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Association Analysis of Polymorphism of *FGFR1* and *EBP41L5* Genes with Kidding Performance of Goats

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ABSTRACT

This study aimed to analyze the association between single nucleotide polymorphisms (SNPs) in the *FGFR1* and *EBP41L5* genes and kidding performance of goats. Mass ARRAY®SNP typing technology was applied to genotype the SNPs of *FGFR1* and *EBP41L5* genes, of which the genetic characteristics of Yunshang Black goat (YS, n=544), Jining Grey goat (JN, n=133) and Liaoning Cashmere goat (LN, n=91) were explored. Then the association was analyzed between *FGFR1* and *EBP41L5* and kidding performance (litter size, litter weight at birth, litter weight at weaning) in different goats. Population genetics statistics showed that *FGFR1* gene g. 12120297A> G and *EPB41L5* gene g. 64237881A> C were low polymorphisms in the three populations (PIC< 0.25); *EPB41L5* gene g. 64266710G> C and g. 64266715G> C were moderately polymorphic in Yunshang Black goat and Liaoning Cashmere goat (0.25<PIC<0.5), and in Jining Grey goat was low polymorphism (PIC< 0.25). The χ^2 test indicated that *EPB41L5* gene g. 64237881A> C locus was under Hardy-Weinberg disequilibrium in Jining grey goat (P< 0.05), and was under Hardy-Weinberg equilibrium in Yunshang Black goat and Liaoning Cashmere goat (P> 0.05). *FGFR1* gene g. 12120297A> G, *EPB41L5* gene g. 64266710G> C, g. 64266715G> C were under Hardy-Weinberg equilibrium in Yunshang Black goat, Jining Grey goat and Liaoning Cashmere goat (P> 0.05). Association analysis indicated that there were no significant correlation between *FGFR1* gene g. 12120297A> G locus and *EPB41L5* gene g. 64266715G> C locus and litter size, litter weight at birth and weaning (P> 0.05). There were significant correlation between *EPB41L5* gene g. 64237881A> C and g. 64266710 G> C and litter size (P< 0.05), and were no significant correlation and litter weight at birth (P> 0.05), there were no significant correlation between *EPB41L5* gene g. 64237881A> C and litter weight at weaning (P> 0.05), there were significant correlation between *EPB41L5* gene g. 64266710C> G and litter weight at weaning (P< 0.05). Therefore, these results suggested that *EPB41L5* gene g. 64237881A> C, g. 64266710 G> C loci were suitable as molecular markers for litter size in YS, and g. 64266710G> C locus was suitable as a selection marker for litter weight at weaning.

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Key words
Goat, Litter size, Litter weight, *FGFR1* gene, *EPB41L5* gene

INTRODUCTION

In the development process of human civilization, goats provided humans with the main means of living such as meat, milk, skin and hair, and played an important role in human agricultural civilization and economic development. The kidding traits of goats were closely related to the reproduction speed and scale of their populations. They were an important factor that affects the economic benefits of goat breeding and were also important economic traits of goat breeding (Zheng *et al*., 2021). However, due to the low heritability of kidding traits, it was relatively slow to use traditional breeding methods to get more obvious genetic improvement of kidding traits, while molecular breeding technology can quickly increase the kidding rate of goats by controlling the major genes of kidding traits. Many scientists have conducted a lot of research on the relationship between single nucleotide polymorphisms and goat kidding traits, hoping to find genes that can be used to improve goat kidding traits (Zhu *et al*., 2021). Fibroblast growth factor receptor 1 (FGFR1) belongs to the fibroblast

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growth factor receptors (FGFRs) family, which includes a total of 4 receptor protein tyrosine kinases FGFR (1-4) (Qin *et al*., 2019). Each receptor contains an extracellular domain composed of three immunoglobulin (Ig)-like domains (domains I to III), a transmembrane domain, and an intracellular tyrosine kinase structure 2 (Turner and Grose, 2010). Fibroblast growth factors (FGFs) combined with FGFRs induce dimerization and phosphorylation of specific cytoplasmic tyrosine residues, and activate cytoplasmic signal transduction pathways (Carter *et al*., 2015). Studies have shown that genetic variation in the FGF/FGFR pathway is significantly related to the risk of ovarian cancer, treatment response and survival rate (Meng *et al*., 2014; Haugsten *et al*., 2010). Most studies have shown that the FGF/FGFR pathway plays a role in ovarian diseases, and it can be seen that the genes on this pathway are expressed in the ovaries. Erythrocyte membrane protein band 4.1 like 5 (*EPB41L5*) is a member of the band 4.1 superfamily (Hirano *et al*., 2008), locate on chromosome 2. It has a FERM domain at its N terminus and an actin-binding domain at its C terminus (Gamblin *et al*., 2018), which was related to cell movement. In addition, it can also participate in the development process of the body and can regulate the expression of downstream cadherin E (E-cadherin) in the process of epithelial cell-mesenchymal transition (EMT transformation) induced by transforming growth factor-β (TGF-β) (Li *et al*., 2019), the EMT transformation process played an important role in embryonic development, chronic inflammation, tissue reconstruction, cancer metastasis, and various fibrotic diseases. At the same time, *EPB41L5* directly controlled the contractility and focal adhesion of actin, and regulated cell proliferation and migration (Hashimoto *et al*., 2016). The role of abnormal expression of *EPB41L5* in malignant tumors has gradually been confirmed (Chen *et al*., 2019). It can be seen that the FGF/FGFR pathway has a certain influence in ovarian diseases. *EPB41L5* was involved in embryonic development, so these two genes may be related to goat kidding performance. Based on the results of re-sequencing of Yunshang black goat (The Yunshang black goat is the first meat purpose black goat breed in China, which is bred from the Yunling goat as the dam and the Nubi goat as the sire (Tao *et al*., 2020a, b)) in the early stage of our laboratory, it is speculated that *FGFR* gene and *EPB41L5* gene may have some influence on kidding performance. A MassARRAY® SNP typing technology was used to detect the genotypes of candidate genes and analyze the association between their polymorphisms and litter size traits. This study aimed to analyze the genetic polymorphisms of *FGFR* and *EPB41L5* in Yunshang black goat, Jining grey goat and Liaoning cashmere goat population and their correlation with kidding performance, and provide more information about genetic markers for improving the prolificacy of goat.

MATERIALS AND METHODS

Animal preparation and sample collection

A total of 768 goats (2~5 years old) were employed in this study, including 544 Yunshang black goats, 133 Jining grey goats, and 91 Liaoning cashmere goats. The higher prolificacy breeds included in this study was Jining Grey goats, and Liaoning Cashmere goats was regarded as lower prolificacy breeds (Wang *et al*., 2020; Chu *et al*., 2013). The breeding conditions of all experimental goats were the same. Yunshang Black goat had records of the number of litters born at least one litter, and some had records of litter weight at birth and litter weight at weaning (3 months old). Jugular blood samples from 768 goats of the three breeds were collected into EDTA-coated tubes and stored at –20°C until used for DNA isolation.

Extraction of blood DNA

Samples of test goat were processed with the phenol–chloroform method for DNA isolation. The concentration of DNA samples were detected by using Nano Drop2000, and the quality of DNA samples were detected by using 1.5% agarose gel electrophoresis.

Genotyping

FGFR1 gene g. 12120297 A>G, *EPB41L5* gene g. 64237881 A>C, g. 64266710 G>C and g. 64266715 G>C sites were used to detect by Sequenom MassARRAY® SNP typing technology. The related primer information was shown in Table I. The typing sample is DNA, the required amount of each sample is 20 μL, and the DNA concentration is 40~80 ng/μL.

Phylogenetic analysis

A phylogenetic tree of *EPB41L5* gene coding sequence in fifteen species (goat, pig, alpaca, human, horse, sheep, dog, cow, Norway rat, mouse, monkey, chicken, duck, fish and cat) were constructed with MEGA software. Sequences were obtained from the NCBI (https://www.ncbi.nlm.nih.gov/) Reference (NC_030809.1, NC_010457.5, NW_021964164.1, NC_000002.12, NC_009161.3, NC_056055.1, NC_051823.1, NC_037329.1, NC_051348.1, NC_000067.7, NC_041765.1, NC_052538.1, NC_051778.1, NC_007120.7, NC_018732.3, respectively). Prediction of the physical and chemical properties of *EPB41L5* protein was performed using ProtParam. The secondary structure of *EPB41L5* was analyzed using SOPMA. Prediction of the mRNA Structure of *EPB41L5* was performed using RNAFOLD.

Statistical analysis

Based on genotyping data, genotype frequency, allele frequency, polymorphic information content (PIC), heterozygosity (He) and effective allele number (Ne) were calculated by using Microsoft Excel 2020 software. Hardy-Weinberg equilibrium test was carried out by Chi-square test. The one-way ANOVA was performed by IBM SPSS Statistics 22.0 software, and the LSD method was used for multiple comparisons. The correlation analysis between goat genotype and kidding phenotype data was carried out, and all data were expressed as mean±standard error.

RESULTS

Population genetic analysis of SNPs in FGFR1 *and* EPB41L5

The genotyping results showed that one SNP in *FGFR1* (g.12120297 A>G) and three SNPs in *EPB41L5* (g.64237881 A>C, g. 64266710 G>C and g. 64266715 G>C) were detected in all three breed of Yunshang Black goat, Jining Grey goat and Liaoning Cashmere goat. Two genotypes at *FGFR1* gene g.12120297 A>G locus, AA and GA, were detected in 759 individuals with a detection rate of 98.8%. Three genotypes at *EPB41L5* gene g. 64237881 A>C locus, AA, CC and CA, were detected in 756 individuals with a detection rate of 98.4%. The *EPB41L5* g.64266710 C>G and g.64266715 G>C loci contained three genotypes, CC, GG and GC. The locus g.64266710 C>G was detected in 762 individuals with a detection rate of 99.2%, and the locus g.64266715 G>C in 748 individuals with a detection rate of 97.4% (Fig. 1).

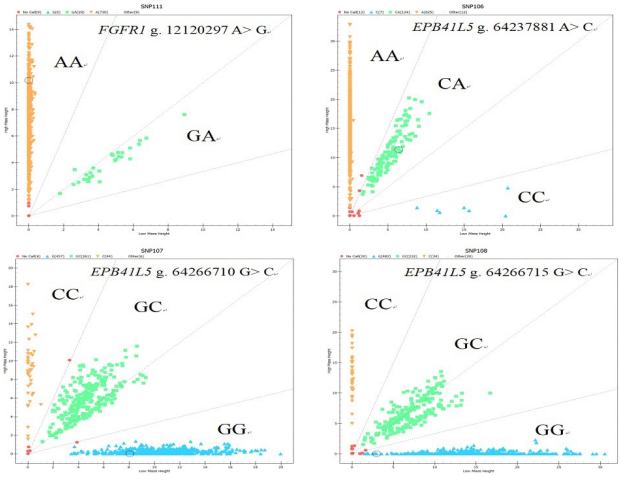

Fig. 1. *FGFR1* and *EPB41L5* gene locus genotyping results.

It can be seen from Table II that the genotype frequency and gene frequency of *EPB41L5* gene g.64237881 A>C, g.64266710 G>C and g.64266715 G>C locus differs between the high- and low-reproduction goat populations to a very significant level (P< 0.01). The dominant genotype of *EPB41L5* gene g.64237881A>C locus in the high and low-reproduction populations was AA, and the dominant allele is A; the dominant genotype of *EPB41L5* gene g. 64266710 G> C and g. 64266715 G> C locus in the high- and low-reproduction populations were GG, and the dominant allele was G. Population genetics statistics showed that there was a genetic polymorphism at *FGFR1* gene g. 12120297 A> G locus in Yunshang Black goat, but there were no gene mutations in Jining Grey goat and Liaoning Cashmere goat. The *FGFR1* gene g. 12120297A> G and *EPB41L5* gene g. 64237881A> C were low polymorphisms in the three population (PIC< 0.25); *EPB41L5* gene g. 64266710G> C and g. 64266715G> C were moderately polymorphic in Yunshang Black goat and Liaoning Cashmere goat (0.25< PIC< 0.5), and in Jining Grey goat were low polymorphism (PIC< 0.25). The χ^2 test indicated that *EPB41L5* gene g. 64237881A> C locus was under Hardy-Weinberg disequilibrium in Jining Grey goat (P< 0.05), and was under Hardy-Weinberg equilibrium in Yunshang Black goat and Liaoning Cashmere goat (P> 0.05). *FGFR1* gene g. 12120297A> G, *EPB41L5* gene g. 64266710G> C, g. 64266715G> C were under Hardy-Weinberg equilibrium in Yunshang Black goat, Jining Grey goat and Liaoning Cashmere goat (P> 0.05).

Association analysis between candidate loci and reproductive performance

Association analysis indicated that there were no significant correlations between *FGFR1* gene g.12120297A>G locus and *EPB41L5* gene g.64266715G>C locus and litter size, litter weight at birth and weaning (*P*> 0.05). There was significant correlation between *EPB41L5* gene g.64237881A>C and g.64266710 G>C and litter size (*P*< 0.05), and there was no significant correlation between the two loci and litter weight at birth (*P*> 0.05). There was no significant correlation between *EPB41L5* gene g.64237881A>C and litter weight at weaning (*P*> 0.05), there were significant correlation between *EPB41L5* gene g.64266710C>G and litter weight at weaning (*P*< 0.05).

Construction of phylogenetic tree of EPB41L5 *gene and other animal species*

A phylogenetic tree of *EBP41L5* gene coding sequence for other animal species was constructed. The results showed that there was the highest homology between goats and sheep, and there was the higher similarity between goats and cattle, and the sequences of the other species showed relatively large differences between them (Fig. 2).

Table I. Primer sequences.

Locus	Classification	Sequences (5'-3')
FGFR1	F	ACGTTGGATGTGGTTTTAGGCAAGCCACTG
g. 12120297 A> G	R	ACGTTGGATGGGTTGGGTTTGTCCTTGTCC
	E	GTCCTTGTCCAGCCCGA
EPB41L5	F	ACGTTGGATGTACCTTTAGAGCAGACCCAC
g. 64237881 A> C	R	ACGTTGGATGAAATGAGAACACTGTGCCCG
	E	TGTTCGCATCAGAGCC
EPB41L5	F	ACGTTGGATGGCATGTGAAGGAGATCTCTG
g. 64266710 G> C	R	ACGTTGGATGATGGAAAGCAGTCCTGTGCC
	E	TGCCAGCTCCTCACCCCAA
EPB41L5	F	ACGTTGGATGGCATGTGAAGGAGATCTCTG
g. 64266715 G> C	R	ACGTTGGATGATGGAAAGCAGTCCTGTGCC
	E	CTGTGCCAGCTCCTCAC

Note: F, Upstream primer; R, Downstream primer; E, Extension primer.

Table II. Genotype frequency and allele frequency of *FGFR1* and *EPB41L5* genes in high and low-reproduction goat breeds.

Locus	Genotype	Genotype frequency		Chi-square test (P value)	Allele	Gene frequency		Chi-square test (P value)
		High fertility population	Low fertility population			High fertility population	low fertility population	
FGFR1	AA	1.00 (n=133)	1.00 (n=90)	-	A	1.00	1.00	-
g. 12120297 A> G	GA	0.00 (n=0)	0.00 (n=0)		G	0.00	0.00	
EPB41L5	AA	0.89 (n=115)	0.67 (n=57)	0.00	A	0.94	0.82	9.60×10^{-5}
g. 64237881 A> C	CC	0.01 (n=1)	0.04 (n=3)		C	0.06	0.18	
	CA	0.10 (n=13)	0.29 (n=25)					
EPB41L5	CC	0.02 (n=3)	0.06 (n=6)	0.00	C	0.10	0.22	0.00
g. 64266710 G> C	GG	0.82 (n=107)	0.63 (n=57)		G	0.90	0.78	
	GC	0.16 (n=20)	0.31 (n=28)					
EPB41L5	CC	0.03 (n=3)	0.10 (n=8)	1.25×10^{-5}	C	0.12	0.31	1.94×10^{-6}
g.64266715 G> C	GG	0.79 (n=100)	0.48 (n=40)		G	0.88	0.69	
	GC	0.18 (n=23)	0.42 (n=35)					

Note: P< 0.05 means the difference is significant; P< 0.01 means the difference is extremely significant.

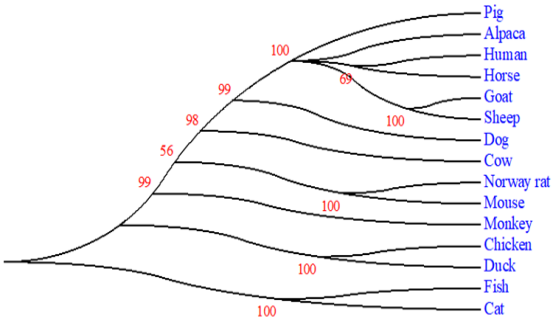

Fig. 2. The phylogenetic tree of the *EBP41L5* gene in different species.

Physical and chemical properties of EPB41L5 *protein*

It can be seen from Table V that the molecular weight, formula, total number of atoms, instability index, aliphatic index and grand average of hydropathicity of the protein before and after the mutation have happened (Table V). It can be seen from the figure that the hydrophilic amino acids are more than the hydrophobic amino acids before and after *EPB41L5* mutation (Fig. 3A, 3B). Therefore, the protein was hydrophobic before and after the mutation, but grand average of hydropathicity has changed. It can be seen that the physicochemical properties of the protein are changed due to the mutation of the three sites, which may cause the function of the protein to change, thereby affecting the kidding traits of the Yunshang Black goat.

Table III. Population genetic analysis of candidate loci in three goat breeds.

Locus	Breeds	Genotype frequency			Allele frequency		PIC	He	Ne	P
FGFR1		AA	AG		A	G				
g. 12120297 A> G	YS (n=536)	0.95 (n=507)	0.05 (n=29)		0.97	0.03	0.06	0.06	1.06	0.52
	JN (n=133)	1.00 (n=133)	0.00 (n=0)		1.00	0.00	0.00	0.00	1.00	-
	LN (n=90)	1.00 (n=90)	0.00 (n=0)		1.00	0.00	0.00	0.00	1.00	-
EPB41L5		AA	CC	CA	C	A				
g. 64237881 A> C	YS (n=542)	0.83 (n=453)	0.01 (n=3)	0.16 (n=86)	0.08	0.92	0.14	0.15	1.17	0.62
	JN (n=129)	0.97 (n=115)	0.02 (n=1)	0.01 (n=13)	0.03	0.97	0.06	0.06	1.06	3.4×10^{-30}
	LN (n=85)	0.67 (n=57)	0.04 (n=3)	0.29 (n=25)	0.18	0.82	0.25	0.30	1.42	0.90
EPB41L5		CC	GG	GC	C	G				
g. 64266710 G> C	YS (n=541)	0.07 (n=35)	0.54 (n=293)	0.39 (n=213)	0.26	0.74	0.31	0.38	1.63	0.65
	JN (n=130)	0.03 (n=3)	0.82 (n=107)	0.15 (n=20)	0.10	0.90	0.16	0.18	1.22	0.10
	LN (n=91)	0.06 (n=6)	0.63 (n=57)	0.31 (n=28)	0.22	0.78	0.28	0.34	1.52	0.33
EPB41L5		CC	GG	GC	C	G				
g.64266715 G> C	YS (n=539)	0.04 (n=23)	0.32 (n=342)	0.64 (n=174)	0.20	0.80	0.27	0.32	1.47	0.88
	JN (n=126)	0.03 (n=3)	0.79 (n=100)	0.18 (n=23)	0.12	0.88	0.19	0.21	1.27	0.24
	LN (n=83)	0.10 (n=8)	0.48 (n=40)	0.42 (n=35)	0.31	0.69	0.34	0.43	1.75	0.93

Note: PIC, HE and NE represent polymorphic information content, heterozygosity and effective number of alleles, respectively; numbers in the parentheses represent the number of detected sheep of each genotype; YS, JN and LN represent Yunshang Black goat, Jining Grey goat, Liaoning Cashmere goat, respectively; $P> 0.05$ indicates the locus was under Hardy-Weinberg equilibrium; $P< 0.05$ indicates the locus was under Hardy-Weinberg disequilibrium.

Table IV. The effect of *FGFR1* and *EPB41L5* on kidding performance of Yunshang Black goat (mean ± standard error).

Locus	Genotype	Number of kidding	Birth weight, kg	Weaning weight, kg
FGFR1 g. 12120297 A> G	AA	2.03±0.02 (n=507)	6.98±0.08 (n=339)	35.95±0.43 (n=339)
	GA	2.18±0.01 (n=29)	7.20±0.30 (n=25)	36.60±1.66 (n=25)
EPB41L5 g. 64237881 A> C	AA	2.10±0.02^{a} (n=453)	6.97±0.09 (n=321)	35.74±0.45 (n=321)
	CC	2.33±0.33^{ab} (n=3)	6.79±0.22 (n=2)	39.89±1.40 (n=2)
	CA	1.88±0.51^{b} (n=86)	7.02±0.16 (n=47)	36.84±7.47 (n=47)
EPB41L5 g. 64266710 G> C	CC	2.24±0.09^{a} (n=35)	7.63±0.40 (n=19)	38.45±2.03^{a} (n=19)
	GG	2.07±0.03^{a} (n=293)	6.97±0.10 (n=208)	36.43±0.56^{ab} (n=208)
	GC	1.95±0.36^{b} (n=213)	6.90±0.13 (n=143)	34.76±0.61^{b} (n=143)
EPB41L5 g.64266715 G> C	CC	2.18±0.06 (n=23)	7.50±0.33 (n=22)	36.34±1.30 (n=22)
	GG	2.00±0.03 (n=342)	7.02±0.10 (n=196)	36.41±0.59 (n=196)
	GC	2.07±0.04 (n=174)	6.83±0.13 (n=149)	35.10±0.63 (n=149)

Note: Numbers in the parentheses next to litter size represent the number of sheep of each genotype; Different lower-case letters in the same group indicate significant difference ($P < 0.05$).

Table V. The physicochemical properties of protein before and after *EPB41L5* mutation.

Physical and chemical properties	Before mutation	After mutation
Number of amino acids	724	724
Molecular weight	80349.07	80378.03
Theoretical pI	6.45	6.45
negatively charged residues (Asp + Glu)	96	96
Positively charged residues (Arg + Lys)	91	91
Formula	$C_{3542}H_{5658}N_{1000}O_{1093}S_{19}$	$C_{3540}H_{5653}N_{1003}O_{1094}S_{19}$
Total number of atoms	11312	11309
Instability index	51.79 (Unstable protein)	52.17 (Unstable protein)
Aliphatic index	82.57	82.03
Grand average of hydropathicity (GRAVY)	-0.460	-0.471

Prediction of the protein and mRNA structure

For two missense mutations found in *EPB41L5* of goat, the secondary structure of the protein and mRNA before and after the mutation at g. 64266710 G> C, g.64266715 G> C and g. 64237881 A>C were predicted, respectively. It can be seen that alpha helix, extended strand, beta turn and random coil in the secondary structure of the protein have changed before and after the mutation (Table VI). The results showed that the secondary structure

of the protein (Fig. 4A, B) and mRNA (Fig. 4C, D) did change significantly after the mutation. And its minimum structural free energy is reduced, from -716.13kJ mol^{-1} to -719.88.kJmol^{-1}.

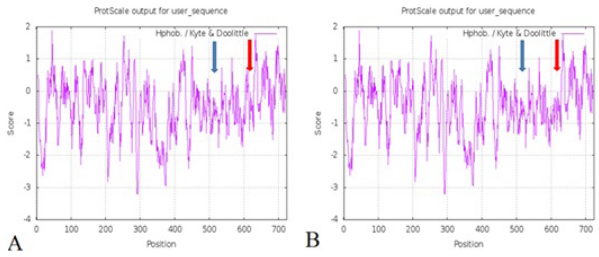

Fig. 3. Hydrophobicity of EPB41L5 protein before and after mutation. (A) The hydrophobicity of the protein before mutation; (B) The hydrophobicity of the protein after mutation. Note: The blue arrow refers to the g. 64266710 G> C and g.64266715 G> C locus; the red arrow refers to the g. 64237881 A> C locus. The ordinate in the figure represents the hydrophobicity score of the protein. The area above the value of 0 is the hydrophobic area, and the area below the value of 0 is the hydrophilic area. The higher the score, the stronger the hydrophobicity of this area; the lower the score, the lower the hydrophobicity of this area. The abscissa represents the position of the amino acid.

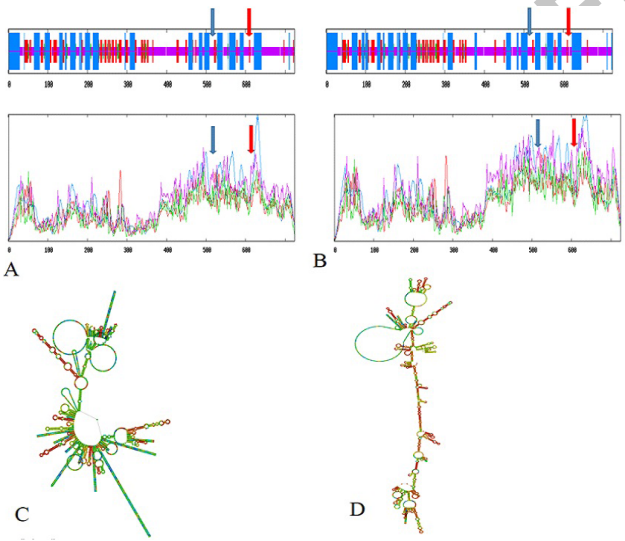

Fig. 4. The secondary structure of the protein and mRNA before and after the mutation at g. 64266710 G> C, g.64266715 G> C and g. 64237881 A> C. (A) Secondary protein structure before the mutation; (B) Secondary protein structure after the mutation; (C) mRNA secondary structure before the mutation; (D) mRNA secondary structure after the mutation. Note: The blue arrow refers to the g. 64266710 G> C and g.64266715 G> C locus; The red arrow refers to the g. 64237881 A> C locus.

Table VI. The secondary protein structures before and after *EPB41L5* mutation.

The secondary protein structures	Before mutation	After mutation
Alpha helix(Hh)	31.91%	33.98%
Pi helix(Ii)	0.00%	0.00%
Beta bridge(Bb)	0.00%	0.00%
Extended strand (Ee)	16.30%	14.50%
Beta turn(Tt)	3.18%	2.90%
Bend region(Ss)	0.00%	0.00%
Random coil(Cc)	48.62%	48.62%

DISCUSSION

Fibroblast growth factor receptor 1 (*FGFR1*) was located on goat chromosome 27. The binding of FGF led to FGFR dimerization and conformational changes, which phosphorylate the tyrosine kinase domain. Fibroblast growth factor receptor substrate 2 was a crucial adaptor protein in the FGFR signaling cascade. The phosphorylated tyrosine residues in turn became its docking site, and its phosphorylation led to adaptor proteins such as the recruitment of SOS and GRB2, activated downstream MAPK, PI3K/AKT and other signaling pathways (Datta *et al*., 2017). The physiological roles of FGF/FGFR signaling pathway included embryonic development, wound healing, angiogenesis and tissue crosstalk (Heinzle *et al*., 2011). In addition, the FGF/FGFR signaling pathway was essential for cell proliferation, migration, differentiation, apoptosis and survival (Dieci *et al*., 2013). Studies have shown that abnormal FGF/FGFR signals are closely related to the pathogenesis of a variety of cancers, including lung cancer, gastric cancer, breast cancer, ovarian cancer, urothelial cancer and endometrial cancer (Dorey and Amaya, 2020). Erythrocyte membrane protein band 4.1 like 5 (*EPB41L5*) also known as BE37, YMO1, had a molecular weight of 85 kD. *EPB41L5* discovered for the first time in November 2007, which was a protein related to cytoskeleton and cell movement (Rhim *et al*., 2012), and it was widely expressed in mammalian embryonic mesoderm and endoderm epithelial tissues (Tepass, 2009). It had a FERM domain at their N-terminus, but lacks an actin binding site at their C-terminus (Hashimoto *et al*., 2016). *EPB41L5* was involved in the regulation of many important biological processes, included the cellular actin skeleton, cell adhesion molecules, EMT, basement membrane formation, cell polarity formation and other processes. Research has shown that the FERM domain contained in *EPB41L5* can cross-link with the

myosin protein containing the myosin tail homology 4 (MyTH4) domains. Involved in regulating the formation of pseudopodia and cell movement, and can be combined with the cytoskeleton protein Talinl, PTK, etc., (Sordella, 2003). Hirano's (Hirano *et al*., 2008) research showed that *EPB41L5* overexpression can reduce Ecadherin on the cell membrane surface through the action of its FERM domain, thereby promoting the EMT process. Gosens *et al*. (2007) found that CRB-MPP5-EPB41L5 complex can regulate a variety of cell adhesion factors, it played an important role in the tight junctions between cells and in the formation of cell polarity, and was closely related to epithelial cell polarity and basement membrane formation. In addition, experiments have shown that *EPB41L5* can affect the expression of cell adhesion molecules and skeletal proteins in embryonic endoderm epithelial cells (Hirano *et al*., 2008). At the same time, *EPB41L5* can regulate cell polarity and was an essential protein for basement membrane formation and embryonic gastrulation (Takakuwa *et al*., 1986). In summary, the expression of *EPB41L5* is related to embryonic development and may affect goat kidding traits. The genotype frequency and allele frequency of *EPB41L5* reached extremely significant levels between the high- and low-reproduction goat populations ($P< 0.01$). Therefore, it is speculated that its mutation may have a certain impact on fecundity, and it needs to be further verified by experiments. Association analysis showed that there were significant correlation between *EPB41L5* gene g.64237881A> C and g.64266710G> C and number of litter size ($P< 0.05$). There were significant correlation between *EPB41L5* gene g. 64266710C> G and litter weight at weaning ($P< 0.05$). It indicates that *EPB41L5* gene g. 64237881A> C and g. 64266710G> C may be critical sites for the number of litter size and litter weight at weaning. Therefore, in order to provide a reference for goat molecular breeding, this study aimed to analyze the genetic polymorphisms of *FGFR* and *EPB41L5* in Yunshang Black goat, Jining Grey goat and Liaoning Cashmere goat population and their association with kidding performance.

Correlation analysis showed that g. 64237881A> C, g. 64266710G> C of *EPB41L5* gene may affect kidding performance. In order to further explore the changes in the structure of the protein before and after the mutation of g. 64237881A> C and g. 64266710G> C, two sites were analyzed by bioinformatics. Bioinformatics analysis of *EPB41L5* gene showed that the gene conforms to the laws of biological evolution. Before and after the mutation, the physical and chemical properties, the secondary structure of the protein and mRNA were changed. Therefore, it is possible that mutations in the site may result in changes in the structure of the protein, which in turn may result in changes in function. Therefore, g. 64237881A> C, g. 64266710G> C of *EPB41L5* gene may be the key site for litter size and litter weight at weaning.

CONCLUSIONS

There was significant correlation between *EPB41L5* gene g. 64237881A> C and g. 64266710G> C and litter size ($P< 0.05$). There was significant correlation between *EPB41L5* gene g. 64266710C> G and litter weight at weaning ($P< 0.05$). Therefore, these results suggested that the *EPB41L5* gene g. 64237881A> C and g. 64266710G> C loci are suitable as a molecular marker for litter size in Yunshang Black goat, and g. 64266710G> C locus is suitable as a selection marker for litter weight at weaning.

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Statement of conflict of interest

All authors have declared no conflicts of interest.

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