



Genetic variations in the sheep *SIRT7* gene and their correlation with body size traits

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Abstract. As a nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylase and ADP ribosyl transferase, the silent information regulator 7 (Sirtuin 7, *SIRT7*) plays a crucial role in regulating the differentiation of adipocytes and myoblasts, lipid metabolism, glucose metabolism, and cellular growth in mammals. It has been hypothesized that *SIRT7* affects growth traits in animals; therefore, in this study, the potential insertion/deletion (indel) of genetic variations within the ovine *SIRT7* gene and their correlation with sheep growth traits were explored. A total of 709 individuals from five Chinese and Mongolian sheep breeds were analyzed. Two novel indel loci of the sheep *SIRT7* gene were detected and were named 5' promoter region-insertion-7 bp (5' promoter region-7 bp) and 3' UTR-insertion-17 bp (3' UTR-17 bp), respectively. In all of the sheep breeds, frequencies of the 5' promoter region-7 bp mutation were low, whereas mutations of 3' UTR-17 bp were high in Tong sheep and Lanzhou fat-tail sheep (LFTS). Furthermore, both indel polymorphisms had significant associations with different growth characteristics ($P < 0.05$). Among these associations, the 3' UTR-17 bp was highly correlated with rump width in small-tail Han sheep (STHS, rams; $P < 0.01$), and individuals with the ID genotype had better chest depth values than those with the II genotype. In this paper, two novel indels within the sheep *SIRT7* gene were identified, and genetic diversity and its effects on body size traits were explored. These findings will potentially provide useful DNA markers for the improvement of economic traits in sheep genetic breeding.

1 Introduction

Silent information regulator 2 (sirtuins2, *SIRT2*) proteins, which belonging to the class III nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylases and ADP ribosyl transferases, are connected to metabolism and life span regulation in lower organisms and are highly conservative from prokaryotes to eukaryotes (O'Callaghan and Vassilopoulos, 2017). In particular, the mammalian sirtuin proteins have been reported to participate in the regulation of energy metabolism (e.g., sugar and lipid metabolism) (Li and Kazgan, 2011; Ye et al., 2017) and stress (Blank and

Grummt, 2017), as well as the maintenance of genomic stability (Bosch-Presegué and Vaquero, 2014), tumor development (Roth and Chen, 2014), cell proliferation, senescence, and apoptosis (O'Callaghan and Vassilopoulos, 2017).

Recently, *SIRT* genes have been shown to regulate the differentiation of adipocytes and myoblasts (Cioffi et al., 2015). Additionally, an increasing number of studies have revealed that the genetic diversity of *SIRT* genes is related to the economic traits of livestock. For instance, single nucleotide polymorphisms (SNPs) of the bovine *SIRT1* gene have been shown to be significantly correlated with carcass

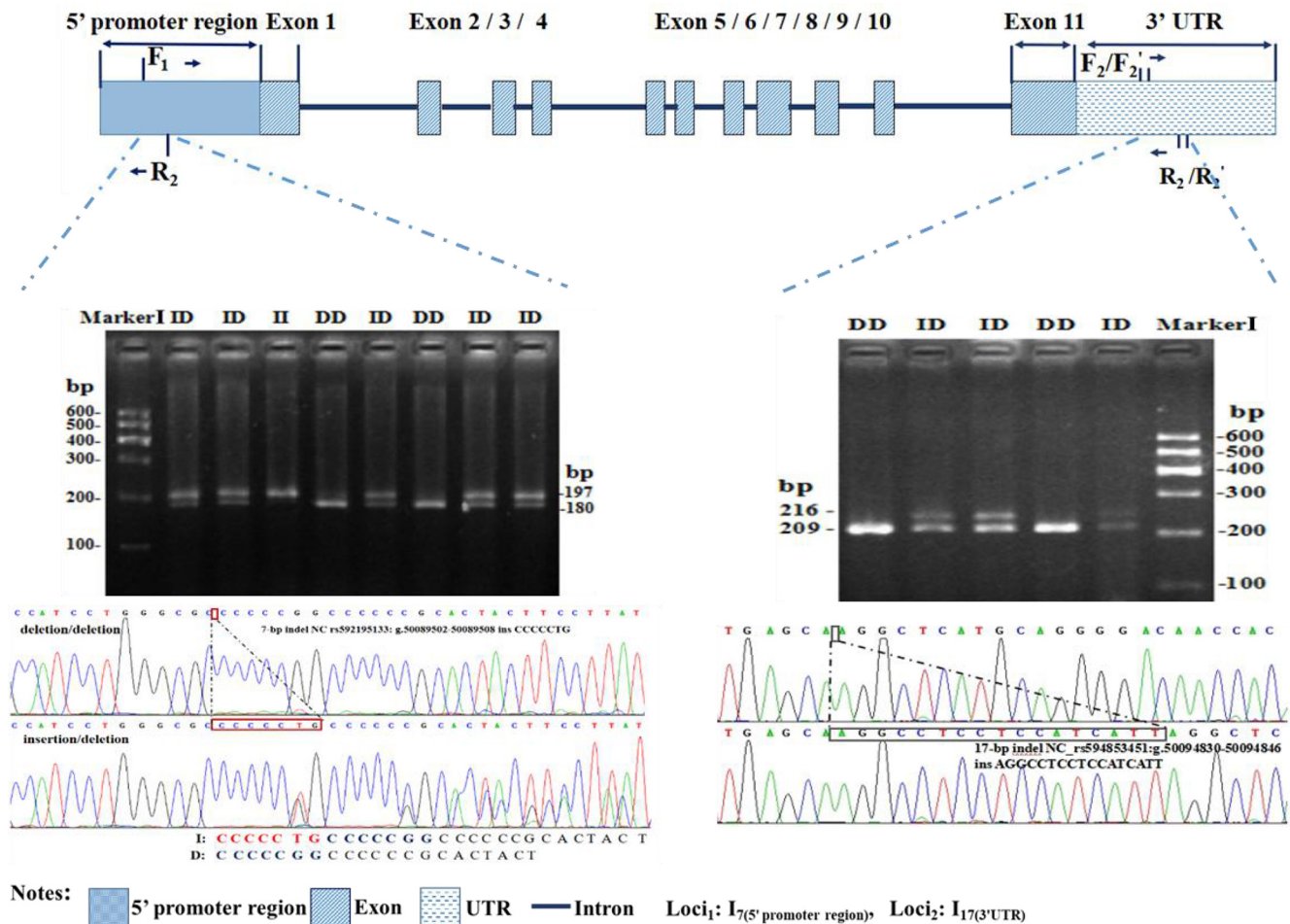


Figure 1. Electrophoresis diagram and sequence of 3' UTR-insertion-17 bp loci and 5' promoter region-insertion-7 bp within the ovine *SIRT7* gene.

traits (such as dressing percentage, meat percentage, and carcass weight) of Chinese Luxi cattle (Liu et al., 2017). Moreover, g.13915A>G within the *SIRT4* gene significantly influences body size traits (body length, chest depth, rump length, and chest circumference) in Chinese Qinchuan cattle (Gui et al., 2016). There are seven members in the mammalian sir-tuin protein family (*SIRT1*–*SIRT7*) that have different sub-cellular localizations. As the only member that is predominantly localized to the nucleolus (Michishita et al., 2005), *SIRT7* has been reported to be involved in cellular growth and metabolism. At the cellular level, *SIRT7* participates in the physiological processes of the cell stress response, proliferation, apoptosis, aging, and so on (Shin et al., 2016; Blank and Grummt, 2017). At the individual level, *SIRT7* can control the progress of glycolysis (Jiang et al., 2017; Ye et al., 2017) and hepatic lipid metabolism by regulating the ubiquitin–proteasome pathway (Yoshizawa et al., 2014; Tang et al., 2015; Ye et al., 2017; Yamagata and Yoshizawa, 2018). Previous studies have revealed that *SIRT7* antagonizes TGF- β signaling to inhibit breast cancer cell metastasis (Tang et

al., 2017) and promotes glioma proliferation and invasion via the activation of the ERK/STAT3 signaling pathway (Mu et al., 2019). Notably, two synonymous SNP mutations of bovine *SIRT7* have been confirmed to be significantly associated with the body length, hip length, back fat thickness, and chest circumference of Qinchuan cattle (Gui et al., 2016). In contrast, there are few related reports regarding ovine *SIRT7*.

The ovine *SIRT7* gene is located on chromosome 11, and includes 11 exons. The molecular and biological properties of the *SIRT7* gene make it a potential candidate gene to affect body size traits in animals; however, the associations between the body size traits of sheep and mutations in the *SIRT7* gene are poorly explored and researched, especially regarding the insertion/deletion (indel) variants. Compared with other molecular markers (including SNPs), indel variants have superiority in terms of detection efficiency (Yang et al., 2016). Therefore, to explore the indel polymorphisms of the ovine *SIRT7* gene, five representative sheep breeds were studied. The Sartuul sheep (SS) is a vital wool/meat dual purpose domestic sheep breed in Mongolia, whereas

Table 1. PCR primer sequences of the sheep *SIRT7* gene for amplification.

| Locus | Primer sequences (5'-3') | Product size (bp) | <i>T_m</i> (°C) | Region |
|------------------------|---|-------------------|---------------------------|--------------------|
| 5' promoter region-7bp | F ₁ : TCGGCTTCGCATGTGTGT | 209/216 | 59.97 | 5' promoter region |
| | R ₁ : GAGGCCAGAAGGAAGACAGC | | 60.39 | |
| 3' UTR-17bp | F ₂ : CTTGCACTGCTGTGGTTGTC | 180/197 | 59.97 | 3' UTR |
| | R ₂ : ATTGGGTTGATGCCCTGAG | | 60.03 | |
| | F ₂ ' : CGGGACTCTTATTCAGGGGC | 192/209 | 58.92 | |
| | R ₂ ' : GGTGTGACTGCCACCTCTTT | | 57.50 | |

the Lanzhou fat-tail sheep (LFTS), the small-tail Han sheep (STHS), the Tong sheep (TS), the and Hu sheep (HS) are representative indigenous sheep breeds in China (Li et al., 2018a, b). The objectives of this study were to investigate the novel indel polymorphisms within the ovine *SIRT7* gene in the five abovementioned sheep breeds and explore the correlations of these novel indel polymorphisms with ovine body size traits. The results of this study could potentially accelerate the progress of sheep breeding.

2 Material and methods

2.1 Animal welfare statement

In this study, all experimental processing was fully consistent with the animal welfare guidelines, laws and policies of Northwest A&F University.

2.2 Collection of DNA samples and data

In total, 737 same-aged sheep from five different breeds were used: four indigenous Chinese breeds, STHS ($n = 187$), TS ($n = 165$), LFTS ($n = 58$), and HS ($n = 189$); and one Mongolian native breed, SS ($n = 138$) (Li et al., 2018a, b; Ma et al., 2018a, b). Using the same standard, the body traits of all of the sheep were measured (Zhao et al., 2017), including the cannon circumference (CaC), body weight (BW), and chest depth (ChD), among others. Thereafter, related growth trait indices, such as the chest width index (ChWI), were calculated according to the method from Lan et al. (2007).

2.3 Construction of genomic DNA pool

Using the high salt extraction method, the sheep genomic DNA was isolated from ear tissue which was preserved in 70% alcohol at -80°C (Lan et al., 2007). The samples were then assayed using a NanoDrop 1000 spectrophotometer (Thermo Scientific, USA); the concentration of all of the diluted DNA samples was $10\text{ ng }\mu\text{L}^{-1}$ (Li et al., 2018b). Additionally, 50 samples of DNA were randomly selected and mixed to detect potential indel loci within the sheep *SIRT7* gene (Yang et al., 2017).

2.4 Mutation loci amplification and production sequencing

On the basis of the Ensembl database (<https://asia.ensembl.org/>, last access: 28 March 2019), two indel loci were selected in the sheep *SIRT7* gene, one was located in the 5' promoter region and the other was located in the 3' UTR (Fig. 1). Referencing the gene sequence of the sheep *SIRT7* gene (GenBank no: NC_019468.2), three pairs of amplification primers were designed using the Primer-BLAST tool in the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome, last access: 28 March 2019) (Table 1) and were synthesized by Tsingke Biotech Company (Xi'an, China).

The amplification reaction was performed in a 13 μL volume system, including 1 μL of genomic DNA ($10\text{ ng }\mu\text{L}^{-1}$), 6.5 μL of 2 \times EasyTaq PCR SuperMix (+dye), 0.5 μL of each primer, and 4.5 μL of ddH₂O. The PCR amplification procedure was touch-down PCR (TD-PCR) using the steps described in previous studies (Yang et al., 2016; Xu et al., 2015; Li et al., 2018a). The amplification products were detected using 3.5% agarose gel electrophoresis; the amplification products of each pair of primers were only sequenced if they were different (Zhang et al., 2015; Cui et al., 2018).

2.5 Statistical analysis

The mutation sequences of the two new indel loci were verified by contrasting and analyzing the sequences using BioEdit software (BioEdit, USA). Frequencies of the genotypes and alleles of the sheep *SIRT7* gene between different breeds were directly calculated using a Chi-square (χ^2) test. Based on the GDIcall Online Calculator (<http://www.msrecall.com/Gdicall.aspx>, last access: 28 March 2019), the polymorphism information content (PIC) was calculated (Li et al., 2018a). In addition, the values of the r^2 test and D' test were also calculated using the SHEsis program (<http://analysis.bio-x.cn>, last access: 28 March 2019) to analyze the linkage disequilibrium of the populations of the two mutation loci (Shi et al., 2005; Li et al., 2009, 2018a; Wang et al., 2017). Furthermore, using an analysis of variance (ANOVA) and independent samples t tests in SPSS software (version 23.0; IBM Corp, USA), the relationships between the indels

Table 2. Genetic diversity parameters of novel polymorphisms of the ovine *SIRT7* gene.

| Locus | Breeds | Sizes | Genotypic frequencies | | | Allelic frequencies | | HWE <i>P</i> values | Population parameters | | | |
|------------------------|--------|-------|-----------------------|-------|-------|---------------------|-------|------------------------|-----------------------|-------|-------|-------|
| | | | DD | ID | II | D | I | | Ho | He | Ne | PIC |
| 5' promoter region-7bp | LFTS | 57 | 0.123 | 0.877 | 0.000 | 0.561 | 0.439 | <i>P</i> <0.05 | 0.508 | 0.492 | 1.970 | 0.371 |
| | STHS | 173 | 0.485 | 0.503 | 0.012 | 0.737 | 0.263 | <i>P</i> <0.05 | 0.612 | 0.388 | 1.633 | 0.313 |
| | TS | 158 | 0.595 | 0.405 | 0.000 | 0.797 | 0.203 | <i>P</i> <0.05 | 0.677 | 0.323 | 1.477 | 0.323 |
| | HS | 135 | 0.593 | 0.407 | 0.000 | 0.796 | 0.204 | <i>P</i> <0.05 | 0.676 | 0.324 | 1.480 | 0.324 |
| | SS | 123 | 0.463 | 0.520 | 0.016 | 0.724 | 0.276 | <i>P</i> <0.05 | 0.600 | 0.400 | 1.667 | 0.400 |
| 3' UTR-17bp | LFTS | 58 | 0.120 | 0.690 | 0.190 | 0.466 | 0.534 | <i>P</i> <0.05 | 0.502 | 0.498 | 1.991 | 0.374 |
| | STHS | 187 | 0.278 | 0.529 | 0.193 | 0.543 | 0.457 | <i>P</i> >0.05 | 0.504 | 0.496 | 1.986 | 0.373 |
| | TS | 165 | 0.261 | 0.400 | 0.339 | 0.461 | 0.539 | <i>P</i> <0.05 | 0.503 | 0.497 | 1.988 | 0.497 |
| | HS | 189 | 0.238 | 0.656 | 0.106 | 0.566 | 0.434 | <i>P</i> <0.05 | 0.509 | 0.491 | 1.966 | 0.371 |
| | SS | 138 | 0.297 | 0.507 | 0.196 | 0.551 | 0.449 | <i>P</i> >0.05 | 0.505 | 0.495 | 1.980 | 0.372 |

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

Table 3. Linkage disequilibrium test (D' and r^2) of two pairs of alleles in the different sheep breeds.

| Breeds | D' test | | r^2 test | |
|--------|-------------------------|--------------|-------------------------|--------------|
| LFTS | 5' promoter region-7 bp | 3' UTR-17 bp | 5' promoter region-7 bp | 3' UTR-17 bp |
| | | 0.830 | | 0.478 |
| STHS | 5' promoter region-7 bp | 3' UTR-17 bp | 5' promoter region-7 bp | 3' UTR-17 bp |
| | | 0.914 | | 0.375 |
| TS | 5' promoter region-7 bp | 3' UTR-17 bp | 5' promoter region-7 bp | 3' UTR-17 bp |
| | | 0.622 | | 0.082 |
| HS | 5' promoter region-7 bp | 3' UTR-17 bp | 5' promoter region-7 bp | 3' UTR-17 bp |
| | | 0.999 | | 0.213 |
| SS | 5' promoter region-7 bp | 3' UTR-17 bp | 5' promoter region-7 bp | 3' UTR-17 bp |
| | | 0.632 | | 0.202 |

of *SIRT7* and the body size traits (e.g., chest width) in different breeds were determined (Li et al., 2018b). The genotypes were considered to be the independent variables and the growth traits were the dependent variables (Li et al., 2018a). If the data did not show homogeneity of variances and a normal distribution, a non-parametric (Kruskal–Wallis) test was used (Cui et al., 2018; Wang et al., 2019). The results were regarded as statistically significant when $P < 0.05$, and all statistical tests were bilateral.

3 Results

3.1 Genotyping of individuals

Two novel indel loci within the ovine *SIRT7* gene, namely the 5' promoter region-insertion-7 bp (5' promoter region-7 bp) and 3' UTR-insertion-17 bp (3' UTR-17 bp), were genotyped and identified via gel agarose electrophoresis (3.5%) and DNA sequencing (Fig. 1).

Polymorphisms of each indel had three different genotypes. For 5' promoter region-7 bp (Fig. 1), genotype II (insertion/insertion) showed one band of 209 bp, genotype DD (deletion/deletion) exhibited one band of 216 bp, whereas the heterozygote genotype ID (insertion/deletion) exhibited two bands (216 and 209 bp). For 3' UTR-17 bp (Fig. 1), three different bands were also exhibited: 180 bp, 197 bp, and 197/180 bp. After contrasting and analyzing mutant sequences, the two new indel loci were verified. The insertion sequences were CCCCCTG for 5' promoter region-7 bp and AGGCCTCCTCCATCATT for 3' UTR-17 bp, which were in agreement with the information in the Ensembl database.

3.2 Genetic parameters calculation

In Table 2, the frequencies of population parameters, genotypes, and alleles for the two indel loci in the five breeds tested are shown. For 5' promoter region-7 bp, the DD and ID genotypes had higher frequencies than the II genotype in

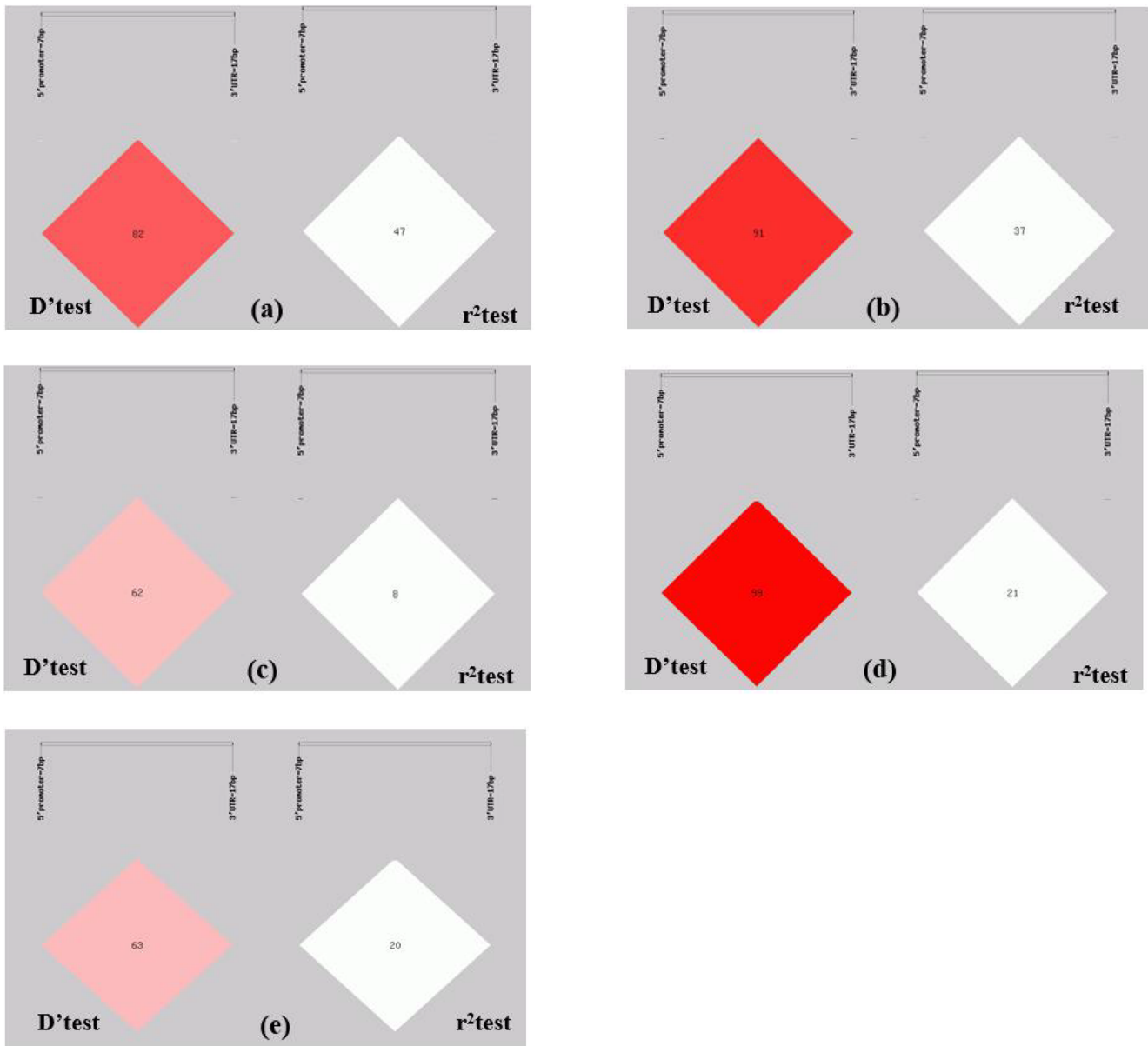


Figure 2. Linkage equilibrium test of two pairs of alleles within the ovine *SIRT7* gene in different populations: (a) Lanzhou fat-tail sheep, (b) small-tail Han sheep, (c) Tong sheep, (d) Hu sheep, and (e) Sartuul sheep. Note: the 5' promoter region-insertion-7 bp (loci1) and 3' UTR-insertion-17 bp loci (loci2) were chosen for haplotype analysis.

all of the breeds analyzed, and the D allele had a higher frequency than the I allele. For 3' UTR-17 bp, the five breeds had a different dominant allelic frequency. In STHS, HS, and SS, the D allele had a higher frequency than the I allele, whereas the opposite was found in the LFTS and TS breeds. Additionally, the results of the population parameters demonstrated that these two indel markers displayed moderate polymorphism, and the PIC among them ranged from 0.313 to 0.497 in all of the breeds studied. Moreover, the 5' promoter region-7 bp locus in all breeds tested, and the 3' UTR-17 bp

locus in the LFTS, TS, and HS breeds, did not conform to the Hardy-Weinberg equilibrium (HWE; $P < 0.05$).

The r^2 test showed that the 5' promoter region-7 bp and the 3' UTR-17 bp loci were at linkage equilibrium in all of the sheep groups tested (Table 3, Fig. 2). However, the D' test showed the opposite: in the SS and TS breeds, the D' test values showed that the two indel loci were in the weak linkage disequilibrium. Furthermore, the haplotype analysis revealed that there were four haplotypes and that “ $D_{5' \text{ promoter region-7 bp}} D_{3' \text{ UTR-17 bp}}$ ” had the highest incidence in all of the sheep groups detected (Fig. 3).

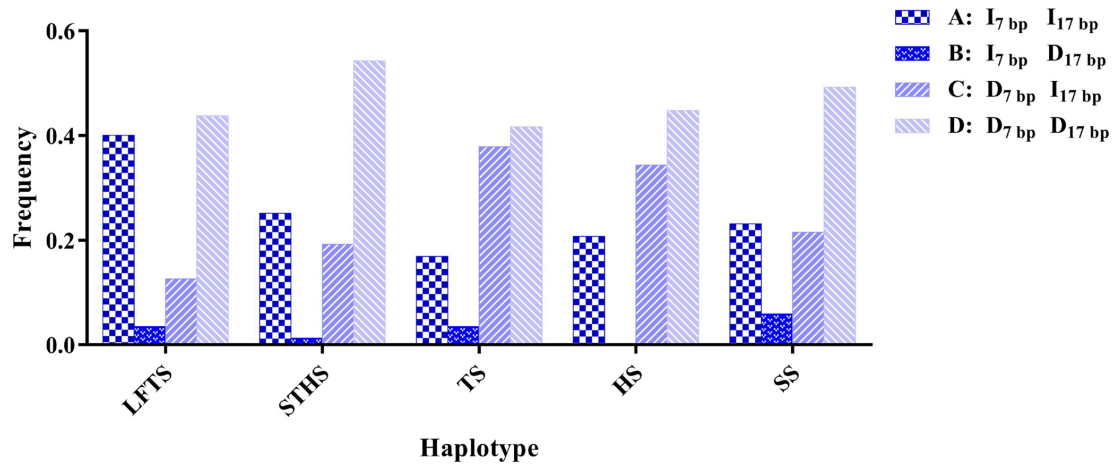


Figure 3. Haplotype frequency of the 5' promoter region-insertion-7 bp and 3' UTR-insertion-17 bp loci of the ovine *SIRT7* gene in different breeds. Note: LFTS – Lanzhou fat-tail sheep; STHS – small-tail Han sheep; TS – Tong sheep; HS – Hu sheep; and SS – Sartuul sheep.

Table 4. Association of the novel indel of the ovine *SIRT7* gene and growth traits in different breeds (LSM^a ± SE).

| Locus | Breeds | Sizes | Growth traits | Observed genotypes (LSM ^a ± SE) | | | P values |
|-------------------------|------------|--------|---------------|--|--------------------------------|---------------------------------|----------|
| | | | | II (n) | ID (n) | DD (n) | |
| 5' promoter region-7 bp | STHS (ram) | 87 | ChWI | 83.21 ^a ± 8.21 (3) | 66.47 ^b ± 1.93 (41) | 68.64 ^{ab} ± 1.34 (43) | 0.035 |
| | STHS (ewe) | 86 | CaC (cm) | – | 7.08 ± 0.12 (45) | 6.70 ± 0.12 (41) | 0.032 |
| | | | CaCI | – | 11.43 ± 0.25 (45) | 10.61 ± 0.20 (41) | 0.013 |
| | TS (ram) | 24 | BL (cm) | – | 68.18 ± 1.46 (8) | 73.47 ± 0.62 (16) | 0.001 |
| HS (ewe) | 135 | RW(cm) | – | 17.89 ± 0.11 (55) | 17.38 ± 0.10 (80) | 0.001 | |
| 3' UTR-17 bp | LFTS (ewe) | 25 | BH (cm) | 71.17 ^b ± 3.11 (9) | 77.48 ^a ± 1.42 (26) | 68.33 ^b ± 3.18 (5) | 0.028 |
| | | | ChWI | 68.45 ^a ± 2.93 (9) | 67.65 ^a ± 2.34 (26) | 55.27 ^b ± 6.14 (5) | 0.035 |
| | STHS (ram) | 96 | ChD (cm) | 26.20 ^c ± 0.69 (16) | 27.85 ^a ± 0.26 (51) | 27.49 ^{ac} ± 0.41 (29) | 0.009 |
| | STHS (ewe) | 91 | CaCI | 10.61 ^b ± 0.31 (20) | 11.44 ^a ± 0.23 (48) | 10.71 ^{ab} ± 0.27 (23) | 0.039 |
| | TS (ewe) | 44 | MFW (cm) | 12.32 ^b ± 0.13 (16) | 12.77 ^a ± 0.12 (22) | 12.68 ^{ab} ± 0.16 (16) | 0.049 |

Note: ChWI – chest width index; CaC – cannon circumference; CaCI – cannon circumference index; BL – body length; RW – rump width; BH – body height; ChD – chest depth; and MFW – maximum forehead width. The different letters (^a and ^b, or ^a and ^c) beside values within the same row signify significance at the $P < 0.05$ and $P < 0.01$ level, respectively.

3.3 Association of the indel polymorphisms and body size traits

The correlation of the two abovementioned novel indels and sheep body size traits were investigated to explore whether the polymorphisms of the ovine *SIRT7* gene were related to sheep growth (Table 4). As shown in Table 4, both indel loci displayed significant relationships with the different sheep body size traits. The polymorphisms of the 5' promoter region-7 bp were significantly associated with the chest width index (ChWI) in STHS (rams), the cannon circumference and cannon circumference index (CaC and CaCI, respectively) in STHS (ewes), the body length and chest depth (BL and ChD, respectively) in TS (rams), and the rump width in HS (ewes). Furthermore, mutations of the 3' UTR-17 bp showed a significant relationship with the body height and ChWI in LFTS (ewes), the ChD in STHS (rams), the

CaCI in STHS (ewes), and the maximum forehead width in TS (ewes). In particular, in STHS (rams), the effects of the 3' UTR-17 bp on the chest depth was highly significant ($P = 0.009$).

4 Discussion

Studies of *SIRT* genes tend to focus on the regulation of the physiological processes of cell stress responses, proliferation, apoptosis, aging, metabolism, and especially on the development of tumors (Li and Kazgan, 2011; Roth and Chen, 2014; O'Callaghan and Vassilopoulos., 2017; Ye et al., 2017). Although, in recent years, *SIRT* genes have also been shown to regulate the differentiation of adipocytes and myoblasts (Cioffi et al., 2015). Furthermore, the genetic mutations of *SIRT* genes have revealed their effects on the production traits of livestock (Gui et al., 2016; Liu et al.,

2017). However, existing studies on the polymorphism of *SIRT* genes have mostly focused on SNP or haplotype mutations in cattle, whereas the study of polymorphisms of ovine *SIRT* genes is lacking. Thus, this study is the first to identify genetic diversities in the ovine *SIRT7* gene and explore the effects of these novel indel polymorphisms in representative Chinese and Mongolian sheep breeds. In this paper, two novel indels, both located in the untranslated region of the ovine *SIRT7* gene, were identified. For the 5' promoter region-7 bp, the D allele had a higher frequency than the mutant allele (I), whereas the sheep breeds tested had different dominant allelic frequencies in 3' UTR-17 bp. Moreover, the indel polymorphisms detected did not conform to the HWE in several of the breeds tested (e.g., 5' promoter region-7 bp in STHS and 3' UTR-17 bp in LFTS). Migration, crossbreeding, small sample numbers, and long-term artificial selection are suspected to be the factors contributing to the deviation from the HWE (Li et al., 2018a).

In mammals, polymorphisms of the *SIRT7* gene are involved in human longevity (Donlon et al., 2018), high-altitude adaptation (Y. Li et al., 2014), and Parkinson's disease (Jesús et al., 2013). Because of the key role of *SIRT7* in regulating lipid metabolism (Yoshizawa et al., 2014; Tang et al., 2015), glucose metabolism (Jiang et al., 2017; Ye et al., 2017), and cell growth or differentiation, the relationship between the polymorphisms tested and sheep growth traits were also analyzed. Through the association analysis, all indel loci detected were found to be significantly associated with sheep body size traits, and several associations were highly significant. For instance, in 3' UTR-17 bp, STHS rams with the ID genotype had a greater ChD than individuals with the II genotype.

Previous studies have revealed that the mutation polymorphisms in the UTR could affect the expression of the gene by affecting the functions of elements of DNA or via microRNA-mediated post-transcriptional regulation mechanisms (Krawczak et al., 1992; Komar, 2007; G. Li et al., 2014; Tak and Farnham, 2015). Nevertheless, in this study, the location of 3' UTR-17 bp was approximately 100 bp upstream of the seed sequence of microRNA. Furthermore, the interactions between the target gene and its adjacent genes were also speculated to contribute to this association (Li et al., 2018a, b). *MAF bZIP transcription factor G (MAFG)*, situated in the upstream 3 kb of *SIRT7* was considered to be a transcriptional repressor whose over expression could inhibit the synthesis and metabolism of bile acid (de Aguiar Vallim et al., 2015; Katsuoka and Yamamoto, 2016). Moreover, *Phosphate cytidylyltransferase 2, ethanolamine (PCYT2)*, located downstream 1 kb of *SIRT7*, could regulate muscle cell differentiation (Zhu et al., 2009). The mutation of UTR in the ovine *SIRT7* gene may influence the expression of the adjacent genes, thereby affecting the growth traits of sheep. However, the specific regulatory mechanisms still require further study.

In summary, two novel indel loci within the ovine *SIRT7* gene, 5' promoter region-insertion-7 bp and 3' UTR-insertion-17 bp, were detected in sheep. Moreover, the results of correlation analyses demonstrated that the indel loci were significantly associated with sheep growth traits, which indicated that the novel indel loci could potentially be useful DNA markers for the genetic improvement of economic traits in sheep breeding.

Data availability. Data sets are available upon request from the corresponding authors.

Author contributions. HZ and XZ contributed equally to this work. JW and XL designed the study. RZ, YC, XC, and JY collected the samples. XZ and JL performed the experiments. This paper was written by HX. JW corrected the paper and gave advice. XL edited and reviewed the paper.

Competing interests. The authors declare that they have no conflict of interest.

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References

- Blank, M. F. and Grummt, I.: The seven faces of SIRT7, *Transcription*, 8, 67–74, <https://doi.org/10.1080/21541264.2016.1276658>, 2017.
- Bosch-Presegué, L. and Vaquero, A.: Sirtuins in stress response: guardians of the genome, *Oncogene*, 33, 3764–3775, <https://doi.org/10.1038/onc.2013.344>, 2014.
- Cioffi, M., Vallespinos-Serrano, M., Trabulo, S. M., Fernandez-Marcos, P. J., Firment, A. N., Vazquez, B. N., Vieira, C. R., Mulero, F., Camara, J. A., Cronin, U. P., Perez, M., Soriano, J., G. Galvez, B., Castells-Garcia, A., Haage, V., Raj, D., Megias, D., Hahn, S., Serrano, L., Moon, A., Aicher, A., and Heesch, C.: MiR-93 controls adiposity via inhibition of Sirt7 and Tbx3, *Cell Rep.*, 12, 1594–1605, <https://doi.org/10.1016/j.celrep.2015.08.006>, 2015.
- Cui, Y., Yan, H. L., Wang, K., Xu, H., Zhang, X. L., Zhu, H. J., Liu, J. W., Qu, L., Lan, X. Y., and Pan, C. Y.: Insertion/Deletion within the KDM6A gene is significantly associated with litter size in goat, *Front. Genet.*, 9, 91, <https://doi.org/10.3389/fgene.2018.00091>, 2018.
- de Aguiar Vallim, T. Q., Tarling, E. J., Ahn, H., Hagey, L. R., Romanoski, C. E., Lee, R. G., Graham, M. J., Motohashi, H., Ya-

- mamoto, M., and Edwards, P. A.: MAFG is a transcriptional repressor of bile acid synthesis and metabolism, *Cell Metab.*, 21, 298–311, <https://doi.org/10.1016/j.cmet.2015.01.007>, 2015.
- Donlon, T. A., Morris, B. J., Chen, R., Masaki, K. H., Allsopp, R. C., Willcox, D. C., Tiirikainen, M., and Willcox, B. J.: Analysis of polymorphisms in 59 potential candidate genes for association with human longevity, *J. Gerontol. A. Biol. Sci. Med. Sci.*, 73, 1459–1464, <https://doi.org/10.1093/gerona/glx247>, 2018.
- Gui, L. S., Xin, X. L., Wang, J. L., Hong, J. Y., and Zan, L. S.: Expression analysis, single nucleotide polymorphisms within *SIRT4* and *SIRT7* genes and their association with body size and meat quality traits in Qinchuan cattle, *J. Integr. Agr.*, 15, 2819–2826, [https://doi.org/10.1016/s2095-3119\(16\)61419-3](https://doi.org/10.1016/s2095-3119(16)61419-3), 2016.
- Jesús, S., Gómez-Garre, P., Carrillo, F., Cáceres-Redondo, M. T., Huertas-Fernández, I., Bernal-Bernal, I., Bonilla-Toribio, M., Vargas-González, L., Carballo, M., and Mir, P.: Genetic association of sirtuin genes and Parkinson's disease, *J. Neurol.*, 260, 2237–2241, <https://doi.org/10.1007/s00415-013-6970-7>, 2013.
- Jiang, L., Xiong, J., Zhan, J., Yuan, F., Tang, M., Zhang, C., Cao, Z., Chen, Y., Lu, X., Li, Y., Wang, H., Wang, L., Wang, J., Zhu, W. G., and Wang, H.: Ubiquitin-specific peptidase 7 (USP7)-mediated deubiquitination of the histone deacetylase *SIRT7* regulates gluconeogenesis, *J. Biol. Chem.*, 292, 13296–13311, <https://doi.org/10.1074/jbc.M117.780130>, 2017.
- Katsuoaka, F. and Yamamoto, M.: Small Maf proteins (MafF, MafG, MafK): History, structure and function, *Gene*, 586, 197–205, <https://doi.org/10.1016/j.gene.2016.03.058>, 2016.
- Komar, A. A.: Genetics. SNPs, silent but not invisible, *Science*, 315, 466–467, <https://doi.org/10.1126/science.1138239>, 2007.
- Krawczak, M., Reiss, J., and Cooper, D. N.: The mutational spectrum of single base-pair substitutions in mRNA splice junctions of human genes: causes and consequences, *Hum. Genet.*, 90, 41–54, <https://doi.org/10.1007/BF00210743>, 1992.
- Lan, X. Y., Pan, C. Y., Chen, H., Zhang, C. L., Li, J. Y., Zhao, M., Lei, C. Z., Zhang, A. L., and Zhang, L. Z.: An AluI PCR-RFLP detecting a silent allele at the goat *POU1F1* locus and its association with production traits, *Small Ruminant Res.*, 73, 8–12, <https://doi.org/10.1016/j.smallrumres.2006.10.009>, 2007.
- Li, G., Pan, T., Guo, D., and Li, L. C.: Regulatory variants and disease: the E-cadherin-160C/A SNP as an example, *Mol. Biol. Int.*, 2014, 967565, <https://doi.org/10.1155/2014/967565>, 2014.
- Li, J., Erdenee, S., Zhang, S. L., Wei, Z. Y., Zhang, M., Jin, Y. Y., Wu, H., Chen, H., Sun, X. Z., Xu, H. W., Cai, Y., and Lan, X. Y.: Genetic effects of PRNP gene insertion/deletion (indel) on phenotypic traits in sheep, *Prion*, 12, 42–53, <https://doi.org/10.1080/19336896.2017.1405886>, 2018a.
- Li, J., Zhang, S. L., Erdenee, S., Sun, X. Z., Dang, R. H., Huang, Y. Z., Lei, C. Z., Chen, H., Xu, H., Cai, Y., and Lan, X. Y.: Nucleotide variants in prion-related protein (testis-specific) gene (PRNT) and effects on Chinese and Mongolian sheep phenotypes, *Prion*, 12, 185–196, <https://doi.org/10.1080/19336896.2018.1467193>, 2018b.
- Li, X. L. and Kazgan, N.: Mammalian sirtuins and energy metabolism, *J. Biol. Sci.*, 7, 575–587, <https://doi.org/10.7150/ijbs.7.575>, 2011.
- Li, Y., Wu, D. D., Boyko, A. R., Wang, G. D., Wu, S. F., Irwin, D. M., and Zhang, Y. P.: Population variation revealed high-altitude adaptation of Tibetan mastiffs, *Mol. Biol. Evol.*, 31, 1200–1205, <https://doi.org/10.1093/molbev/msu070>, 2014.
- Li, Z., Zhang, Z., He, Z., Tang, W., Li, T., Zeng, Z., He, L., and Shi, Y.: A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (<http://analysis.bio-x.cn>), *Cell Res.*, 19, 519–523, <https://doi.org/10.1038/cr.2009.33>, 2009.
- Liu, G., Zhao, H., Tan, X., Cheng, H., You, W., Wan, F., Liu, Y., Song, E., and Liu, X.: *SIRT1* gene polymorphisms associated with carcass traits in Luxi cattle, *Arch. Anim. Breed.*, 60, 27–32, <https://doi.org/10.5194/aab-60-27-2017>, 2017.
- Ma, L., Li, Z. Z., Cai, Y., Xu, H. W., Yang, R. L., and Lan, X. Y.: Genetic variants in fat and short tailed sheep from high-throughput RNA-sequencing data, *Anim. Genet.*, 49, 483–487, <https://doi.org/10.1111/age.12699>, 2018a.
- Ma, L., Zhang, M., Jin, Y. Y., Sarantsetseg, E., Hu, Li. Y., Chen, H., Cai, Y., and Lan, X. Y.: Comparative transcriptome profiling of mRNA and lncRNA related to tail adipose tissues of sheep, *Front. Genet.*, 9, 365, <https://doi.org/10.3389/fgene.2018.00365>, 2018b.
- Michishita, E., Park, J. Y., Burneskis, J. M., Barrett, J. C., and Horikawa, I.: Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins, *Mol. Biol. Cell.*, 16, 4623–4635, <https://doi.org/10.1091/mbc.e05-01-0033>, 2005.
- Mu, P., Liu, K., Lin, Q., Yang, W., Liu, D., Lin, Z., Shao, W., and Ji, T.: Sirtuin 7 promotes glioma proliferation and invasion through activation of the ERK/STAT3 signaling pathway, *Oncol. Lett.*, 17, 1445–1452, <https://doi.org/10.3892/ol.2018.9800>, 2019.
- O'Callaghan, C. and Vassilopoulos, A.: Sirtuins at the crossroads of stemness, aging, and cancer, *Aging Cell*, 16, 1208–1218, <https://doi.org/10.1111/accel.12685>, 2017.
- Roth, M. and Chen, W. Y.: Sorting out functions of sirtuins in cancer, *Oncogene*, 33, 1609–1620, <https://doi.org/10.1038/ncr.2013.120>, 2014.
- Shi, Y. Y. and He, L.: SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci, *Cell Res.*, 15, 97–98, <https://doi.org/10.1038/sj.cr.7290272>, 2005.
- Shin, J. Y. and Chen, D.: Molecular, Cellular, and Physiological Characterization of Sirtuin 7 (*SIRT7*), *Methods Mol. Biol.*, 1436, 271–277, https://doi.org/10.1007/978-1-4939-3667-0_18, 2016.
- Tak, Y. G. and Farnham, P. J.: Making sense of GWAS: using epigenomics and genome engineering to understand the functional relevance of SNPs in non-coding regions of the human genome, *Epigenetics Chromatin*, 8, 57, <https://doi.org/10.1186/s13072-015-0050-4>, 2015.
- Tang, B. L.: *SIRT7* and hepatic lipid metabolism, *Front. Cell. Dev. Biol.*, 3, 1–3, <https://doi.org/10.3389/fcell.2015.00001>, 2015.
- Tang, X., Shi, L., Xie, N., Liu, Z., Qian, M., Meng, F., Xu, Q., Zhou, M., Cao, X., Zhu, W. G., and Liu, B.: *SIRT7* antagonizes TGF- β signaling and inhibits breast cancer metastasis, *Nat. Commun.*, 8, 318, <https://doi.org/10.1038/s41467-017-00396-9>, 2017.
- Wang, X. Y., Yang, Q., Wang, K., Zhang, S. H., Pan, C. Y., Chen, H., Qu, L., Yan, H. L., and Lan, X. Y.: A novel 12-bp indel polymorphism within the *GDF9* gene is significantly associated with litter size and growth traits in goats, *Anim. Genet.*, 48, 735–736, <https://doi.org/10.1111/age.12617>, 2017.
- Wang, X. Y., Yang, Q., Wang, K., Yan, H. L., Pan, C. Y., Chen, H., Liu, J. W., Zhu, H. J., Qu, L., and Lan, X. Y.: Two strongly linked single nucleotide polymorphisms

- (Q320P and V397I) in GDF9 gene are associated with litter size in cashmere goats, *Theriogenology*, 125, 115–121, <https://doi.org/10.1016/j.theriogenology.2018.10.013>, 2019.
- Xu, X. C., Li, B. B., Wei, X., Yang, Y. X., Wang, X. L., and Chen, Y. L.: Differential expression of peroxisome proliferator-activated receptor γ , fatty acid synthase, and hormone-sensitive lipase in fat-tailed and thin-tailed sheep breeds, *Genet. Mol. Res.*, 14, 15624–15633, <https://doi.org/10.4238/2015.December.1.14>, 2015.
- Yamagata, K. and Yoshizawa, T. Transcriptional Regulation of Metabolism by SIRT1 and SIRT7, *Int. Rev. Cell Mol. Biol.*, 335, 143–166, <https://doi.org/10.1016/bs.ircmb.2017.07.009>, 2018.
- Yang, Q., Zhang, S. H., Liu, L. L., Cao, X. K., Lei, C. Z., Qi, X. L., Lin, F. P., Qu, W. D., Qi, X. S., Liu, J. M., Wang, R., Chen, H., and Lan, X. Y.: Application of mathematical expectation (ME) strategy for detecting low frequency mutations: an example for evaluating 14 bp insertion/deletion (indel) within the bovine PRNP gene, *Prion*, 10, 409–419, <https://doi.org/10.1080/19336896.2016.1211593>, 2016.
- Yang, Q., Yan, H. L., Li, J., Xu, H., Wang, K., Zhu, H. J., Chen, H., Qu, L., and Lan, X. Y.: A novel 14-bp duplicated deletion within goat GHR gene is significantly associated with growth traits and litter size, *Anim. Genet.*, 48, 499–500, <https://doi.org/10.1111/age.12551>, 2017.
- Ye, X., Li, M. T., Hou, T. Y., Gao, T., Zhu, W. G., and Yang, Y.: Sirtuins in glucose and lipid metabolism, *Oncotarget*, 8, 1845–1859, <https://doi.org/10.18632/oncotarget.12157>, 2017.
- Yoshizawa, T., Karim, M. F., Sato, Y., Senokuchi, T., Miyata, K., Fukuda, T., Go, C., Tasaki, M., Uchimura, K., Kadomatsu, T., Tian, Z., Smolka, C., Sawa, T., Takeya, M., Tomizawa, K., Ando, Y., Araki, E., Akaike, T., Braun, T., Oike, Y., Bober, E., and Yamagata, K.: SIRT7 controls hepatic lipid metabolism by regulating the ubiquitin-proteasome pathway, *Cell Metab.*, 19, 712–721, <https://doi.org/10.1016/j.cmet.2014.03.006>, 2014.
- Zhang, S. H., Sun, K., Bian, Y. N., Zhao, Q., Wang, Z., Ji, C. N., and Li, C. T.: Developmental validation of an X-Insertion/Deletion polymorphism panel and application in HAN population of China, *Sci. Rep.*, 5, 18336, <https://doi.org/10.1038/srep18336>, 2015.
- Zhao, H., He, S., Zhu, Y., Cao, X., Luo, R., Cai, Y., Xu, H., and Sun, X.: A novel 29 bp insertion/deletion (indel) variant of the *LHX3* gene and its influence on growth traits in four sheep breeds of various fecundity, *Arch. Anim. Breed.*, 60, 79–85, <https://doi.org/10.5194/aab-60-79-2017>, 2017.
- Zhu, L., Michel, V., and Bakovic, M.: Regulation of the mouse CTP: Phosphoethanolamine cytidylyltransferase gene *Pcyt2* during myogenesis, *Gene*, 447, 51–59, <https://doi.org/10.1016/j.gene.2009.07.014>, 2009.