Detection of a 4 bp Mutation in the 3′UTR Region of Goat Sox9 Gene and Its Effect on the Growth Traits

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Received: 4 March 2020; Accepted: 8 April 2020; Published: 13 April 2020

Simple Summary: The sex determining region Y (SRY)-type high mobility group (HMG) box 9 (Sox9) gene is critically important in the formation and development of cartilage and is considered the “main regulator” of chondrogenesis. Additionally, a large number of studies have shown that mutations in a single allele of human Sox9 can lead to campomelic dysplasia syndrome. Therefore, the mutations of Sox9 have been the subject of increasing interest among researchers. However, no studies to date have examined the association between Sox9 gene variants and growth traits in goats. Here, we detected a 4 bp indel in the 3′UTR region of Sox9 in Shaanbei white cashmere (SBWC) goats (n = 1109) and studied the association between this indel and growth traits. The 4 bp indel of Sox9 was significantly associated with body length, heart girth, hip width, and all body measurement indexes (p < 0.05) in SBWC goats. Thus, this deletion could be used as an effective molecular marker for maximizing the growth traits of goats in breeding programs.

Abstract: The SRY-type HMG box 9 (Sox9) gene plays an important role in chondrocyte development as well as changes in hypertrophic chondrocytes, indicating that Sox9 can regulate growth in animals. However, no studies to date have examined the correlation between variations in Sox9 and growth traits in goats. Here, we found a 4 bp indel in the 3′UTR of Sox9 and verified its association with growth traits in Shaanbei white cashmere goats (n = 1109). The frequencies of two genotypes (ID and II) were 0.397 and 0.603, respectively, and polymorphic information content (PIC) values showed that the indel had a medium PIC (PIC > 0.25). The 4 bp indel was significantly correlated with body length (p = 0.006), heart girth (p = 0.001), and hip width (p = 4.37 × 10^{-4}). Notably, individuals with the ID genotype had significantly superior phenotypic traits compared with individuals bearing the II genotype. Hence, we speculated that the 4 bp indel is an important mutation affecting growth traits in goat, and may serve as an effective DNA molecular marker for marker-assisted selection in goat breeding programs.

Keywords: growth performance; chondrocyte; Sox9; polymorphism; goat

1. Introduction

The sex determining region Y (SRY)-type high mobility group (HMG) box 9 (Sox9) gene is an important member of the SRY-type HMG box (Sox) gene family. It plays an essential role
in cell differentiation in multiple tissues during embryonic development and in adult [1]. As a transcription factor, Sox9 gene plays a pivotal role in mammalian sex determination during embryonic development [2]. In addition, Sox9 is crucial for regulating reproduction. Previously, we have shown that the expression of Sox9 was significantly associated with pig reproduction traits, and that it plays a critical role in testes development [3].

Meanwhile, Sox9 has also been documented as playing a role in chondrocyte reproduction, differentiation, and the cartilage-specific extracellular matrix, thereby facilitating chondrogenesis [4,5]. The molecular mechanism underlying the regulation of chondrogenesis involves the binding of the HMG box of Sox9 to a specific sequence on the DNA groove [6]. Next, the DNA strand is bent, and the double helix structure is unwound, leading to the transcription of the target gene [7,8]. Additionally, previous research has confirmed protein encoded by Sox9, which is a powerful activator of transcription both in vivo and in vitro, can bind intron 1 of Col2al (II collagen gene) at a special site and directly regulate the expression of this gene, which is specifically expressed in cartilage cells [9–11]. In addition, the differentiation of undifferentiated mesenchymal stem cells (MSCs) and polymerization of mesenchymal cells require the presence of Sox9 in the early stages of chondrogenesis. Chondrocyte and MSCs can develop into the body’s skeleton during the late development of individuals, and Sox9 plays an important role in this process [12]. Therefore, we hypothesize that Sox9 is a candidate gene that could affect growth trait.

The goat industry, which is one of the most ancient and productive livestock industries, plays an important role in the Chinese economy. However, the current state of goat breeding in China, in particular its low efficiency and the imbalance between demand and supply, has become a major problem requiring immediate attention [13–16]. In northern Shaanxi, China, Shaanbei white cashmere (SBWC) goats are a crucial breed used for wool and meat, and are cold-tolerant, highly adaptable, and feedstuff-tolerant. However, SBWC goats still have the problem of poor growth traits in their actual production [17]. Thus, the way in which to improve the production performance of goats needs to be solved urgently [18]. There are many factors that can affect growth traits of goats, among which the genetic factor plays a key regulatory role [19]. The main genetic variations are insertion/deletions (indels), SNPs, and CNVs, among others. Among them, insertion/deletions (indels) are easily identified by simple PCR amplification and agarose gel electrophoresis [20], and extensively exist in eukaryotic genomes. Indels were used in marker-assisted selection (MAS), forming a convenient and efficient method for breeding selection that is not affected by the environment [21]. From the previous studies, we found that indels of a gene are closely related to certain traits [22], including the growth trait of cashmere goats [23], such as Glutaminyl-peptide cyclotransferase-like (QPCTL) [24], Cell division cycle 25A (CDC25A) [25], and Cyclin-dependent kinase inhibitor 3 (CDKN3) [26].

However, few studies have examined the association between Sox9 and growth traits in SBWC goats. Herein, we aimed to identify indels in the Sox9 that could enhance the process of goat-selective breeding. To increase the reliability of this experiment and characterize the impact of the 4 bp indel on the growth traits, 1109 individuals of SBWC goats were analyzed. These findings could identify molecular markers for MAS programs that could be used to improve the growth traits of local breeds in the goat industry.

2. Materials and Methods

All experiments involving animals were approved by the Faculty Animal Policy and Welfare Committee of Northwest A&F University (protocol number NWAFAC1008). Furthermore, the care and use of experimental animals was completely in accord with the local animal welfare laws and policies.  

2.1. DNA Samples and Data Collection

All tested female goats (12 to 18 months of age) were just reaching physical maturity when they were selected randomly ($n = 1109$). All goats in the SBWC Breeding Farm received the same diet and were reared under the same set of standard conditions after weaning in Yulin, Shaanxi, China.
Ear tissue samples were collected for subsequent DNA analyses. Data on SBWC growth-related traits were collected by staff in farms, including body height (BH), height across the hip (HH), body length (BL), heart girth (HG), Cannon circumference (CC), chest depth (CD), chest width (CW), and hip width (HW) [27,28].

2.2. DNA Isolation and DNA Pool Construction

Genomic DNA was extracted from the goat ear tissues using a high-salt extraction protocol [29,30]. The extracted DNA samples were quantified by Nanodrop 1000 (Thermo Fisher Scientific Inc., Wilmington, DE, USA), and then diluted to 20 ng/µL and stored at −20 °C [19,23]. Next, a DNA pool of 50 DNA samples was constructed. The 50 DNA samples were selected randomly from the experimental samples to explore the variation in the goat Sox9 gene. Then, according to the polymorphism of this result, we could judge whether to continue to expand the sample at this site for analysis.

2.3. Primer Design and Genotyping

On the basis of the Ensembl indel database (http://www.ensembl.org/index.html), four potential indel sites were detected in the goat Sox9 gene, which were used to design four primers with the software Primer Premier 5 to test the indel of Sox9 (Table 1). Subsequently, PCR-based agarose gel electrophoresis amplification of fragment length polymorphism was used to score the genotype of the indel. Indel identification and genotyping were performed by using touch-down PCR in a 13 µL reaction mixture containing 50 ng of genomic DNA [28]. Each PCR product was electrophoresed on a 3.5% agarose gel stained with ethidium bromide to identify the indel locus for subsequent sequencing [19,31].

| Table 1. Primers for PCR amplification of SRY-type HMG box 9 (Sox9) gene. |
|---------------------------------------------------------------|
| **Primers Name** | **Sequences (5’-3’)** | **Sizes (bp)** | **Function** | **Location** | **Note** |
| P1 | F: GCTATTTCTTGTGGGCGCCCTGTG | 112/108 | Indel detection | 3’UTR | Original design |
| R: TCTCGGAGGCAACTAAGCTCGTCG | | | | |
| P2 | F: AGTGCCCTTTTCCTCTCTCA | 166/162 | Indel detection | 3’UTR | Original design |
| R: TGACCTCCTCCTCACTCATCCTT | | | | |
| P3 | F: CCTTTTCTCTCATTACACCGAT | 172/168 | Indel detection | 3’UTR | Original design |
| R: CCCTTTTCCTGTCGATACCAAATA | | | | |
| P4 | F: TCTTTGGGTCTGGGTATTC | 242/238 | Indel detection | 3’UTR | Original design |
| R: AAGGCCCAAAAGACTGCTTAACG | | | | |

F is the upstream primer, and R is the downstream primer.

2.4. Statistical Analysis

Genotypic frequencies, allelic frequencies, and Hardy–Weinberg equilibrium (HWE) of the indel locus in Sox9 were calculated using the SHEsis program (http://analysis.bio-x.cn/myAnalysis.php) [32]. Population indexes (heterozygosity, He; homozygosity, Ho; polymorphic information content, PIC) were calculated online using Nei’s methods (http://www.msrcall.com/Gdicall.aspx) [33]. The χ² test was conducted to evaluate HWE using the SHEsis online platform (http://analysis.bio-x.cn/myAnalysis.php). According to the published linear model [23,31], the association between the indel locus and growth traits in SBWC goats was analyzed using a t-test in SPSS software (version 24.0) (International Business Machines Corporation, New York, NY, USA).

3. Results

3.1. Identification of a 3’UTR 4 bp Indel within Sox9

PCR was performed with the four synthesized primers using a mixed DNA pool of samples from SBWC goats (50 samples) to verify the indels in Sox9 (Table 1). Ultimately, a novel 4 bp indel of the Sox9 3’UTR region (NC_030826.1: g.58090106-58090109delTCGC; rs649476917) was detected in SBWC goats using primer 2. Agarose gel electrophoresis of the PCR products and sequence diagrams of the
novel indel showed that the 4 bp indel was polymorphic and had two genotypes (Figures 1 and 2). Specifically, genotype II (homozygote) showed one band (166 bp), and genotype ID (heterozygote) exhibited two bands (166 and 162 bp), whereas the homozygous genotype DD (Deletion/Deletion) was not detected (Figures 1 and 2). Meanwhile, the indel sequence that we detected was consistent with the sequence registered at the available Ensembl indel database.

![Agarose electrophoresis of goat Sox9-4 bp insertion/deletion (indel).](image1)

**Figure 1.** Agarose electrophoresis of goat Sox9-4 bp insertion/deletion (indel).

![The sequencing chromas for the 4 bp indel in the goat Sox9 gene.](image2)

**Figure 2.** The sequencing chromas for the 4 bp indel in the goat Sox9 gene. Sequencing chromas showed homozygotic insertion type (II) and heterozygote type (ID).

3.2. Analysis of Genotype and Allele Frequencies

The genotype and allele frequencies of this polymorphism in SBWC goats \((n = 1109)\) were calculated (Table 2). The frequencies of genotypes ID and II were 0.397 and 0.603, respectively. Subsequently, the population indices (Ho, He, Ne, and PIC) were calculated in the current locus on the basis of the frequency numbers of the different genotypes (Table 2). It showed that the numerical value of Ho was more than 0.500, and the PIC values were greater than 0.25, indicating that the indel had medium PIC. Additionally, the genotype distribution was not in HWE \((p < 0.05)\) in totality.

3.3. Association Analysis between Indel Genotypes and Growth Traits in SBWC Goats

The body traits that we measured are a direct reflection of skeleton structure and are also related to the physiological function and the production performance of goats [15]. The associations between the 3′UTR 4 bp indel and growth-related traits were listed in the Table 3. We found that the 4 bp indel was extremely significantly related to body length \((p = 0.006)\), heart girth \((p = 0.001)\), and hip width \((p = 4.37 \times 10^{-4})\). These growth traits can thus be used to improve the production of livestock. Additionally, individuals with the ID genotype had superior values of all phenotypes relative to II individuals for all of the significantly associated characteristics. Surprisingly, all of the body
measurement indexes were significantly correlated ($p < 0.05$); for example, the correlation between body length and chest circumference index was extremely significant ($p < 0.01$). Thus, this method can be used to analyze the body measurement of animals, which determines whether each body part is fully developed and whether body part is symmetrical and conforms to the characteristics of a certain production type and variety.

4. Discussion

In this paper, we detected a genetic polymorphism in a 4 bp indel located in the 3'UTR region among the four possible variants with the following two genotypes: II (homozygote insertion type) and ID (heterozygote type). Mutations in the Sox9 can lead to campomelic dysplasia syndrome, a rare and lethal congenital skeletal dysplasia syndrome characterized by an endochondral osteogenesis disorder [1,34,35]. In addition, we hypothesized that this site is linked to other unknown genes; thus, this site might have been subjected to genetic drift or disease because of the linkage imbalance. This hypothesis would explain the apparent absence of the DD genotype. To further explore the correlation between the 4 bp indel and growth traits, we analyzed the association in SBWC goats ($n = 1109$). We found that the genotype and allele frequencies at this locus were not in HWE, which may be explained by migration, genetic drift, and artificial selection [36,37]. In addition, on the basis of the PIC value ($0.25 < p < 0.50$), this locus was in a medium polymorphism, indicating that it has a high potential value for selection in goat breeds [38,39]. The 4 bp indel was shown to be strongly correlated with growth traits (e.g., BL, HG, and HW) in SBWC goats. Therefore, we expected that this 4 bp indel could be used as a molecular marker to improve the reproduction and production performance in goats via MAS breeding.

### Table 2. Genetic parameters of the indel within Sox9 in Shaanbei white cashmere goat.

| Observed Genotypes (N = 1109) | Frequencies | Genotypes | Alleles | Ho | He | PIC | $\chi^2$ (p-Value) |
|-------------------------------|-------------|-----------|---------|----|----|-----|------------------|
| DD (0)                        | 0           | 0.198(D)  | 0.682   | 0.318 | 0.268 | 67.916 (p = 0.0001) |
| ID (440)                      | 0.397       | 0.802(I)  | 0.603   | 0.279 | 0.312 |                 |                  |
| II (669)                      |             |           |         | 0.279 | 0.312 |                 |                  |

Note: Ho, homozygosity; He, heterozygosity; PIC, polymorphism information content.

### Table 3. Associations of the indel with growth traits in SBWC goats.

| Traits | Genotypes (bp) | ID | II | $p$-Values |
|--------|----------------|----|----|------------|
| BH (cm)       | 54.78 ± 0.18 (n = 439) | 55.01 ± 0.14 (n = 668) | 0.329 |
| BL (cm)       | 66.84 ± 0.25 (n = 439) | 65.92 ± 0.22 (n = 668) | 0.006 |
| HH (cm)       | 57.77 ± 0.18 (n = 439) | 57.87 ± 0.15 (n = 667) | 0.708 |
| CW (cm)       | 19.13 ± 0.14 (n = 439) | 19.12 ± 0.11 (n = 668) | 0.974 |
| CD (cm)       | 27.67 ± 0.14 (n = 439) | 27.20 ± 0.11 (n = 668) | 0.010 |
| HG (cm)       | 83.15 ± 0.48 (n = 439) | 81.13 ± 0.41 (n = 668) | 0.001 |
| CC (cm)       | 8.05 ± 0.03 (n = 440) | 7.95 ± 0.03 (n = 668) | 0.041 |
| HW (cm)       | 16.71 ± 0.16 (n = 440) | 15.92 ± 0.14 (n = 668) | 4.37 × 10$^{-4}$ |
| BTI          | 124.33 ± 0.50 (n = 439) | 122.80 ± 0.50 (n = 668) | 0.026 |
| BLI          | 122.40 ± 0.52 (n = 439) | 120.10 ± 0.40 (n = 668) | 3.83 × 10$^{-4}$ |
| CCI          | 152.19 ± 0.90 (n = 439) | 147.48 ± 0.73 (n = 668) | 5.2 × 10$^{-5}$ |
| TCI          | 14.75 ± 0.08 (n = 439) | 14.46 ± 0.06 (n = 668) | 0.004 |
| CWWI         | 69.28 ± 0.45 (n = 439) | 70.49 ± 0.34 (n = 668) | 0.029 |
| HWII         | 119.86 ± 1.53 (n = 439) | 125.56 ± 1.16 (n = 668) | 0.003 |

Note: BH: body height, BL: body length, HH: height at hip cross, CW: chest width, CD: chest depth, HG: heart girth, CC: cannon circumference, HW: hip width, BTI: body trunk index, BLI: body length index, CCI: chest circumference index, TCI: tube confining index, CWE: chest width index, HWI: hip width index. The mean values with different superscripts (a, b, c, d) within the same row differ significantly at the $p < 0.05$ level; (b, c) within the same row extremely different significantly at the $p < 0.01$ level. II = insertion genotype; ID = heterozygote genotype.
Sox9 plays a critical role in chondrogenesis, bone formation, and the treatment for cartilage diseases [40]. This gene comprises an HMG-box (high mobility group-box) region that can specifically combine with the minor groove of DNA and ultimately modulate the expression of the relevant genes. For example, Sox9 can initiate chondrogenic differentiation by regulating the expression of SRY-type HMG box5 (Sox5) and SRY-type HMG box6 (Sox6) [41,42]. Additionally, it is a key chondrogenic transcription factor, and the entire network of regulatory mechanisms depends on and affects Sox9 expression and activity [43].

There are several possible mechanisms by which Sox9 might inhibit chondrocyte hypertrophy. Studies have shown that Sox9, as a major transcriptional activator, maintains the chondrocyte through a phosphoinositide 3-kinase (PI3K) -AKT pathway [44,45], as well as preventing chondrocytes from becoming hypertrophic [12]. In addition, Sox9 could block Wnt signaling through inducing the degradation of β-catenin to inhibit chondrogenic hypertrophy [46,47]. Moreover, Sox9 also blocks the activity of Runt-related transcription factor 2 (Runx2) [48], which plays a crucial role in inducing chondrocyte maturation.

Several previous studies have shown that 3′UTR variants can affect gene transcription [49,50], and structural changes in the 3′UTR region are closely related to livestock and poultry production performance [51–53]. The 3′UTR is generally thought to regulate gene expression by affecting the stability of mRNA. Changes in mRNA abundance are a key means by which post-transcriptional gene expression is regulated. It can cause changes in protein levels of related genes in the upstream and downstream, thereby affecting the expression of gene. Thus, the 4 bp indel of the 3′UTR region may play an important role in regulating the Sox9 gene. Although the exact mechanism was not clear, association analysis of large samples (n = 1109) showed that the 4 bp indel of Sox9 is closely related to the growth traits of goats. Hence, we speculated that the 4 bp indel might affect the expression process of Sox9, thereby resulting in the change in growth traits.

Sox9 is not only involved in the expression of genes in specific cartilage tissue, but also in the regulation of the expression of mesenchymal stem cells. Moreover, it plays an important role in regulating the hypertrophy and ossification of chondrocytes. Ultimately, we speculate that Sox9 may mediate changes in growth traits in goats by affecting the growth of bone. Further study is required to determine whether this 4 bp indel would affect the expression of Sox9 gene in different tissues and influences the binding of miRNA.

5. Conclusions

Briefly, the 4 bp indel of the Sox9 gene in the 3′UTR region was proven to be strongly associated with growth traits (body length and heart girth), suggesting that this indel might be a potential DNA marker in goat MAS breeding in connection with production performance. These results provide a scientific basis for the development of growth traits and genetic resources in goat.

Author Contributions: Conceptualization, L.H., Y.B., R.W.; methodology, L.H., Y.B.; software, L.H.; validation, C.P., H.C.; formal analysis, L.H., Y.B.; investigation, X.L.; resources, L.Q., X.L., C.P.; data curation, X.L.; writing—original draft preparation, L.H.; writing—review and editing, L.H., Y.B., X.L.; visualization, C.P.; supervision, L.H., Y.B., L.Q., X.L.; project administration, C.P., H.C., L.Q., X.L.; funding acquisition, H.C., L.Q., C.P., X.L. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the National Scientific and Technological Innovation Project of Undergraduate of Northwest A&F University (201910712070), the National Natural Science Foundation of China (31760650), and Provincial Key Projects of Shaanxi (2014KTDZ02-01).

Acknowledgments: We greatly thank the staff of Shaanbei white cashmere (SBWC) goat breeding farm, Shaanxi province, China, for their collecting samples. We are also very grateful to the Life Science Research Core Services (LSRCS), Northwest A&F University, for the equipment.

Conflicts of Interest: We confirm that this manuscript has not been published in whole or in part and is not being considered for publication elsewhere. There are no ethical conflicts of interest for all authors. The corresponding authors Dr. X.Y. Lan and Dr. Lei Qu take responsibility on behalf of all authors for the authorship, authenticity, and integrity of this manuscript, and affirm that all authors and acknowledged contributors have read and approved this manuscript.
References

1. Jo, A.; Denduluri, S.; Zhang, B.; Wang, Z.; Yin, L.; Yan, Z.; Kang, R.; Shi, L.L.; Mok, J.; Lee, M.J.; et al. The versatile functions of Sox9 in development, stem cells, and human diseases. Genes Dis. 2014, 1, 149–161. [CrossRef] [PubMed]

2. Croft, B.; Ohnesorg, T.; Hewitt, J.; Bowles, J.; Quinn, A.; Tan, J.; Corbin, V.; Pelosi, E.; van den Bergen, J.; Sreenivasan, R.; et al. Human sex reversal is caused by duplication or deletion of core enhancers upstream of SOX9. Nat. Commun. 2018, 9, 5319. [CrossRef] [PubMed]

3. Chen, M.; Wang, J.; Liu, N.; Cui, W.; Dong, W.; Xing, B.; Pan, C. Pig SOX9: Expression profiles of Sertoli cell (SCs) and a functional 18 bp indel affecting testis weight. Theriogenology 2019, 138, 94–101. [CrossRef] [PubMed]

4. Akiyama, H.; Lefebvre, V. Unraveling the transcriptional regulatory machinery in chondrogenesis. J. Bone Miner. Metab. 2011, 29, 390–395. [CrossRef]

5. Henry, S.P.; Liang, S.; Akdemir, K.C.; de Crombrugghe, B. The postnatal role of Sox9 in cartilage. J. Bone Miner. Res. 2012, 27, 2511–2525. [CrossRef]

6. Mertin, S.; Mcdowall, S.G.; Harley, V.R. The DNA-binding specificity of SOX9 and other SOX proteins. Nucleic Acids Res. 1999, 27, 1359–1364. [CrossRef]

7. Lefebvre, V.; Huang, W.; Harley, V.R.; Goodfellow, P.N.; de Crombrugghe, B. SOX9 is a potent activator of the chondrocyte-specific enhancer of the pro alpha1(II) collagen gene. Mol. Cell. Biol. 1997, 17, 2336–2346. [CrossRef]

8. Ng, L.J.; Wheatley, S.; Muscat, G.E.; Conway-Campbell, J.; Bowles, J.; Wright, E.; Bell, D.M.; Tam, P.P.; Cheah, K.S.; Koopman, P. SOX9 binds DNA, activates transcription, and coexpresses with type II collagen during chondrogenesis in the mouse. Dev. Biol. 1997, 183, 108–121. [CrossRef] [PubMed]

9. Zhou, G.; Lefebvre, V.; Zhang, Z.; Eberspaecher, H.; de Crombrugghe, B. Three high mobility group-like sequences within a 48-base pair enhancer of the Col2a1 gene are required for cartilage-specific expression in vivo. J. Biol. Chem. 1998, 273, 14989–14997. [CrossRef]

10. Akiyama, H.; Kamitani, T.; Yang, X.; Kandyil, R.; Bridgewater, L.C.; Fellous, M.; Mori-Akiyama, Y.; de Crombrugghe, B. The transcription factor Sox9 is degraded by the ubiquitin-proteasome system and stabilized by a mutation in a ubiquitin-target site. Matrix Biol. 2005, 23, 499–505. [CrossRef]

11. Shi, S.; Wang, C.; Acton, A.J.; Eckert, G.J.; Trippel, S.B. Role of sox9 in growth factor regulation of articular chondrocytes. J. Cell. Biochem. 2015, 116, 1391–1400. [CrossRef] [PubMed]

12. Lui, J.C.; Yue, S.; Lee, A.; Kikani, B.; Temnycky, A.; Barnes, K.M.; Baron, J. Persistent Sox9 expression in hypertrophic chondrocytes suppresses transdifferentiation into osteoblasts. Bone 2019, 125, 169–177. [CrossRef] [PubMed]

13. Wang, K.; Yan, H.; Xu, H.; Yang, Q.; Zhang, S.; Pan, C.; Chen, H.; Zhu, H.; Liu, J.; Qu, L.; et al. A novel indel within goat casein alpha S1 gene is significantly associated with litter size. Gene 2018, 671, 161–169. [CrossRef]

14. Yang, Q.; Chang, S.; Li, J.; Wang, X.; Peng, K.; Lan, X.; Pan, C. Development of a touch-down multiplex PCR method for simultaneously rapidly detecting three novel insertion/deletions (indels) within one gene: An example for goat GHR gene. Anim. Biotechnol. 2019, 30, 366–371. [CrossRef]
19. Wang, X.; Yang, Q.; Wang, K.; Yan, H.; Pan, C.; Chen, H.; Liu, J.; Zhu, H.; Qu, L.; Lan, X. Two strongly linked single nucleotide polymorphisms (Q320P and V397T) in GDF9 gene are associated with litter size in cashmere goats. *Theriogenology* 2019, 125, 115–121. [CrossRef]

20. Naicy, T.; Venkatachalapathy, R.T.; Aravindakshan, T.V.; Radhika, G.; Raaghavan, K.C.; Mini, M.; Shyama, K. Nerve Growth Factor gene ovarian expression, polymorphism identification, and association with litter size in goats. *Theriogenology* 2016, 86, 2172–2178. [CrossRef] [PubMed]

21. Gregersen, V.R.; Sorensen, K.K.; Christensen, O.F.; Busch, M.E.; Vingborg, R.K.; Velander, I.H.; Lund, M.S.; Bendixen, C. Identification of QTL for dorso-caudal chronic pleuritis in 12 crossbreed porcine families. *Anim. Genet.* 2010, 41, 509–514. [CrossRef] [PubMed]

22. Liang, K.; Wang, X.; Tian, X.; Geng, R.; Li, W.; Jing, Z.; Han, R.; Tian, Y.; Liu, X.; Kang, X.; et al. Molecular characterization and an 80-bp indel polymorphism within the prolactin receptor (PRLR) gene and its associations with chicken growth and carcass traits. *3 Biotech* 2019, 9, 296. [CrossRef] [PubMed]

23. Zhang, S.; Jiang, E.; Wang, K.; Zhang, Y.; Yan, H.; Qu, L.; Chen, H.; Lan, X.; Pan, C. Two Insertion/Deletion Variants within SPAG17 Gene Are Associated with Goat Body Measurement Traits. *Animals 2019*, 9, 379. [CrossRef] [PubMed]

24. Ren, T.; Li, W.; Liu, D.; Liang, K.; Wang, X.; Li, H.; Jiang, R.; Tian, Y.; Kang, X.; Li, Z. Two insertion/deletion variants in the promoter region of the QPCTL gene are significantly associated with body weight and carcass traits in chickens. *Anim. Genet.* 2019, 50, 279–282. [CrossRef]

25. Cui, W.; Liu, N.; Zhang, X.; Zhang, Y.; Qu, L.; Yan, H.; Lan, X.; Dong, W.; Pan, C. A 20-bp insertion/deletion (indel) polymorphism within the CDC25A gene and its associations with growth traits in goat. *Arch. Anim. Breed.* 2019, 62, 353–360. [CrossRef]

26. Li, W.; Liu, D.; Tang, S.; Li, D.; Han, R.; Tian, Y.; Li, H.; Li, G.; Li, W.; Liu, X.; et al. A multiallelic indel in the promoter region of the Cyclin-dependent kinase inhibitor 3 gene is significantly associated with body weight and carcass traits in chickens. *Poult. Sci.* 2019, 98, 556–565. [CrossRef]

27. Wang, X.; Yang, Q.; Wang, K.; Zhang, S.; Pan, C.; Chen, H.; Qu, L.; Yan, H.; Lan, X. A novel 12-bp indel polymorphism within the GDF9 gene is significantly associated with litter size and growth traits in goats. *Anim. Genet.* 2017, 48, 735–736. [CrossRef]

28. Kang, Z.; Zhang, S.; He, L.; Zhu, H.; Wang, Z.; Yan, H.; Huang, Y.; Dang, R.; Lei, C.; Chen, H.; et al. A 14-bp functional deletion within the CMTM2 gene is significantly associated with litter size in goat. *Theriogenology* 2019, 139, 49–57. [CrossRef]

29. Lan, X.Y.; Penagaricano, F.; Dejung, L.; Weigel, K.A.; Khatib, H. Short communication: A missense mutation in the PROPI (prophet of Pit 1) gene affects male fertility and milk production traits in the US Holstein population. *J. Dairy Sci.* 2003, 96, 1255–1257. [CrossRef]

30. Wang, K.; Kang, Z.; Jiang, E.; Yan, H.; Zhu, H.; Liu, J.; Qu, L.; Lan, X.; Pan, C. Genetic effects of DSCAML1 identified in genome-wide association study revealing strong associations with litter size and semen quality in goat (Capra hircus). *Theriogenology* 2020, 146, 20–25. [CrossRef]

31. Wang, Z.; Zhang, X.; Jiang, E.; Yan, H.; Zhu, H.; Chen, H.; Liu, J.; Qu, L.; Pan, C.; Lan, X. InDelS within caprine IGF2BP1 intron 2 and the 3′-untranslated regions are associated with goat growth traits. *Anim. Genet.* 2020, 51, 117–121. [CrossRef] [PubMed]

32. Lachance, C.; Leclerc, P. Mediators of the Jak/STAT signaling pathway in human spermatozoa. *Biol. Reprod.* 2011, 85, 1222–1231. [CrossRef] [PubMed]

33. Menchaca, A.; Pinczak, A.; Rubianes, E. Follicular recruitment and ovulatory response to FSH treatment initiated on day 0 or day 3 postovulation in goats. *Theriogenology* 2002, 58, 1713–1721. [CrossRef]

34. Wagner, T.; Wirth, J.; Meyer, J.; Zabel, B.; Held, M.; Zimmer, J.; Pasantes, J.; Bricarelli, F.D.; Keutel, J.; Hustert, E.; et al. Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene SOX9. *Cell* 1994, 79, 1111–1120. [CrossRef]

35. Von Bohlen, A.E.; Bohm, J.; Pop, R.; Johnson, D.S.; Tolmie, J.; Stucker, R.; Morris-Rosendahl, D.; Scherer, G. A mutation creating an upstream initiation codon in the SOX9 5′ UTR causes acampomelic campomelic dysplasia. *Mol. Genet. Genom. Med.* 2017, 5, 261–268. [CrossRef]

36. Zhang, X.; Wu, X.; Jia, W.; Pan, C.; Li, X.; Lei, C.; Chen, H.; Lan, X. Novel Nucleotide Variations, Haplotypes Structure and Associations with Growth Related Traits of Goat AT Motif-Binding Factor (ATBF1) Gene. *Asian Australas. J. Anim. Sci.* 2015, 28, 1394–1406. [CrossRef]
37. Liu, S.; He, S.; Chen, L.; Li, W.; Di, J.; Liu, M. Estimates of linkage disequilibrium and effective population sizes in Chinese Merino (Xinjiang type) sheep by genome-wide SNPs. *Genes Genom.* 2017, 39, 733–745. [CrossRef]

38. Chen, M.; Yan, H.; Wang, K.; Cui, Y.; Chen, R.; Liu, J.; Zhu, H.; Qu, L.; Pan, C. Goat SPEF2: Expression profile, indel variants identification and association analysis with litter size. *Theriogenology* 2019, 139, 147–155. [CrossRef]

39. Yang, W.; Yan, H.; Wang, K.; Cui, Y.; Zhou, T.; Xu, H.; Zhu, H.; Liu, J.; Lan, X.; Qu, L.; et al. Goat PDGFRB: Unique mRNA expression profile in gonad and significant association between genetic variation and litter size. *R. Soc. Open Sci.* 2019, 6, 180805. [CrossRef]

40. Lefebvre, V.; Dvir-Ginzberg, M. SOX9 and the many facets of its regulation in the chondrocyte lineage. *Connect. Tissue Res.* 2017, 58, 2–14. [CrossRef]

41. Akiyama, H.; Chaboissier, M.C.; Martin, J.F.; Schedl, A.; de Crombrugghe, B. The transcription factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of Sox5 and Sox6. *Genes Dev.* 2002, 16, 2813–2828. [CrossRef] [PubMed]

42. Im, G.I.; Kim, H.J.; Lee, J.H. Chondrogenesis of adipose stem cells in a porous PLGA scaffold impregnated with plasmid DNA containing SOX trio (SOX-5, -6 and -9) genes. *Biomaterials* 2011, 32, 4385–4392. [CrossRef] [PubMed]

43. Kozhemyakina, E.; Lassar, A.B.; Zelzer, E. A pathway to bone: Signaling molecules and transcription factors involved in chondrocyte development and maturation. *Development* 2015, 142, 817–831. [CrossRef] [PubMed]

44. Dy, P.; Wang, W.; Bhattaram, P.; Wang, Q.; Wang, L.; Ballock, R.T.; Lefebvre, V. Sox9 directs hypertrophic maturation and blocks osteoblast differentiation of growth plate chondrocytes. *Dev. Cell.* 2012, 22, 597–609. [CrossRef] [PubMed]

45. Hartmann, C.; Tabin, C.J. Dual roles of Wnt signaling during chondrogenesis in the chicken limb. *Development* 2000, 127, 3141–3159.

46. Topol, L.; Chen, W.; Song, H.; Day, T.F.; Yang, Y. Sox9 inhibits Wnt signaling by promoting beta-catenin phosphorylation in the nucleus. *J. Biol. Chem.* 2009, 284, 3323–3333. [CrossRef]

47. Zhou, G.; Zheng, Q.; Engin, F.; Munivez, E.; Chen, Y.; Sebald, E.; Krakow, D.; Lee, B. Dominance of SOX9 function over RUNX2 during skeletogenesis. *Proc. Natl. Acad. Sci. USA* 2006, 103, 19004–19009. [CrossRef] [PubMed]

48. Ozmen, O.; Kul, S.; Unal, E.O. Polymorphism of sheep POU1F1 gene exon 6 and 3′UTR region and their association with milk production traits. *Iran J. Vet. Res.* 2014, 15, 331–335. [PubMed]

49. Sandberg, R.; Neilson, J.R.; Sarma, A.; Sharp, P.A.; Burge, C.B. Proliferating cells express mRNAs with shortened 3′untranslated regions and fewer microRNA target sites. *Science* 2008, 320, 1643–1647. [CrossRef]

50. Hou, J.; An, X.; Song, Y.; Gao, T.; Lei, Y.; Cao, B. Two Mutations in the Caprine MTHFR 3′UTR Regulated by MicroRNAs Are Associated with Milk Production Traits. *PLoS ONE* 2015, 10, e0133015. [CrossRef] [PubMed]

51. Miltiadou, D.; Hager-Theodorides, A.L.; Symeou, S.; Constantinou, C.; Psifidi, A.; Banos, G.; Tzamaloukas, O. Variants in the 3′ untranslated region of the ovine acetyl-coenzyme A acyltransferase 2 gene are associated with dairy traits and exhibit differential allelic expression. *J. Dairy Sci.* 2017, 100, 6285–6297. [CrossRef] [PubMed]