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The genetic diversity of both *FecB* gene and microsatellite *GC101* is associated with reproduction selection in sheep

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Abstract: The aims of study were to reveal the changes in genetic diversity in reproduction selection of sheep. Genetic polymorphisms of microsatellite *GC101* locus and its linkage with the *FecB* gene were detected in Chinese local sheep (Small Tail Han and Hu sheep) and western sheep (Texel and Dorset) breeds. There was the same *FecB* mutation (A746G) in both Small Tail Han and Hu sheep as that in Booroola Merino ewes, but not in Texel and Dorset sheep. Seven alleles, from 192 bp to 210 bp, and 20 genotypes were detected at the *GC101* locus in 447 individuals totally. The preponderant allele was 200 bp for Chinese sheep and 202 bp for western sheep. Linkage analysis showed that there was strong linkage disequilibrium between the 200-bp allele of the *GC101* locus and the B allele of the *FecB* gene ($r^2 = 0.639$). These results preliminarily implied that both the 200-bp allele of the *GC101* locus and the B allele of *FecB* had been reinforced in reproduction selection of Chinese sheep. The differentiation of *GC101* allele distribution between Chinese sheep and western sheep also indicated that the *FecB* of Chinese sheep might be derived from Chinese local areas.

Key words: Sheep, *GC101*, *FecB* gene, prolificacy, reproduction selection

1. Introduction

Many factors, such as genetic drift, artificial selection, and natural selection, can influence genetic diversity. Microsatellite *GC101* is closely linked with the *FecB* (*Fec* = fecundity, *B* = Booroola) gene, a major gene responsible for high prolificacy in sheep (1), and so the genetic diversity of *GC101* may be changed in reproduction selection.

The *FecB* gene was first identified in Booroola Merino sheep based on segregation studies on litter size and ovulation rate (2,3). It is located in ovine chromosome 6 corresponding to human chromosome 4q22-23 that contains the bone morphogenetic protein receptor 1B (*BMPR1B*) gene, which encodes a member of the transforming growth factor β (TGFβ) receptor family (1,4). One point mutation at base 746 of the coding region (746A→G) in the highly conserved intracellular kinase signaling domain of *BMPR1B* caused an amino acid change (249Q→R). The genotypes of *FecB* gene in ewes have been classified as homozygous noncarriers (++), with the ovulation rate of two or less; heterozygous carriers (B+), with the ovulation rate of three to four; and homozygous carriers (BB), with more than five ovulations per estrous cycle (5,6). *FecB* mutation was present in many sheep breeds, such as Booroola Merino (Australia) (1,4), Garole (India) (7), Javanese (Indonesia) (7), Kendrapada (India) (8), Bonpala (India) (9), Kalehkoohi (Iran) (10), Small Tail Han (China) (11,12), Hu (China) (12,13), prolific strains of Chinese Merino (China) (14), Duolang (China) (15), Cele (China) (16), Wadi (China) (17), Altay (China) (18), and Bayanbulak sheep (China) (19).

Now the *FecB* is one of most important fecundity markers widely used in sheep breeding. However, in ancient times, our ancestors selected and improved sheep reproductive performance according to phenotypic characters. Microsatellite *GC101* links with the prolificacy gene, and its genetic diversity might reveal the reproduction selection that happened in the past years. Information on *GC101* is scant. Guan et al. (20) found low genetic diversity (200/200 bp, 200/238 bp) of *GC101* present in Hu sheep. Small Tail Han sheep and Hu sheep are excellent local breeds in China for their significant characteristics of hyperprolificacy and year-round estrus. The mean live litter sizes of Small Tail Han, Hu, Dorset, and Texel sheep have been reported to be 2.61, 2.29 (21), 1.45, and

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1.41 (22), respectively. This study compared and analyzed the polymorphisms of microsatellite *GC101* and the *FecB* gene in Chinese sheep (Small Tail Han and Hu sheep) and western sheep (Texel and Dorset). Linkage disequilibrium analysis between the *FecB* gene and microsatellite locus *GC101* was also conducted in Small Tail Han sheep. The results contribute to the elucidation of the processing of fertility evolution in sheep.

2. Materials and methods

2.1. Experimental materials

Venous jugular blood samples (10 mL per ewe) were collected from 293 Small Tail Han ewes (Jiaxiang Sheep Breeding Farm, Shandong Province, China), 59 Hu ewes (Yuhang Hu Sheep Breeding Farm, Hangzhou, China), and 48 Dorset ewes and 47 Texel ewes (Beijing Aoxin Stud Farm Co. Ltd., Beijing, China) using acid citrate dextrose as an anticoagulant. Genomic DNA was extracted from whole blood using the phenol-chloroform method, dissolved in TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA [pH 8.0]), and kept at –20 °C.

2.2. Microsatellite *GC101* analysis

The primers of microsatellite *GC101*, as were cited from Mulsant et al. (1), are based on the sequences of XM_004009688 in GenBank, forward: 5'-ATCCTCACCCTTCAAACAG-3'; reverse: 5'-CTGGGGAGTTTTCTCTGAC-3'. The primers were synthesized by Shanghai Invitrogen Biotechnology Limited Corporation (Shanghai, China).

Polymerase chain reactions were carried out in volumes of 25 μL containing 1.0 μL of 5 μM of each primer (forward and reverse), 2.5 μL of 10X PCR buffer, 2.5 μL of 200 μmol L^{-1} dNTPs, 0.5 mL of 2.0 U μL^{-1} Taq DNA polymerase, 1.5 mL of 20 mM Mg^{2+}, and 3.0 μL of 50 ng μL^{-1} DNA template, and the rest was ddH_2O. Amplification conditions were as follows: initial denaturation at 94 °C for 7 min, followed by 32 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 10 min on a Mastercycler 5333 (Eppendorf AG, Hamburg, Germany). The PCR products were kept at 4 °C and detected by electrophoresis on 1.5% agarose gels (Promega, Madison, WI, USA).

The PCR product was loaded onto 12% nondenaturing polyacrylamide gel (acrylamide:bisacrylamide = 29:1) for electrophoresis. The electrophoresis was carried out at 200 V for 2 h, and the gel was subsequently silver-stained and then photographed and analyzed using AlphaImager 2200 and 1220 Documentation and Analysis Systems (Alpha Innotech Corporation, San Leandro, CA, USA).

2.3. PCR-RFLP analysis

The *FecB* genotyping was conducted by PCR-RFLP technique as described by Chu et al. (11). The PCR products were digested by *AvaII* restriction enzyme (NEB, Beijing, China) at 37 °C overnight. After restriction enzyme digestion, the products were detected by 2% agarose gels and genotyped using AlphaImager 2200 and 1220 Documentation and Analysis Systems (Alpha Innotech Corporation).

2.4. Statistical analysis

Genotype frequency, allele frequency, and allele number (N) were determined by direct counting and calculating. Polymorphism information content (PIC), number of effective alleles (E), and heterozygosity (h) of microsatellites were estimated following Botstein et al. (23) and van Zeveren et al. (24).

The linkage disequilibrium (LD) analysis between microsatellite *GC101* and the *FecB* gene was performed using Arlequin 3.1 software. Standardized linkage disequilibrium coefficient D′ and the coefficient of determination r^2 were calculated as per Pritchard and Przeworski (25).

3. Results

3.1. Polymorphism analysis of sheep microsatellite *GC101*

As shown in Figure 1, the PCR product of microsatellite *GC101* was a specific fragment of about 200 bp in length without nonspecific amplification bands. It was used for microsatellite *GC101* polymorphism analysis directly, and the results of the main genotypes of microsatellite *GC101* are shown in Figure 2.

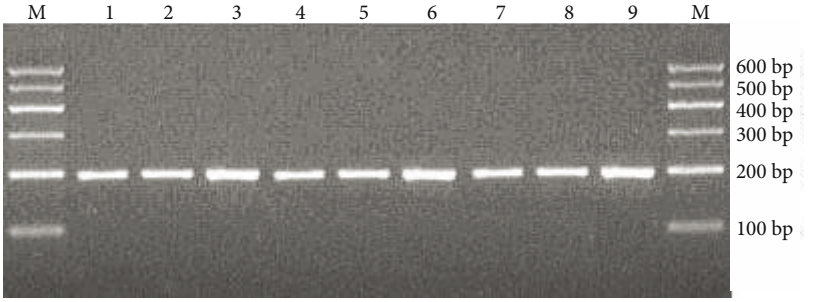

Figure 1. PCR products of microsatellite *GC101* in sheep. 1–9: PCR products of microsatellite GC101; M: 600-bp DNA marker.

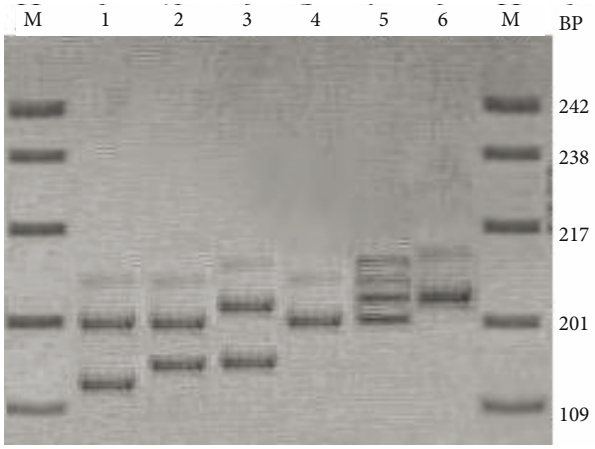

Figure 2. The main genotypes of microsatellite *GC101* in sheep. 1: 192 bp/200 bp; 2: 196 bp/200 bp; 3: 196 bp/202 bp; 4: 200 bp/200 bp; 5: 200 bp/202 bp; 6: 202 bp/202 bp; M: *pBR322/Msp*I.

The genotypes and allele frequencies of *GC101* in four sheep breeds are given in Table 1. Seven alleles from 192 bp to 210 bp and 20 genotypes of microsatellite *GC101* were detected in the present study. The number of the genotypes and alleles was 17, 3, 6, and 9 and 6, 3, 4, and 5, and the preponderant allele was 200 bp (0.686), 200 bp (0.966), 202 bp (0.478), and 202 bp (0.677) in the four breeds (Small Tail Han, Hu, Texel, Dorset sheep), respectively. These results indicated that *GC101* genotype distribution between Chinese (Small Tail Han and Hu sheep) and western breeds (Texel and Dorset) was highly different.

Genetic parameters of microsatellite *GC101* and litter size in four sheep breeds are presented in Table 2. The polymorphisms of *GC101* were abundant in these sheep breeds except Hu sheep (PIC = 0.065). The allele distribution of *GC101* was even in Texel and Dorset sheep, and it was uneven in Small Tail Han sheep (N = 6; E = 1.934).

3.2. Polymorphism analysis of *FecB* gene

Three genotypes, BB (110 bp / 110 bp), B+ (110 bp / 140 bp), and ++ (140 bp / 140 bp), were detected in the present study. Allele and genotype frequencies of the *FecB* gene are presented in Table 3. There were three genotypes (BB, B+, and ++) in Small Tail Han sheep, two genotypes (BB and B+) in Hu sheep, and only the ++ genotype in Texel and Dorset sheep. B allele frequency in prolific Small Tail Han and Hu sheep was 0.677 and 0.898, respectively. The results indicated that the B allele of the *FecB* gene only existed in two prolific sheep breeds.

3.3. Microsatellite *GC101* distribution in different genotypes of the *FecB* gene in Small Tail Han and Hu sheep

Based on *FecB* genotyping results, Small Tail Han sheep were divided into three groups, BB, B+, and ++, and Hu sheep were divided into two groups, BB and B+. The microsatellite *GC101* genotype and allele distribution in these flocks are summarized in Table 4. In five populations (BB, B+, and ++ groups from Small Tail Han sheep; BB and B+ groups from Hu sheep), the number of genotypes and alleles was 5, 9, 12, 1, and 3 and 4, 6, 6, 1, and 3,

Table 1. Allele and genotype frequencies of microsatellite *GC101* in four sheep breeds.

Breed	Genotype (frequency)	Allele (frequency)
Small Tail Han sheep (n = 293)	192/192 (0.003), 192/196 (0.008), 192/198 (0.003), 192/200 (0.020), 192/202 (0.003), 196/196 (0.003), 196/200 (0.058), 196/202 (0.055), 196/210 (0.008), 198/200 (0.003), 198/202 (0.008), 198/210 (0.003), 200/200 (0.515), 200/202 (0.242), 200/210 (0.017), 202/202 (0.048), 202/210 (0.003)	192 bp (0.020) 196 bp (0.067) 198 bp (0.009) 200 bp (0.686) 202 bp (0.203) 210 bp (0.015)
Hu sheep (n = 59)	196/200 (0.051), 200/200 (0.932), 200/202 (0.017)	196 bp (0.025) 200 bp (0.966) 202 bp (0.009)
Texel sheep (n = 47)	196/196 (0.255), 196/198 (0.021), 196/200 (0.043), 196/202 (0.319), 200/202 (0.085), 202/202 (0.277)	196 bp (0.447) 198 bp (0.011) 200 bp (0.064) 202 bp (0.478)
Dorset sheep (n = 48)	194/196 (0.042), 196/196 (0.021), 198/200 (0.021), 200/200 (0.042), 200/202 (0.042), 194/202 (0.083), 196/202 (0.250), 200/202 (0.021), 202/202 (0.478)	194 bp (0.063) 196 bp (0.167) 198 bp (0.010) 200 bp (0.083) 202 bp (0.677)

Table 2. Genetic parameters of microsatellite *GC101* in four sheep breeds.

Breed	Number	Litter size	Genetic parameter			
			PIC	h	N	E
Small Tail Han sheep	293	2.56 ± 0.73[a]	0.439	0.483	6	1.934
Hu sheep	59	2.45 ± 0.52[a]	0.065	0.066	3	1.071
Texel sheep	47	1.42 ± 0.44[b]	0.472	0.567	4	2.312
Dorset sheep	48	1.38 ± 0.41[b]	0.465	0.502	5	2.009

Litter sizes are presented as mean ± SEM; a and b refer to significant differences ($P < 0.05$).

Table 3. Allele and genotype frequencies of *FecB* gene in four sheep breeds.

Breed	Number	Allele frequency		Genotype frequency		
		B	+	BB	B+	++
Small Tail Han sheep	293	0.677	0.323	0.478 (140)	0.399 (117)	0.123 (36)
Hu sheep	59	0.898	0.102	0.797 (47)	0.203 (12)	0.000 (0)
Texel sheep	47	0	1	0 (0)	0 (0)	1 (47)
Dorset sheep	48	0	1	0 (0)	0 (0)	1 (48)

The numbers in parentheses are the individuals that belong to the respective genotypes.

Table 4. Allele and genotype frequencies of microsatellite *GC101* in different genotypes of *FecB* in Small Tail Han and Hu sheep.

Breed	Genotype of *FecB*	Genotype (frequency)	Allele (frequency)
Small Tail Han sheep	BB (n = 140)	196/210 (0.007), 200/200 (0.893), 200/202 (0.079), 200/210 (0.014), 202/202 (0.007)	196 bp (0.004) 200 bp (0.939) 202 bp (0.046) 210 bp (0.011)
	B+ (n = 117)	192/200 (0.051), 196/200 (0.145), 196/202 (0.026), 198/200 (0.009), 200/200 (0.214), 200/202 (0.504), 200/210 (0.026), 202/202 (0.016), 202/210 (0.009)	192 bp (0.026) 196 bp (0.086) 198 bp (0.004) 200 bp (0.581) 202 bp (0.286) 210 bp (0.017)
	++ (n = 36)	192/192 (0.028), 192/196 (0.055), 192/198 (0.028), 192/202 (0.028), 196/196 (0.028), 196/202 (0.361), 196/210 (0.028), 198/202 (0.055), 198/210 (0.028), 200/200 (0.028), 200/202 (0.028), 202/202 (0.305)	192 bp (0.083) 196 bp (0.250) 198 bp (0.056) 200 bp (0.042) 202 bp (0.542) 210 bp (0.027)
Hu sheep	BB (n = 47)	200/200 (1.000)	200 bp (1.000)
	B+ (n = 12)	196/200 (0.250), 200/200 (0.667), 200/202 (0.083)	196 bp (0.125) 200 bp (0.833) 202 bp (0.042)

and the preponderant allele was 200 bp (0.939), 200 bp (0.581), 202 bp (0.542), 200 bp (1.000), and 200 bp (0.833), respectively. The results showed that the 200-bp and 202-bp allele of *GC101* was the preponderant allele in the BB and ++ groups, respectively.

Genetic parameters of microsatellite *GC101* and litter sizes in five groups are given in Table 5. The polymorphisms of *GC101* in these groups were rather low, except B+ and ++ from Small Tail Han sheep (PIC = 0.510, 0.585). The distribution of *GC101* in the BB population from Small Tail Han sheep was extremely uneven.

3.4. Linkage disequilibrium analysis between microsatellite *GC101* and the *FecB* gene in Small Tail Han sheep

Linkage disequilibrium parameter D′ (r^2) and P-values between the *FecB* gene and microsatellite *GC101* in Small Tail Han sheep are shown in Table 6. Linkage analysis indicated that there was strong linkage disequilibrium between the 200-bp allele of the *GC101* locus and the B allele of the *FecB* gene (D′ = 0.816, r^2 = 0.639), certain linkage disequilibrium between the 196-bp and 202-bp alleles of the *GC101* locus and + allele of the *FecB* gene (D′= 0.962, 0.777), and complete linkage between the 192-bp and 198-bp alleles of the *GC101* locus and + allele of the *FecB* gene (D′ = 1.000).

4. Discussion

The genetic diversity of microsatellites is widely distributed in sheep. In this study, seven alleles from 192 bp to 210 bp and 20 genotypes were detected at the *GC101* locus. Most of the alleles were novel, except the 200-bp allele, as compared with Guan et al. (20). This novel variation could be used in the research of population diversity.

Both Small Tail Han and Hu sheep were derived from Mongolian sheep (breeds with low litter sizes), which underwent breeding for high prolificacy about 1000 years ago. Texel, a cultivated breed from the Netherlands with medium fertility, was formed in the beginning of the 20th century. Dorset, a cultivated breed from Australia with medium fertility, was formed in the 1950s. All of them

Table 5. Genetic parameters of microsatellite *GC101* in different genotypes of *FecB* in Small Tail Han and Hu sheep.

Breed	Genotype of *FecB*	Number	Litter size	Genetic parameter			
				PIC	h	N	E
Small Tail Han sheep	BB	140	2.89 ± 0.93[a]	0.112	0.116	4	1.131
	B+	117	2.41 ± 0.43[ab]	0.510	0.572	6	2.338
	++	36	1.75 ± 0.54[b]	0.585	0.631	6	2.712
Hu sheep	BB	47	2.56 ± 0.58	0	0	1	1
	B+	12	2.02 ± 0.32	0.264	0.289	3	1.406

Litter sizes are presented as mean ± SEM; a and b refer to significant differences (P < 0.05).

Table 6. Linkage disequilibrium parameters D′ (r^2) and P values between *FecB* gene and microsatellite *GC101* in Small Tail Han sheep.

Allele	*GC101* microsatellite locus					
	192 bp	196 bp	198 bp	200 bp	202 bp	210 bp
B	–1.000 (0.044) P = 0.000 N=0	–0.962 (0.139) P = 0.000 N=1	–1.000 (0.018) P = 0.001 N=0	0.816 (0.639) P = 0.000 N=374	-0.777 (0.322) P = 0.000 N=18	-0.344 (0.004) P = 0.132 N=4
+	1.000 (0.044) P = 0.000 N=12	0.962 (0.139) P = 0.000 N=38	1.000 (0.018) P = 0.001 N=5	–0.816 (0.639) P = 0.000 N=28	0.777 (0.322) P = 0.000 N=101	0.344 (0.004) P = 0.132 N=5

N is the number of haplotypes.

had been involved in reproductive artificial selection. Interesting, as for the four breeds (Small Tail Han, Hu, Texel, Dorset sheep), the preponderant allele was 200 bp (0.686), 200 bp (0.966), 202 bp (0.478), and 202 bp (0.677), respectively. It seemed that the preponderant allele was 200 bp for Chinese sheep and 202 bp for western sheep. These results implied that Chinese sheep and western sheep were cultivated from different selection systems in reproduction. The 200-bp allele of *GC101* was dominant in Chinese sheep, had very low frequency in Texel, and was absent in Dorset, indicating that this allele might have come from Asian sheep, not European or Australian sheep. The 202-bp allele of *GC101* existed in both western and Chinese sheep, meaning that this allele might have come from a common ancestor.

In the past, our ancestors selected good livestock based on phenotype. Thus, some prolificacy genes accompanied with some linkage microsatellites were selected and enhanced. As the selection force was enhanced, the number of haplotypes decreased. The high diversity of *GC101* in Small Tail Han sheep reflected the shortage of breeding caused by free-range farming in China, and the low diversity in Hu sheep accorded with the fact that strong selection for fertility had happened in recent years (26,27). The medium diversity of *GC101* in western sheep implied that less selection was conducted on these sheep.

The polymorphism of the *FecB* gene had been of interest all over the world. In the last few years, our group devoted its attention to its polymorphism detection (28,29) and demonstrated that it had rich polymorphism. In this study, there was the same *FecB* mutation (A746G) of the *BMPR1B* gene in both Small Tail Han and Hu sheep as in Booroola Merino ewes, and none in Texel and Dorset sheep. The genotype frequencies of BB, B+, and ++ were 0.478, 0.399, and 0.123 in Small Tail Han sheep and were 0.797, 0.203, and 0.000 in Hu sheep, respectively. Linkage analysis indicated that there was strong linkage disequilibrium between the 200-bp allele of the *GC101* locus and the *B* allele of the *FecB* gene ($D' = 0.816$, $r^2 = 0.639$). This implied that the selection site of fertility in ancient China was *FecB*. It also indicated that the mutation (*FecB*) might have happened in the *GC101* 200-bp allele linkage chromosome when humans domesticated the animals, and then the 200-bp allele of the *GC101* locus accompanied with the *B* allele was reinforced in reproductive selection. Above all, the distribution of the 200-bp allele of *GC101* of Chinese sheep and western sheep indicated that the *FecB* of Chinese sheep might have derived from Chinese local areas.

In conclusion, Chinese sheep and western sheep were bred from different selection systems in reproduction. The diversity of *GC101* in Small Tail Han sheep reminded us that its degeneration had already happened. The selection of *FecB* in China had been undertaken for a long time, and this mutation might have originated from the processing of animal domestication.

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