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# Detection of insertions/deletions (InDels) within the goat *Runx2* gene and their association with litter size and growth traits

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**ABSTRACT**

*Runt-related transcription factor 2* (*Runx2*) is characterized by its critical functions in osteoblastic and ovulatory processes. The goal of this study was to explore the insertion/deletion (indel) variants of this gene and to evaluate their association with productive traits. Herein, a 12 bp and 6 bp insertion within the *Runx2* gene was uncovered in Shaanbei white cashmere goats (SBWC; $n = 1200$). Chi-square analysis revealed that the 12 bp insertion was related to litter size ($p < 0.01$). Further association analysis also found this insertion was significantly associated with litter size ($p = 1.1E\text{-}5$). Interestingly, this insertion was also significantly associated with chest circumference ($p = 0.018$). Additionally, the 6 bp insertion was associated with body length ($p = 0.003$), chest width ($p = 0.011$), and chest circumference ($p = 0.005$). Furthermore, diplotype associations also uncovered that the combined genotypes of these two indels also significantly affected litter size and growth traits ($p < 0.05$). These findings suggested that these two insertions within the *Runx2* gene were significantly associated with reproduction and growth traits, which would make them beneficial functional DNA markers that can be used in goat breeding.

**KEYWORDS**

Goat; *Runx2*; insertion; litter size; growth traits; association

## Introduction

In the ruminant industry, productive performance cannot increase without improvement of individual traits. Although artificial selection is especially important to this progress, which can increase the frequency of beneficial alleles and decrease the frequency of harmful alleles.[1] Marker-assisted selection (MAS) can be supplemented to promote the efficiency of artificial selection. The Shaanbei white cashmere (SBWC) goat is a breed developed from crossing the Liaoning Cashmere goat (male parent) and Shaanbei black goat (female parent).[2] It is characterized by resistance to rough feeding, cold, wind, and disease.[2,3] However, low litter size and poor growth traits have become restraining factors to its breeding at large scales. Thus, SBWC goats have been frequently selected to improve litter size and growth traits.[4] It is particularly essential to find markers that can both affect reproduction and growth traits in MAS.[5–7] Many genes involved in growth and reproduction of pigs and chickens have been regarded as candidates for MAS, but only several indels in these genes have been shown to significantly influence goat productive traits, including *GDF9*,[8] *KDM6A*,[9] *PRNP*,[10] *MARCH1*,[5] and *CMTM2*[11]. Similarly, considering its remarkable functions, the *runt-related transcription factor 2* (*Runx2*) gene is regarded as an important candidate gene for potential study in MAS to improve the goat industry.

*Runx2* is a member of the *Runx* family, also containing transcription factors *Runx1* and *Runx3*[12–14] and contacts a variety of signal pathways,[15] such as *TGF*β/*BMP2* and *Wnt*.[16,17] Thus, it influences many physiology processes of animals, such as osteoblastic differentiation[18,19] and ovulatory processes.[20,21] In osteoblastic differentiation, *Runx2* is always considered a marker gene because of its outstanding functions.[22,23] In mice, *Runx2* can regulate mesenchymal stem cells to differentiate into preosteoblasts and further into mature osteoblasts.[24] As an upstream transcription factor, *Runx2* not only regulates expression of bone matrix genes, such as *Col1a1*, *Ibsp*, and *Spp1*,[25] but also transcription factors,

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such as *Sp7*, which is essential for the maturation of osteoblasts.[13] Temporal expression of these genes ensures a smooth osteoblastic differentiation. Studies have also demonstrated that *Runx2*-deficient mice cannot generate bone or even osteoblasts,[26] which also demonstrates that *Runx2* is an important gene for osteoblast differentiation.

Furthermore, *Runx2* also plays a key role in reproduction. The expression of *Runx2* is induced by the luteinizing hormone (LH) surge and increased in ovulatory follicles.[20,27] Within the LH surge, the ovulatory follicle undergoes morphological and physiological changes that culminate in ovulation or follicular atresia.[28] In this process, *Runx2* regulates the expression of some luteal- and follicle-specific genes, such as *Edn2* and *Ptgs1*.[29] Additionally, *Runx2* can downregulate *Runx1*, *Ptgs2*, and *Tnfaip6* in luteinizing granulosa cells of rats.[30,31] All of these facts suggest that *Runx2* acts as a crucial regulator in ovulation. When the expression of *Runx2* is reduced in granulosa cells, mice exhibited subfertility, or in severe cases, infertility, which caused follicles to fail to ovulate.[20] Interestingly, *Runx2* serves as a marker gene of follicular atresia in pigs, because of its differential expression between small healthy follicles and small atretic follicles.[32] In goats, the expression of *Runx2* is regulated hormonally as well, and it is involved in progesterone production and promotes granulosa cell proliferation.[33]

Based on the function of *Runx2*, it is hypothesized that this gene may influence animal reproduction and growth traits. However, current studies on polymorphisms of this gene have mostly focused on humans. In humans, SNPs of the *Runx2* gene are often related to skeleton defects, such as osteonecrosis of the femoral head[28,34] and temporomandibular joint osteoarthritis.[35] In chickens, SNP g.124,883A > G in the *Runx2* gene can influence many physical traits.[36] However, the relationship between polymorphism of the *Runx2* gene and production traits remain largely unexplored in goats. Hence, this study was conducted to detect possible indels within *Runx2* and evaluate their effects on litter size and growth traits in SBWC goats ($n = 1200$), which would be beneficial for building DNA markers for goat MAS, as well as the development of the cashmere goat industry in China.

## Materials and methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Northwest A and F University (NWAFU). The use of experimental animals was permitted by local animal welfare laws, guidelines, and policies.

### DNA samples and related data collection

Ear tissue samples were obtained from 1200 adult female Shaanbei white cashmere (SBWC) goats.[11,37,38] They were reared at adjacent farms with similar management and feeding programs in Yulin, Shaanxi, China.[3,8,39] Data such as litter size (LS) of the first birth and growth traits, including body height (BH), body length (BL), chest width (CW), chest circumference (CC), and chest depth (CD) were also obtained from these goats.[3,37,40]

### Genomic DNA isolation and DNA Pool construction

Genomic DNA samples were isolated from ear tissue samples by the standard salt-chloroform extraction method.[37,41,42] Then, they were quantified using a Nanodrop 1000 (Thermo Scientific, Waltham, MA, USA) and diluted to 50 ng/μL as working solutions. The genomic DNA pool, which was made up of 50 random DNA samples, was used to explore genetic variation in the *Runx2* gene.[11,37]

### Primer design and PCR amplification

Information on a total of 13 potential indels of the *Runx2* gene was obtained from NCBI. Indels were designed using Primer Premier 5.0 software based on the reference sequence (GenBank accession number NC_030813.1) (Table 1). All primer pairs were synthesized by Sangon Biotech (Xian, Shaanxi, China). Touch down polymerase chain reaction (TD-PCR) was performed in a 12.5 μL reaction volume, containing 1.5 μL (10 ng/μL) of genomic DNA, 0.5 μL of each primer, 6.0 μL 2X *Taq* Master Mix, 4.0 μL $ddH_2o$. The thermal cycling program was 5 min at 95 °C for predenaturation, then 18 cycles at 94 °C for 30 s, then annealing for 30 s at 68 °C (with a decrease of 1 °C per cycle), 15 s at 72 °C, and a final extension at 72 °C for 10 min.[9] Finally, 4.0 μL PCR products were directly assayed by electrophoresis on 3.0% agarose gels stained with ethidium bromide.

### Statistical analyses

Genotypic and allelic frequencies were calculated manually, including the Hardy–Weinberg equilibrium (HWE). Population genetic parameters: heterozygosity

(He), homozygosity (Ho), effective allele number (Ne), and the polymorphism information content (PIC) were calculated by PopGene version 1.3.1 (Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, AB, Canada).[43] Linkage disequilibrium (LD) and haplotype analysis were performed by the SHEsis program (http://analysis.bio-x.cn).[2] In the LD analyses, the case of D′=1 or $r^2=1$ is known as complete LD. Values of D′ < 1; $r^2 > 0.33$ indicate that the complete ancestral LD has been disrupted, but strong.[37] Distribution differences for genotypic frequencies between the mothers of a single lamb and multi lambs were analyzed by the $\chi^2$ test using SPSS software (version 18.0, IBM, USA). The association test of indel polymorphism with several traits was calculated by independent *t*-test using SPSS software. The results are expressed as the means ± standard error (SE). Differences between the means were considered significant at $p < 0.05$.

**Table 1.** Primers used for detecting *Runx2* indel variants.

| Indel names | Locus | Primer sequences (5′ to 3′) | Sizes (bp) |
|---|---|---|---|
| rs637856923 | P1 | F1: AGCACGAAAGAGTATGTGCCC | 148 |
| | | R2: TGCAATCTACAGAAACAGCCTC | |
| rs638047165 | P2 | F2: TCTTCAGGAGAACGGTCTATGC | 156 |
| | | R2: AGGGGACCATCACGTTTGAAG | |
| rs639514493 | P3 | F3: CAGGGAGCCTTACTCAACCC | 169 |
| | | R3: TTGGAACTTGCCTGCTCCTT | |
| rs639817839 | P4 | F4: GAAGCATTAACTGAGTCTTG | 192 |
| | | R4: ACCACAAACGGTAGAAA | |
| rs647405170 | P5 | F5: CTCACCAGCTTTTCCCCCTC | 135 |
| | | R5: ACCGTGAATCCCAAGACAGC | |
| rs648686495 | P6 | F6: GGAGTCGCCATAAGGTACAG | 155 |
| | | R6: GGTGGAAACACCCCATGAAAG | |
| rs655201764 | P7 | F7: CCTCGGAGGCCCCACTTATC | 163 |
| | | R7: AACTGCCAGCGCCAGATGT | |
| rs655425647 | P8 | F8: CCGTGAGGCATCTGGTTTTATC | 200 |
| | | R8: CACTCAATGCTCTGATGGTGAC | |
| rs637856932 | P9 | F9: GTCTTTGGGCTCCAAGTGTG | 144 |
| | | R9: CCCTGAGAATACACTGGATGTGA | |
| rs637856933 | P10 | F10: TAACCGCTAGTTTGCTTTGT | 227 |
| | | R10: GAGTCAGGGAAGAACCACAG | |
| rs637856934 | P11 | F11: TGGCCTGCCTACACCTATTAAC | 180 |
| | | R11: GACAGAATTGCCCACGGTAG | |
| rs637856935 | P12 | F12: TATCCGACAAAAGGACTCACC | 147 |
| | | R12: GGACGTGACCTTCGTTCAAA | |
| rs637856937 | P13 | F13: CTGTGTCTGAAATGCTTTCCTGC | 187 |
| | | R13: TTTAGGCAAGGGTGCTCCA | |
| rs648686495 | P6 | F14: GGAGTCGCCATAAGGTACAGT | 276 |
| | | R14: GGAGTCGCCATAAGGTACAGT | |
| rs637856937 | P13 | F15: TCCCCGCCTTACCTCTTACA | 328 |
| | | R15: GGGCAGGTCAGATGCTGAAT | |

## Results

### Identification of two indel variations

In this study, a 6 bp (loci P6) and a 12 bp (loci P13) insertion were uncovered in the 5th intron of the goat *Runx2* gene, which was described as NC_030813.1: g. 30094437ins TGTCTC and NC_030813.1: 30094869ins TATTTTCTGGTC. They both had two genotypes. Electrophoresis showed that in the 6 bp insertion, the DD genotype exhibited one band (155 bp) and the ID genotype exhibited two bands (161 bp and 155 bp) (Figure 1). In the 12 bp insertion, the DD genotype exhibited one band (187 bp) and the ID genotype exhibited three bands (199 bp, 187 bp, and additional non-target fragment)[40] (Figure 2).

### Genetic parameter analysis and linkage disequilibrium analysis of two indels

The frequency distributions of the two genotypes and two alleles with these two loci are summarized in Table 2, as well as gene $H_O$, He, Ne, and PIC. The frequency of the 'D' allele were higher than that of the 'I' allele at these two loci, which were not in HWE ($p < 0.05$). The value of PIC showed that it had medium genetic diversity at the 6 bp insertion locus and had low genetic diversity at the 12 bp insertion locus.

The linkage disequilibrium between Locus P6 and Locus P13 is shown in Figure 3. There were four haplotypes found in the analyzed sample: Haplotype1, Haplotype2, Haplotype3, and Haplotype4 with frequencies of 0.724, 0.032,0.232, and 0.012, respectively

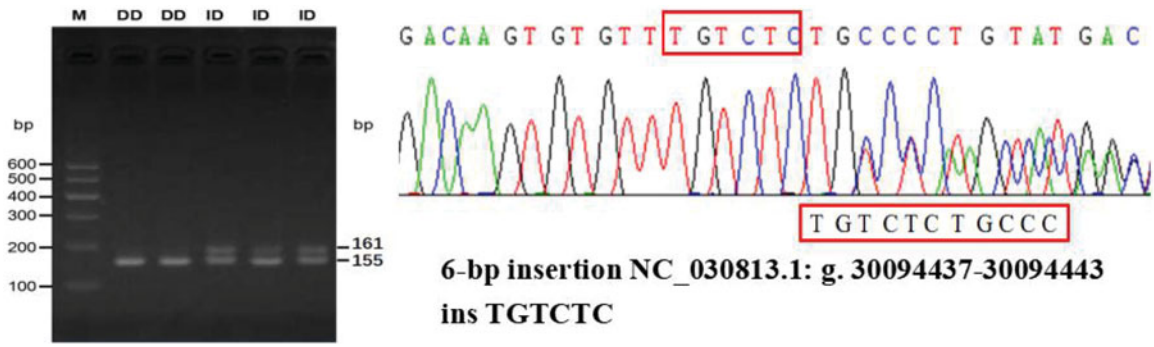


**Figure 1.** Agarose gel electrophoresis and DNA sequencing of goat *Runx2* gene Loci P6.

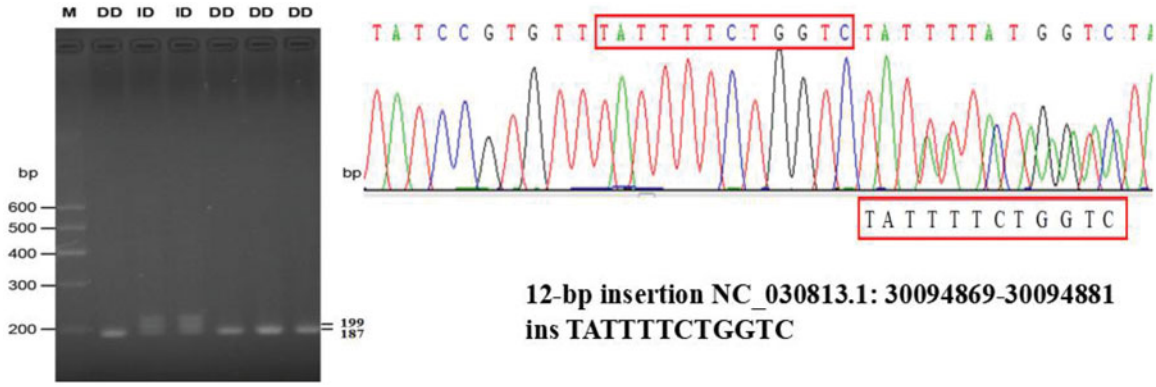


**Figure 2.** Agarose gel electrophoresis and DNA sequencing of goat *Runx2* gene Loci P12.

**Table 2.** Genotypic and allelic frequencies and population genetic indexes of *Runx2* gene in shaanbei white cashmere (SBWC) goats.

| | | Genotypes frequencies | | Alleles frequencies | | | Population genetic indexes | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Locus | Indel size (bp) | ID | DD | I | D | *p* value (HWE) | Ho | He | Ne | PIC |
| P6 (6-bp indel) | 6 | 507 | 498 | 0.25 | 0.75 | 1.09E-26 | 0.62 | 0.38 | 1.61 | 0.31 |
| P13 (12-bp indel) | 12 | 81 | 970 | 0.04 | 0.96 | <1.00E-30 | 0.93 | 0.07 | 1.08 | 0.07 |

**Note:** HWE: Hardy–Weinberg equilibrium; Ho: observed homozygosity; He: heterozygosity; Ne: effective allele numbers; PIC: polymorphism information content.

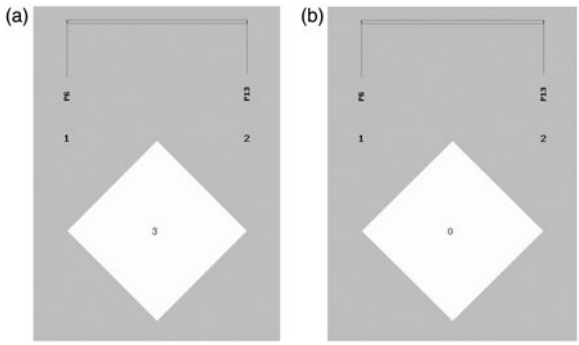


**Figure 3.** Linkage disequilibrium (LD) between Loci P6 and Loci P13 in the goat *Runx2* gene. Note: (a) D′ and (b) *r*2 of LD analyze between Loci P6 and Loci P13.

**Table 3.** Haplotypic frequencies within the *Runx2* gene in SBWC goats.

| Haplotypic names | Haplotypic types | Haplotypic frequencies |
|---|---|---|
| Haplotype1 | $D_{6\text{-bp}}$ $D_{12\text{-bp}}$ | 0.724 |
| Haplotype 2 | $D_{6\text{-bp}}$ $I_{12\text{-bp}}$ | 0.032 |
| Haplotype 3 | $I_{6\text{-bp}}$ $D_{12\text{-bp}}$ | 0.232 |
| Haplotype 4 | $I_{6\text{-bp}}$ $I_{12\text{-bp}}$ | 0.012 |

Note: $D_{6\text{-bp}}$: 'D' allele in loci P6; $I_{6\text{-bp}}$: 'I' allele in loci P6; $D_{12\text{-bp}}$: 'D' allele in loci P13; $I_{12\text{-bp}}$: 'I' allele in loci P13. All those frequency < 0.01 will be ignored in analysis.

(Table 3). The D′ value was 0.038 and the $r^2$ value was 0, suggesting that there was little recombination between these two loci.

### *Intragroup analysis of single-kid and multi-kids individuals in SBWC goats*

The independent intra-group $\chi^2$ test for 1012 female goats (Table 4) showed that litter size was significantly correlated with different genotypic frequencies ($\chi^2$=26.50, df = 1, $p < 1.0E\text{-}30$) at the 12 bp insertion locus. However, there was no significant difference at the 6 bp insertion.

### *Association analysis between indel genotypes and litter size in SBWC goats*

For litter size, the association between the 12 bp insertion and litter size is shown in Table 5; individuals with the genotype of ID had significantly lower litter sizes than did individuals with the DD genotype ($p = 1.1E\text{-}5$). The relationship between different diplotypes and litter size in SBWC goats also showed the similar result: the two diplotypes ($I_{6\text{-bp}}D_{6\text{-bp}}$ – $D_{12\text{-bp}}$ $D_{12\text{-bp}}$ and $D_{6\text{-bp}}D_{6\text{-bp}}$ – $D_{12\text{-bp}}D_{12\text{-bp}}$) had the largest litter size ($p = 0.01$) (Table 6).

**Table 4.** The genotypes distribution between mothers of single-lamb and multi-lamb in shaanbei white cashmere (SBWC) goats within *Runx2* loci P13.

| | | | Genotypes | | Genotypes frequencies | | |
|---|---|---|---|---|---|---|---|
| Locus | Types | Sample sizes | ID | DD | ID | DD | χ2 (df), *p* value |
| P6 (6-bp indel) | Single-kid | 559 | 282 | 277 | 0.50 | 0.50 | $\chi^2 = 0.005(df = 1)$ $p = 0.944$ |
| | Multi-kids | 365 | 185 | 180 | 0.51 | 0.49 | |
| **P13** (12-bp indel) | Single-kid | 602 | 70 | 532 | 0.12 | 0.88 | $\chi^2 = 26.50(df = 1)$ $\mathbf{p < 1.0E\text{-}30}$ |
| | Multi-kids | 410 | 11 | 399 | 0.02 | 0.98 | |

Note: Single-kid, individuals who give single lamb; Multi-kid, individuals who give more than one lambs.
Bolded values indicate the values $p < 0.05$.

**Table 5.** The relationship between two genotypes within *Runx2* litter sizes in shaanbei white cashmere (SBWC) goats (mean ± SE).

| Locus | ID ($n = 408$) | DD ($n = 430$) | *p* Value |
|---|---|---|---|
| P6 (6-bp indel) | $1.40 \pm 0.02$ | $1.41 \pm 0.02$ | $p = 0.45$ |
| **P13** (12-bp indel) | $1.18 \pm 0.05^{B}$ | $1.43 \pm 0.02^{A}$ | $\mathbf{p = 1.1E\text{-}5}$ |

Note: Cells with different letters (a,b/A,B) differed significantly ($p < 0.05/p < 0.01$).
Bolded values indicate the values $p < 0.05$.

**Table 6.** The relationship between different diplotypes and litter size in Shaanbei white cashmere goats (mean ± SE).

| Diplotypes | Sample size (*n*) | Litter size (Mean ± SE) | *p* Values |
|---|---|---|---|
| $I_{6\text{-bp}}\ D_{6\text{-bp}} - I_{12\text{-bp}}\ D_{12\text{-bp}}$ | 38 | $1.21 \pm 0.09^{B}$ | $p = 0.01$ |
| $I_{6\text{-bp}}\ D_{6\text{-bp}} - D_{12\text{-bp}}\ D_{12\text{-bp}}$ | 364 | $1.41 \pm 0.03^{A}$ | |
| $D_{6\text{-bp}}\ D_{6\text{-bp}} - I_{12\text{-bp}}\ D_{12\text{-bp}}$ | 37 | $1.08 \pm 0.06^{B}$ | |
| $D_{6\text{-bp}}\ D_{6\text{-bp}} - D_{12\text{-bp}}\ D_{12\text{-bp}}$ | 389 | $1.41 \pm 0.03^{A}$ | |

Note: Cells with different letters (a,b/A,B) differed significantly ($p < 0.05/p < 0.01$).

### *Association analysis between indel genotypes and growth traits in SBWC goats*

For growth traits, the association between these insertions and some growth traits is shown in Table 7. Results showed that the 6 bp insertion was significantly associated with body length ($p = 0.003$), chest width ($p = 0.011$), and chest circumference ($p = 0.005$). In addition, the 12 bp insertion was significantly associated with chest width ($p = 0.018$). Furthermore, the association between diplotypes and growth traits were also analyzed. It can be seen from Table 8 that deficient diplotypes can significantly affect most growth traits ($p < 0.05$), except chest circumference (CC).

## Discussion

*Runx2* contacts important signal pathways *TGFβ/BMP2* and *Wnt*,[13,15,18,19] suggesting this gene may affect reproduction and growth traits. Hence, this study explored indel diversity of the *goat Runx2* gene using a robust sample size ($n = 1200$), revealing a 6 bp and 12 bp insertion associated with productive traits, such as litter size and body height. The 6 bp insertion locus showed intermediate polymorphism and the 12 bp insertion showed low polymorphism. These two loci both had a high frequency of 'D' alleles (0.75–0.95), which could be seen as a protogene. Especially, in the locus of the 12 bp insertion, the frequency of the 'I' allele was very low (0.04), which may indicate that this insertion was generated more recently. Interestingly, there were no II genotypes at these two loci. We suggest the hypothesis that II homozygous might be lethal to embryos. It was shown that the *Runx2* gene plays an essential role in embryonic development, especially in the process of osteoblastic differentiation[21] and skeletal formation.[45] Thus, dysfunction of the *Runx2* gene may reduce the survival of embryos and individuals. For example, *Runx2*-deleted homozygous mice cannot form bones and heterozygotes cannot develop clavicles.[46,48] For this reason, homozygous oosperm with insertions in the *Runx2* gene cannot develop into a fetus to a large degree, which reflects the importance of the *Runx2* gene in terms of development. Furthermore, the tested SBWC population was not in accordance with the HWE, indicating that it might be undergoing artificial selection, migration, or genetic drift.[4,48]

To further explore relationships between the *Runx2* gene and reproduction and growth traits, several methods were utilized. Firstly, the results of the intra-group chi-square test proved the genotype frequency of 'DD' was associated with litter size in the 12 bp insertion in multi-kid individuals had a higher allelic frequency of 'D' than did single-kid individuals. On the contrary, the 6 bp insertion was not associated with litter size. Secondly, an association between the different genotypes and litter size was determined by independent *t*-test. The consensus was that individuals with the 'DD' genotype had larger litter sizes ($p = 1.1$ E-5) with the 12 bp insertion. Then, the combined genotype analysis showed that diplotypes '$I_{6\text{-bp}}D_{6\text{-bp}}$–$D_{12\text{-bp}}D_{12\text{-bp}}$' and '$D_{6\text{-bp}}D_{6\text{-bp}}$-$D_{12\text{-bp}}D_{12\text{-bp}}$' corresponded to larger litter size than did the others ($p = 0.01$). All of the above indicated that existence of the '$I_{12\,bp}$' allele may correspond to a smaller litter size. The exact molecular mechanism needs further verification. Thirdly, an independent *t*-test was also

**Table 7.** The relationship between different variants and growth traits in shaanbei white cashmere goats (mean ± SE).

| Locus | Traits | Genotypes | | p Values |
|---|---|---|---|---|
| | | ID (*n*) | DD (*n*) | |
| P6 (6-bp indel) | BH (cm) | $57.17 \pm 0.22$ ($n = 502$) | $57.75 \pm 0.22$ ($n = 491$) | $p = 0.062$ |
| | **BL (cm)** | $64.81 \pm 0.27^{B}$ ($n = 501$) | $65.93 \pm 0.27^{A}$ ($n = 490$) | $\mathbf{p = 0.003}$ |
| | CC**(cm)** | $86.33 \pm 0.45$ ($n = 501$) | $87.22 \pm 0.42$ ($n = 489$) | $p = 0.152$ |
| | **CD(cm)** | $28.36 \pm 0.13^{B}$ ($n = 479$) | $28.87 \pm 0.13^{A}$ ($n = 464$) | $\mathbf{p = 0.005}$ |
| | **CW(cm)** | $18.48 \pm 0.16^{b}$ ($n = 479$) | $19.04 \pm 0.15^{a}$ ($n = 465$) | $\mathbf{p = 0.011}$ |
| P13 (12-bp indel) | BH**(cm)** | $57.80 \pm 0.57$ ($n = 79$) | $57.62 \pm 0.15$ ($n = 941$) | $p = 0.745$ |
| | BL**(cm)** | $64.03 \pm 0.55$ ($n = 79$) | $65.66 \pm 0.61$ ($n = 943$) | $p = 0.443$ |
| | CC**(cm)** | $84.71 \pm 1.04$ ($n = 79$) | $87.46 \pm 0.76$ ($n = 943$) | $p = 0.297$ |
| | CD**(cm)** | $27.88 \pm 2.68$ ($n = 79$) | $28.52 \pm 0.10$ ($n = 943$) | $p = 0.072$ |
| | **CW(cm)** | $18.14 \pm 0.34^{b}$ ($n = 79$) | $19.03 \pm 0.10^{a}$ ($n = 943$) | $\mathbf{p = 0.018}$ |

Note: BH: body height; BL: body length; CC: chest circumference; CD: chest depth; CW: chest width; SBWC, Shannbei white cashmere goat. Cells with different letters (a, b/A, B) differed signifificantly ($p < 0.05/p < 0.01$).
Bolded values indicate the values $p < 0.05$.

**Table 8.** The relationship between different diplotypes (between loci 6 and Loci13) and growth traits in Shaanbei white cashmere goats (mean ± SE).

| Diplotypes | BH (cm) | BL (cm) | CC (cm) | CD (cm) | CW (cm) |
|---|---|---|---|---|---|
| $I_{6\text{-bp}}$ $D_{6\text{-bp}}$ – $I_{12\text{-bp}}$ $D_{12\text{-bp}}$ | $57.66 \pm 0.74$ ($n = 37$) | $63.80 \pm 0.76^{B}$ ($n = 37$) | $86.03 \pm 1.64^{AB}$ ($n = 37$) | $27.86 \pm 0.37^{B}$ ($n = 37$) | $18.41 \pm 0.51^{AB}$ ($n = 37$) |
| $I_{6\text{-bp}}$ $D_{6\text{-bp}}$ – $D_{12\text{-bp}}$ $D_{12\text{-bp}}$ | $57.37 \pm 0.24$ ($n = 376$) | $64.90 \pm 0.30^{B}$ ($n = 377$) | $87.71 \pm 0.49^{A}$ ($n = 376$) | $28.61 \pm 0.15^{B}$ ($n = 377$) | $19.01 \pm 0.17^{AB}$ ($n = 377$) |
| $D_{6\text{-bp}}$ $D_{6\text{-bp}}$ – $I_{12\text{-bp}}$ $D_{12\text{-bp}}$ | $57.61 \pm 0.87$ ($n = 37$) | $64.12 \pm 0.87^{B}$ ($n = 37$) | $83.80 \pm 1.42^{B}$ ($n = 37$) | $27.92 \pm 0.51^{B}$ ($n = 37$) | $18.00 \pm 0.52^{B}$ ($n = 37$) |
| $D_{6\text{-bp}}$ $D_{6\text{-bp}}$ – $D_{12\text{-bp}}$ $D_{12\text{-bp}}$ | $57.99 \pm 0.21$ ($n = 399$) | $66.03 \pm 0.27^{A}$ ($n = 399$) | $88.37 \pm 0.41^{A}$ ($n = 398$) | $29.04 \pm 0.14^{A}$ ($n = 399$) | $19.43 \pm 0.16^{A}$ ($n = 399$) |
| *p* Values | $p = 0.296$ | $\mathbf{p = 0.005}$ | $\mathbf{p = 0.014}$ | $\mathbf{p = 0.009}$ | $\mathbf{p = 0.046}$ |

**Note:** BH: body height; BL: body length; CC: chest circumference; CD: chest depth; CW: chest width; SBWC: Shannbei white cashmere goat. Cells with different letters (a, b/A, B) differed significantly ($p < 0.05/p < 0.01$).
Bolded values indicate the values $p < 0.05$.

utilized to explore relationships between the *Runx2* gene and growth traits. The 12 bp insertion affected chest width ($p = 0.018$) and the 6 bp insertion affected body length ($p = 0.003$), chest width ($p = 0.011$), and chest circumference ($p = 0.005$), implying these indels can influence reproduction and growth development. These genetic effects were consistent with the functions of the *Runx2* gene.

Firstly, the *Runx2* gene plays an essential role in skeletal formation and development.[49] In this process, *Runx2* regulates initial mesenchymal progenitor cell differentiation into mature osteocytes.[50] For example, phosphorylation generation in different sections of the Runx2 protein can regulate osteoblast differentiation by influencing some signaling molecules, such as *BMP-2*, *IGF-1*, and *MAPK*.[51] Thus, the *Runx2* gene always serves as a maker for osteoblast differentiation.[52] Furthermore, as an intersection of transforming TGF-β and BMP osteogenic signaling pathways, the Runx2 protein does not only interact with a variety of proteins, it also serves as a platform protein providing a place for other signaling molecules to make contact.[53] Because the *Runx2* gene contacts multiple signaling pathways directly and indirectly, insertions in it may influence growth traits. Secondly, the *Runx2* gene plays key roles in the ovulatory process. *Runx2* cannot only regulate expression levels of relevant genes, such as *RUNX1*, *PTGS2*, *TNFAIP6*, and *HAPLN1*,[22,30] but also hormones, such as hCG.[28] Furthermore, the *Runx2* gene plays an essential role in the BMP osteogenic signaling pathway. Interestingly, many members, such as *BMP-2*,[54] *BMP-4*,[55,56] *BMP-6*,[57] *BMP-15*,[58,59] and *BMP-9*[60] can also influence osteoblastic differentiation and the ovulatory process. All of this indicates that *Runx2* is important to the ovulatory process and may explain the reason why insertions in the *Runx2* gene can influence litter size.

Indels within a critical gene, even in the intron, often affect productive traits. For example, a 14 bp duplicated deletion in the 1st intron of the goat *GHR* gene can influence litter size and growth traits.[3] A 12 bp deletion in 1st intron of the goat *LHX4* gene can also affect litter size.[2] Many studies have shown that introns play roles of regulators, which are essential to gene transcription, mRNA processing, alternative splicing, and even contain kinds of noncoding RNA.[61] Therefore, these two insertion locations in the intron within the *Runx2* gene may also affect economic traits.

Briefly, these two insertions within the goat *Runx2* gene were significantly associated with litter size and growth traits, which could be used as effective molecular markers for improving economic traits in the goat industry.

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