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Research Article

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Insertion/deletion variants within the *IGF2BP2* gene identified in reported genome-wide selective sweep analysis reveal a correlation with goat litter size

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Abstract: Insulin-like growth factor 2 mRNA-binding protein 2 (*IGF2BP2*, also called *IMP2*) plays an essential role in the development and maturation of germ cells and embryos and is a candidate gene for goat litter size, based on a previous genome-wide selective sweep analysis. In this study, the mRNA expression level of *IGF2BP2* was found to be significantly higher in a single-lamb group than in a multi-lamb group. Insertions/deletions (indels) within the goat *IGF2BP2* gene, including P4-Ins-13bp and P5-Del-12bp, were verified in 918 Shaanbei White Cashmere (SBWC) female goats. The minor allelic frequencies (MAFs) of P4-Ins-13bp and P5-Del-12bp loci were 0.349 and 0.295, respectively. Analysis using the Chi-square (χ^2) test showed that the genotype (χ^2=14.479, P=0.006) distribution of P4-Ins-13bp was significantly different between the single-lamb and multi-lamb groups. Correlation analysis demonstrated that P4-Ins-13bp was significantly associated with goat litter size (P=0.022), and individual goats with the homozygous deletion/deletion (DD) genotype produced more litters than other goats. Therefore, considered as a potential molecular marker significantly related to lambing traits, the P4-Ins-13bp mutation of the goat *IGF2BP2* gene can be used in goat breeding with practical molecular marker-assisted selection (MAS) to optimize female reproduction and improve economic efficiency in the goat industry.

Key words: Goat; Insulin-like growth factor 2 mRNA-binding protein 2 (*IGF2BP2*); Litter size; Indel; Marker-assisted selection (MAS)

1 Introduction

Litter size among domestic animals is a complex quantitative and economic trait and serves as a key factor in determining the economy of the goat-rearing industry (Bünger et al., 2005; Abdoli et al., 2019; Lu and Miller, 2019; Li et al., 2021). To increase economic efficiency, improvements in goat reproductive quality should be prioritized. Marker-assisted selection (MAS), which is characterized as an alternative selection of DNA mutations, is not affected by the microenvironment. In addition, compared with traditional breeding techniques, MAS is a more accurate, efficient, and convenient genetic selection method (Collard and Mackill, 2008; Kang et al., 2019b; Hui et al., 2020). However, identifying effective target markers associated with favorable outcomes remains necessary for practical application of MAS to the breeding process.

In recent years, the rapid development of genome-wide selective sweep analysis and genome-wide association study (GWAS) have identified many candidate genes associated with the litter sizes of livestock, such as the porcine spermatogenesis-associated 19 (*SPATA19*), ovine follicle-stimulating hormone receptor (*FSHR*), and bovine 2',5'-oligoadenylate synthetase 1 (*OAS1*) (Wang et al., 2012; Pan et al., 2014; Alex et al., 2018). In goats, previous studies have identified growth differentiation factor 9 (*GDF9*), membrane-associated

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ring-CH-type finger 1 (*MARCH1*), and DNA methyltransferase 3β (*DNMT3B*) as genes that affect fecundity (Kang et al., 2019a; Wang et al., 2019; Hui et al., 2020).

A previous genome-wide selective sweep analysis performed in Dazu Black goats has identified insulin-like growth factor 2 (IGF2) mRNA-binding protein 2 (*IGF2BP2*, also called *IMP2*) as a candidate gene associated with litter size (E et al., 2019). *IGF2BP2* is a member of the IGF2 mRNA-binding protein family (IGF2BP, also known as IMP), which includes *IGF2BP1*, *IGF2BP2*, and *IGF2BP3*, and is a highly conserved post-transcriptional regulatory factor involved in RNA processing, localization, stability, and translation. The IMP family is expressed ubiquitously throughout the embryonic development process, and subsequently plays a crucial role during the normal growth and development of embryos (Ruggiu et al., 1997; Chennathukuzhi et al., 2003; Biswas et al., 2019). Recently, one study demonstrated that 5-bp and 9-bp insertions/deletions (indels) in the *IGF2BP1* gene were related to litter size during the second gestation in Australian White sheep (Liu et al., 2021). It has been reported that the involvement of *IGF2BP2* in the HMGA2-IMP2 pathway plays an important role in mouse embryonic development (Brants et al., 2004). In addition, *IGF2BP3* is highly expressed in human placental trophoblasts during early pregnancy and plays a vital role in embryo implantation and placenta formation (Wang et al., 2016). *IGF2BP3* is closely related to the RNA-binding protein (RBP), Vg1 RBP (also known as Vera), and is essential for mesoderm induction and left–right axis formation (Li et al., 2014). These data suggest that the IMP family may play key roles in follicular maturation and embryo development; however, thus far, whether mutations in the IMP family member *IGF2BP2* affect goat reproductive traits and which genotype is advantageous remain unknown.

To confirm the effects of *IGF2BP2* polymorphisms on female goat reproductive capacity, we examined Shaanbei White Cashmere (SBWC) goats (n=918) in this study. The primary aims of the study were to verify the relationship between indels in *IGF2BP2* and SBWC goat litter sizes and to select the favorable alleles. We also wanted to lay a foundation for the application of MAS in goat breeding, to improve the economic efficiency of the goat livestock industry.

2 Materials and methods

2.1 Bioinformatics analysis

Nucleotide sequences from *Capra hircus* (XM_005675253.3), *Ovis aries* (XM_004003071.4), *Bos taurus* (NM_001192504.2), *Sus scrofa* (XM_021069998.1), *Homo sapiens* (NM_001007225.3), *Mus musculus* (NM_183029.2), and *Gallus gallus* (XM_015277139.2) were obtained from the National Center for Biotechnology Information (NCBI) database. Nucleotide sequence similarity was detected using the Basic Local Alignment Search Tool (BLAST) database (BLASTN) (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and the MEGA7 MUSCLE program was used to compare multiple nucleotide sequences and perform evolutionary analysis (Kumar et al., 2016).

2.2 Animal sample collection

Female adult goats of a representative local breed of SBWC (n=918), which produce high-quality cashmere and meat, were randomly selected from a group with resistance to coarse feeding, wind, sand, and disease. Ear tissue samples were collected from healthy, unrelated adult female goats with similar ages. First-born litter sizes were recorded for 885 individuals. Ovarian tissue samples were collected from adult female goats, and three samples were collected from single-lamb (first-born litter size=1) and multi-lamb (first-born litter size>1) groups maintained by an SBWC goat farm in Shaanxi Province, China.

2.3 DNA extraction, RNA isolation, and cDNA synthesis

DNA was extracted from the ear tissue samples using a previously described high-salt method (Bi et al., 2020). The extracted DNA was stored at −80 °C and then diluted to a standard concentration (10 ng/μL), after which it was stored at −40 °C.

The procedure used to isolate total RNA from ovarian tissue was described in a previous study (Tang et al., 2021). After isolation, 1% (0.01 g/mL) agarose gel was used to verify RNA integrity. Complementary DNA (cDNA) was synthesized using a PrimeScript™ RT Reagent kit (TaKaRa, Dalian, China), according to the manufacturer's instructions.

2.4 Primer design and DNA pooling

Based on the goat *IGF2BP2* (GenBank number: NC_030808.1) gene reference sequence obtained from the NCBI database and the variant table information obtained from the Ensembl database (http://asia.ensembl.org/index.html), six potential indel loci were selected according to the length and location of the variants, and primers were designed using NCBI Primer Blast software (Table 1). A total of 30 DNA samples from SBWC individuals were randomly selected to construct a genomic DNA pool as the template for polymerase chain reaction (PCR) amplification to detect the presence of non-specific primer amplification, after which 50 random SBWC individuals were selected to detect the presence of indels at the six loci of the *IGF2BP2* gene.

2.5 Amplification, genotyping, and DNA sequencing

In this study, the PCR reaction volume (13 μL) and amplification steps (touch down-PCR) were the same as described (Wang et al., 2020). The genotypes of different individuals were identified by 3% (0.03 g/mL) agarose gel electrophoresis, and sequencing was performed by Tsingke Biological Technology (Shaanxi, China) to verify mutations.

2.6 Quantitative real-time PCR

We analyzed the expression levels of goat *IGF2BP2* in the single-lamb group (n=3) and the multi-lamb group (n=3) by quantitative real-time PCR (qRT-PCR). For this purpose, we used a 10-μL reaction system in an FTC-3000 qRT-PCR thermocycler (Funglyn Biotech, Toronto, Canada), applying the same procedure described in a previous study (Tang et al., 2021). For the assessment of *IGF2BP2* gene (*IGF2BP2*-Q-F: CCTGGGGGCTCTTCTCAG; *IGF2BP2*-Q-R: GGATGTCTACCCGGGACTG) expression levels in goat ovarian tissue, we used glyceraldehyde-3-phosphate dehydrogenase (*GAPHD*) (*GAPDH*-F: AAAGTGGACATCGTCGCCAT; *GAPDH*-R: CCGTTCTCTGCCTTGACTGT) as an internal control.

2.7 Statistical analyses and prediction of potential transcription factor-binding sites

Allelic and genotypic frequencies of the goat *IGF2BP2* gene were analyzed in terms of Hardy-Weinberg equilibrium (HWE) using the Chi-square (χ^2) test. We calculated genetic diversity indices using a web-based tool (http://www.msrcall.com/Gdicall.aspx) (Jia et al., 2015). A polymorphism information content (PIC) of >0.50 indicates a high level of polymorphism, whereas 0.25<PIC≤0.50 and PIC≤0.25 represent moderate and low levels of polymorphism, respectively. In addition, we performed linkage disequilibrium (LD) analysis when two different loci were identified within the same gene, using the SHEsis platform (http://analysis.bio-x.cn) (Yong and He, 2005). We used a mixed-method linear model to determine the association between two indel mutations and goat litter size among the 885 SBWC goats with litter size information. The least-square means test was used to determine the correlation between different indel genotypes and litter size using the following formula: $Y_{ij}=\mu+S_i+G_i+e_{ij}$,

Table 1 Primers used for detecting indel loci of the goat insulin-like growth factor 2 mRNA-binding protein 2 (*IGF2BP2*) gene

Variant ID	Locus name	Primer sequence (5'→3')	Product length (bp) (insertion/deletion)
rs655740526	P1-Ins-16bp	F1: CTTCAAGGTATAATTCGCTGTGCAT	260/244
		R1: GATATGCAACAAGCAACAAAGGT	
rs637951471	P2-Del-14bp	F2: TGATACTTGGGAGGGGATGT	197/183
		R2: TGAAGCACATGTGGCAAAAGA	
rs642405889	P3-Ins-13bp	F3: GAATATTGGAAACAGCTGCTCAT	196/183
		R3: AAAAGTTTGAAAATCCCACTCACA	
rs660766378	P4-Ins-13bp	F4: CTGTGGTCCAGACCTCCTCA	163/150
		R4: TACAGACACTGTGAGAGGCG	
rs655441618	P5-Del-12bp	F5: CCAGTTCAGTGACCCATTCG	140/128
		R5: TGCAAGAAGCATCTGTCTGCTA	
rs649205677	P6-Del-6bp	F6: GTGCCTAAGACTGTGTAATGCCA	208/202
		R6: CTGGGATCAAACCTGGCACAT	

F, forward; R, reverse; Ins, insertion; Del, deletion.

where Y_{ij} represents the phenotypic value of each litter size; μ represents the overall average; S_i represents the year of kidding; G_i represents the fixed effect of the genotype; and e_{ij} represents random error (Wang et al., 2020). Using SPSS 20.0 software (IBM, USA), we analyzed the relationship between variants in the goat *IGF2BP2* gene and litter size with analysis of variance (ANOVA) and the χ^2 test. All results were considered significant when P was <0.05, and all statistical tests were performed as two-sided tests. In addition, we employed the JASPAR (http://jaspar.genereg.net/collection/core) and Alibaba2 (http://gene-regulation.com/pub/programs/alibaba2/index.html) online databases to predict the combination of transcription factors (TFs) and mutation region sequences.

3 Results

3.1 Gene conservation analysis

NCBI BLAST analysis showed that the nucleotide sequence of *C. hircus IGF2BP2* shared homology values with the sequences from *O. aries*, *B. taurus*, *S. scrofa*, *H. sapiens*, *M. musculus*, and *G. gallus* (99.44%, 99.22%, 94.10%, 91.26%, 85.05%, and 83.47%, respectively) (Fig. 1). In addition, phylogenetic tree analysis showed that the *IGF2BP2* gene from *C. hircus* was most closely related to those from *O. aries* and *B. taurus*, whereas the relationship with *G. gallus* was the most distant (Fig. 1).

3.2 Association of expression of *IGF2BP2* mRNA in ovaries with different fertility levels

In ovarian tissue obtained from adult female goats, the mRNA expression level of *IGF2BP2* in the single-lamb group was significantly higher than that in the multi-lamb group (Fig. 2).

3.3 Indel selection and sequencing validation

After the DNA pooling test, we detected polymorphisms at two mutation sites among the six potential sites, for which primer pairs were designed (Table 1), including P4-Ins-13bp (13-bp insertion in *IGF2BP2*) and P5-Del-12bp (12-bp deletion in *IGF2BP2*). Both of these mutation sites in *IGF2BP2* were associated with three potential genotypes, including homozygous insertion/insertion (II), heterozygous insertion/deletion

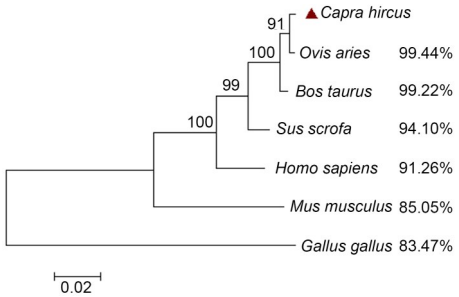

Fig. 1 Phylogenetic tree and percent identity of insulin-like growth factor 2 mRNA-binding protein 2 (*IGF2BP2*) nucleotide sequences among different species.

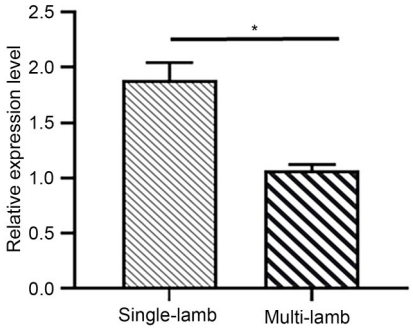

Fig. 2 *IGF2BP2* gene mRNA expression level in the ovarian tissue of Shaanbei White Cashmere (SBWC) goats in the single-lamb group (n=3) and the multi-lamb group (n=3). The data are expressed as mean±standard deviation. * P<0.05.

(ID), and homozygous deletion/deletion (DD), which were identified by 3% agarose gel electrophoresis (Figs. 3a and 3c).

We selected two PCR products demonstrating the presence of indel mutations for Sanger sequencing, which identified two indels located in intron 2. The 12-bp deletion from the *IGF2BP2* gene (12-bp indel NC_rs655441618: g. 81 116 754–81 116 765, Del AGGGTTTGCTGT) was consistent with the predicted indel information obtained from the NCBI database (Fig. 3d). The 13-bp insertion in the *IGF2BP2* gene (13-bp indel: g. 81 163 744–81 163 745, Ins TTTCCTT TTCTGG) was not completely consistent with that reported by the NCBI database (Fig. 3b).

3.4 Genotypic and allelic frequencies and population indices

For the P4-Ins-13bp, the frequency of the wild-type genotype (DD) was higher than those for the II and ID genotypes (Table 2). Similarly, the frequency of the wild-type P5-Del-12bp genotype (II) was higher than those for the other genotypes. The two indel loci

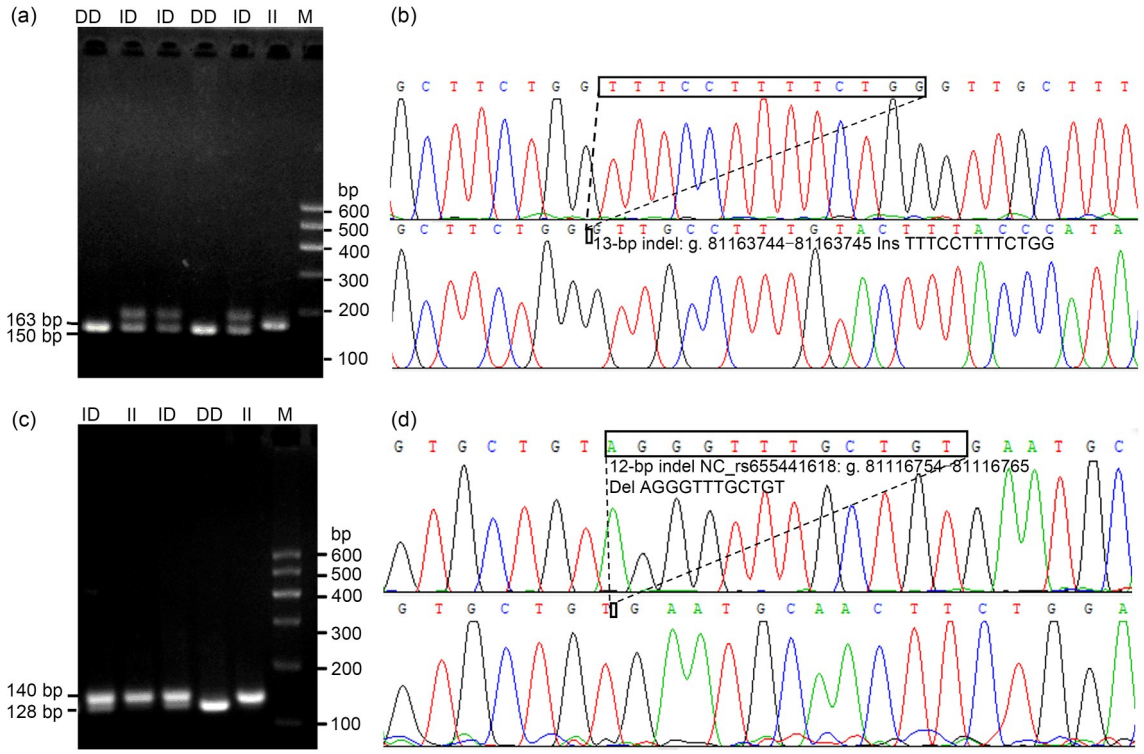

Fig. 3 Agarose electrophoresis of insulin-like growth factor 2 mRNA-binding protein 2 (*IGF2BP2*) P4-Ins-13bp indel (a) and P5-Del-12bp indel (c), and sequencing chromas for the 13-bp indel (b) and 12-bp indel (d) in the *IGF2BP2* gene. Sequencing chromas showed homozygotic insertion/insertion (II) type and homozygotic deletion/deletion (DD) type.

Table 2 Genetic parameters of *IGF2BP2* gene in SBWC goats

Locus	Genotypic frequency			Allelic frequency		HWE	Population parameter			
	II	ID	DD	D	I	*P* value	Ho	He	Ne	PIC
P4-Ins-13bp	0.177 (*n*=162)	0.345 (*n*=317)	0.478 (*n*=439)	0.651	0.349	<0.05	0.545	0.455	1.833	0.351
P5-Del-12bp	0.524 (*n*=325)	0.363 (*n*=223)	0.113 (*n*=70)	0.295	0.705	<0.05	0.585	0.416	1.711	0.329

IGF2BP2, insulin-like growth factor 2 mRNA-binding protein 2; SBWC, Shaanbei White Cashmere goat; II, homozygous insertion/insertion; ID, heterozygous insertion/deletion; DD, homozygous deletion/deletion; I, insertion; D, deletion; HWE, Hardy-Weinberg equilibrium; Ho, homozygosity; He, heterozygosity; Ne, effective allele number; PIC, polymorphism information content.

identified in *IGF2BP2* did not conform to the HWE ($P<0.05$). According to the PIC values, the two detected *IGF2BP2* mutations in SBWC goats were characterized as moderate polymorphisms ($0.25<PIC\leqslant0.50$) with PIC values ranging from 0.329 to 0.351.

3.5 Linkage disequilibrium and haplotype analyses

To further explore whether the *IGF2BP2* variant loci follow Mendelian inheritance patterns, we performed LD analysis using the SHEsis online platform. The D' and r^2 values for the relationship between P5-Del-12bp and P4-Ins-13bp in the *IGF2BP2* gene were 0.015 and 0.000, respectively, which indicated that these two mutations were not strongly linked (Fig. 4). The results of haplotype analysis for *IGF2BP2* generated four haplotypes, and $D_{P4\text{-}Ins\text{-}13bp}$-$I_{P5\text{-}Del\text{-}12bp}$ had the highest frequency (Fig. 5).

3.6 Association analysis between *IGF2BP2* indels and litter size

To further evaluate the practical value of the detected mutations, the χ^2 test and correlation analyses were performed to assess the relationships between mutations and litter size. The χ^2 test results showed that the genotype (χ^2=14.479, P=0.006) distribution of the P4-Ins-13bp locus in the *IGF2BP2* gene was significantly

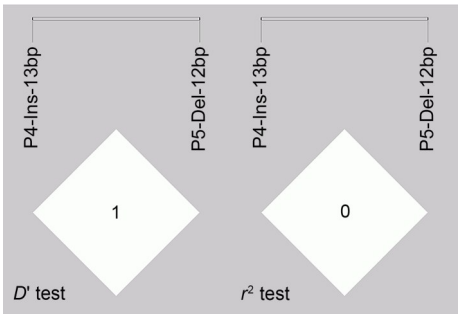

Fig. 4 Linkage equilibrium analysis of P4-Ins-13bp and P5-Del-12bp in SBWC goats. SBWC, Shaanbei White Cashmere goat; Ins, insertion; Del, deletion.

different between the single-lamb and multi-lamb groups (Fig. 6a), whereas no significant differences were observed for the P5-Del-12bp locus (Fig. 6c). At the same time, there was no significant difference in the distribution of I and D alleles at the two loci of *IGF2BP2* between single-lamb and multi-lamb groups (Figs. 6b and 6d), The association analysis showed that the P5-Del-12bp site of *IGF2BP2* had no significant effect on litter size in SBWC goats. Importantly, at the P4-Ins-13bp site of *IGF2BP2*, the litter size of

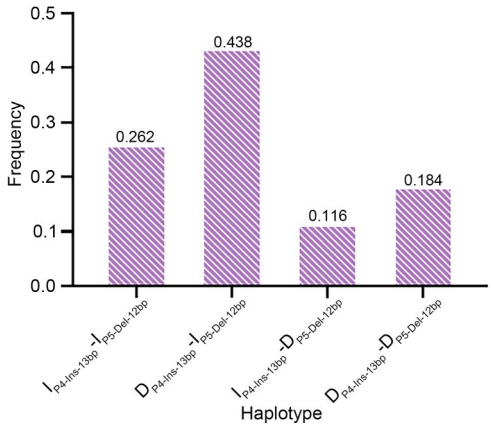

Fig. 5 Haplotype frequencies of the *IGF2BP2* gene in SBWC goats. *IGF2BP2*, insulin-like growth factor 2 mRNA-binding protein 2; SBWC, Shaanbei White Cashmere goat; Ins, insertion; Del, deletion.

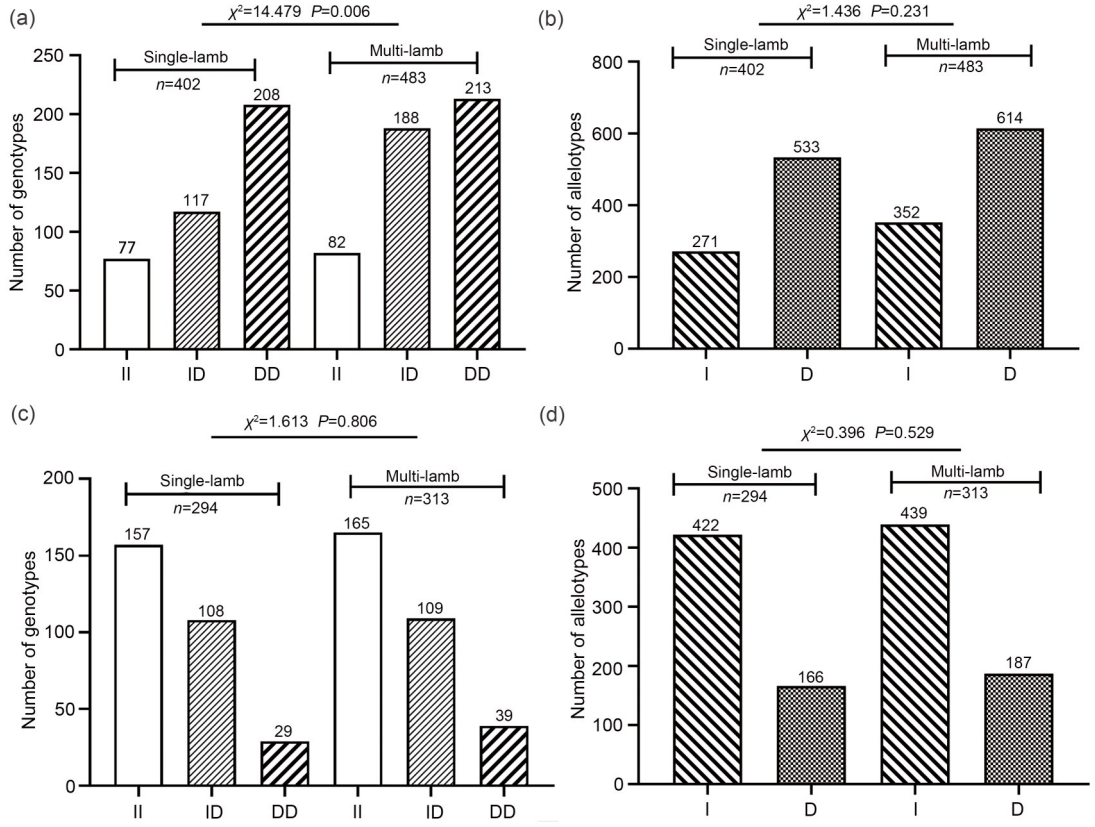

Fig. 6 Chi-square (χ^2) test was used to compare the distribution of genotypes in single-lamb and multi-lamb groups of SBWC goats. (a) II, ID, and DD genotype numbers of P4-Ins-13bp; (b) I and D allelotype numbers of P4-Ins-13bp; (c) II, ID, and DD genotype numbers of P5-Del-12bp; (d) I and D allelotype numbers of P5-Del-12bp in single-lamb and multi-lamb groups. SBWC, Shaanbei White Cashmere goat; II, homozygous insertion/insertion; ID, heterozygous insertion/deletion; DD: homozygous deletion/deletion; I, insertion; D, deletion.

individuals with the DD genotype was significantly higher than that of individuals with the ID and II genotypes (P<0.05; Table 3). Therefore, for the P4-Ins-13bp indel in *IGF2BP2*, the DD genotype was the favorable genotype.

3.7 Prediction of potential transcription-binding factors

The JASPAR online database predicted that the TFs, GATA-binding factor 2 (GATA2) [MA0036.3] and forkhead box L1 (FOXL1) [MA0033.2], might bind to the P4-Ins-13bp and P5-Del-12bp insertion sequences in the *IGF2BP2*, respectively (Fig. 7).

4 Discussion

Recent studies have identified *IGF2BP2* as a candidate gene associated with litter size in goats, based on genome-wide selective sweep analysis (E et al., 2019). Additionally, *IGF2BP2* expression has been detected in both resting and growing oocytes and granulocytes in mature mouse and human ovaries (Hammer et al., 2005). In this study, qRT-PCR analysis of adult female goat ovarian tissue indicated that the *IGF2BP2* mRNA expression level in the single-lamb group was significantly higher than that in the multi-lamb group. This result indicates that *IGF2BP2* might play a vital role in germ cells, embryonic development, and maturation, directly affecting fertility in goats. Thus far, most studies examining *IGF2BP2* polymorphisms have focused on the effects of these polymorphisms on the risk of type 2 diabetes mellitus (T2DM), breast cancer, and lung

Table 3 Association analysis of *IGF2BP2* indels with litter sizes in SBWC goats

Locus	Observed genotype			*P* value
	II	ID	DD	
P4-Ins-13bp	1.52±0.04^{b} (n=159)	1.52±0.03^{b} (n=305)	1.62±0.03^{a} (n=421)	0.022
P5-Del-12bp	1.52±0.03 (n=322)	1.57±0.06 (n=217)	1.51±0.04 (n=68)	0.348

The data are expressed as mean±standard deviation. Values with different letters (a, b) within the same row differ significantly at P<0.05 or P<0.01. *IGF2BP2*, insulin-like growth factor 2 mRNA-binding protein 2; SBWC, Shaanbei White Cashmere goat; II, homozygous insertion/insertion; ID, heterozygous insertion/deletion; DD, homozygous deletion/deletion; I, insertion; D, deletion.

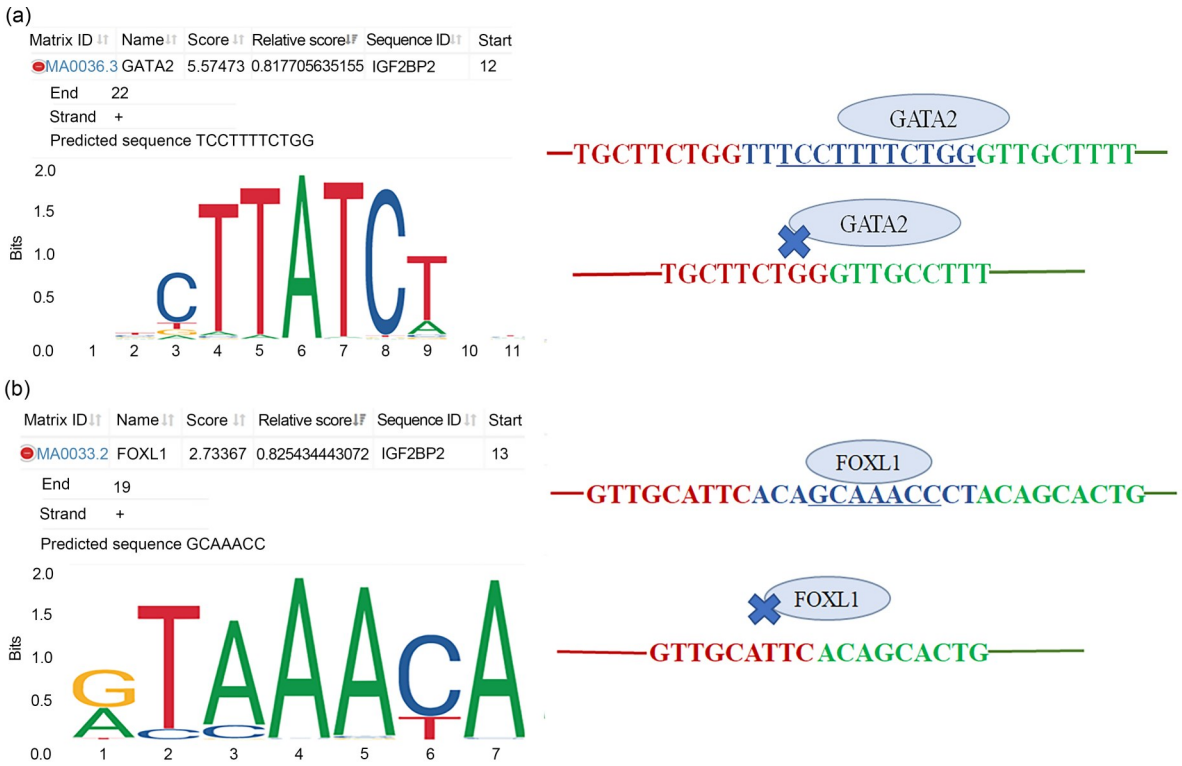

Fig. 7 Predicted combination of transcription factors (TFs) and sequence of the P4-Ins-13bp (a) and P5-Del-12bp (b) of the insulin-like growth factor 2 mRNA-binding protein 2 (*IGF2BP2*) gene. Ins, insertion; Del, deletion.

cancer (Zhao et al., 2012; Liu et al., 2015; Rao et al., 2016; Chen et al., 2018). However, the association between *IGF2BP2* indel polymorphisms and litter size in goats had not yet been examined; therefore, we explored the relationship in SBWC goats.

In this study, two indel loci were detected in *IGF2BP2* by sequencing, with PIC values ranging from 0.329 to 0.351, which indicated that these were moderate polymorphisms. However, the two indel loci in *IGF2BP2* did not conform to the HWE ($P<0.05$), which might be due to genetic drift, artificial selection, or migration (Wu et al., 2019). To further explore the relationship between the identified mutations and litter size, we performed the χ^2 test, which showed that the genotypic distributions of the *IGF2BP2* P4-Ins-13bp mutation differed significantly between the single-lamb and multi-lamb groups. Correlation analysis showed that the 13-bp indel in *IGF2BP2* was significantly associated with litter size in SBWC goats. Previous studies have reported that the absence of *IGF2BP2* in mouse embryos resulted in the arrest of early embryonic development at the 2-cell-stage in vitro and the downregulation of cell division cycle and apoptosis regulator 1 (*Ccar1*) and ribosomal protein S14 (*Rps14*) expression, which are essential for early embryonic development (Liu et al., 2019). Additionally, *IGF2BP2* has been reported to promote the translation of *IGF2* mRNA, and *IGF2BP2* overexpression increases the level of IGF2 protein in cell lines (Dai et al., 2011; Mu et al., 2015). IGF2 is a key regulator of embryo development and follicular maturation (Hammer et al., 2005), and polymorphisms in IGF2 exert significant impacts on the litter sizes of pigs of different parities (Muñoz et al., 2010). These data suggest that *IGF2BP2* may be involved in animal reproduction and that polymorphisms in this gene might influence the litter sizes of goats by influencing *IGF2BP2* gene expression. However, the mechanism through which *IGF2BP2* mutations alter goat reproduction requires further study.

The two indels identified in this study were both located in intron 2 of *IGF2BP2*, indicating that mutations in non-coding regions can affect mammalian reproductive traits. For instance, a 13-bp indel within the lysine methyltransferase 2A (*KMT2A*) gene and a novel 12-bp indel within the *GDF9* gene are significantly associated with goat litter size (Wang et al., 2017; Tang et al., 2021). The single-nucleotide polymorphism (SNP) mutation (c.2366G>A) in the 3' untranslated region (UTR) of bone morphogenetic protein 15 (*BMP15*) has also been demonstrated to significantly affect the reproductive traits of Large White pigs (Yin et al., 2019). To date, many studies have shown that non-coding regions can influence gene expression by altering the binding of TFs. For example, xeroderma pigmentosum group A (XPA)-binding protein 2 (*XAB2*) gene deletion leads to the retention of RNA polymerase II subunit A (*POLR2A*) introns, weakening overall transcription and promoting cell senescence (Hou et al., 2019). The bioinformatics analysis described in this study showed that the 13-bp indel sequence might alter GATA2 TF binding. GATA2 is essential for the differentiation of endometrial stromal cells (ESCs) (Kin et al., 2015), and both GATA2 and GATA4 are involved in transcriptional regulation of gonadotropin genes (Zheng et al., 2015). In summary, *IGF2BP2* may bind GATA2 through the P4-Ins-13bp insertion sequence, reducing the mRNA expression of *IGF2BP2* in the multi-lamb group. Therefore, we speculate that the DD genotype at the P4-Ins-13bp site of the *IGF2BP2* gene is the favored genotype, which can be selected for during goat breeding to improve the economic efficiency of the goat livestock industry.

5 Conclusions

A novel 13-bp indel in *IGF2BP2* was found to be significantly correlated with the litter size of SBWC goats and may serve as a molecular marker for MAS-guided breeding to accelerate the goat breeding process.

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Author contributions

Xianyong LAN and Dongyun XIN designed the experiment; Haijing ZHU, Lei QU, and Libang HE provided goat samples and data records; Dongyun XIN and Yangyang BAI carried out all the experiments; Dongyun XIN prepared all figures and tables and drafted the manuscript; Yi BI, Yuxin KANG, Hong CHEN, Xianyong LAN, and Chuanying PAN checked and revised the manuscript. All authors have read and approved the final manuscript and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Dongyun XIN, Yangyang BAI, Yi BI, Libang HE, Yuxin KANG, Chuanying PAN, Haijing ZHU, Hong CHEN, Lei QU, and Xianyong LAN declare that they have no conflict of interest.

The animal participants involved in this experiment were maintained in strict accordance with the "Regulations on the Management of Experimental Animal Affairs" (Ministry of Science and Technology of China, 2004) and this study was approved by the International Animal Care and Use Committee (IACUC) of Northwest A&F University, Yangling, China (Protocol No. NWAFAC1008).

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