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Two strongly linked blocks within the *KIF16B* gene significantly influence wool length and greasy yield in fine wool sheep (*Ovis aries*)

Haiyu Zhao, Ruixue Hu, Fadi Li, Xiangpeng Yue *

State Key Laboratory of Grassland Agro-ecosystems, Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs, Engineering Research Center of Grassland Industry, Ministry of Education, College of Pastoral Agriculture Science and Technology, School of Life Sciences, Lanzhou University, No. 222 South Tianshui Road, Lanzhou 730000, Gansu Province, PR China

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ABSTRACT

Background: A previous genome-wide association study (GWAS) identified the kinesin family member 16B (KIF16B) as a candidate gene related to sheep wool production. In this work, DNA pool sequencing and SNPscanTM high-throughput genotyping methods were used to detect single-nucleotide polymorphisms (SNPs) in the sheep KIF16B gene. The correlations between the SNPs and wool length and greasy wool yield were systematically assessed.

Results: Forty-five SNPs were identified and 37 of them were genotyped, including 10 exon mutations, 26 intron mutations, and 1 promoter region mutation. Most of the SNPs were of medium genetic diversity and at Hardy-Weinberg equilibrium (HWE). Among them, 10 SNPs were associated with greasy wool yield and 28 SNPs impact the wool length. Five specific SNPs were found to exert significant effects on the wool length in all body parts analyzed in this study. Furthermore, linkage disequilibrium (LD) analysis was conducted among SNP loci and they were found to be significantly associated with economically important traits. Two strongly linked SNP blocks were identified within these SNPs and they might exert significant impacts on the greasy wool yield and wool length.

Conclusions: The identified SNPs exert significant effects on wool production and could be considered as potential DNA markers for selecting the individuals with superior phenotypes.

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1. Introduction

The growth status of wool from sheep relates to several factors, such as heredity, environmental factors, gender, age, and nutrition [1] among which heredity is one of the most decisive factors affecting the overall production and quality of wool [2]. For identifying candidate genes linked to phenotypes, genome-wide association study (GWAS)—which is aimed at searching for single-nucleotide polymorphism (SNPs) related to traits of organisms [3]—was first applied in human disease gene research. It has been used to identify susceptible genomic areas, related genes, and SNPs of many diseases [4]. With the decline of sequencing costs, GWAS also has been used in the detection of major genes related to economically-important traits in livestock and poultry [5,6,7]. A recent GWAS study of 765 Chinese merino sheep found 28 genome-wide association significant SNPs for fiber diameter, fiber diameter coefficient of variation, fineness dispersion, and crimp traits. Among these SNPs, 43% were located on the *YWHAZ*, *KRTCAP3*, *TSPEAR*, *PIK3R4*, *KIF16B*, *PTPN3*, *GPRC5A*, *DDX47*, *TCF9*, *TPTE2*, *EPHA5*, and *NBEA* genes [3]. Following this, we speculated that the *KIF16B* gene could be a candidate gene affecting wool traits in sheep because of the key functions of kinesin superfamily proteins (KIFs).

KIFs serve as molecular motors on microtubule systems and transport various cellular proteins, macromolecules, and organelles [8]. As a member of the kinesin-3 family (KIF3), the 16B (*KIF16B*) gene transports early endosomes (EE) of the positive Rab5 and the terminal kinesin of Rab14 vesicles in non-neuronal cells [9]. The KIF16B has a conserved motor domain and three coiled-coil and PX domains in the stem domain. In human cervical cancer cells, the *KIF16B* gene regulates the recovery and degradation of the epidermal growth factor (EGF) receptor by controlling

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* Corresponding author.
E-mail address: lexp@lzu.edu.cn (X. Yue).

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Table 1
The information of experimental animals and wool samples collection.

Farms	Population	Number	Age	Sex	Measured economic traits
XT	SG	40	Yearling	Male	Wool length
MX	SSG	90	Yearling	Male	Wool length
RM	SSG	16	Yearling	Male	Wool length
SYS	SG	207	Adult	Female	Wool length and the greasy wool weight
XM	SSG	103	Yearling	Male	Wool length
XM	SSG	188	Adult	Female	The greasy wool weight

Note: XT, Tianzhu Xingtai Agriculture and Animal Husbandry Technology Ltd; MX, Tianzhu Muxing Breeding Professional Cooperative; RM, Tianzhu Rongmu Breeding Professional Cooperative; SYS, Tianzhu Sanyangsheng Bioengineering Company; XM, Tianzhu Xumu Breeding Professional Cooperative.

Table 2
The primer information of PCR amplification.

Names	Forward Primer sequences (5′>3′)	Reverse Primer sequences (5′>3′)	Region	Sizes (bp)	Tm (°C)
P1	AGGGTGGATTCCATAACT	TTTACCAAATGGCTGTCC	Exon1	439	56.2
P2	GCCACATTCAGCCGAGAC	GTGCGTCAGAACCAAGAG	Exon1	522	62.8
P3	ACTGACAGGGCAGCCAATG	AGGTCCCGCTGTGAGGTT	Exon3 and part Intron 2, 3	484	56.3
P4	CAGAAGAGCGATGAAGGGAC	GGAACATCAAAGCCAGACAT	Exon4 and part Intron 3	404	62
P5	AAGGCAACCCTATCAATC	GTGCTAAATTATCCCAAG	Exon5 and part Intron4	465	52
P6	TTCGCTCACCTTCACCAT	GAGTCTCCCTTCTGTTCC	Part Exon6	352	56.2
P7	ATCTTGTATAGAGCCCTTCA	TTGTCTGGGTCTCCTGTG	Exon7 and part Intron6	340	56.9
P8	TGCCTTCGCTTGAACTCT	TGATCCATCACCGCTTTAC	part Exon8 and Intron8	601	61.8
P9	CCAATACAGGCACCCACA	AACCTAACAGCCAACTCT	Exon9 and part Intron8, 9	701	58
P10	ATCGCTATGGCTGACTTGA	TCCTGGTCTCCCTGCTCCTC	Exon10 and part Intron9, 10	563	55
P11	GATTCCACGCTGCTCACC	GACCCTACGCTTTGTTTG	Exon11 and part Intron10, 11	453	56
P12	CCCATTACCTAGCCAACT	CATCTGTCCCTTCCATTT	Exon12 and part Intron11, 12	540	53.2
P13	ATCAAGATTCTCACCGATAT	CTCCCAAACAGAGTAGCC	Exon13 and part Intron12, 13	531	52
P14	GTTCCAGGTAAGCAGTTTC	GCCAGGATTTCAGCACGA	Exon14 and part Intron13, 14	551	54.7
P15	CCCAACCTAAGCAAAGCA	GACCCAAAGAATAATCAG	Exon15 and part Intron14, 16	531	54.5
P16	TAAGCACCTTCCCAGAGT	GATTTCGTGGTGACTAACA	Exon16 and part Intron15	743	59
P17	CTCCCGAGATGAGTGTCT	ACCTTTCCTATGAGTCCC	Exon17 and part Intron16, 17	428	58.5
P18	TTTCCGCCGAGGCTATCT	ATGTATGGCTGAGTTGCT	Exon19 and part Intron18, 19	794	59.5
P19	TCAGTGGCAGCCCGTGTA	GAGGCAGTTTGGTCAGTGGT	Exon21 and part Intron20, 21	567	63.8
P20	GACTTCCAACCAGCACAG	CCGACCGAAACATGACTA	Exon22 and part Intron21, 22	763	56.2
P21	CCTCCTCTGCCGCCTATT	TTGGCACAGATGTCGTGAAGT	Exon23 and part Intron22	675	58.9
P22	AGCCTTCAACAAGTCATC	GGATTTGAGTCAGGGTAT	Exon24 and part Intron23, 24	839	54.7
P23	CACTTGTCACCCATTTGC	GTCCTCGTGTTTATGTTCTA	Exon25 and part Intron24, 25	411	54.7
P24	CTTGCCAGCATTCCTTCA	AATGGGTAACGAAACAGG	Part Exon1 and promoter	798	60.8

Table 3
SNPs scanning results of the *KIF16B* gene.

Names	HGVS names	Position	Mutation type	Name	HGVS names	Position	Mutation type
SNP1	g .9536869 A > G	Exon1	Arginine → Glycine	SNP24	g .9680510 A > G	Intron9	–
SNP2	g .9537002 T > C	Exon1	Cysteine → Arginine	SNP25	g .9702360 G > A	Exon12	–
SNP3	g .9537032 G > T	Exon1	Alanine → Serine	SNP26	g .9702423 G > A	Intron12	–
SNP4	g .9537857 A > G	Exon1	Methionine → Alanine	SNP27	g .9702567 T > C	Intron12	–
SNP5	g .9537858 T > C	Exon1	–	SNP28	g .9705304 G > A	Intron14	–
SNP6	g .9537870 C > T	Exon1	Arginine → Stop	SNP29	g .9778233 A > G	Intron15	–
SNP7	g .9537973 A > G	Intron1	–	SNP30	g .9778314 T > C	Intron15	–
SNP8	g .9538022 T > C	Intron1	–	SNP31	g .9784117 T > C	Intron17	–
SNP9	g .9538065 G > C	Intron1	–	SNP32	g .9784129 C > T	Intron17	–
SNP10	g .9595606 T > C	Exon3	Methionine →Threonine	SNP33	g .9795744 G > A	Intron21	–
SNP11	g .9595630 T > C	Intron3	–	SNP34	g .9795813 T > C	Intron21	–
SNP12	g .9595682 A > C	Intron3	–	SNP35	g .9795859 T > C	Intron21	–
SNP13	g .9595711 A > C	Intron3	–	SNP36	g .9795903 A > G	Intron21	–
SNP14	g .9595721 T > C	Intron3	–	SNP37	g .9796525 G > C	Intron22	–
SNP15	g .9595726 A > G	Intron3	–	SNP38	g .9796553 C > T	Intron22	–
SNP16	g .9595760 T > C	Intron3	–	SNP39	g .9796550 G > A	Intron22	–
SNP17	g .9595803 A > G	Intron3	–	SNP40	g .9796787 C > G	Intron22	–
SNP18	g .9595878 T > C	Intron3	–	SNP41	g .9798272 A > T	Intron22	–
SNP19	g .9595881 C > G	Intron3	–	SNP42	g .9798321 A > G	Intron22	–
SNP20	g .9595914 C > G	Intron3	–	SNP43	g .9536061 A > C	Promoter	–
SNP21	g .9616389 G > A	Intron4	–	SNP44	g .9536301 C > T	Exon1	Serine → Proline
SNP22	g .9616434 G > A	Intron4	–	SNP45	g .9536340 C > T	Exon1	Threonine →Threonine
SNP23	g .9616567 C > T	Intron4	–				

the position and function of EE [10]. In mouse embryonic fibroblasts, KIF16B transports vesicles from the Golgi apparatus to the plasma membrane through the EGF receptor [9]. It has been reported that KIF16B took part in the formation and division of microtubules by inducing early endosomal fusion and mediated transferrin receptor transcytosis to the top of epithelial cells to

Table 4
Genetic parameters of 37 successfully genotyped SNPs in the sheep *KIF16B* gene.

SNP Loci	Genotypic frequencies			Ho	He	Ne	PIC	HWE *P*-values
	D	H	R					
SNP1	0.57	0.37	0.06	0.63	0.37	1.59	0.30	1.000
SNP2	0.17	0.49	0.34	0.51	0.49	1.94	0.37	0.933
SNP3	0.55	0.38	0.07	0.61	0.39	1.63	0.31	0.752
SNP4	0.22	0.51	0.27	0.50	0.50	1.99	0.37	0.621
SNP5	0.11	0.43	0.46	0.56	0.44	1.79	0.34	0.458
SNP8	0.22	0.51	0.27	0.50	0.50	2.00	0.37	0.567
SNP9	0.23	0.52	0.25	0.50	050	2.00	0.37	0.513
SNP10	0.60	0.35	0.05	0.65	0.35	1.53	0.29	0.8133
SNP11	0.75	0.24	0.01	0.77	0.23	1.30	0.20	0.477
SNP12	0.58	0.37	0.05	0.64	0.36	1.57	0.30	0.498
SNP14	0.41	0.46	0.13	0.54	0.46	1.85	0.35	0.859
SNP16	0.60	0.35	0.05	0.66	0.34	1.52	0.28	0.632
SNP17	0.13	0.47	0.40	0.54	0.46	1.85	0.35	0.791
SNP18	0.04	0.35	0.61	0.66	0.34	1.53	0.29	0.637
SNP19	0.32	0.51	0.17	0.51	0.49	1.96	0.37	0.277
SNP20	0.03	0.32	0.65	0.70	0.30	1.44	0.26	0.138
SNP21	0.46	0.46	0.06	0.57	0.43	1.74	0.34	0.068
SNP22	0.42	0.44	0.14	0.54	0.46	1.85	0.35	0.330
SNP23	0.32	0.48	0.20	0.51	0.49	1.96	0.37	0.507
SNP25	0.30	0.55	0.15	0.51	0.49	1.96	0.37	0.005*
SNP26	0.04	0.23	0.73	0.74	0.26	1.36	0.23	0.005*
SNP27	0.06	0.35	0.59	0.64	0.36	1.56	0.30	0.428
SNP29	0.20	0.48	0.32	0.51	0.49	1.96	0.37	0.562
SNP30	0.32	0.48	0.20	0.51	0.49	1.96	0.37	0.620
SNP31	0.66	0.31	0.03	0.70	0.30	1.42	0.25	0.166
SNP32	0.56	0.40	0.04	0.64	0.36	1.57	0.30	0.009*
SNP33	0.37	0.44	0.19	0.52	0.48	1.94	0.37	0.028*
SNP34	0.26	0.49	0.25	0.50	0.50	2.00	0.37	0.684
SNP35	0.38	0.48	0.14	0.53	0.47	1.89	0.36	0.862
SNP36	0.39	0.47	0.14	0.53	0.47	1.89	0.36	0.862
SNP37	0.15	0.41	0.45	0.55	0.45	1.83	0.35	0.015*
SNP40	0.04	0.36	0.60	0.66	0.34	1.52	0.28	0.284
SNP41	0.28	0.55	0.17	0.51	0.49	1.98	0.37	0.017*
SNP42	0.61	0.34	0.05	0.66	0.34	1.51	0.28	0.904
SNP43	0.39	0.46	0.15	0.53	0.47	1.88	0.36	0.794
SNP44	0.15	0.47	0.38	0.53	0.47	1.89	0.36	0.862
SNP45	0.41	0.46	0.13	0.54	0.46	1.86	0.36	0.930

Note: D, homozygous wild type genotype; H, heterozygous mutant genotype; R, homozygous mutant genotype; Ho, homozygosity; He, heterozygosity; Ne, number of effective allele; PIC, polymorphism information content; HWE, Hardy-Weinberg equilibrium.

recirculate endosomes [11]. Additionally, the characteristics of KIF16B in neurons reveals a new molecular "stem inhibition" which can enhance the ability of selective somatic dendritic localization in the early stage [12].

There is no documentation of the impact of the *KIF16B* gene on wool production and quality. In this study, we identified SNP loci within the sheep *KIF16B* gene and analyzed potential effects on wool production and quality to provide candidate DNA molecular markers.

2. Materials and methods

All experimental protocols were reviewed and approved by the Ethics Committee of the College of Pastoral Agriculture Science and Technology in Lanzhou University (Ethic approval file No: 2010-1 and 2010-2). All efforts were taken to minimize animal suffering.

2.1. Sample collection and DNA isolation

A total of 644 individuals of South African mutton merino Gansu alpine fine wool crossbred sheep (SG) and South African mutton merino SG crossbred sheep (SSG) were randomly selected from five sheep breeding farms (Table 1). Detailed records of the greasy wool weight and wool length in body parts (shoulder, side, thigh, notum, and abdomen) were available for all selected individuals.

Genomic DNA was isolated from blood leukocytes according to a published method [13]. The quality of genomic DNA was assayed by Thermo NanoDrop 2000 based on the 1.8<OD260/280<2.0 standard. Qualified DNA samples were diluted to the concentration of 50 ng/μL and stored at 20°C for the downstream experiments.

2.2. Primer design, SNP screening and SNPscan genotyping

Based on the sheep *KIF16B* gene sequence (GenBank accession NO. NC_040264.1) in the NCBI database (https://www.ncbi.nlm.nih.gov/), 24 pairs of primers (Table 2) were designed to amplify the full length of this gene using Primer Premier 6.0. A DNA pooling sequencing method was used to screen putative SNPs in each amplified fragment. A total of 30 DNA pools were mixed and each pool was composed of DNA from 20 sheep individuals. The PCR was performed in a 25 μL reaction condition containing 1.0 μL of pooled DNA, 0.4 μL of each forward and reverse primer (10 pmol/μL), 12.5 μL of 2 × Taq PCR Super MIX (TranGen), and 10.7 μL of ddH2O. The PCR amplification protocol contained a pre-denaturation at 94°C for 5 min and denaturation at 94°C for 30 s, followed by annealing for 30 s at the optimal temperature, 30 cycles of elongation at 72°C for 30 s, and a final extension at 72°C for 5 min with subsequent cooling to 4°C. Thereafter, PCR products were directly sequenced by the Liuhe Huada Gene Technology Company (Beijing, China) and Aoke Dingsheng Biotechnology Company (Beijing, China), and sequence alignments were carried out using DNAstar (DNAstar, USA) and chromas (Technely-

Table 5
The association analysis of SNPs within *KIF16B* gene with greasy wool weigh and natural wool length.

SNP Loci	Greasy wool weigh	Natural wool length				
		Shoulder	Side	Thigh	Notum	Abdomen
SNP1	0.978	0.831	0.246	0.446	0.760	0.144
SNP2	0.103	**0.001**	0.235	0.113	**0.025**	0.279
SNP3	0.517	0.849	0.154	0.267	0.448	0.137
SNP4	**0.004**	**2.45E-5**	0.270	**0.011**	**0.40E-3**	0.343
SNP5	0.253	0.879	0.291	0.178	0.260	0.055
SNP8	**0.005**	**1.25E-5**	0.114	**0.009**	**0.20E-3**	0.310
SNP9	**0.005**	**9.42E-7**	**0.047**	**0.005**	**3.51E-5**	0.230
SNP10	0.311	0.399	**0.004**	0.088	0.102	**0.025**
SNP11	**0.048**	0.410	**0.002**	0.166	**0.048**	0.155
SNP12	0.084	0.433	**1.49E-5**	**0.002**	**0.0013**	**0.004**
SNP14	0.837	**0.014**	0.056	0.072	**0.023**	0.201
SNP16	0.316	0.575	**0.002**	0.067	0.082	**0.030**
SNP17	0.855	**0.008**	**0.043**	0.068	**0.016**	0.196
SNP18	0.316	0.713	**0.004**	0.067	0.113	0.054
SNP19	0.218	**0.028**	**0.044**	**0.015**	**0.0048**	0.077
SNP20	0.437	0.644	**0.002**	0.105	0.096	**0.050**
SNP21	**0.80E-3**	**5.02E-5**	**0.009**	**0.002**	**6.18E-6**	**0.024**
SNP22	**0.50E-3**	**2.81E-9**	**0.006**	**0.2E-3**	**4.61E-5**	**0.015**
SNP23	**0.012**	**0.003**	**0.038**	**0.004**	**0.004**	**0.008**
SNP25	**0.021**	**0.013**	**0.023**	**0.008**	**0.70E-3**	0.211
SNP26	0.522	**0.003**	0.062	**0.9E-3**	**0.003**	0.759
SNP27	0.066	**0.010**	0.665	**0.012**	**0.007**	0.147
SNP29	**0.019**	0.199	**0.002**	0.318	0.284	0.520
SNP30	**0.013**	0.189	**0.002**	0.321	0.277	0.530
SNP31	0.612	0.121	0.168	0.167	0.076	**0.041**
SNP32	0.979	**0.003**	**0.008**	**0.012**	**1.40E-3**	**0.006**
SNP33	0.589	**2.21E-7**	0.181	**0.20E-3**	**0.001**	0.290
SNP34	0.060	0.549	0.522	0.080	0.231	0.106
SNP35	0.141	0.302	0.016*	0.532	0.051	0.492
SNP36	0.119	0.268	0.010*	0.487	**0.045**	0.241
SNP37	0.076	0.114	0.122	0.167	0.065	0.333
SNP40	0.714	**0.019**	0.141	0.219	0.399	0.057
SNP41	0.460	0.688	0.745	0.410	0.658	0.495
SNP42	0.090	**9.61E-9**	**0.011**	**0.30E-3**	**2.63E-7**	**0.006**
SNP43	0.725	0.783	0.333	0.998	0.971	0.573
SNP44	0.156	0.267	0.249	0.772	0.348	0.779
SNP45	0.114	0.439	0.299	0.741	0.475	0.963

Note: the bold numbers indicate the *P*-values with significance ($P < 0.05$ or $P < 0.01$).

Table 6
The results of haplotype construction with strong chain SNPs in greasy wool weight.

SNP4&SNP8&SNP9	SNP22&SNP23	SNP29&SNP30
GCC (0.513)	AC (0.358)	AC (0.442)
ATG (0.477)	GT (0.438)	GT (0.558)

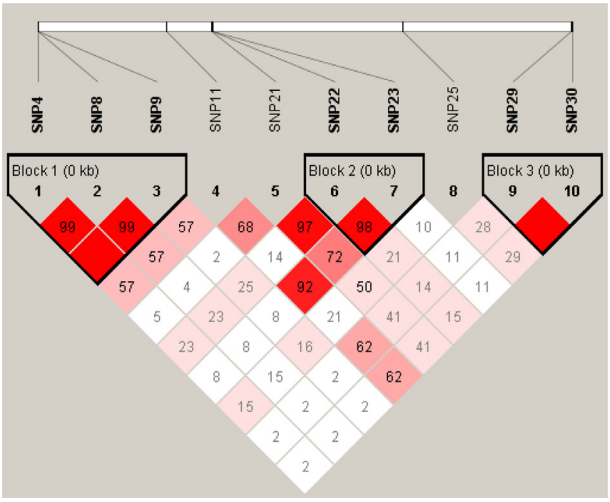

Fig. 1. LD analysis of SNPs with greasy wool weight.

Table 7
The correlation analysis between combined genotype of strong chain SNPs and greasy wool weight.

Combined SNPs	SNP4&SNP8	Greasy wool weight	*P*-values
SNP4&SNP8	AATT (65)	3.86 ± 0.79	0.008**
	GACT (165)	3.55 ± 0.80	
	GGCC (96)	3.50 ± 0.63	
SNP4&SNP9	AAGG (65)	3.86 ± 0.79	0.009**
	GAGC (165)	3.55 ± 0.79	
	GGCC (95)	3.50 ± 0.63	
SNP8&SNP9	CCCC (95)	3.50 ± 0.63	0.015*
	CTGC (165)	3.55 ± 0.80	
	TTGG (66)	3.85 ± 0.79	
SNP4&SNP8&SNP9	AATTGG (65)	3.86 ± 0.79	0.016**
	GACTGC (165)	3.55 ± 0.80	
	GGCCCC (95)	3.50 ± 0.63	
SNP22&SNP23	AACC (43)	3.93 ± 0.81	0.024*
	GACC (52)	3.65 ± 0.66	
	GACT (97)	3.62 ± 0.76	
	GGCC (11)	3.49 ± 0.75	
	GGCT (68)	3.48 ± 0.70	
	GGTT (54)	3.41 ± 0.82	

Note: Values with different asterisk (*/**) means significantly at $P < 0.05$/ $P < 0.01$, respectively.

sium Pty Ltd., South Brisbane, Queensland, Australia) software to screen candidate SNPs. Subsequently, quality control analysis was performed on the candidate SNPs, and the qualified SNPs were genotyped using the SNPscanTM method [14]. Both steps were carried out by the Tianhao Biotechnology Company (Shanghai, China).

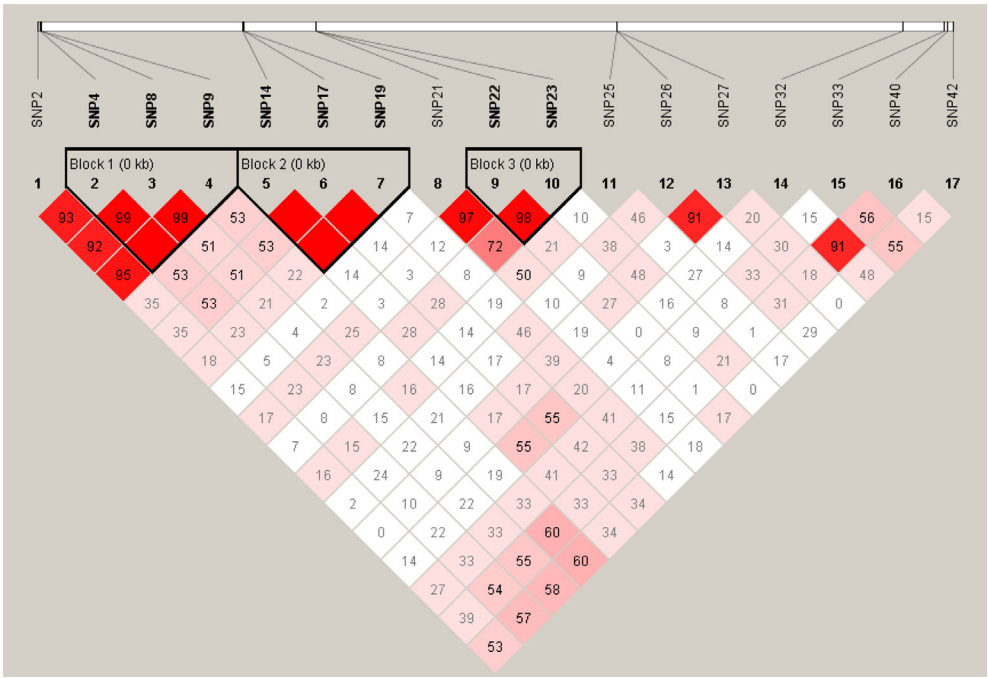

Fig. 2. LD analysis of SNPs with shoulder wool length.

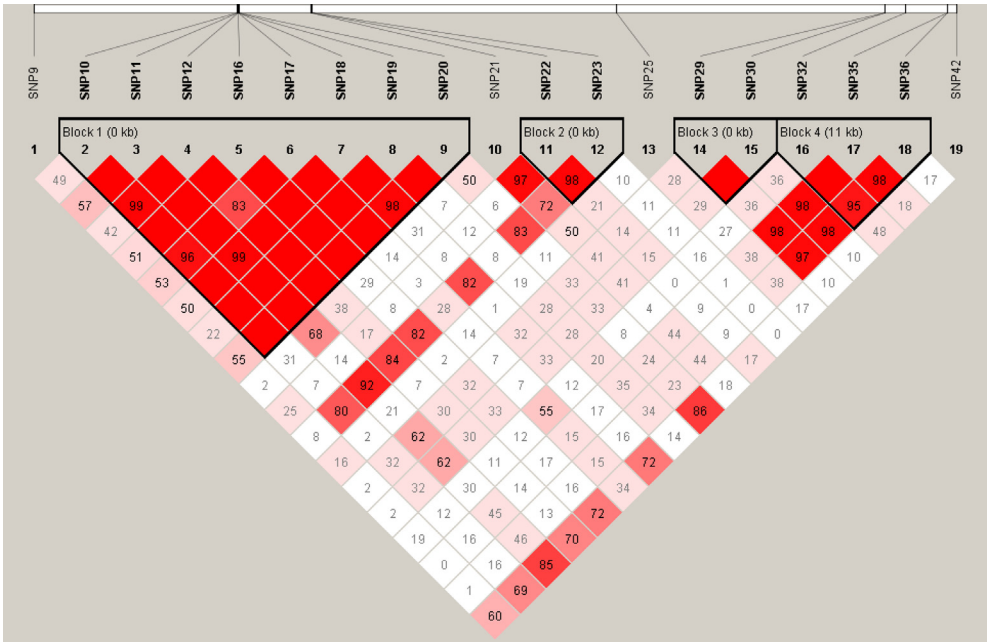

Fig. 3. LD analysis of SNPs with side wool length.

2.3. Statistical analysis

The genotype and allele frequencies, genetic parameters (including homozygosity [Ho], heterozygosity [He], effective allele numbers [Ne], polymorphism information content [PIC]), and Hardy-Weinberg equilibrium (HWE) were calculated using Nei's method [15]. R software was used to analyze the association between the individual genotype and the phenotype of the wool traits and the least-squares method was set to fit the linear model (LSE) for comparison. In consideration of the effects of gender, field, and variety, a statistical analysis model was established as $Y = \mu + G + s + p + b + e$, where Y refers to the measured value of the phenotypic trait, μ represents the population mean, G is the genotype effect, s is the gender effect, p is the field effect, b is the variety effect, and e is the random residual. Haploview 4.2 software (Broad Institute, MIT and Harvard, USA) [16] was used for the haplotype analysis and linkage disequilibrium (LD) analysis. A statistical model was established for the combined genotype analysis, $Y12 = \mu + G1 + G2 + G12 + s + p + b + e$, where Y12 refers to the measured value of the phenotypic trait, μ represents the popula-

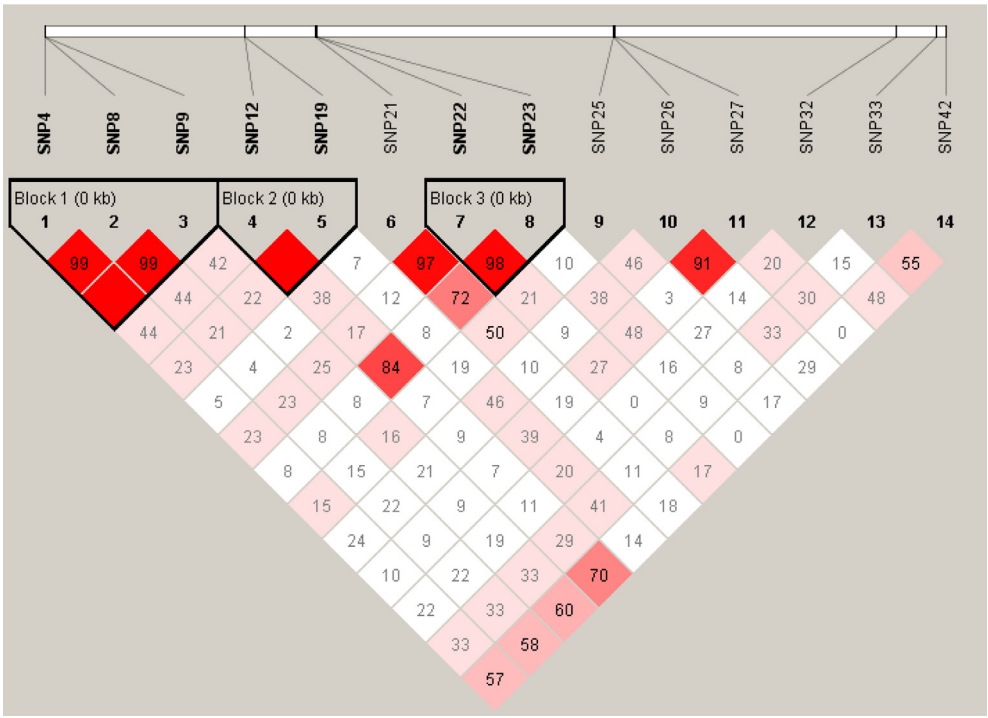

Fig. 4. LD analysis of SNPs with thigh wool length.

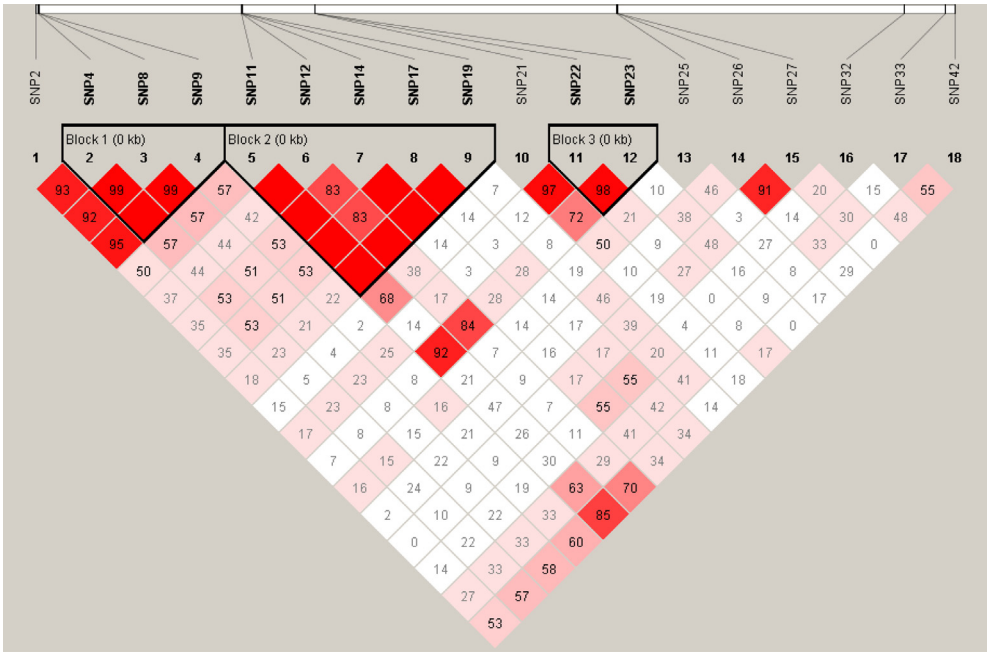

Fig. 5. LD analysis of SNPs with notum wool length.

tion mean, G1 is the genotype effect at the first locus, G2 is the genotype effect at the second locus, G12 is the interaction effect between the two loci, s is the gender effect, p is the field effect, b is the variety effect, and e is the random residual. Analysis of variance (ANOVA) was conducted to analyze the association between SNP loci and wool production/quality traits.

3. Results

3.1. SNP identification and genetic parameter analysis

Based on the DNA pool sequencing results, 45 SNPs in the *KIF16B* gene were identified (Table 3; Fig. S1). Since 8 SNPs were

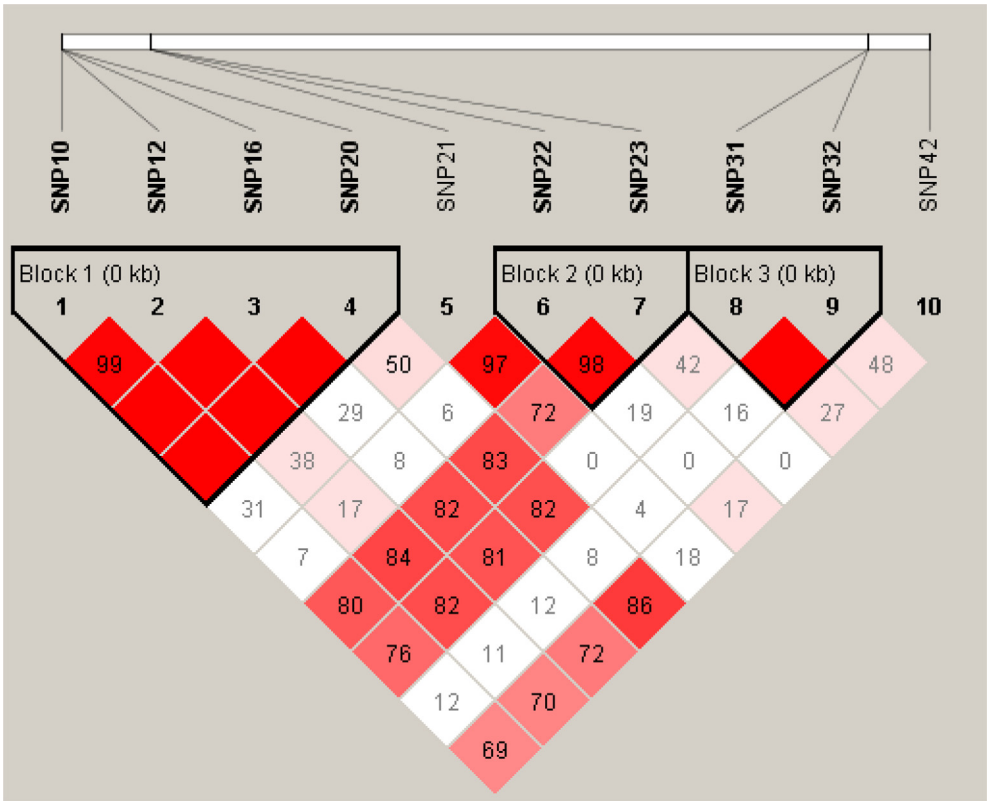

Fig. 6. LD analysis of SNPs with abdomen wool length.

not successfully genotyped using the SNPscanTM method, only 37 SNP loci were analyzed in this study. Among them, 1 SNP locus is in the promoter region, 10 SNP loci are located in the exons (exon 1, 3, and 12) and the others are distributed in the introns (intron 1, 3, 4, 9, 12, 14, 15, 17, 21, and 22). Among the 10 SNPs in the exon region, SNP1 (arginine to glycine), SNP2 (cysteine to arginine), SNP3 (alanine to serine), SNP4 (methionine to alanine), SNP6 (arginine to stop codon), SNP10 (methionine to threonine), and SNP44 (serine to proline) were missense mutations. All these SNPs had three genotypes: the homozygous wild type, heterozygous genotype, and homozygous mutant genotype.

Among the 37 SNP loci in the *KIF16B* gene, SNP11 and SNP26 displayed low genetic diversity (PIC < 0.25), and the other SNPs all had medium genetic diversity (0.25 < PIC < 0.5). Furthermore, except for SNP25, SNP26, SNP32, SNP33, SNP37, and SNP41, the SNP loci met the Hardy-Weinberg principle ($P > 0.05$) (Table 4).

3.2. Association analysis between SNPs and greasy wool weight

To explore the effect of the SNPs on wool production, association analyses between the 37 SNPs and the greasy wool weight were carried out. The results demonstrated that SNP4 ($P = 0.004$), SNP8 ($P = 0.005$), SNP9 ($P = 0.005$), SNP11 ($P = 0.048$), SNP21 ($P = 0.80E-3$), SNP22 ($P = 0.50E-3$), SNP23 ($P = 0.012$), SNP25 ($P = 0.021$), SNP29 ($P = 0.019$), and SNP30 ($P = 0.013$) were significantly associated with the greasy wool weight (Table 5). The linkage disequilibrium analysis showed that there were three strongly linked blocks, SNP4&SNP8&SNP9, SNP22&SNP23, and SNP29&SNP30, among which 7 haplotypes were identified (Table 6 and Fig. 1). Subsequently, association analysis between the combined genotype of the three blocks and the greasy wool weight was performed, and the results demonstrated that all the SNP genotypes exerted significant effects on the greasy wool weight ($P < 0.05$ or $P < 0.01$) (Table 7).

3.3. Association analysis between SNPs and the wool length

We also assessed the effects of genetic variation on the wool length of different body parts (shoulder, side, thigh, notum, and abdomen). Five SNPs, namely SNP21 (Pshoulder-21 = 5.02E-5, Pside-21 = 0.009, Pthigh-21 = 0.002, Pnotum-21 = 6.18E-6, Pabdomen-21 = 0.024), SNP22 (Pshoulder-22 = 2.81E-9, Pside-22 = 0.006, Pthigh-22 = 0.20E-3, Pnotum-22 = 4.61E-5, Pabdomen-22 = 0.015), SNP23 (Pshoulder-23 = 0.003, Pside-23 = 0.038, Pthigh-23 = 0.004, Pnotum-23 = 0.004, Pabdomen-23 = 0.008), SNP32 (Pshoulder32 = 0.003, Pside-32 = 0.008, Pthigh-32 = 0.012, Pnotum-32 = 1.40E-3, Pabdomen-32 = 0.006), and SNP 42 (Pshoulder-42 = 9.61E-9, Pside-42 = 0.011, Pthigh-42 = 0.30E-3, Pnotum-42 = 2.63E-7, Pabdomen-42 = 0.006) were identified to be significantly associated with the wool length of the five body parts (Table 5).

Next, we performed linkage disequilibrium analysis on those SNP loci which had significant effects on the wool length of each body part (Fig. 2, Fig. 3, Fig. 4, Fig. 5 and Fig. 6). Three strongly linked SNP blocks, namely SNP4&SNP8&SNP9, SNP12&SNP19, and SNP22&SNP23, were identified and the SNP loci within these three blocks were significantly associated with the wool length of at least three body parts. For the SNP4&SNP8&SNP9 block, two haplotypes, GCC and ATG, were established and their frequencies were 0.513 and 0.477, respectively. For the SNP12&SNP19 block, three haplotypes (AG, AC, and CC) were found with the frequencies being 0.424, 0.339, and 0.237, respectively. The SNP22&SNP23 block also had three haplotypes (GT, AC, and GC), and the frequencies were 0.438, 0.358, and 0.204, respectively (Table 8, Table 9, Table 10, Table 11, Table 12).

Finally, we performed a combination genotype analysis between the three specific blocks and the wool length of five body parts. The results showed that the combination genotypes of SNP4&SNP8, SNP4&SNP9, and SNP22&SNP23 were significantly

Table 8
The results of haplotype construction with strong chain SNPs in body side wool length.

SNP10&SNP11&SNP12&SNP16&SNP17 &SNP18&SNP19&SNP20		SNP22&SNP23	SNP29&SNP30	SNP32&SNP35&SNP36
TTATGCGG (0.425)	CTCCGTCC (0.054)	GT (0.438)	AT (0.442)	CCG (0.377)
TTATACGG (0.338)	CTCCGTCG (0.034)	AC (0.358)	GT (0.558)	CTA (0.382)
CCCCGTCC (0.127)	TTCTACCG (0.022)	GC (0.202)	GC (0.204)	TTA (0.241)

Table 9
The results of haplotype construction with strong chain SNPs in thigh wool length.

SNP4&SNP8&SNP9	SNP12&SNP19	SNP22&SNP23	SNP48&SNP50&SNP51	
GCC (0.513)	AG (0.424)	GT (0.438)	CTG (0.365)	TAT (0.240)
ATG (0.477)	AC (0.0339)	AC (0.358)	TAG (0.309)	CAG (0.086)
–	CC (0.237)	GC (0.204)	–	–

Table 10
The results of haplotype construction with strong chain SNPs in notum wool length.

SNP4&SNP8SNP9	SNP11&SNP12&SNP14&SNP17&SNP19		SNP22&SNP23	SNP48&SNP51
GCC (0.513)	TATGC (0.425)	TCTGC (0.089)	GT (0.438)	GG (0.451)
ATG (0.476)	TACAC (0.339)	TCCAC (0.020)	AC (0.358)	TG (0.309)
–	CCTGC (0.127)	–	GC (0.204)	TT (0.240)

Table 11
The results of haplotype construction with strong chain SNPs in abdomen wool length.

SNP10&SNP12&SNP16&SNP2	SNP22&SNP23	SNP31&SNP32	SNP48&SNP50&SNP51
TATG (0.762)	GT (0.425)	TC (0.583)	CTG (0.365)
CCCC (0.187)	AC (0.339)	TT (0.237)	TAG (0.309)
CCCG (0.034)	GC (0.204)	CC (0.180)	TAT (0.240)
TATG (0.017)	–	–	CAG (0.086)

Table 12
The results of haplotype construction with strong chain SNPs in shoulder wool length.

SNP57&SNP61	SNP4&SNP8&SNP9	SNP14&SNP17&SNP19	SNP22&SNP23	SNP48&SNP50&SNP51
CT (0.635)	GCC (0.513)	TGG (0.424)	GT (0.438)	CTG (0.365)
TG (0.365)	ATG (0.477)	CAC (0.360)	AC (0.358)	TAG (0.309)
–	–	TGC (0.216)	GC (0.204)	TAT (0.240)
–	–	–	–	CAG (0.086)

associated with the wool length in the shoulder, side, thigh, and notum ($P < 0.05$). The combination genotypes of SNP4&SNP8&SNP9 exerted a significant influence on the wool length in the shoulder, side, and thigh ($P < 0.05$), and the combination genotypes of SNP12&SNP19 could significantly affect the wool length in the side, thigh, notum, and abdomen ($P < 0.05$) (Table 13).

4. Discussion

Kinesin superfamily proteins (KIFs) play an important role in transporting various cellular proteins, the microtubule system, organelles, and macromolecules, implying that this family gene possibly influences hair or wool development [8]. The *KIF16B* gene has been identified as a candidate gene related to wool traits in a previous sheep GWAS analysis [3] but further analysis is needed. In this study, we systematically analyzed the distribution of 37 SNP loci within the *KIF16B* gene in a fine wool sheep population and their effects on wool production traits. Among the 37 SNPs, only SNP11 and SNP26 displayed low genetic diversity and the others had medium genetic diversity. Additionally, most of these SNPs met the HWE principle; SNP25, SNP26, SNP32, SNP33, and SNP41 did not, possibly due to population stratification, selection, or genetic drift [17].

The association between the 37 SNPs loci and the wool production and quality traits was assessed. SNP21, SNP22, SNP23, SNP32, and SNP42 were identified to be significantly associated with the wool length of all body parts. Among these 5 SNPs, (SNP21, SNP22, and SNP23) not only exerted significant impacts on the wool length but also the greasy wool weight. To explain this, it necessary to describe the role of the *KIF16B* gene influencing the recovery and degradation of EGFR by regulating the location of EE [10]. The EFGR, as a member of the ErbB family, can promote protein phosphorylation and function by combining ligands [18]. A previous study showed that EGFR and its ligands contributed to the change of hair-related phenotypes in mice [19] and we spec-

Table 13
The correlation analysis between combined genotype of strong chain SNPs and natural wool length.

Combined SNPs	Natural wool length				
	Shoulder	Side	Thigh	Notum	Abdomen
SNP4&SNP8	AATT (88)	AATT (88)	AATT (88)	AATT (88)	AATT (88)
	7.29 ± 1.39	7.32 ± 1.49	7.10 ± 1.31	7.39 ± 1.48	5.10 ± 1.22
	GACT (205)	GACT (205)	GACT (205)	GACT (205)	GACT (205)
	6.91 ± 1.38	7.29 ± 1.64	6.92 ± 1.21	7.21 ± 1.54	4.94 ± 1.23
	GGCC (102)	GGCC (102)	GGCC (102)	GGCC (102)	GGCC (102)
	6.56 ± 1.16	7.01 ± 1.23	6.66 ± 1.05	6.75 ± 1.14	4.88 ± 1.67
	***P* = 2.92E-8**	***P* = 0.005**	***P* = 0.009**	***P* = 5.29E-5**	*P* = 0.698
SNP4&SNP9	AAGG (88)	AAGG (88)	AAGG (88)	AAGG (88)	AAGG (88)
	7.29 ± 1.39	7.32 ± 1.49	7.10 ± 1.31	7.39 ± 1.48	5.10 ± 1.22
	GAGC (205)	GAGC (205)	GAGC (205)	GAGC (205)	GAGC (205)
	6.91 ± 1.39	7.27 ± 1.64	6.90 ± 1.18	7.18 ± 1.51	4.93 ± 1.22
	GGCC (103)	GGCC (103)	GGCC (103)	GGCC (103)	GGCC (103)
	6.54 ± 1.56	6.96 ± 1.21	6.64 ± 1.06	6.72 ± 1.13	4.83 ± 1.17
	GGGC (5)	GGGC (5)	GGGC (5)	GGGC (5)	GGGC (5)
	8.28 ± 1.47	9.24 ± 2.47	7.64 ± 1.12	8.38 ± 1.42	5.84 ± 0.94
	***P* = 4.82E-8**	***P* = 0.60E-3**	***P* = 0.20E-3**	***P* = 1.65E-7**	*P* = 0.080
SNP8&SNP9	CCCC (102)	CCCC (102)	CCCC (102)	CCCC (102)	CCCC (102)
	6.52 ± 1.44	6.97 ± 1.22	6.64 ± 1.07	6.71 ± 1.13	4.85 ± 1.17
	CCGC (6)	CCGC (6)	CCGC (6)	CCGC (6)	CCGC (6)
	7.97 ± 1.37	7.90 ± 0.89	7.17 ± 0.55	7.73 ± 0.96	5.40 ± 0.97
	CTGC (208)	CTGC (208)	CTGC (208)	CTGC (208)	CTGC (208)
	6.91 ± 1.38	7.30 ± 1.68	6.91 ± 1.18	7.18 ± 1.52	4.94 ± 1.22
	TTGG (90)	TTGG (90)	TTGG (90)	TTGG (90)	TTGG (90)
	7.29 ± 1.38	7.30 ± 1.48	7.08 ± 1.30	7.39 ± 1.46	5.10 ± 1.21
	***P* = 1.20E-6**	*P* = 0.200	*P* = 0.424	***P* = 0.50E-3**	*P* = 0.424
SNP4&SNP8&SNP9	AATTGG (88)	AATTGG (88)	AATTGG (88)	AATTGG (88)	AATTGG (88)
	7.29 ± 1.39	7.32 ± 1.49	7.10 ± 1.31	7.39 ± 1.48	5.10 ± 1.22
	GACTGC (203)	GACTGC (203)	GACTGC (203)	GACTGC (203)	GACTGC (203)
	6.89 ± 1.47	7.27 ± 1.64	6.90 ± 1.18	7.18 ± 1.51	4.93 ± 1.23
	GGCCCC (102)	GCCCC (102)	GGCCCC (102)	GGCCCC (102)	GGCCCC (102)
	6.52 ± 1.14	6.97 ± 1.22	6.64 ± 1.06	6.71 ± 1.13	4.85 ± 1.66
	***P* = 3.36E-9**	***P* = 1.27E-5**	***P* = 6.00E-5**	*P* = 0.232	*P* = 0.232
SNP12&SNP19	AACC (51)	AACC (51)	AACC (51)	AACC (51)	AACC (51)
	6.98 ± 1.21	6.95 ± 1.24	6.85 ± 1.20	7.17 ± 1.41	4.69 ± 1.01
	AAGC (120)	AAGC (120)	AAGC (120)	AAGC (120)	AAGC (120)
	6.96 ± 1.26	7.09 ± 1.41	6.78 ± 1.16	7.01 ± 1.35	4.91 ± 1.16
	AAGG (72)	AAGG (72)	AAGG (72)	AAGG (72)	AAGG (72)
	6.63 ± 1.40	6.91 ± 1.36	6.66 ± 1.05	6.78 ± 1.28	4.72 ± 1.23
	CACC (66)	CACC (66)	CACC (66)	CACC (66)	CACC (66)
	6.93 ± 1.43	7.50 ± 1.68	7.14 ± 1.28	7.32 ± 1.56	5.09 ± 1.27
	CAGC (88)	CAGC (88)	CAGC (88)	CAGC (88)	CAGC (88)
	7.04 ± 1.53	7.46 ± 1.66	6.96 ± 1.20	7.28 ± 1.53	5.24 ± 1.18
	CCCC (15)	CCCC (15)	CCCC (15)	CCCC (15)	CCCC (15)
	7.02 ± 1.42	8.15 ± 1.91	7.43 ± 1.47	7.78 ± 1.69	5.07 ± 1.53
	P = 0.176	***P* = 0.30E-3**	***P* = 0.010**	***P* = 0.004**	***P* = 0.016**
SNP22&SNP23	AACC (50)	AACC (50)	AACC (50)	AACC (50)	AACC (50)
	7.51 ± 1.57	7.44 ± 1.51	7.16 ± 1.27	7.55 ± 1.71	5.24 ± 1.08
	GACC (50)	GACC (50)	GACC (50)	GACC (50)	GACC (50)
	7.02 ± 1.32	7.62 ± 1.61	7.21 ± 1.30	7.38 ± 1.66	5.16 ± 1.24
	GACT (120)	GACT (120)	GACT (120)	GACT (120)	GACT (120)
	7.15 ± 1.23	7.33 ± 1.53	6.98 ± 1.22	7.25 ± 1.45	5.00 ± 1.23
	GGCC (14)	GGCC (14)	GGCC (14)	GGCC (14)	GGCC (14)
	6.70 ± 1.21	6.86 ± 1.01	6.79 ± 0.96	7.06 ± 1.13	4.86 ± 0.81
	GGCT (80)	GGCT (80)	GGCT (80)	GGCT (80)	GGCT (80)
	6.38 ± 1.32	7.03 ± 1.49	6.64 ± 1.06	6.78 ± 1.14	4.93 ± 1.25
	GGTT (95)	GGTT (95)	GGTT (95)	GGTT (95)	GGTT (95)
	6.78 ± 1.30	7.03 ± 1.53	6.71 ± 1.17	6.93 ± 1.39	4.70 ± 1.19
	***P* = 2.20E-9**	***P* = 0.008**	***P* = 0.004**	***P* = 0.001**	***P* = 0.016**

ulated that the mutation of the *KIF16B* gene might affect wool growth by regulating the EGFR. SNP4 in the exon region may regulate the expression of the *KIF16B* gene by changing the corresponding amino acid of the encoded protein, thereby influencing the function and regulation of the *KIF16B* gene. As for intron mutations that do not contribute to the alternation of the amino acids, they might give rise to the changes in the regulatory regions and intron sequences, thereby affecting the transcription initiation, mRNA splicing/editing, nuclear/cytoplasm transportation, and other processes and thus influencing the translation efficiency of *KIF16B* [20].

Given that traits are affected by minor polygenic effects involving multiple genes and loci [21] the interactions between multiple genes or the combined effects of multiple SNP sites may have impacts on wool production and quality traits in sheep. Several SNP loci related to the phenotype might not directly contribute to the causal mutation of the phenotype—they are in linkage disequilibrium (LD) with a causal mutation that mediates changes in amino acids and proteins thereby leading to the phenotypic changes. Therefore, we completed the LD analysis on the SNP loci which exerted significant effects on the wool production and quality traits and then assessed the effect of the strongly linked blocks

on these traits. Two strongly linked blocks, SNP4&SNP8&SNP9 and SNP22&SNP23, were found for their significant impact on both the greasy wool weight and the wool length of all five body parts. Thus, in further selective breeding, the SNP loci within the two strongly linked blocks could be the potential DNA molecular markers applied to identify individuals with superior wool production and quality. However, the specific mechanism(s) warrants further study.

5. Conclusions

Herein, 45 SNP loci within the sheep *KIF16B* gene were identified by DNA pool sequencing and SNPscan™ high-throughput genotyping and individual SNP locus as well as two strongly linked SNP blocks (SNP4&SNP8&SNP9 and SNP22&SNP23) were found to be significantly associated with both the wool greasy production and wool length. These findings can be considered as valuable molecular markers for the marker-assisted selection (MAS) breeding of sheep.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejbt.2021.05.003.

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