Journal of Animal & Veterinary Advances
MEDWELL PUBLICATIONS

Correlation Analysis Between Polymorphism of *CMTM2* Promoter and Growth Measurements of Shaanbei White Cashmere Goats

[1]Jing Xiaoqi, [1]QU Cheng, [1]XU Zhengyu, [1, 3]Song Xiaoyue, [1]SHI Lei, [4]LIU Jiaorong, [1]Dong Shuwei, [4]LIU Jinwang, [1]QU Lei and [1, 2, 3]ZHU Haijing

[1]*Shaanxi Province Shaanbei Cashmere Goats Engneering and Technology Research Center, Yulin, Shaanxi, 719000 PR China*

[2]*Shaanxi Haoli Cashmere Goat Technology Development Co., Ltd, Yulin, Shaanxi, 719000 P.R. China*

[3]*Shaanxi Province "Four Subjects One Union" Sheep and Goat Engineering and Technology University and Enterprise Alliance Research Center, Yulin, Shaanxi, 719318 P.R. China*

[4]*Animal Husbandry Station of Jia County Tong Town, Yulin, Shaanxi, 719200 P.R. China*

Key words: *CMTM2* gene, cashmere goat, InDel, growth traits, PCR

Corresponding Author:
QU Lei
Shaanxi Province Shaanbei Cashmere Goats Engneering and Technology Research Center, Yulin, Shaanxi, 719000 PR China

Page No.: 134-141
Volume: 20, Issue 5, 2021
ISSN: 1680-5593
Journal of Animal and Veterinary Advances

Abstract: To determine the correlation between the 14-bp insertion/deletion (InDel) polymorphism in the promoter of *CMTM2* gene and the growth traits of Shaanbei white cashmere goats. Shaanbei white cashmere nannies population (n = 1105 including 621 bred goats and 484 adult goats) were chosen. The body weight and body measurements of the goats were measured and ear tissues were collected. After the genomic DNA were extracted, PCR amplification were conducted, agarose gel electrophoresis and DNA sequencing were carried out to detect the InDel mutation in the promoter of the *CMTM2* gene. The correlation between the InDel characteristics and growth traits of cashmere goats was analyzed using SPSS 23.0 Software. In Shaanbei white cashmere goats' population, three genotypes of II, ID and DD were detected at the mutation site of *CMTM2* promoter. The frequency of allele "I" (0.863) is higher than that of allele "D" (0.137), this InDel mutation conformed to Hardy Weinberg equilibrium ($p>0.05$) and showed low polymorphism (PIC<0.25). The 14-bp InDel site in the promoter region was extremely significantly correlated with the body height, hip width and height at hip cross in adult goats ($p<0.01$); further association analysis results showed that this site was significantly related with body height and the height at hip cross ($p<0.05$) and extremely significantly related to the hip width ($p<0.01$) in Shaanbei white cashmere goat population. The 14-bp InDel in the promoter of *CMTM2* gene is significantly related to the body height, hip width and height at hip cross of Shaanbei

white cashmere goats. This site can be used for Marker Assisted Selection (MAS) in Shaanbei white cashmere goats. Also, it provided a theoretical and practical basis for the breeding of Shaanbei white cashmere goats.

INTRODUCTION

Shaanbei white cashmere goat is one kind of artificially cultivated breed that produces cashmere and meat. It owns the characteristics of strong stress resistance, rough feeding and great cold adaptability[1, 2]. As one of the important domestic cashmere goat breeds, Shaanbei white cashmere goat has gradually became a leading industry for rural economic development in Shaanbei due to its excellent characteristics related to the livelihood and economic income of millions people. However, some goats were weak and grew slowly for the unique geographical conditions of Shaanbei[3, 4] which may be caused by the differences of genetic and feeding level among individuals. In particular, the genetic factors may be dominant[5-7]. Therefore, in the breeding process of Shaanbei white cashmere goats, screening candidate genes that affect the growth traits and establish an efficient and rapid selecting method means great. Molecular Aiding Selection (MAS) is one kind of simple, fast, accurate, low-cost and automated method (including DNA extraction, molecular marker detection and data analysis) that can be used to screen the most conductive traits of growth and development associated with Shaanbei white cashmere goats[8]. As the Insertion/Deletion (InDel) molecular labeling technology has been widely reported in recent years, researches on goats seems to be extensive and worthy of further exploration[9].

CMTM gene family (chemokine-like factor superfamily) is a gene family first reported in 2003[10]. It was found that the *CMTM* gene family may regulate *PDL1*, *EGFR*, *VE-Cadheirn* and other membrane molecule stability and transport gene's expression[11]. The proteins of the gene family play important roles in activating and chemotaxis of immune cells to tumor cell proliferation and invasion, regulating cell growth and development[10, 12, 13]. *CMTM2* acted as a member of the *CMTM* gene family, mainly expressed in testis and bone marrow tissues and a small amount expressed in peripheral blood leukocytes and pancreas[10, 14]. In testis, *CMTM2* is specifically expressed in germ cells during meiosis and anaphase and located in the endoplasmic reticulum near the Golgi apparatus[15, 16] and associated with spermatogenesis[17-20]. In bone marrow tissue, *CMTM2* promoted the binding of androgen and its receptor, acted as a co-activator to regulate androgen expression[15]. Androgen Receptor (AR) promoted the differentiation of bone marrow mesenchymal stem cells[21, 22] which means that *CMTM2* has an important regulatory effect on bone cell proliferation and differentiation. Reports also showed that there existed a 14-bp deletion in the promoter of *CMTM2* and it significantly affected the lambing traits of Shaanbei white cashmere goats[6], body length and tube circumference[7]. These studies indicated that *CMTM2* may affect the growth and development of the Shaanbei white cashmere goat. However, whether other growth traits were affected by the InDel existed in the promoter of *CMTM2* has not been reported.

In this study, 1105 Shaanbei white cashmere nannies were selected to explore the relationship between the 14-bp InDel in the promoter of *CMTM2* and body weight, body measurements of Shaanbei white cashmere goats. Analyzing the growth and development laws of Shaanbei white cashmere goats and making proper use of them will provide a theoretical and scientific basis for the selection and breeding of cashmere goat.

MATERIALS AND METHODS

Sample collection and body scale data collection: The body size data of 1105 Shaanbei white cashmere nannies in a breeding company in Zizhou County, Shaanxi province were collected including 621 bred nannies and 484 adult nannies which were of similar age at the same stage. Body scale data included body weight (kg), body height (cm), body length (cm), chest circumference (cm), hip width (cm), tube circumference (cm), height at hip cross (cm), chest depth (cm), chest width (cm). All nannies were healthy and fed at the same condition. A few ear tissue was collected, placed in a centrifuge tube containing 1 mL 70% ethanol, brought back to the laboratory on ice as soon and stored at -80°C. All the procedure carried out was approved by Yulin University.

DNA extraction and concentration determination: DNA was extracted by high salt method[23]. The concentration of DNA samples was determined by Nanodrop 2000 spectrophotometer (Shanghai Danding International Trade Co., Ltd.) and diluted to 20 ng μL^{-1}, then stored at -20°C for use.

Primers design and synthesis: *CMTM2* gene (accession number: NC-030825.1) promoter region 14 bp (NC-030825.1.g: 35582961-35582974, del TAATGCCCCAATGG), InDel site information comes from the report of Kang *et al*.[7]. The primers, PCR Master Mix and Marker I were all synthesized by Xi'an Kinko Zexi Biotechnology Co., Ltd.

Polymerase Chain Reaction (PCR) detection: Prepare a 20 μL PCR reaction system including PCR Master Mix 10 μL, upstream and downstream primers (10 μmol L^{-1})

0.4 µL each, template DNA (concentration adjusted to 20 ng μL^{-1}) 1 µL and ddH_2O to make up to 20 µL. PCR amplification was conducted as: 95°C pre-denaturation 5 min; 94°C denaturation 30 s, 68-54°C (decrease 1°C per cycle, each cycle), 72°C extension for 18 sec, 15 cycles; 94°C denaturation for 30 sec, 54°C annealing for 30 sec, 72°C extension 18 sec, 38 cycles, 72°C extended for 10 min finally. The PCR products were electrophoresed in 3% Ethidium Bromide (EB) agarose gel to identify genotyping.

Statistics and analysis of data: The online platform SHEsis (https://analysis.bio-x.cn) was used to analyze the Hardy-Weinberg equilibrium constant and genotype frequency of the 14-bp InDel polymorphic site of *CMTM2* gene in population subjects. Applying General Linear Model to Analyze the Effects of Different Parameters on Traits: Yijk = +Gi+Eij where, Yijk is the population mean value, Gi stands for genotype fixed effect and Eij stands for random error. SPSS 23.0 Software was adapted to analyze the correlation between different genotypes of *CMTM2* 14-bp InDel locus and traits such as body weight and body size. The results were expressed as "means±SE" and $p<0.05$ means significant difference. Then, the phenotypic values of each trait were averaged and the correlation between different genotypes and the growth traits of Shaanbei white cashmere goat population (n = 1105) was analyzed by using "phenotypic value- population average" and single factor analysis method. The results were expressed by "means±SE" and the comparison between groups was performed by LSD test.

RESULTS

PCR amplification of *CMTM2* gene: The genomic DNA of Shaanbei white cashmere goats were amplified using the primer listed in Table 1 and electrophores were carried out after the genomic DNA amplified, the results were shown in Fig. 1. There existed three genotypes: insertion type (II), heterozygous type (ID) and deletion type (DD) at the locus. The length of target fragment is 145 bp (I) and 131 bp (D) (Fig. 1). The sequencing results confirmed that the InDel we detected was consistent with the predicted results (Fig. 2).

Genetic parameter analysis of *CMTM2* gene mutation sites: There existed three (II, ID and DD) genotypes of mutant in Shaanbei white cashmere goats. In Shaanbei white cashmere breeding goats (n = 621), the allele frequencies were I = 0.863 and D = 0.137; the allele frequencies in adult goats were I = 0.840 and D = 0.160. The heterozygosity of the 14-bp InDel site of *CMTM2* gene in the breeding and adult populations of Shaanbei white cashmere goats was 0.236 and 0.269, respectively and both were in accordance with the Hardy-Weinberg equilibrium state ($p>0.05$); the information content of polymorphism is 0.208 (PIC<0.25) and 0.233 (PIC<0.25), both of which are low polymorphism (Table 2).

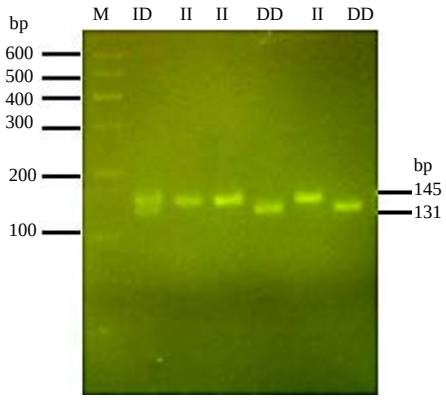

Fig. 1: The InDel site of *CMTM2* gene by gel electrophoresis; M = Marker 1; II = Insertion/Insertion; ID = Insertion/Deletion; DD = Deletion/Deletion

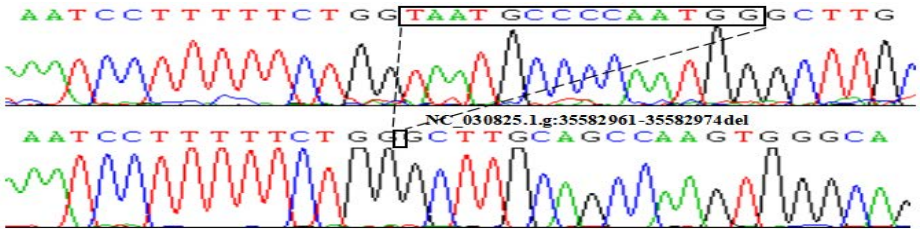

Fig. 2: The InDel site of *CMTM2* determined by DNA sequencing

Table 1: PCR primers used for detecting InDel mutation loci of goat *CMTM2*

Primers	Sequences 5'-3'	Position	RS Number	Length bp
CMTM2	F:GTTGATGAGGCAGGAGCCTT R:GATGCCAGTTTTGTGCCTGG	Promoter	rs664189991	145/131

Table 2: Genetic parameters of *CMTM2* gene polymorphism in Shaanbei white cashmere goat population

	Sample		Genotype frequencies		Allelic frequencies			Population Parameters			
Periods	N	II	ID	DD	I	D	p-values	Ho	He	Ne	PIC
Breeding	621	0.752(467)	0.222(138)	0.026(16)	0.863	0.137	p>0.05	0.764	0.236	1.309	0.208
Adult	484	0.709(343)	0.262(127)	0.029(14)	0.840	0.160	p>0.05	0.731	0.269	1.367	0.233

Ho = Homozygosity; He = Heterozygosity; Ne = Effective allele number; PIC = Polymorphic Information Content

Table 3: The Genetic parameters of *CMTM2* gene polymorphism in the whole population of Shaanbei white cashmere goat

	Sample		Genotype frequencies		Allelic frequencies			Population Parameters			
Periods	N	II	ID	DD	I	D	p-values	Ho	He	Ne	PIC
Breeding+Adult	1105	0.730(807)	0.243(268)	0.027(30)	0.863	0.137	p>0.05	0.747	0.253	1.338	0.221

Ho = Homozygosity; He = Heterozygosity; Ne = Effective allele number; PIC = Polymorphic Information Content

Table 4: Relationship between polymorphism of *CMTM2* gene and growth-related traits of breeding nannies

	(Genotypes mean±SE)			
Growth traits	II (467)	ID (138)	DD (16)	p-values
Body weight (kg)	33.26±0.71 (111)	34.38±1.40 (34)	35.11±4.54 (4)	0.456
Body height (cm)	53.15±0.16 (467)	53.17±0.30 (138)	53.66±0.60 (16)	0.556
Body length (cm)	62.11±0.13 (466)	62.36±0.37 (137)	61.73±1.04 (16)	0.656
Heart girth (cm)	72.48±0.36 (454)	72.57±0.55 (131)	74.00±1.65 (15)	0.426
Hip width (cm)	12.83±0.08 (454)	13.05±0.15 (132)	13.50±0.58 (15)	0.206
Cannon circumference (cm)	7.43±0.03 (454)	7.34±0.06 (131)	7.44±0.15 (15)	0.229
Height at hip cross (cm)	55.36±0.15 (453)	55.15±0.31 (132)	56.45±1.25 (15)	0.161
Chest depth (cm)	25.87±0.14 (454)	25.89±0.24 (131)	26.30±0.65 (15)	0.562
Chest width (cm)	18.81±0.12 (454)	18.78±0.24 (131)	19.50±0.67 (15)	0.305

II = Insertion/Insertion; ID = Insertion/Deletion; DD = Deletion/Deletion; in the same column a, b means significant difference (p<0.05)

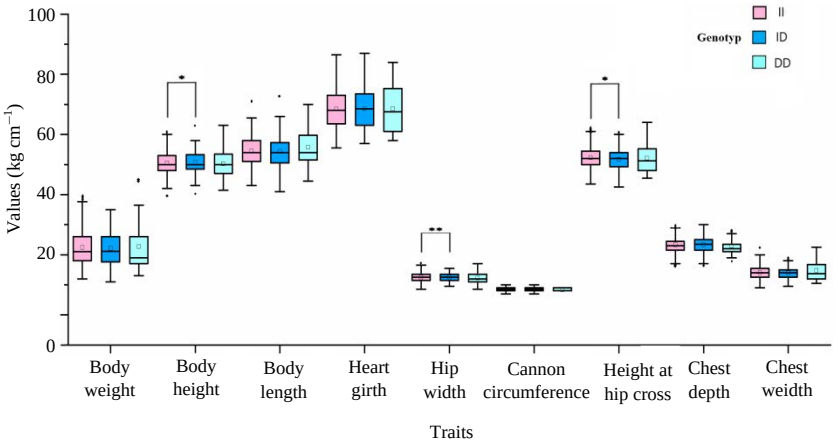

Fig. 3: Relationship between 14-bp InDel mutation of *CMTM2* gene and growth traits of breeding nannies; II = Insertion genotype; ID = Heterozygote genotype; DD = Deletion genotype; * = p<0.05

The 14-bp InDel locus of *CMTM2* gene in Shaanbei white cashmere goat population (n = 1105) allele frequencies were I = 0.863 and D = 0.137 which were in Hardy-Weinberg equilibrium (p>0.05), the polymorphism information content was 0.221 (PIC<0.25) which showed a low degree of polymorphism (Table 3).

Correlation analysis of *CMTM2* gene polymorphism and growth traits in breeding goats and adult goats: Analyzed the correlation between polymorphism of *CMTM2* gene and the growth traits of Shaanbei white cashmere goats with SPSS 23.0, the results showed that the 14-bp InDel locus of *CMTM2* gene were not associated with the growth traits of breeding nannies (Fig. 3 and Table 4). However, it was extremely significantly correlated with the body height, hip width and height at hip cross of adult Shaanbei white cashmere goats (p<0.01) (Fig. 4 and Table 5).

The analysis of dominant genotypes showed that in adult goats, individuals with ID genotype had better growth traits. However, the dominant genotypes was II in breeding goats (mainly showed through

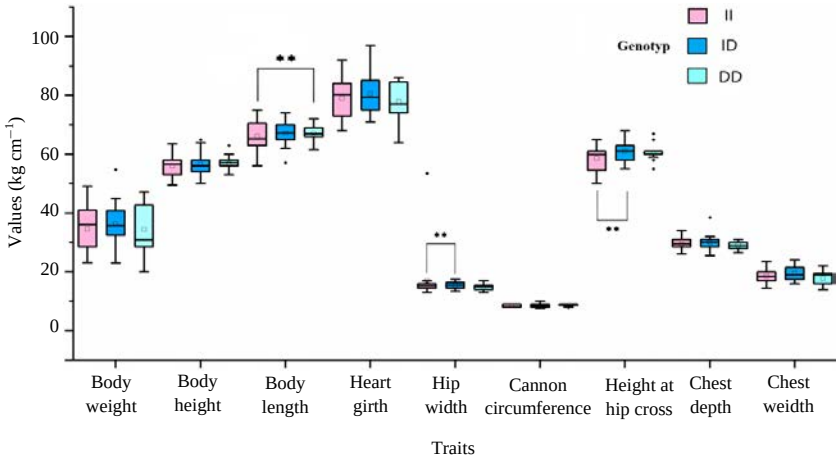

Fig. 4: The relationship between 14-bp InDel mutation of *CMTM2* gene and growth traits of Shaanbei white cashmere goats. II means insertion genotype, ID means heterozygote genotype and DD means deletion genotype; * = p<0.05

Table 5: The relationship between polymorphism of *CMTM2* gene and growth-related traits of adult Shaanbei white cashmere goats

	(Genotypes mean±SE)			
Growth traits	II (346)	ID (128)	DD (14)	p-values
Body weight (kg)	42.48±0.63 (326)	44.02±0.99 (119)	40.63±2.98 (13)	0.202
Body height (cm)	54.68^{B}±0.24 (340)	56.08^{A}±0.39 (125)	54.68^{AB}±1.30 (14)	0.003
Body length (cm)	68.22±0.31 (340)	68.61±0.48 (125)	68.04±1.46 (14)	0.515
Heart girth (cm)	83.74±0.49 (341)	84.29±0.72 (126)	80.93±1.76 (14)	0.171
Hip width (cm)	14.99^{B}±0.11 (340)	15.57^{A}±0.19(127)	14.75^{AB}±0.40 (14)	0.006
Cannon circumference (cm)	8.26±0.04 (340)	8.32±0.07 (127	7.89±0.18 (14)	0.053
Height at hip cross (cm)	57.85^{B}±0.26 (340	59.36^{A}±0.36 (124)	58.00^{AB}±1.26 (14)	0.002
Chest depth (cm)	29.58±0.18 (340)	29.97±0.25 (126)	29.35±0.80 (14)	0.236
Chest width (cm)	21.08±0.21 (340)	21.31±0.35 (126)	20.36±0.84 (14)	0.391

II = Insertion/Insertion; ID = Insertion/Deletion; DD = Deletion/Deletion; in the same column a, b means significant difference (p<0.05)

Table 6: The relationship between *CMTM2* gene polymorphism and the growth-related traits of adult Shaanbei white cashmere goats

	(Genotypes mean±SE)			
Growth traits	II (807)	ID (268)	DD (30)	p-values
Body weight (kg)	-0.33±0.50 (431)	1.22±0.83 (152)	-1.28±2.48 (17)	0.111
Body height (cm)	-0.17^{b}±0.14 (805)	0.47^{a}±0.24 (266)	0.05^{ab}±0.68 (30)	0.022
Body length (cm)	-0.08±0.19 (804)	0.21±0.30 (266)	-0.37±0.86 (30)	0.425
Heart girth (cm)	-0.07±0.29 (795)	0.23±0.44 (261)	-0.66±1.25 (29)	0.567
Hip width (cm)	-0.08^{B}±0.06 (795)	0.32^{A}±0.12 (263)	0.14^{AB}±0.36 (29)	0.003
Cannon circumference (cm)	0.00±0.03 (794)	-0.02±0.04 (262)	-0.20±0.12 (29)	0.157
Height at hip cross (cm)	-0.14^{b}±0.14 (793)	0.45^{a}±0.24 (260)	0.44^{ab}±0.88 (29)	0.043
Chest depth (cm)	-0.07±0.11 (794)	0.15±0.17 (261)	0.04±0.51 (29)	0.312
Chest width (cm)	-0.001±0.11 (794)	0.07±0.21 (261)	0.00±0.54 (29)	0.756

II = Insertion/Insertion; ID = Insertion/Deletion; DD = Deletion/Deletion; in the same column a, b means significant difference (p<0.05)

cannon circumference, height at hip cross and chest depth) and ID was the dominant genotype for the other traits.

Correlation analysis of *CMTM2* gene polymorphism and growth traits in Shaanbei white cashmere goats' population: Treat breeding goats and adult goats as a group, the phenotypic value-group average is used to obtain the difference between individual and group and Single factor analysis was used to analyze the correlation between different genotypes and growth traits of Shaanbei white cashmere goat population (n = 1105), it can be seen that the 14-bp InDel site of *CMTM2* gene is significantly related to the overall body height and height at hip cross of the Shaanbei white cashmere goats population (p<0.05); this locus was significantly correlated with hip bone width (p<0.01), Genotype ID was dominant growth trait (Fig. 5 and Table 6).

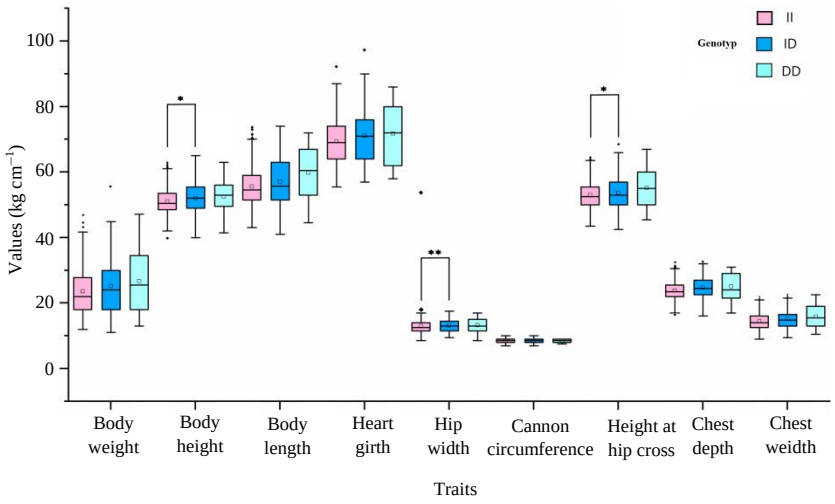

Fig. 5: The relationship between the 14-bp InDel mutation of *CMTM2* gene and the growth traits of Shaanbei white cashmere goats; II = Insertion genotype; ID = Heterozygote genotype; DD = Deletion genotype; * = p< 0.05

DISCUSSION

Shaanbei white cashmere goat is famous for its fine cashmere and meat local goat breed[24]. The mutton of Shaanbei white cashmere goat not only have excellent quality, high protein, low fat, unique flavor and other advantages but also owning significant health care functions. It was known as "ginseng in meat" and widely favored by people[25, 26]. Generally speaking, growth traits are often proportional to body weight. The better the growth traits, the heavier the carcass weight, the higher the economic benefits[27]. Shaanbei white cashmere goat has become an important part of the economic development in Shaanxi province. However, Shaanbei white cashmere goats still face some disadvantages such as small body size and slow growth rate. How to improve production performance of Shaanbei cashmere goat became a research hotspot. Our study demonstrated that *CMTM2* can be used as molecular marker to select the offsprings of Shaanbei white cashmere goat to improve its growth performance and economic characteristics.

CMTM gene family encode protein with special structure which are between chemokines and quaternary transmembrane proteins and play a crucial role in reproductive and immune systems[28, 29]. *CMTM2*, a member of this gene family has a latent four-fold transmembrane structure which is highly expressed in testis, bone marrow, prostate and peripheral blood cells[10, 28, 30]. *CMTM2* gene can not only accelerate myotubular formation and DNA synthesis through C2C12 myoblast differentiation but also promote the secretion and synthesis of Androgen Receptor (AR) to stimulate the proliferation and regeneration of skeletal muscle cells, thus, regulating bone metabolism[15, 31, 32]. Aboveall, *CMTM2* gene has a certain regulatory role in bone metabolism. Meanwhile, in the process of promoting the secretion and synthesis of AR, *CMTM2* gene enhances the AR protein level by promoting dHT-mediated trans-activation of androgen receptor[15]. The higher the level of AR protein and the more beneficial androgen is to combine with AR secretion to regulate growth, development and metabolism as well as secretion and proliferation of skeletal muscle cells. Meanwhile, as a member of the *CMTM* family, *CMTM1* protein has a broad spectrum of chemotactic effects *in vitro* and can also promote colony formation of bone marrow cells and proliferation of skeletal muscle cells[33]. Therefore, we speculated that *CMTM2* may regulate the proliferation of cashmere skeletal muscle cells which may affect the growth and development of cashmere goats. In this study, result showed that the *CMTM2* 14-bp InDel locus was significantly correlated with the body height and cross height of the Shaanbei white cashmere goat ewe population ($p<0.05$) and was strongly significantly correlated with the hip bone width ($p<0.01$) which confirmed that this locus may have a regulatory effect on the growth and development of goats. Previous studies have confirmed that the height at hip cross can significantly affect the adult livestock weight[4]. Though the site that there was no significant correlation with the weight, the weight and body height, hip width and other important growth character has the same trend of performance. And studies have shown that the 14bp deletion in the *CMTM2* promoter region is significantly related to the number of goats. show the site as a molecular marker selection of site to select good development performance of goat is of great significance. In this study, result found that 14-bp InDel site in *CMTM2* gene was in accord with hardy Weinberg equilibrium ($p>0.05$) and in a low polymorphism, illustrating the locus

in Shaanbei white cashmere goats population having low variability. The side reflection shows that the intensity of artificial selection must be increased in order to improve the breeding of Shaanbei white cashmere goats. By the correlation analysis, result showed that the *CMTM2* 14-bp InDel locus was significantly correlated with the body height and height at hip cross of the Shaanbei white cashmere goats ewe population ($p>0.05$) and was strongly significantly correlated with the hip bone width ($p<0.01$), II genotypes had better growth traits than others but the site mutations were no correlation with growth traits of breeding goats. We suspect that may be caused by different growth stages or with some pathological state duration is different. The results showed that the heterozygosity of adult goats was higher than that of breeding goats, it shows that the genetic variation of adult goats population is greater, this may be one of the reasons that the mutation site of this gene is significantly related to the number of multiple traits in the adult goats population.

CONCLUSION

In conclusion, the 14-bp InDel mutation in the promoter region of *CMTM2* gene was significantly associated with body height, hip width and height at hip cross of Shaanbei white cashmere goats. In this study, the 14-bp mutation site of *CMTM2* gene can be used as a candidate gene for molecular marker-assisted breeding of cashmere goats which provides a theoretical basis and scientific basis for the breeding of Shaanbei white cashmere goats.

ACKNOWLEDGMENTS

This work was supported by the National Natural and Science Foundation of China (Reference: 31702115), the Shaanxi Agricultural Science and Technology Innovation and Public Relations Project (Reference: 2016NY-105) and the Yulin University High-Level Talent Research Fund Project (Reference: 16GK06). We are grateful to the College of Animal Science and Technology, Northwest A&F University, Yangling City, Shaanxi Province, P.R. China.

REFERENCES

01. Han, C., Q. Fang, H. Li, S. Hu, H.J. Zhu and J.H. Hu, 2018. Study on the development of spermatogenic cells of Shaanbei white cashmere goats at different ages. J. Livest. Ecol., 4: 43-48.
02. Zhang, Y., Y.H. Bai, R.B. Wang, M.L. Wang, T.T. Gao and X. Li, 2019. Investigation on the production performance of Shaanbei white cashmere goats in Yulin city. J. Anim. Husb. Vet. Med., 5: 45-48.
03. Yan, H.L., Y.Y. Bai, Z. Wang, X.F. Chen and R.H. Deng *et al*., 2019. Relationship between 9-bp InDel of GHR gene and weight and growth traits of Shaanbei white cashmere goats. J. Livest. Ecol., 40: 27-31.
04. Zhu, H.J., Y.J. Wei, Z.H. Kang, Y.R. Jiang and L.P. Li *et al*., 2019. The relationship between the 12-bp InDel of GDF9 gene and the growth traits of Shaanbei white cashmere goats. Adv. Vet. Med., 40: 55-59.
05. Liu, A., 2018. Research progress on the performance of Shaanbei white cashmere goats. Chin. Herbivore Sci., 38: 65-68.
06. He, L., Z. Kang, Y. Kang, W. Xiang and C. Pan *et al*., 2020. Goat *CMTM2*: MRNA expression profiles of different alternative spliced variants and associations analyses with growth traits. 3 Biotech, 10: 1-10.
07. Kang, Z., S. Zhang, L. He, H. Zhu and Z. Wang *et al*., 2019. A 14-bp functional deletion within the *CMTM2* gene is significantly associated with litter size in goat. Theriogenology, 139: 49-57.
08. Shen, X.L. and T.Z. Zhang, 2013. The progress and prospects of research on molecular marker-assisted selection breeding of crops. High Technol. Lett., 2: 105-110.
09. Huang, J., P.H. Zhang and S.Q. Huang, 2017. Progress in sheep breeding technology. Hunan Anim. Husb. Vet. Med., 4: 38-39.
10. Ding, Y. and J.T. Jiang, 2019. The biological function and mechanism of CMTM family. Chin. J. Cancer Biother., 26: 463-467.
11. Shao, L., T. Li, X. Mo, O. Majdic and Y. Zhang *et al*., 2010. Expressional and functional studies of CKLF1 during dendritic cell maturation. Cell. Immunol., 263: 188-195.
12. Han, W., P. Ding, M. Xu, L. Wang and M. Rui *et al*., 2003. Identification of eight genes encoding chemokine-like factor superfamily members 1-8 (CKLFSF1-8) by in silico cloning and experimental validation. Genomics, 81: 609-617.
13. Kong, J. and S.K. Tan, 2019. Research progress of CMTM4 and tumor. Chin. J. Cancer Prevention Treat., 11: 85-88.
14. Liu, G., Z.C. Xin, L. Chen, L. Tian and Y.M. Yuan *et al*., 2007. Expression and localization of CKLFSF2 in human spermatogenesis. Asian J. Andrology, 9: 189-198.
15. Liu, D., C. Yin, Y. Zhang, L. Tian and T. Li *et al*., 2009. Human *CMTM2*/CKLFSF2 enhances the ligand-induced transactivation of the androgen receptor. Chin. Sci. Bull., 54: 1050-1057.
16. Shi, S., M. Rui, W. Han, Y. Wang and X. Qiu *et al*., 2005. CKLFSF2 is highly expressed in testis and can be secreted into the seminiferous tubules. Int. J. Biochem. Cell Biol., 37: 1633-1640.

17. Zaneveld, L.J., 1978. The biology of human spermatozoa. Obstetrics Gynecology Annu., 7: 15-40.
18. Liu, G., Z.C. Xin, L. Chen, L. Tian, Y.M. Yuan and Y.L. Guo, 2006. Regulation of gene developmental expression and protein expression localization of chemokine-like factor superfamily in mouse testis. Chin. J. Andrology, 9: 7-9.
19. Sun, T., Z.C. Zhang, J. Peng, G. Liu, B.X. Liu, Z. Jin, Y.Q. Gong and Z.C. Xin, 2008. Gene expression and protein localization of CKLFSF2a in mouse testis development. Chin. J. Andrology, 6: 1-4.
20. Zhang, X., Z. Chen, N. Huang, L. Kuang and H. Yin *et al*., 2016. *CMTM2* is essential for spermiogenesis in mice. Int. J. Clin. Exp. Pathol., 9: 12431-12438.
21. Wang, P.J., J.R. McCarrey, F. Yang and D.C. Page, 2001. An abundance of X-linked genes expressed in spermatogonia. Nat. Genet., 27: 422-426.
22. Fu, S.T. and X.H. Wang, 2018. Research progress in the regulation and mechanism of androgen and androgen receptor on bone metabolism. Chin. J. Cell Biol., 6: 1041-1048.
23. Aljanabi, S.M. and I. Martinez, 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acid Res., 25: 4692-4693.
24. Ma, C.Y., Y.L. Chen, X.B. Feng, L. Ai, L. Zhang, G.Y. Lu and A Ls, 2019. Estimation of heritability of main economic traits of Shaanbei white cashmere goats after housing. J. Livestock Ecol., 40: 75-77.
25. Tong, J.J., 2017. Looking at the development of Shaanbei white cashmere goats from the supply-side reform. Proceedings of the 14th 2017 China Sheep Industry Development Conference, October 3-6, 2017, China Animal Husbandry Association, Bayannaoer, China, pp: 39-40.
26. Zou, H., Y.Y. Cui, Q. Xia, X.F. Cheng and Y.J. Wu *et al*., 2018. Research progress on genes related to goat lambing traits. Heilongjiang Anim. Sci. Vet. Med., 11: 63-67.
27. Lin, M., Q. Qin, Q. Yang, M. Zhang and H. Zhao *et al*., 2017. Associations of six SNPs of POU1F1-PROP1-PITX1-SIX3 pathway genes with growth traits in two Chinese indigenous goat breeds. Ann. Anim. Sci., 17: 399-411.
28. Liu, Z.H., X.W. Zhang and T. Xu, 2010. The role of *CMTM2* in the male reproductive system. J. Med. Mol. Biol., 7: 173-175.
29. Ya-Bo, Z., J. Ye, P. Bing-Xin, W. Qian-Qian, L.U. Jia, H. Jie and L. Wei, 2015. The expression and role of CMTM family in tumor. J. Int. Oncol., Vol. 42,
30. Hu, F.Z., Z.Z. Sheng, C.P. Qin and T. Xu, 2016. Research progress of chemokine-like factor superfamily member 3. J. Chin. Acad. Med. Sci., 38: 360-363.
31. Xia, D., X. Li, Y. Lou, W. Han and P. Ding *et al*., 2002. Overexpression of chemokine-like factor 2 promotes the proliferation and survival of C2C12 skeletal muscle cells. Biochim. Biophys. Acta (BBA)-Mol. Cell Res., 1591: 163-173.
32. Han, W., Y. Lou, J. Tang, Y. Zhang and Y. Chen *et al*., 2001. Molecular cloning and characterization of chemokine-like factor 1 (CKLF1), a novel human cytokine with unique structure and potential chemotactic activity. Biochem. J., 357: 127-135.
33. Liu, P., X.J. Zhang, B. Sun, J. Zhang and L.N. Zhao, 2006. Superfamily of chemokine-like factors: A new human gene family. Chem. Life, 4: 339-341.