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Article

Single nucleotide polymorphisms in the 3′ UTR of follistatin-like 4 and scavenger receptor class B member 1 are associated with Dazu black goat litter size

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Abstract

The untranslated regions (UTRs) of genes play crucial roles in regulating gene expression at the post-transcriptional level such as affecting mRNA stabilization. In this study, 26 single nucleotide polymorphisms (SNPs) and one deletion located in UTR were genotyped from 186 Dazu black goats via SNaPshot, and the correlation between genotype and litter size was analyzed. The results indicated that two SNP loci, SNP_chr17-20182525 and SNP_chr7-65652612, which were located at the 3′ UTR of scavenger receptor class B member 1 and follistatin-like 4, were significantly ($P < 0.05$) correlated with the litter size of first parity goats. SNP_chr7-65652612 was also significantly associated with the total litter size of first and second parity offspring ($P < 0.05$). In conclusion, SNP_chr7-65652612 and SNP_chr17-20182525 have correlation with the litter size of Dazu black goat and they are potential genetic markers for litter size breeding.

Key words: goat, litter size, untranslated regions, correlation, single nucleotide polymorphism

Résumé

Les séquences non traduites (UTR — « untranslated regions ») des gènes jouent un rôle primordial dans la régulation de l'expression de gènes du point de vu post transcriptionnel, comme celui de la stabilisation de l'ARNm. Dans cette étude, 26 polymorphismes mononucléotidiques (SNP — « single nucleotide polymorphism ») et une délétion localisés dans les UTR de 186 chèvres noires Dazu ont été génotypés par SNaPshot, et la corrélation entre le génotype et la taille de portée a été analysée. Les résultats indiquent qu'il y a une corrélation significative ($P < 0,05$) entre deux loci SNP, SNP_chr17-20182525 et SNP_chr7-65652612, qui se trouvent à l'extrémité 3′ UTR du récepteur scavenger de classe B, type 1 et apparenté à la follistatine 4, et la taille de portée des chèvres à la première parité. SNP_chr7-65652612 est aussi associé de façon significative à la taille totale de la portée de la progéniture aux premières et deuxièmes parités ($P < 0,05$). En conclusion, il y a corrélation entre SNP_chr7-65652612 et SNP_chr17-20182525 et la taille de portée des chèvres noires Dazu et les SNPs sont des marqueurs génétiques potentiels de taille de portée en reproduction. [Traduit par la Rédaction]

Mots-clés : chèvre, taille de portée, séquences non traduites, corrélation, polymorphisme mononucléotidique

Introduction

Rapid development in economy and living standards has remarkably changed the human dietary structure. The current demand for mutton has been gradually increasing due to its low cholesterol and high contents of protein, amino acid, and trace elements.

Dazu black goat, originated in Dazu County, Chongqing, China, is formed by breeding and selection with excellent meat quality. In addition, Dazu black goats are also well known for reproduction traits with high fecundity, big litter size, and precocious sexual maturity. Thus, it can be used as a valuable material to search for genetic markers related to economic traits. Litter size is widely recognized as one of the most important economic parameters for mutton sheep farming. Fortunately, some major genes and genetic variations associated with litter size, including growth differentiation factor 9 (Wang et al. 2019), bone morphogenetic protein receptor, type IB (Shokrollahi and Morammazi 2018), and gonadotropin-releasing hormone receptor (Bemji et al. 2018), have been identified. The development of the whole-genome resequencing technique provided a useful tool to elaborate on genetic factors underlying the formation of

Table 1. Twenty-seven variants with significant divergence between high and low yields of litter size in Dazu black goats based on a genome-wide selection data set.

Marker name	Location			Ref	Mut	Variation type	Structure type	Structure gene	*P*-value (Fisher's Exact Test)
	chr	Start	End						
SNP_2–31139315	2	31139315	31139315	T	C	SNP	UTR3	IGFBP5	0.033707
SNP_11–9668275	11	9668275	9668275	C	G	SNP	UTR5	HK2	0.011783
SNP_11–92672606	11	92672606	92672606	G	A	SNP	UTR3	LHX6	0.031813
SNP_11–70706856	11	70706856	70706856	A	G	SNP	UTR5	SPDYA	0.031813
SNP_10–16748873	10	16748873	16748873	T	G	SNP	UTR3	TMED10	0.039588
SNP_22–14670534	22	14670534	14670534	A	G	SNP	UTR5	HHATL	0.033486
SNP_4–27004016	4	27004016	27004016	C	G	SNP	UTR3	SMO	0.019112
SNP_7–65652612	7	65652612	65652612	C	G	SNP	UTR3	FSTL4	0.031813
SNP_16–32401875	16	32401875	32401875	C	A	SNP	UTR5	SDCCAG8	0.045114
SNP_4–49493714	4	49493714	49493714	C	A	SNP	UTR3	LOC102173997	0.023573
SNP_4–21310795	4	21310795	21310795	A	G	SNP	UTR3	LOC102175184	0.012464
SNP_12–74985766	12	74985766	74985766	C	T	SNP	UTR3	NAA16	0.001411
SNP_10–80912375	10	80912375	80912375	AC	–	Deletion	UTR3	CD276	0.03383
SNP_17–20182525	17	20182525	20182525	G	A	SNP	UTR3	SCARB1	0.020887
SNP_22–50560282	22	50560282	50560282	C	T	SNP	UTR5	AMIGO3	0.031813
SNP_3–27655090	3	27655090	27655090	C	T	SNP	UTR3	SLC1A7	0.037681
SNP_17–86461	17	86461	86461	G	A	SNP	UTR5	SLC2A11	0.045833
SNP_3–99056046	3	99056046	99056046	G	T	SNP	UTR5	ANKRD35	0.011783
SNP_13–75297781	13	75297781	75297781	A	G	SNP	UTR3	ZMYND8	0.026434
SNP_21–60734283	21	60734283	60734283	G	C	SNP	UTR3	PAPOLA	0.0309
SNP_10–91662100	10	91662100	91662100	C	T	SNP	UTR3	LHFPL2	0.048202
SNP_15–41595324	15	41595324	41595324	G	A	SNP	UTR3	DKK3	0.028115
SNP_8–53491804	8	53491804	53491804	G	A	SNP	UTR5	GNA14	0.003032
SNP_2–10084855	2	10084855	10084855	C	T	SNP	UTR3	WASF2	0.031813
SNP_2–10086943	2	10086943	10086943	C	T	SNP	UTR3	WASF2	0.011783
SNP_13–37221670	13	37221670	37221670	G	A	SNP	UTR3	PCSK2	0.006783
SNP_11–48684304	11	48684304	48684304	T	C	SNP	UTR3	ATOH8	0.031813

Note: chr is the number of chromosome, Ref is the marker genotype in reference genome, Mut is the mutation genotype of marker, and Structure gene is where the marker was located.

economic traits at the whole-genome level. Several studies have been carried out on goat litter size with the whole-genome resequencing (E et al. 2019; Zhang et al. 2019). However, studies on the polymorphisms in untranslated regions (UTRs) are limited (Zhang et al. 2018; Kang et al. 2019; Quan et al. 2019; Yang et al. 2019), and the associations with litter size remain unclear.

UTRs, including 5′ and 3′ UTRs, play crucial roles in regulating gene expression at multiple levels including transcriptional regulation, pre-mRNA processing, mature mRNA transportation, mRNA stability, and translational control. Variations in UTR sequences can affect gene expression and thus phenotypic expression. The association between the polymorphisms in the UTR and goat litter size should be evaluated to further understand the relevant molecular mechanisms. Follistatin-like 4 (*FSTL4*) is a member of the follistatin gene family and it belongs to transforming growth factor (*TGF*)-β superfamily inhibitors. *FSTL4*, ubiquitously expressed in tissues including heart, lung, kidney, testis, neurons, muscle, and intestine, plays multiple roles in biological processes (Tsuchida et al. 2000). Recent studies showed that *FSTL4* was a candidate gene for the reproduction ability in dairy cows (Lu et al. 2021). Scavenger receptor class B member 1 (*SCARB1*), also known as *SR-BI*, plays an important role in reproduction activity. *SCRAB1* is expressed in cells of developing follicles such as theca, granulosa, and cumulus cells. Mice carrying null mutation of the *SCARB1* gene (*SCARB1*$^{-/-}$) had ovaries with small corpora lutea (Jiménez et al. 2010).

In this study, based on the data of the published article (E et al. 2019) and expanded the sample size, we explore the association of 26 single nucleotide polymorphisms (SNPs) and one deletion in the UTRs with litter size in Dazu black goats and determine the potential for these polymorphisms as genetic markers in the marker-assisted selection of goat.

Materials and methods

Ethical statement

The experimental conditions in this study were approved by the Committee on the Ethics of Animal Experiments of Southwest University and the Animal Protection Law of China

Table 2. Polymorphisms of 27 variants within the Dazu black goat population.

Marker	H_O	H_E	PIC	*P*-value (*d*HWD)
SNP_chr22-50560282	0.2097	0.2527	0.2202	0.03481*
SNP_chr2-31139315	0.3092	0.4407	0.3428	0.00039*
SNP_chr11-9668275	0.1022	0.1068	0.1009	0.45018
SNP_chr22-14670534	0.3656	0.3506	0.2885	0.67669
SNP_chr10-80912375	0.2366	0.2333	0.2056	1.00000
SNP_chr11-70706856	0	0	0	/
SNP_chr8-53491804	0.2944	0.3675	0.2994	0.01388*
SNP_chr13-37221670	0.6613	0.4996	0.3741	0.00002*
SNP_chr10-91662100	0.1568	0.1718	0.1566	0.20666
SNP_chr13-75297781	0.9948	0.5013	0.375	0.00000*
SNP_chr11-92672606	0.043	0.0422	0.0412	1.00000
SNP_chr17-20182525	0.2043	0.2411	0.2116	0.05714
SNP_chr12-74985766	0	0	0	/
SNP_chr4-49493714	0.3817	0.4363	0.3405	0.09154
SNP_chr11-48684304	0.2312	0.2602	0.2258	0.15450
SNP_chr21-60734283	0.9946	0.5013	0.375	0.00000*
SNP_chr7-65652612	0.1022	0.1068	0.1009	0.45023
SNP_chr4-27004016	0.3871	0.4132	0.3272	0.47424
SNP_chr2-10086943	0.3011	0.2639	0.2286	0.05271
SNP_chr3-99056046	0.1667	0.1709	0.1559	0.66523
SNP_chr16-32401875	0.4892	0.477	0.3626	0.75965
SNP_chr3-27655090	0.4624	0.422	0.3323	0.22429
SNP_chr17-86461	0.4677	0.4874	0.368	0.64956
SNP_chr10-16748873	0.4839	0.49	0.3693	0.88137
SNP_chr4-21310795	0.0269	0.4543	0.3504	0.00000*
SNP_chr2-10084855	0.2043	0.1839	0.1666	0.22429
SNP_chr15-41595324	0.2312	0.2372	0.2086	0.75575

Note: * means the SNP significant deviations of Hardy–Weinberg equilibrium (dHWD).

(this study did not directly carry out any relevant experiments on animals).

Animals and genomic DNA extraction

A total of 186 individual goats were sampled from the Dazu black goat breeding farm of Southwest University (Chongqing, China). No close kinship did exist between individuals within 2–3 generations. The animals were bred under the same feeding conditions and environment. The lambing records of all animals and the litter size of first and second parity animals were collected. Exactly 1 mL of venous blood was collected, and whole blood genomic DNA was extracted using the TIANamp Blood DNA kit (Tiangen, Beijing, China). Optical density was detected using a NanoDrop2000 instrument, and DNA quality was tested by 1.25% agarose gel electrophoresis. The DNA samples were stored at −20 °C for further analysis.

Primer design and genotyping

The detailed primer information is described in Table S1 and SNaPshot was used to analyze genotype. The polymerase chain reaction (PCR) was performed and the reaction volume and thermal profile were given in Table S2. The PCR product was treated with alkaline phosphatase remove free dNTPs (Table S3). Single base extension reaction was conducted and the detailed information was given in Table S4. Finally, SNaPshot reaction product was purified as described in Table S5. The 27 variant loci of all individuals were genotyped using SNaPshot and the ABI3730XL sequencing platform (AB, Waltham, USA). The expected and observed heterozygosities (H_E and H_O, respectively) and polymorphic information content (PIC) of each SNP were estimated using GenePop (http://www.genepop.curtin.edu.au/).

Statistical analyses

Although the limitations of using a small sample size for case-control studies were reported (Clarke et al. 2011), the goat litter size is a strong associated trait with high heritability. Many studies have been proved that relative small sample size could be used to detect key candidate markers (Lai et al. 2016; Islam et al. 2020; Tao et al. 2020; Smołucha et al. 2021). In accordance with the Genome Variant Map data (GVM000039) and litter size record of Dazu goats (E et al. 2019), the genotype frequencies of 27 variants located at the 5′ and 3′ UTRs were measured by Fisher's test with R script. The genetic effects (GEs) of minimum allele frequency (MAF) were measured via a general linear model with efficient mixed-model association eXpedited (EMMAX, Kang et al. 2010; Legarra et al. 2018). On the basis of the limited number of markers in this study, $P < 0.05$ was used to filter sig-

Table 3. Correlation analysis of 24 SNP variations with litter size in Dazu black goats.

	Litter size of first parity (LF)			Litter size of second parity (LS)			Sum litter size of first and second parity (SL)		
Marker	*P*-value	MAF	GE	*P*-value	MAF	GE	*P*-value	MAF	GE
SNP_chr10-16748873	0.8057	0.4247	0.0244	0.6232	0.4290	−0.0607	0.8354	0.4290	−0.0362
SNP_chr10-80912375	0.7108	0.1344	0.0418	0.4147	0.1392	−0.1106	0.7407	0.1392	−0.0633
SNP_chr10-91662100	0.5815	0.0968	−0.0647	0.6485	0.0994	−0.0638	0.6245	0.0994	−0.0967
SNP_chr11-48684304	0.5547	0.1532	0.0533	0.7609	0.1563	−0.0329	0.7730	0.1563	0.0440
SNP_chr11-92672606	0.2491	0.0215	−0.2834	0.3306	0.0227	−0.2816	0.1806	0.0227	−0.5474
SNP_chr11-9668275	0.3761	0.0565	−0.1361	0.2385	0.0597	0.2144	0.6780	0.0597	0.1064
SNP_chr13-37221670	0.1266	0.4704	−0.1436	0.0833	0.4659	0.1988	0.8895	0.4659	0.0224
SNP_chr15-41595324	0.1775	0.1371	0.1432	0.4366	0.1364	0.1022	0.2110	0.1364	0.2321
SNP_chr16-32401875	0.5899	0.3898	−0.0628	0.7302	0.3892	−0.0485	0.4625	0.3892	−0.1459
SNP_chr17-20182525	**0.0472***	0.1398	−0.1908	0.6388	0.1420	0.0538	0.3477	0.1420	−0.1519
SNP_chr17-86461	0.1298	0.4167	−0.1429	0.6291	0.4233	0.0547	0.7652	0.4233	−0.0477
SNP_chr2-10084855	0.7261	0.1022	−0.0429	0.8328	0.1023	−0.0310	0.6506	0.1023	−0.0939
SNP_chr2-10086943	0.8941	0.1559	−0.0140	0.3434	0.1563	−0.1196	0.4721	0.1563	−0.1280
SNP_chr21-60734283	0.4678	0.4973	0.4833	0.3957	0.4972	0.6647	0.3036	0.4972	1.1365
SNP_chr22-14670534	0.5279	0.2258	0.0571	0.5760	0.2216	0.0636	0.5298	0.2216	0.1008
SNP_chr22-50560282	0.2992	0.1478	−0.0969	0.0922	0.1534	−0.1881	0.1053	0.1534	−0.2552
SNP_chr2-31139315	0.4118	0.3575	0.0747	0.7600	0.3438	−0.0341	0.9791	0.3438	−0.0041
SNP_chr3-27655090	0.3932	0.3011	0.0743	0.9240	0.3040	−0.0099	0.6609	0.3040	0.0641
SNP_chr3-99056046	0.9101	0.0941	−0.0132	0.1693	0.0852	−0.2005	0.2571	0.0852	−0.2330
SNP_chr4-21310795	0.8528	0.3468	−0.0322	0.9413	0.3551	0.0152	0.9318	0.3551	−0.0250
SNP_chr4-27004016	0.3879	0.2903	−0.0685	0.4185	0.2869	0.0786	0.9589	0.2869	−0.0071
SNP_chr4-49493714	0.7974	0.3199	−0.0191	0.5061	0.3267	−0.0593	0.5003	0.3267	−0.0848
SNP_chr7-65652612	**0.0493***	0.0565	−0.2860	0.1261	0.0540	−0.2721	**0.0118***	0.0540	−0.6357
SNP_chr8-53491804	0.8742	0.2500	0.0119	0.4714	0.2585	0.0650	0.5089	0.2585	0.0840

Note: MAF, minor allele frequency; GE: genetics effects. Bold values indicate significance.

Table 4. Genotype frequency and litter size differences between genotypes of candidate SNPs.

Marker	Genotype	Frequency of genotype (%)	Mean litter size of first parity	Sum litter size of first and second parity (SL)
SNP_chr17-20182525	A/A	3.80	1.±857 ± 0.261 ^{A}	3.714 ± 0.359 ^{A}
	A/G	20.4	1.816 ± 0.112 ^{A}	3.250 ± 0.171 ^{A}
	G/G	75.8	1.610 ± 0.054 ^{A}	3.278 ± 0.099 ^{A}
SNP_chr7-65652612	C/C	89.2	1.627 ± 0.050 ^{A}	3.209 ± 0.089 ^{A}
	C/G	10.2	1.947 ± 0.162 ^{B}	4.059 ± 0.201 ^{B}
	G/G	0.60	–	

Note: Only one individual carries G/G genotype in SNP_chr7-65652612, so we did not estimated the significant difference of litter size with G/G.

nificant markers. All markers were placed into fixed effect estimated matrix to explain the phenotype variances:

$$\boldsymbol{Y} = \boldsymbol{\mu} + \boldsymbol{\alpha}\mathrm{X} + \mathrm{e}$$

where $\boldsymbol{Y}$ is a vector of phenotype values; $\boldsymbol{\mu}$ is the average vector; the length is the same as the number of individuals (n); $\boldsymbol{\alpha}$ is a vector of the markers' estimated effect; **X** is a designed matrix in which the dimension is $n \times m$, where m is the number of markers; and **e** is the residual matrix.

Deviations of SNPs from the Hardy–Weinberg equilibrium (HWE) within populations were identified using Arlequin software version 3.5.1.3 (Excoffier and Lischer 2010).

Results and discussion

In this study, the genotype frequencies of 27 variants located at the 5′ and 3′ UTRs were significantly different between high and low yields of litter size by Fisher's test according to previously published wide-genome data set (Table 1, Genome Variant Map data: GVM000039). This finding suggests that the series of SNPs have a strong correlation with goat litter size. In addition, the genotype data of 27 variants of all individuals were uploaded to European Variation Archive (Project_PRJEB35784, Analyses_ERP118896). Based on the data analysis, we further explore the correlation between 27 variant loci and litter size by expanding the sample size of Dazu goat. Analysis of all 27 variant loci showed that the majority of variants carry high levels of polymorphism, as revealed by H_E, H_O, and PIC. Among these variants, SNP_chr11-70706856 and SNP_chr12-74985766 were homozygous and excluded from the further study (Table 2). According to HWE, seven SNPs within the population deviated from equilibrium, thus suggesting that these SNPs may be subject to artificial selection. The dominant genotype frequencies of the relevant trait-determining sites were fixed due to artificial selection within the population during long-term breeding.

Correlation analysis showed that two SNP loci, namely, SNP_chr17-20182525 ($P = 0.0472$, GE [MAF] $= -0.1908$) and SNP_chr7-65652612 ($P = 0.0493$, GE [MAF] $= -0.2860$), which were located at the 3′ UTR of *SCARB1* (XM_018061401.1: c.∗403G > A) and *FSTL4* (XM_018050393.1: c.∗741C > G), respectively (Table 3), were significantly correlated with the litter size of first parity goats (LF). No SNP was significantly associated with the litter size of second-born animals (LS). The sum litter size of first and second parity animals (SL) was evaluated, and correlation analysis of all SNPs with the first and second parity litter size was performed. The results showed that SNP_chr7-65652612 was significantly associated with SL ($P = 0.0118$, GE (MAF) $= -0.6357$, Table 3).

SCARB mediates the liver uptake and bile secretion of high-density lipoprotein cholesterol, thereby promoting reverse cholesterol transport and slow lipoprotein aggregation; thus, *SCARB* has antiatherosclerotic effects (Takiguchi et al. 2018; Lee et al. 2019). Previous studies have shown that the SNP rs5888 in the *SCARB1* gene is associated with serum lipid levels in a sex-specific manner (Morabia et al. 2004). *SCARB1* has been involved in osteoblast differentiation (Tourkova et al. 2019), the molecular immune mechanism of allicin (Toma et al. 2019), and reproduction performance (Jiménez et al. 2010). However, no direct evidence supports the involvement of *SCARB1* in goat litter size.

SNP_chr7-65652612 in the 3′ UTR of *FSTL4* was not only significantly associated with the litter size of first parity goats and the total litter size of first and second parity goats but it was also a factor promoting significant differences in litter size between different genotypes (Table 4). *FST* is a monoaffinity glycoprotein that binds to many members of the *TGF-β* superfamily via paracrine and autocrine signaling, hence critical to female animal reproduction activity (Passos et al. 2013; Cannarella et al. 2019). A series member of *FSTL* has been found to play a role in reproduction. *FSTL3* levels could be attributed to pre-eclampsia, and they are associated with an increased likelihood of developing pre-eclampsia (Founds et al. 2015). Moreover, *FSTL3* could limit the age-related degeneration and size of testicles (Oldknow et al. 2013). *FSTL4* was recently shown to be a candidate for the reproduction performance in dairy cows (Lu et al. 2021).

Natural selection is the main cause of genetic polymorphism. The diversity of genetic variation in population is the basis for phenotype difference. In this study, 27 gene polymorphisms may lead to changes in protein function, resulting in differences in litter size of Dazu black goat. At present, many studies have concluded that genetic variation is related to economic traits of animals. *IGF1R* c.654g > a variation had significant effects on many economic traits of Polish colored Merino sheep (Grochowska et al. 2020). The variation of *FABP4* is related to the milk production traits of Greek Sfakia sheep (Ibrahim et al. 2019). The sample size in the study is a key pa-

rameter for detection of candidate marker. Although the sample size in this study is much larger than other reported studies (Lai et al. 2016; Islam et al. 2020; Tao et al. 2020; Smołucha et al. 2021), expansion of sample size will surely enhance detection power and reduce false positive rate.

In conclusion, this study explored 26 SNPs and one deletion variation in the UTR and found that two SNPs were associated with high fecundity of Dazu black goats. The results could contribute to revealing the genetic mechanism underlying the high reproduction performance of Dazu goats.

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Conflicts of interest

The authors declare no conflicts of interest.

Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/CJAS-2020-0170.

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