	CC	T	C	P-values
GZ	235	0.963	0.037	0.000	0.981	0.019	P>0.05
HNBG	284	0.544	0.266	0.190	0.677	0.323	P<0.01
SNP3	N	TT	TC	CC	T	C	P-values
GZ	235	0.549	0.440	0.011	0.769	0.231	P<0.05
HNBG	284	0.965	0.035	0.000	0.983	0.017	P>0.05
SNP4*	N	TT	TC	CC	T	C	P-values
GZ	235	0.000	0.014	0.986	0.007	0.993	P>0.05
HNBG*	284	0.194	0.493	0.313	0.440	0.560	P>0.05
SNP5	N	AA	AG	GG	A	G	P-values
GZ	235	0.224	0.118	0.658	0.283	0.717	P<0.01
HNBG	284	0.906	0.067	0.027	0.940	0.060	P<0.01
SNP6	N	AA	AT	TT	A	T	P-values
GZ	235	0.025	0.525	0.450	0.287	0.713	P<0.05
HNBG	284	0.091	0.497	0.412	0.340	0.660	P>0.05

At SNP4 locus, the genotype frequencies of TT (P=0.194), TC (P=0.493), CC (P=0.313), allele frequencies of T (P=0.440), C (P=0.560) and HWE (P>0.05) in HNBG breed were cited from Xu et al. (2010).

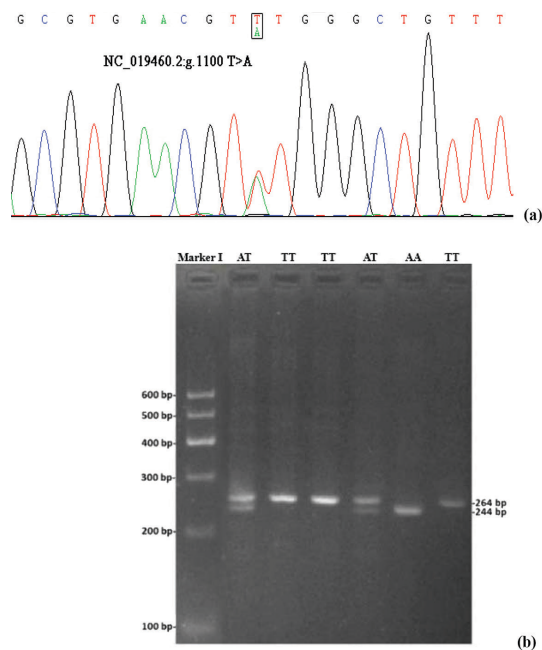


Figure 1. Sequencing results of mutation in the *SLX3* gene (a). Electrophoresis pattern of the novel genetic variation of goat *SLX3* gene (b)

Genetic indices (Ho, He, Ne, and PIC) in the GZ and HNBG breeds were presented in Table 4. The values of Ho exceeded 0.500 at current SNPs. The values of Ne were approaching 2.000. The maximum and minimum PIC values were 0.375 and 0.013. The χ^2 test showed that the GZ breed was not at Hardy-Weinberg equilibrium in SNP3, SNP5 and SNP6 loci ($P < 0.05$), the HNBG breed was not at Hardy-Weinberg equilibrium only in SNP2 and SNP5 loci ($P < 0.05$) (Table 3).

Table 4. Ho, He, Ne and PIC for the SNPs within the *POU1F1-PROPI-PITX1-SIX3* pathway genes in GZ and HNBG goat

Loci	Breed	Ho	He	Ne	PIC
SNP1	GZ	0.727	0.273	1.375	0.236
	HNBG	0.501	0.499	1.997	0.375
SNP2	GZ	0.964	0.036	1.038	0.036
	HNBG	0.563	0.437	1.777	0.342
SNP3	GZ	0.645	0.355	1.551	0.292
	HNBG	0.966	0.034	1.035	0.034
SNP4*	GZ	0.987	0.013	1.014	0.013
	HNBG*	0.507	0.493	1.972	0.371
SNP5	GZ	0.594	0.406	1.683	0.323
	HNBG	0.886	0.114	1.129	0.108
SNP6	GZ	0.590	0.410	1.694	0.326
	HNBG	0.551	0.449	1.814	0.348

At SNP4 locus, the Ho (Ho=0.507), He (He=0.493), Ne (Ne=1.972), PIC (PIC=0.371) in HNBG breed were cited from Xu et al. (2010).

Association between genetic variations of *POU1F1-PROPI-PITX1-SIX3* pathway genes and growth performance

Statistical analysis revealed that only the four SNPs showed significant associations with growth traits (Tables 5, 6). At the SNP1 locus, different genotypes in the GZ breed were found to significantly associate with HuW and HuWI ($P < 0.05$). Individuals with genotype TG and GG demonstrated significantly inferior HuW when compared with the genotype TT. Genotype TT had significantly higher HuWI than those genotypes TG and GG. The TT homozygotes at the SNP2 locus had significantly higher BW, ChC, ChW, HuW, CaC and TI than genotypes TC ($P < 0.05$) in HNBG breed. According to our previous report (Xu et al., 2010), at the SNP4 locus, the HNBG breed with genotype CC had significantly lower ChW, CWI and HuWI than those genotypes TT and TC ($P < 0.05$). The AA at SNP6 locus had a lower ChW than TT and AT in the GZ goat breed. Moreover, the different genotypes in the HNBG breed were found to be significantly associated with CWI.

Table 5. Associations of the SNP1 and SNP2 within *POU1F1* gene and growth traits in GZ and HNBG goat (LSM \pm SD)

Loci/breed	Traits	TT	TG	GG	P-values
SNP1/GZ	HuW (cm)	17.35 \pm 0.21 b	22.83 \pm 3.61 a	23.44 \pm 2.95 a	P<0.05
	HuWI (%)	166.09 \pm 15.89 a	122.24 \pm 19.60 b	124.34 \pm 20.98 b	P<0.05
SNP2/HNBG	BW (kg)	29.11 \pm 6.24 a	26.31 \pm 7.05 b	28.37 \pm 7.86 ab	P<0.05
	ChC (cm)	72.95 \pm 6.00 a	70.04 \pm 6.30 b	71.48 \pm 7.36 ab	P<0.01
	ChW (cm)	15.06 \pm 1.73 a	14.30 \pm 1.95 b	14.86 \pm 2.13 ab	P<0.05
	HuW (cm)	13.84 \pm 1.19 a	13.30 \pm 1.36 b	13.62 \pm 1.50 ab	P<0.05
	CaC (cm)	7.96 \pm 0.64 a	7.49 \pm 0.73 b	7.67 \pm 0.75 b	P<0.01
	TI (%)	130.75 \pm 10.02 a	126.40 \pm 9.62 b	128.06 \pm 6.03 ab	P<0.01

a, b – values in rows with different letters differ significantly (P<0.05).

Table 6. Associations of the SNP6 with growth traits in GZ and HNBG goat (LSM \pm SD)

Breed	Traits	AA	AT	TT	P-values
GZ	ChW (cm)	24.70 \pm 3.25 b	28.42 \pm 2.33 a	28.07 \pm 2.58 a	P<0.05
HNBG	CWI (%)	53.59 \pm 5.16 b	56.92 \pm 5.87 a	57.94 \pm 5.37 a	P<0.05

a, b – values in rows with different letters differ significantly (P<0.05).

Discussion

In this study, *POU1F1-PROPI-PITX1-SIX3* pathway genes polymorphisms and their associations with growth performance were detected. A novel SNP (NC_019460.2:g.1100T>A, named SNP6) within *SIX3* gene was identified in intron 1. Although the intronic genetic variants do not change amino acids, many papers still have powerfully proved that intronic polymorphisms are significantly associated with quantitative traits varied from human to animals (Chang et al., 2015; Clark et al., 2015; Maitra et al., 2014; Palagano et al., 2015; Sun et al., 2015; Wang et al., 2015; Zhao et al., 2013; Zhou et al., 2016). For SNP6 locus, goats with genotype TT or AT had superior ChW and CWI compared to those with genotype AA in GZ and HNBG breeds, respectively. *SIX3* gene played a critical role in affecting goat growth traits from the above mentioned description. Although mutation was detected at intron 1 region, the mutation might be linked to another mutation in the coding or regulatory regions of the gene, which was responsible for the growth performance (Lan et al., 2011).

The result revealed significant relationships between the SNP1 and HuW, HuWI in GZ breed, SNP2 and BW, ChC, ChW, HuW, CaC and TI in HNBG breed, suggesting that the *POU1F1* gene had significant effects on growth traits. Overall, in HNBG breed, goats with genotype TT or CC had superior growth traits compared to those with genotype TC at SNP2 locus. The SNP1 and SNP2 were identified silent allele. For *POU1F1* gene, although silent mutation did not change amino acid sequence, it possibly resulted in the change of Serine synonymous codon usage frequency. It

could have an indirect effect on goat growth traits (Lan et al., 2007 a, b). The SNP4 locus of the other related gene *PROPI* has been found to be associated with growth traits (Xu et al., 2010). During pituitary development, several critical transcription factors involved in *POUIF1*, *PROPI*, *PITX1*, *SIX3*, have their own temporal and spatial expression, and play key roles in affecting growth and development by affecting functional hormones, such as, GH, PRL and TSH β (Castinetti et al., 2011; Pfäffle and Klammt, 2011; Savage et al., 2003). Therefore, pituitary transcription factors are responsible for a phenotype of combined pituitary hormone deficiency involving in GH, PRL and TSH β , which indicates that these genes are associated with growth traits in livestock (Li et al., 1990). Taken together, *POUIF1-PROPI-PITX1-SIX3* pathway genes are critical for selection and breeding in goat growth performance.

The associations of the goat *POUIF1-PROPI-PITX1-SIX3* pathway genes polymorphisms and growth traits exhibited that these genes can be regarded as genetic marker genes correlating with growth traits. Therefore, these primary findings about the significant relationships between SNPs of the *POUIF1-PROPI-PITX1-SIX3* pathway genes and growth traits will potentially contribute to the goat industry and these genes were given as critical candidate genes for selection and breeding in goat growth performance through MAS.

Besides, almost all of PIC values at the above polymorphic loci are more than 0.250, except SNP1, SNP2, SNP4 loci in GZ breed, SNP3 and SNP5 loci in HNBBG breed, implying that these five loci possess low genetic diversity in GZ or HNBBG breed. Therefore, these loci are not suggested to be effective in evaluating genetic resources of goat industry. The GZ and HNBBG breeds were not at Hardy-Weinberg equilibrium in some loci ($P < 0.05$). Genetically, it was possibly due to limited sample sizes or artificial selection, which complied with the background of GZ and HNBBG culture. Of course, the genetic drift function and migration also might account for its disequilibrium.

To summarize, six SNPs extended the spectrum of genetic variation of the goat *POUIF1-PROPI-PITX1-SIX3* pathway genes, and their significant association with growth performance would contribute to implementing MAS in breeding and genetics in goats.

Conclusion

In conclusion, a novel SNP extended the spectrum of genetic variation of caprine *SIX3* gene. The associations of the goat *POUIF1-PROPI-PITX1-SIX3* pathway genes polymorphisms and growth traits exhibited that these genes can be regarded as a genetic marker gene correlating with growth traits.

Competing interests

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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