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Polymorphism of *SYNE2* Gene and its Association with Litter Size in Small Tail Han Sheep

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YW, ZT and MC designed the study. JT and ZT conducted the experiments. ZT analyzed the data and drafted the manuscript. YW, ZT, JS and MC helped in preparation of the manuscript.

Key words
Sheep, *SYNE2* gene, SNP, Genotyping, Litter size

ABSTRACT

To elucidate the association between polymorphism of *SYNE*2 and litter size in sheep and provide a new locus for marker-assisted selection of high fecundity traits in sheep. A total of 384 small tail han sheep (STH) were sampled to detect single nucleotide polymorphism (SNP), and Sequenom Mass ARRAY® SNP assay was applied to genotype SNP loci of the *SYNE*2 gene. In this study, four SNPs were identified and that SNPs were identified that involved in amino acid changes. Population genetic analysis indicated that *SYNE*2 gene g.73310578G>A, g.73312791A>G showed moderate polymorphism (0.25<PIC<0.5) in Small Tail Han sheep. Furthermore, g.73310578G>A and g.73312791A>G loci were closely linked in STH ($r^2 > 0.33$). Association analysis results showed that g.73310578G>A and g.73312791A>G SNPs significantly affected litter size ($P < 0.05$). In addition, the litter size of individuals with the combined genotype AA/AG was greater than that of individuals with AA/GG, GA/AG, and GG/AA genotypes in the third parity ($P < 0.05$). In summary, the *SYNE*2 gene had a positive influence on the litter size of STH sheep. The linkage of g.73310578G>A and g.73312791A>G could be used in the marker-assisted selection of the litter size of STH.

INTRODUCTION

Litter size plays a vital role in the livestock economy (Rothschild *et al*., 1996). The litter size in sheep is a complex trait that is influenced by many factors, such as genetic background (Chu *et al*., 2007), nutritional level (Mellor, 1983), and feeding management. The genetic experience principally includes the number of ovulation (Chu *et al*., 2007), fertilization efficiency (Edwards *et al*., 2016), and estrus (Sánchez-Dávila *et al*., 2015). Among them, the ovulation is particularly important, which can affect the number of lambs per year in the sheep. Identification of the candidate genes that are responsible for variation in continuous traits or quantitative traits has been a challenge in modern genetics. So far, there have been some studies of a candidate gene, such as *FecB*, *BMP15,* and *GDF9* on reproductive traits in sheep, which revealed that candidate gene plays an important role in sheep reproduction. The *FecB* gene is crucial in the regulation of prolificacy phenotype in sheep (Mulsant *et al*., 2001).

Nuclear envelope spectrin repeat proteins (Nesprins) are the latest identified members of the spectrin repeat (SR)-containing protein family (Zhou *et al*., 2018a). Nesprin-1/2 giant isoforms localize at the outer nuclear membrane and form the L Inker of Nucleoskeleton-and-Cytoskeleton (LINC) complex via associations between their KASH domains and the SUN domains of SUN1/2 in the perinuclear space (Sosa *et al*., 2012; Sosa *et al*., 2013). The LINC complex tethers the nuclear envelope to cytoskeletal elements, including actin filaments and the microtubule network (Gimpel *et al*., 2017; Wilson and Holzbaur, 2015). This molecular linking network is pivotal in regulating nuclear integrity, maintaining nuclear-cytoskeleton coupling, and participating in mechanotransduction, nuclear migration and positioning uniquely in muscle cell differentiation (Mellad *et al*., 2011; Stroud *et al*., 2014; Zhou *et al*., 2018a). Previous studies

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have suggested that Nesprin-2 regulates the Wnt/β-catenin signaling pathway (Sascha *et al*., 2010; Zhang *et al*., 2016). Several reports have shown that the Wnt/β-catenin pathway plays an essential role in follicular development, granulosa cell growth, and oocyte maturation (Gustin *et al*., 2016). Most studies on the *SYNE*2 gene have focused on diseases in the human (Baumann *et al*., 2017; Marina *et al*., 2015) and the mouse (Zhou *et al*., 2018a). However, few studies have investigated the effect of the *SYNE*2 gene on litter size in sheep. Therefore, the objectives of the present research were to detect SNPs associated with the litter size in small tail han (STH) sheep and identify a genetic marker conceivably valuable for marker-assisted selection.

MATERIALS AND METHODS

All the experimental procedures mentioned in the present study were approved by the Science Research Department (in charge of animal welfare issue) of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (IAS-CAAS) (Beijing, China). Ethical approval on animal survival was given by the animal ethics committee of IAS-CAAS (No. IASCAAS-AE-03, 12 December 2016).

Animals selection, blood sampling, and DNA extraction

As detailed in Table I, 726 ewes from six sheep breeds were selected for genotyping. Jugular vein blood samples (10 mL blood per ewe) were collected using citrate glucose as an anticoagulant. Genomic DNA was extracted by the phenol-chloroform method (Deininger, 1983), dissolved in ddH_2O and stored at -20℃.

Primer design and genotyping

Four pairs of primers were designed according to the ovine *SYNE*2 sequence from Ensemble (ENSOART00000023042.1). Primer sequence, product size and annealing temperature are presented in Table II. All primers were synthesized by Beijing Tianyihuiyuan Biotechnology Co. Ltd. (Beijing, P.R. China). PCR was carried out in 50 μL volume containing 25 μL of 2×GC Buffer Ⅰ, 8 μL of 2.5 mmol/L each dNTP, 0.5 μL of 5 U/μL TaKaRa LA *Taq*, 2 μL of 40 ng/μL genomic DNA, and 1 μL of 10 μmol/L each primer, the rest was ddH_2O. Amplification conditions were as follows: initial denaturation at 95℃ for 5 min; followed by 34 cycles of denaturation at 95℃ for 30 s, annealing for 30 s, extension at 72℃ for 1 min with a final extension at 72℃ for 5 min.

All of the PCR products were sent to Sangon Biotech Co, Ltd. (Shanghai, China). The sequencer software Chromas Pro 2 was used to identify SNPs. Genotyping of *SYNE*2 SNPs by Sequenom MassARRAY® SNP as described by Zhou (Zhou *et al*., 2018b). Genotyping primer sequence and product size are presented in Table II.

Statistical analysis

Allelic frequencies, heterozygosity (*He*), polymorphism information content (*PIC*) and the Hardy-Weinberg equilibrium tests were calculated using Pop gene (version 1.31). Linkage disequilibrium was analyzed using Haploview. Statistical analysis was performed by univariate analysis in a General Linear Model procedure of SAS (V. 8.1) (SAS Institute Inc., Cary, NC, USA). Multiple comparisons of means were performed using the least significant difference method. The applied model was expressed as follows: $y_{ijn} = \mu + P_i + G_j + I_{PG} + e_{ijn}$, where y_{ijn} is the phenotypic value of litter size; μ is the population mean; P_i is the fixed effect of the *i*th parity (i = 1, 2, 3); G_j is the fixed effect of the *j*th genotype (j=1, 2, 3); I_{PG} is the interaction effect of parity and genotype; and e_{ijn} is the random residual.

RESULTS

Polymorphisms of the coding region of the SYNE2 *gene*

In this study, sequencing of the amplicons of different primer pairs identified four polymorphic nucleotide sites in sheep *SYNE*2 gene. The g.73310578G>A mutation was in the 112 exons and the g.73312892G>A, g.73312791A>G and g.73314606G>A mutations were in the 114 exons (Fig. 1). Four SNPs (g.73310578G>A, g.73312892G>A, g.73312791A>G and g.73314606G>A) were genotyped in STH sheep (Fig. 2). At g.73312892G>A locus, the *PIC* was 0.07 in STH (Fig. 2). At g.73310578G>A, g.73314606G>A and g.73312791A>G locus, the *PIC* was 0.18~0.30 in STH. Genotypic distribution and allelic frequencies of four SNPs are shown in Figure 2. It was shown that STH sheep were in Hardy-Weinberg equilibrium at four-locus ($p > 0.05$) (Fig. 2). To reveal the linkage relationships between the four SNPs, the linkage disequilibrium was estimated at in STH sheep (Fig. 3). If $r^2 > 0.33$ and $D' > 0.5$ the linkage disequilibrium was considered strong (Ardlie *et al*., 2002). Following the result, both g.73310578G>A and g.73312791A>G loci were closely linked in STH sheep.

Population genetic analysis of polymorphism in the SYNE2 *gene*

Besides STH sheep (Fig. 2), population genetic characteristics of four SNPs in the other five sheep breeds were also analyzed, the results were listed in Table III. It revealed that the g.73314606G>A and g.73312791A>G loci were moderately polymorphic (0.25 <PIC<0.5) in the Sunite sheep and Hu sheep, respectively.

Table I. Information of six sheep breeds selected for genotyping.

Breed	Number	District
Small tail han sheep	384	Yuncheng, Shandong Province, China
Hu sheep	101	Xuzhou, Jiangsu Province, China
Cele black sheep	68	Cele, Hetian, Xinjiang Uigur Autonomous Region, China
Prairie Tibetan sheep	80	Dangxiong, Tibet Autonomous Region, China
Sunite sheep	70	Wulatezhongqi, Bayannaoer, Inner Mongolia Autonomous Region, China
Tan sheep	23	Yanchi, Ningxia Hui Autonomous Region, China

Table II. Information of primer in sheep *SYNE2* gene.

Primer name	Primer sequence	Tm	product size	Amplified region
*SYNE*2-1	F: CATCACTGTTTTCAGAGTGCCT R: ATACCTCTTCTCCCACCCACG	59.5	342 bp	Exon112
SYNE2-2	F: TTCCTGTCTAGATGATGCCAG R: GCTGCAAGGACACTAAGTCT	58.9	352 bp	Exon114
*SYNE*2-3	F: AAGCTGATTCCGGCCACAC R: CAGGGCCATAACGTAGCTTT	60.6	341 bp	Exon114
*SYNE*2-4	F: CATGCTGGCTCTAGTCCCCT R: TAGAAGGACCTGGCAAAGTTGT	61.1	332 bp	Exon114
SYNE2-1-S	F:ACGTTGGATGAGCTGGCTGACTCTATCTTG R:ACGTTGGATGTCTCTGTCAACGTGAACAGC	60.0	102 bp	PCR for g.73310578G>A
SYNE2-1-E	5’- TGACTCTATCTTGGAGTTCT -3’			Extension reaction
SYNE2-2-S	F: ACGTTGGATGCCTAGCAACTGGAAAAGGAG R: ACGTTGGATGTAAGCAGGGTGCTGGAAATC		98 bp	PCR for g.73312791A>G
SYNE2-2-E	5’- AAAGGAGCTAGTGGAAC -3’			Extension reaction
SYNE2-3-S	F: ACGTTGGATGGGAGAAGACTACATTGAGGC R: ACGTTGGATGGGGACACTTGCTCAAGTAAC		99 bp	PCR for g.73312892G>A
SYNE2-3-E	5’- aggaTGAAGAGAAGGTCCATGTTATC -3’			Extension reaction
SYNE2-4-S	F: ACGTTGGATGAGCTCTCACCTCCTCTGTTG R: ACGTTGGATGTGATCACCCGAGAAAGGAAG		109 bp	PCR for g.73314606G>A
SYNE2-4-E	5’- tgaCCGATCCCGCTGCCCCC -3’			Extension reaction

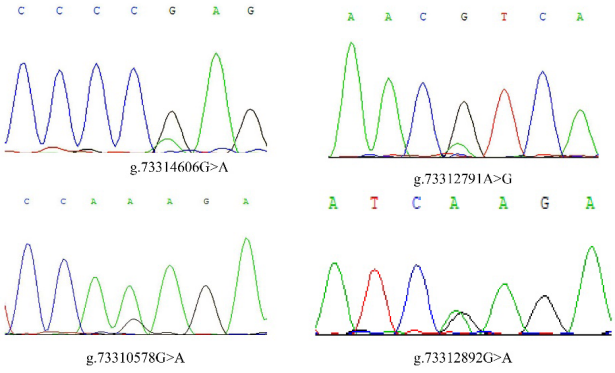

Fig. 1. Identification SNP of *SYNE*2 gene in small tail han sheep.

The chi-square test indicated that all SNPs under Hardy Weinberg equilibrium (P>0.05) in six sheep breeds. Besides, we classified six breeds into two categories, Multiparous and uniparous, based on the litter size characteristics, the results of the comparison of the population genetic analysis were shown in Table IV.

Association analysis of SNPs with litter size

At the g.73312892G>A locus in the STH sheep, individuals with the GG genotype higher litter size than did those with AA genotypes in each parity (Table V). However, it did not reach a significant level (P > 0.05). At other loci, no significant differences in each parity litter size between different genotypes were found. The results of association analysis of the combined genotypes showed

Table III. Population genetic analysis of four loci of *SYNE2* in five sheep breeds.

Locus	Breed	Genotype frequency			Allele frequency		PIC	HE	NE	Chi-Square test (P-value)
g.73312892G>A		GG	AG	AA	G	A				
	Hu sheep	0.90	0.09	0.01	0.95	0.05	0.10	0.10	1.11	0.18
	Prairie Tibetan sheep	0.84	0.16	0.00	0.92	0.08	0.13	0.14	1.17	0.29
	Cele black sheep	0.98	0.02	0.00	0.99	0.01	0.02	0.02	1.02	0.94
	Sunite sheep	0.81	0.19	0.00	0.90	0.10	0.16	0.17	1.21	0.63
	Tan sheep	0.86	0.14	0.00	0.93	0.07	0.12	0.13	1.15	0.73
g.73310578G>A		AA	GA	GG	A	G				
	Hu sheep	0.69	0.29	0.02	0.84	0.16	0.24	0.27	1.38	0.61
	Prairie Tibetan sheep	0.77	0.20	0.03	0.87	0.13	0.20	0.23	1.29	0.12
	Cele black sheep	0.73	0.23	0.04	0.85	0.15	0.23	0.26	1.35	0.41
	Sunite sheep	0.67	0.33	0.00	0.83	0.17	0.24	0.28	1.38	0.36
	Tan sheep	0.82	0.18	0.00	0.91	0.09	0.15	0.17	1.20	0.64
g.73314606G>A		AA	GA	GG	A	G				
	Hu sheep	0.04	0.28	0.68	0.18	0.82	0.25	0.29	1.41	0.59
	Prairie Tibetan sheep	0.04	0.22	0.74	0.15	0.85	0.22	0.25	1.34	0.13
	Cele black sheep	0.02	0.12	0.86	0.08	0.92	0.14	0.15	1.17	0.06
	Sunite sheep	0.00	0.38	0.62	0.19	0.81	0.26	0.31	1.45	0.28
	Tan sheep	0.00	0.27	0.73	0.14	0.86	0.21	0.24	1.31	0.46
g.73312791A>G		AA	GA	GG	A	G				
	Hu sheep	0.02	0.38	0.60	0.21	0.79	0.28	0.33	1.49	0.15
	Prairie Tibetan sheep	0.03	0.20	0.77	0.13	0.87	0.20	0.23	1.29	0.12
	Cele black sheep	0.04	0.23	0.73	0.15	0.85	0.23	0.26	1.35	0.41
	Sunite sheep	0.00	0.29	0.71	0.14	0.86	0.21	0.24	1.32	0.44
	Tan sheep	0.00	0.18	0.82	0.09	0.91	0.15	0.17	1.20	0.64

Note: PIC, HE and NE represent polymorphism information content, heterozygosity and effective number of alleles, respectively; $p>0.05$ indicates the locus was under Hardy-Weinberg equilibrium.

Table IV. Genotype and allele frequencies of four loci in *SYNE2* gene of sheep with different litter size characteristics.

Loci	Characteristics of litter size	Genotype frequency			Allele frequency		Chi-square test (P-value)
		GG	AG	AA	G	A	
g.73312892G>A	Polytocous sheep	0.92	0.08	-	0.96	0.04	0.00
	Monotocous sheep	0.84	0.16	-	0.92	0.08	
		AA	GA	GG	A	G	
g.73310578G>A	Polytocous sheep	0.62	0.32	0.06	0.78	0.22	0.00
	Monotocous sheep	0.76	0.21	0.03	0.87	0.13	
		AA	GA	GG	A	G	
g.73314606G>A	Polytocous sheep	0.02	0.21	0.77	0.12	0.88	0.34
	Monotocous sheep	0.02	0.25	0.73	0.15	0.85	
		AA	GA	GG	A	G	
g.73312791A>G	Polytocous sheep	0.06	0.35	0.21	0.23	0.77	0.00
	Monotocous sheep	0.02	0.21	0.77	0.13	0.87	

Table V. Analysis of different loci and litter size at *SYNE2* gene in small tail han sheep.

Loci	Genotype	1st parity litter size	2nd parity litter size	3rd parity litter size
g.73312892G>A	AG	1.77±0.16(30)	1.96±0.18(28)	2.00±0.35(13)
	GG	2.05±0.05(312)	2.24±0.06(299)	2.39±0.10(147)
g.73310578G>A	AA	2.01±0.06(197)	2.20±0.07(188)	2.33±0.13(93)
	GG	2.17±0.16(30)	2.21±0.18(28)	2.34±0.36(12)
	GA	2.08±0.08(120)	2.23±0.09(115)	2.32±0.17(55)
g.73312791A>G	AA	2.08±0.17(26)	2.13±0.20(24)	2.19±0.38(11)
	AG	2.07±0.08(125)	2.27±0.09(120)	2.31±0.16(59)
	GG	2.02±0.07(180)	2.22±0.07(171)	2.46±0.14(84)
g.73314606G>A	AA	2.23±0.21(3)	2.37±0.16(3)	2.40±0.21(2)
	AG	2.10±0.11(66)	2.27±0.12(66)	2.29±0.23(31)
	GG	2.02±0.05(258)	2.18±0.06(258)	2.39±0.11(127)

Note: Numbers in the parentheses next to litter size represent the amount of sheep of each genotype.

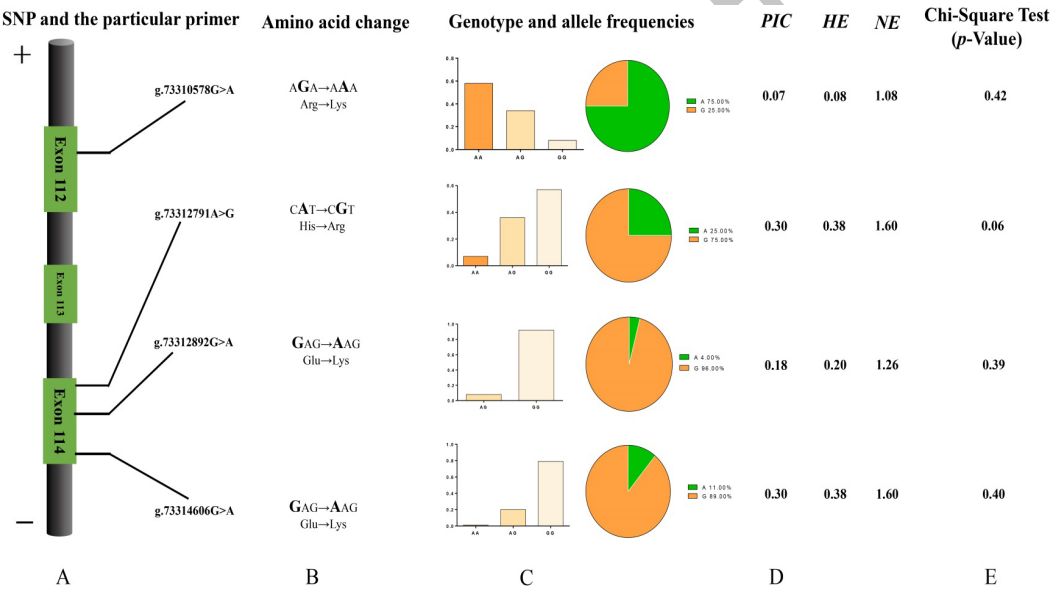

Fig. 2. Polymorphisms of the *SYNE2* gene in small tail han Sheep. A, the detected SNP and particular primer. A total of 4 exons are included in the *SYNE2* gene of sheep; B, the change of amino acids; C, the genotype and allele frequencies of the *SYNE2* gene; D, the information of PIC, HE and NE; E, the test of HWE, $P > 0.05$ indicates the locus was in Hardy-Weinberg equilibrium.

Table VI. Association analysis of *SYNE*2 haplotype and litter size in small tail han Sheep.

Genotype	Number	1st parity litter size	2nd parity litter size	3rd parity litter size
AAAG	8	2.00±0.30	2.63±0.32b	2.75±0.32b
AAGG	176	2.08±0.06	2.21±0.07a	2.25±0.07a
GAAG	113	2.11±0.08	2.15±0.09a	2.19±0.09a
GGAA	25	2.06±0.17	2.12±0.18a	2.15±0.18a

Note: Different small letters in the same group mean a significant difference ($p < 0.05$).

that individuals in the STH sheep with the AA/AG genotype had larger litter sizes than did those with AA/GG, GA/AG and GG/AA genotypes in the second and third parity ($P < 0.05$; Table VI).

DISCUSSION

Several reports have shown that nesprins (nuclear envelope spectrin repeat proteins) are the latest identified members of the spectrin repeat (SR)-containing protein family (Zhang *et al*., 2001). To date, six genes encoding

for different KASH domain-containing proteins named as nesprins-1, -2, -3, -4, lymphoid-restricted membrane protein (LRMP) and KASH5 have been identified in mammals (Zhou *et al*., 2018a). Nesprins play pivotal roles in the maintenance of NE integrity (Luke *et al*., 2008), nuclear positioning (Zhang *et al*., 2007) and anchorage to the cytoskeleton and the centrosome (Roux *et al*., 2009). Previous studies have suggested that Nesprin-2 regulates the Wnt/β-catenin signaling pathway (Sascha *et al*., 2010; Zhang *et al*., 2016). Several reports have shown that the Wnt/β-catenin pathway plays an important role in follicular development, granulosa cell growth and oocyte maturation (Gustin *et al*., 2016). WNT families consist of local–acting glycoproteins. They can regulate a wide range of biological processes, which include cell fate determination, proliferation, differentiation, apoptosis and embryogenesis (Fan *et al*., 2010). Therefore, we want to know that the *SYNE*2 gene is related to sheep reproduction or not and then detect SNPs of the *SYNE2* gene in STH sheep and identify a genetic marker conceivably valuable for marker-assisted selection (MAS).

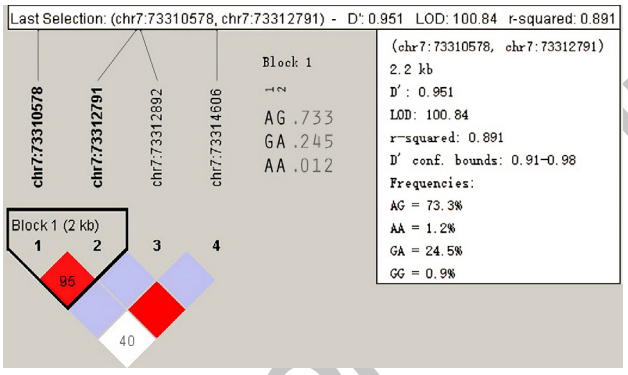

Fig. 3. The g.73310578G>A and g.73312791A>G loci linkage disequilibrium.

In this study, a total of four SNPs were identified and that SNPs were identified as that involved in amino acid change all SNPs were in Hardy-Weinberg disequilibrium in six sheep (P>0.05). Previous studies have demonstrated that *Ne* and *PIC* are important genetic parameters that indicate the level of intra-population genetic variation (Botstein *et al*., 1980). The results of the present study show that the g.73314606G>A and g.73312791A>G loci were moderately polymorphic (0.25<PIC<0.5) in the Sunite sheep and Hu sheep, respectively. These results indicated that the g.73314606G>A and g.73312791A>G loci have a higher level of intra-population genetic variation. The results of this study show that we found g.73312892G>A, g.73310578G>A, g.73312791A>G, g.73314606G>A are all missense mutations. There are many studies that missense mutations change sheep reproductive traits, such as *FecB*, *BMP15,* and *GDF9* (Chong *et al*., 2018; Zhou *et al*., 2018b). Several studies indicated that ewes carrying FecB-mutation have significantly higher ovulation rates if compared with their wild-type contemporaries (Mulsant *et al*., 2001; Qiuyue *et al*., 2015). Six mutations (*FecX*^{I}, *FecX*^{H}, *FecX*^{G}, *FecX*^{B}, *FecX*^{L}, *FecX*^{R}) of bone morphogenetic protein 15 (*BMP15*) can increase ovulation rate in heterozygotes and cause complete sterility in homozygotes. However, homozygous ewes with mutations (*FecX*^{Gr}, *FecX*^{O}) of BMP15 had increased ovulation rate without causing sterility (Qiuyue *et al*., 2015). Five mutations (*FecG*^{H}, *FecG*^{T}, *FecG*^{E}, *FecG*^{F}, *FecG*^{V}) in growth differentiation factor 9 (*GDF9*) associated with sheep prolificacy where FecG^{E} and FecG^{F} have additive an effect on ovulation rate and litter size (Qiuyue *et al*., 2015). When amino acid changes, the spatial structure of the protein changes, but its function may not change. The association analysis has shown that the four SNPs have no significant differences in each parity litter size between different genotypes. Further research is required to verify the mechanism of the impact of the SNPs on the parity litter size in STH sheep. Reproductive traits are complex quantitative traits involving multiple genes, loci and their interactions. Therefore, the combined effects of multiple genes or loci on reproductive traits should be analyzed. Association analysis revealed that mutations at g.73310578G>A and g.73312791A>G had a significant impact on litter size in STH sheep, which is consistent with the linkage disequilibrium result. The interesting finding was that of association analysis of the combined genotypes showed that individuals in the STH sheep with the AA/AG genotype had larger litter sizes than did those with AA/GG, GA/AG and GG/AA genotypes in the second and third parity. This result may be explained by the fact that this mutation in linkage disequilibrium with other responsible mutations or this mutation may change some events of *SYNE2* in term of the post-transcriptional regulation (Oerum *et al*., 2017; Zhang *et al*., 2019).

CONCLUSIONS

In Summary, the current study explored the genetic polymorphisms in the coding region of the *SYNE*2 gene, indicating that the AAAG haplotypes of *SYNE*2 gene g.73310578G>A and g.73312791A>G linkage loci could influence the third parity litter size in STH sheep. Therefore, it could be useful in the marker-assisted selection of the second and third parity litter size in STH sheep.

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Statement of conflict of interest

All authors declare no conflicts of interest

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