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A Novel Mutation at Exon 4 of IGF-1 Gene in Three Indigenous Goat Breeds in China

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ABSTRACT

This study was designed to analyze the association between polymorphisms of insulin-like growth factor-1 (IGF-1) and cashmere production traits (fineness, thickness and yield) and body weight. PCR-SSCP (single-strand conformational polymorphism) and DNA sequencing were used to detect polymorphisms of IGF-1 gene in 776 samples of three Xinjiang local goat breeds (Xinjiang, Bogeda Cashmere and Nanjiang Cashmere Goat). The obtained results showed that the frequencies of genotype AA were 0.414, 0.591 and 0.319. Genotype AB were 0.000, 0.126 and 0.029. Genotype BB were 0.586, 0.241 and 0.597 and genotype AC were 0.000, 0.042 and 0.055 for Xinjiang, Bogeda Cashmere and Nanjiang Cashmere Goat breeds, respectively. The mutation was detected at IGF-1-P1 locus, a novel of SNP was revealed in exon 4 (Genebank D26119: c.1617 G >A, c.1620 C>T) and they belong to silent mutation, which was the first reported in international goat. In addition, a novel of SNP at IGF-1-P1 locus is significantly associated with cashmere production traits. In Nanjiang Cashmere Goat population, the cashmere fineness of AA genotype individual was significantly ($p<0.05$) lower than that of AB genotype. The body weight of AC genotype individual was significantly ($p<0.05$) higher than that of BB genotype.

Key words: Mutation, exon 4, IGF-1 gene, PCR-SSCP, goat

INTRODUCTION

In animal industry, growth traits of animal are always of primary concern during breeding for its determinant economical value (Zhang *et al.*, 2005). With the development of molecular biology and biotechnology, scientists are able to achieve more accurate and efficient selection goal.

The IGFs signaling system, which composed of IGF-I, IGF-II, IGF-I receptor, IGF-II receptor and six binding proteins (IGFBP-1~IGFBP-6), play an important role in development, growth and reproduction as well as ageing (Bale and Conover, 1992; Hastie *et al.*, 2004; Duan and Xu, 2005). In addition, IGF-1 is only one component of the complex IGF superfamily (Hwa *et al.*, 1999) that plays an essential role in mammalian reproduction (Liu *et al.*, 1993; Baker *et al.*, 1996; Zhou *et al.*, 1997; Kadakia *et al.*, 2001). The conventional wisdom regarding the actions of IGF-1 in mammals is that IGF-1 mainly a mitogenic/differentiation response *in vivo* (Siddle *et al.*, 2001). Some researches add to findings that the IGF-1 protein can accelerate the proliferation and differentiation of the cells in the skin (Zhang *et al.*, 2005; Philpott *et al.*, 1994) and play an important role in the control of hair cycles (Philpott *et al.*, 1995; Nixon *et al.*, 1997).

Recently, though few molecular researches in goat of IGF-1 gene have been reported (Palsgaard *et al.,* 2009; Velazquez *et al.,* 2008), but they were not related to production traits. The present study was designed to analyze the genetic variations of IGF-1 gene in 776 samples of three Xinjiang local goat breeds (Xinjiang, Bogeda Cashmere and Nanjiang Cashmere Goat) in China by PCR-SSCP and DNA sequencing methods. In addition, the association between the polymorphism of IGF-1 gene and cashmere production traits was analyzed, through these, provide reference for early selection and MAS breeding for Xinjiang local goat breeds in China.

MATERIALS AND METHODS

Animal source: In this research, genomic DNA samples were obtained from 776 individuals belonging to three Xinjiang local goat breeds (Xinjiang, Bogeda Cashmere and Nanjiang Cashmere Goat) from April 10, 2008 to April 25, 2008. Among them, a total of 207 Xinjiang Goat were from the breeding centre of Kuerle of Xinjiang in China; a total of 279 Bogeda Cashmere Goat were from Breeding Farm of Bogeda Goat and a total of 290 Nanjiang Cashmere Goat were from AkeSu Goat Research Center of Xinjiang. The traits evaluated including cashmere production traits (fineness, thickness and yield) and body weight. Approximate 3~5 mL blood per goat was collected aseptically from the jugular vein and kept in a tube containing anticoagulant ACD. All samples were delivered back to the laboratory in an ice box.

DNA preparation and primer design: The genomic DNA of 776 cashmere goat was isolated from 2% heparin-treated blood samples and stored at -80°C for use, following standard procedures according to Sambrook and Russell (2001). Based upon the IGF-1 gene sequences (GenBank accession: D26119), one pair of PCR primers was designed to amplify the goat IGF-1 gene with Primer 5.0 software, as follow:

- Forward: 5'- AAAATCTTTGCCCTGTCG -3'
- Reverse: 5'- CTACCGGGCATGAAGACAC -3'

They were used to amplify 233 bp PCR products, containing for Capra IGF-1 gene exon 4 and partial of intron 4 locus (P1 locus).

PCR amplification: All PCR reactions were performed in a 25 μL mixture containing 50 ng genomic DNA, 0.5 μM of each primer, 1×Buffer (including1.5 mM $MgCl_2$), 200 μM dNTPs and 0.625 units of Taq DNA polymerase (MBI). The PCR was performed using the following program: 94°C for 5 min, 35 cycles of 94°C for 40 sec, annealing 62°C for 35 sec, 72°C for 35 sec and a final extension at 72°C for 10 min.

PCR-SSCP and DNA sequencing: Aliquots of 5 μL PCR products were mixed with 5 μL denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled on ice (Sun *et al.,* 2002). Denatured DNA was subjected to PAGE (80×73×0.75 mm) in 1×TBE buffer and constant voltage (140~180 V) for 14~16 h. The gel was stained with 0.1% silver nitrate.

The PCR fragments from different SSCP patterns in the three goat breeds were subcloned by the pair of primers and sequenced in both directions by ABI PRIZM 233 DNA sequencer (PerkinElmer). The sequences were analyzed with BioXM software (version 2.6).

Statistical methods and analysis: Statistical analysis was performed on the basis of records of cashmere production traits in Xinjiang Goat (n = 207), Bogeda Cashmere Goat (n = 279) and Nanjiang Cashmere Goat (n = 290).

In three Xinjiang local goat breeds, population genetic indexes, such as He (gene heterozygosity), Ho (gene homozygosity), Ne (effective allele numbers) and PIC (Polymorphism Information Content) were calculated according to Nei and Roychoudhury (1974) and Nei and Li (1979), respectively.

$$H_o = \sum_{i=1}^{n} P_i^2,\ H_e = 1 - \sum_{i=1}^{n} P_i^2$$

In addition, the differences among genotypic frequencies at IGF-1-P1 locus were analyzed using a χ^2-test, which were performed by SPSS software (version 16.0) according to Norušis (2008).

All analyses were done in two steps, first using a full animal model and then using a reduced animal model. The full animal model included fixed effects of marker genotype, age of ram, ewe, sex, farm, body weight, after combed cashmere fineness, down cashmere thickness, cashmere yield and random effects of animal. The reduced model was used in the final analysis (Boldman *et al.,* 1993; Zhao *et al.,* 2004). The software SPSS (version 16.0) was used to analyze the relationship between the genotypes and cashmere traits in goat. The reduced linear model with fixed effects was established and included effects of ewe, ram within ewe, age and genotype, as well as interaction between ram and genotype was involved. Reduced linear model:

$$Y_{ijklm} = u + S_i + D_{ij} + A_k + G_l + (SG)_{il} + E_{ijklm}$$

where, Y_{ijklm} was the trait measured on each of the ijklmth animal, u was the overall population mean, S_i was the fixed effect associated with the ith ram, D_{ij} was the fixed effect associated with jth ewe with ram i, A_k was fixed effect due to the kth age, G_l was the fixed effect associated with lth genotype (IGF-1/AA, AB and BB genotype), $(SG)_{il}$ was interaction between the ith ewe and the lth genotype and E_{jiklm} was the random error.

An effect associated with farm, sex were not matched in the linear model, as the preliminary statistical analyses indicated that these effect did not have a significant influence on variability of traits in analyzed populations. The Least Square Means estimates (LSM) with standard errors for three genotypes of IGF-1 gene and growth traits were used (Zhao *et al.,* 2004; Liu *et al.,* 1993).

RESULTS

In this study, DNA sequencing analysis showed, at the P1 locus, the sequence of PCR products of IGF-1 gene is the same to sequence (D26119) in Genebank. Then, at IGF-1-P1 locus, there were two mutations (c.1617 G > A and c.1620 C > T) in exon 4 region (Fig. 1), further analysis indicates that they all belong to silent mutation. The 233 bp PCR products including Capra IGF-1 gene exon 4 and partial of intron4 region (P1 locus) were amplified by one pair of PCR primers (Fig. 2). In addition, it was demonstrated that there were four genotypes (named as genotype AA, AB, AC and BB) by PCR-SSCP method (Fig. 3).

From the community genetics angle, the genotypic frequencies and allelic frequencies of different genotype in three goat breeds were calculated (Table 1). Table 1 indicated that frequencies

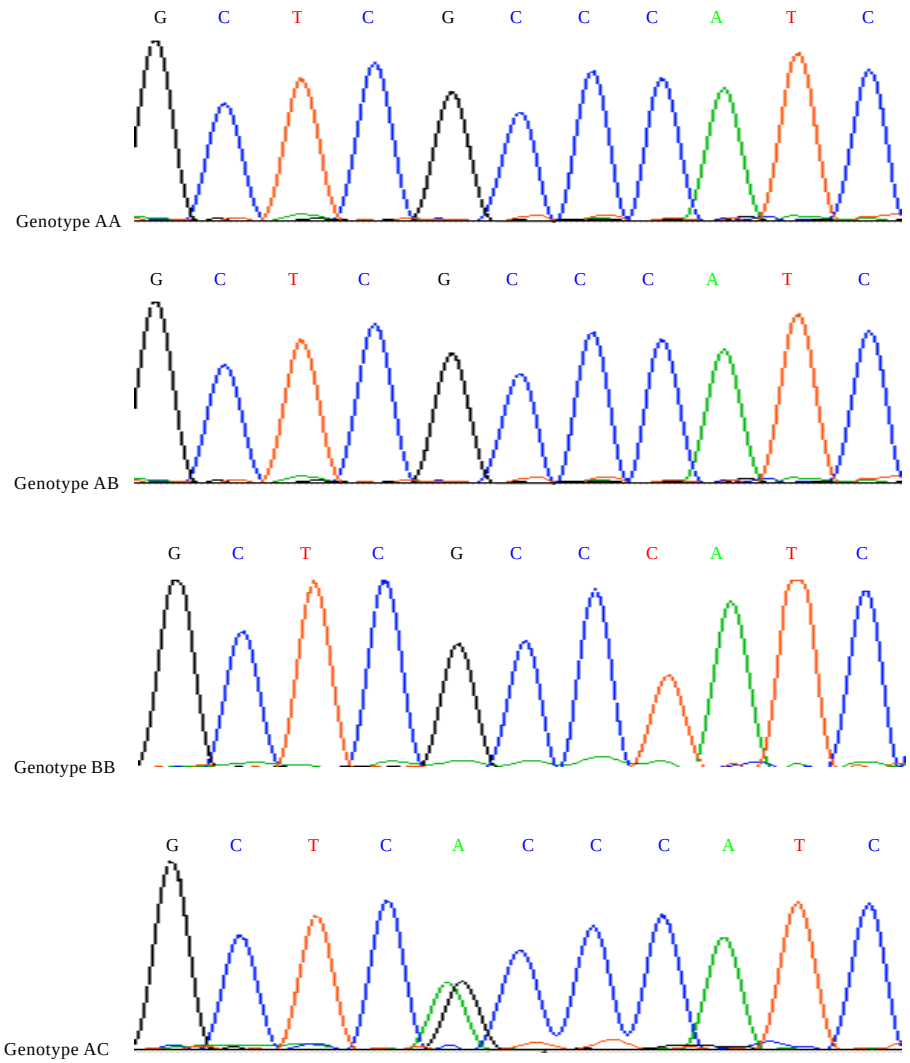

Fig. 1: Sequencing maps from different genotypes in goat breeds IGF1-P1 locus

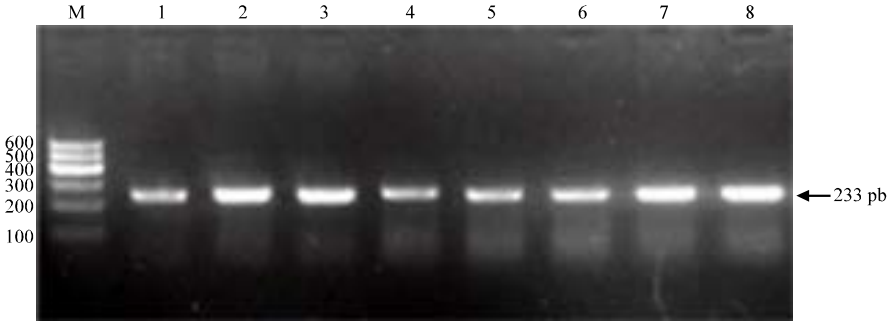

Fig. 2: Detection of PCR product of the IGF1-P1 locus M:DNA molecular weight marker is Marker I

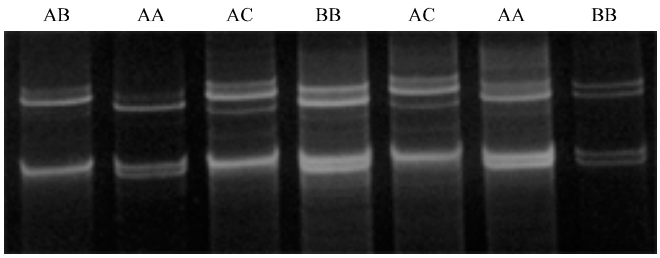

Fig. 3: Detection of the PCR-SSCP at the IGF1-P1 locus

Table 1: Genotype distribution and allelic frequencies at the IGF1-P1 locus

	Genotype and genotype frequencies					Allele frequencies		
Breeds	AA	AB	BB	AC	No.	A	B	C
Xinjiang goat	89/0.414	0	118/0.586	0	207	0.414	0.586	0
Bogeda cashmere goat	165/0.591	35/0.126	68/0.241	11/0.042	279	0.675	0.304	0.021
Nanjiang cashmere goat	91/0.319	8/0.029	177/0.597	14/0.055	290	0.361	0.611	0.027

Table 2: He, Ho, PIC and Ne of IGF1-P1 locus in goat breeds

Breeds	Ho	He	Ne	PIC
Xinjiang goat	0.515	0.485	1.942	0.3674
Bogeda cashmere goat	0.548	0.452	1.824	0.3669
Nanjiang cashmere goat	0.505	0.495	1.980	0.3967

Ho: Gene homozygosity, He: Gene heterozygosity, Ne: Effective allele numbers, PIC: Polymorphism information content

of haplotype IGF-1-P1-A, B, C were 0.414, 0.586, 0.000 in Xinjiang Goat (n = 207), 0.675, 0.304, 0.021 in Bogeda Cashmere Goat (n = 279) and 0.361, 0.611, 0.027 in Nanjiang Cashmere Goat (n = 290). B haplotype and BB genotype were predominant in Xinjiang Goat. A haplotype and AA genotype were predominant in Bogeda Cashmere Goat. B haplotype and BB genotype were predominant in Nanjiang Cashmere Goat. The χ^2-test showed that the genotype distributions of IGF-1-P1 loci were in disagreement with Hardy-Weinberg equilibrium in three breeds.

According to Nei's methods, the population genetic indexes (namely, gene homozygosity (Ho), gene heterozygosity (He), effective allele numbers (Ne) and Polymorphism Information Content (PIC)) were calculated (Table 2). Table 2 showed that Ho varied from 0.505 (Nanjiang Cashmere Goat) to 0.548 (Bogeda Cashmere Goat) and Ne ranged from 1.824 (Bogeda Cashmere Goat) to 1.980 (Nanjiang Cashmere Goat). The minimum and maximum PIC values were 0.3674 and 0.3967. Due to the classification of PIC (low polymorphism if PIC value<0.25, median polymorphism if 0.25<PIC value<0.5 and high polymorphism if PIC value>0.5), IGF-1 gene in three Xinjiang local goats was at median polymorphic level.

Moreover, analyses of variance (Table 3) indicated that various genotypes in the three Xinjiang goat breeds were highly significant ($p<0.01$). Table 4 demonstrated that the polymorphism of IGF-1 gene was not associated with cashmere production traits in Xinjiang Goat. However, at this P1 loci, the polymorphism of IGF-1 gene was associated with cashmere production traits in Nanjiang Cashmere Goat ($p<0.05$) (Table 5). The cashmere fineness of AA genotype individual was significantly lower than that of AB genotype ($p<0.05$). The body weight of AC genotype individual was significantly higher than that of BB genotype ($p<0.05$).

Table 3: Chi-Square analysis of genotype distribution at IGF1-P1 locus

Breeds	Xinjiang goat	Nanjiang cashmere goat	Bogeda cashmere goat
Xinjiang goat		0.000	0.000
Nanjiang cashmere goat	21.666**		0.000
Bogeda cashmere goat	82.375**	87.497**	

Above diagonal data showed p-value of genotype distribution, below diagonal data showed χ^2 of genotype; Value with *and** differ significantly at $p<0.05$ and $p<0.01$, respectively

Table 4: Least square means for genotype of IGF1-P1 locus in xinjiang goat

	Genotypes (Mean±SE)	
Cashmere traits	AA	BB
Cashmere fineness (μm)	15.5±0.2	15.5±0.2
Cashmere thickness (cm)	3.5±0.2	3.1±0.2
Cashmere yield (g)	142.1±7.5	156.0±7.4
Body weight (kg)	29.4±1.1	30.0±1.1

Estimates are given as Mean±SE. Data with a different letter (a, b, c) within the same line differ significantly at $0.01<p<0.05$. Values with different superscripts within the same line differ significantly at $p>0.05$. SE: Standard error of means

Table 5: Least square means for genotype of IGF1-P1 locus in nanjiang cashmere goat

	Genotypes (Mean±SE)			
Cashmere traits	AA	AB	AC	BB
CD (μm)	$15.6±0.9^{a}$	$16.2±0.3^{b}$	$15.9±0.2^{ab}$	$15.7±0.1^{ab}$
CT (cm)	4.7±0.1	5.0±0.2	4.8±0.2	4.7±0.1
CY (g)	463.9±10.4	481.1±34.6	442.4±25.1	469.3±7.6
BWC (kg)	$21.6±0.3^{ab}$	$21.8±0.9^{ab}$	$22.5±0.6^{a}$	$21.1±0.2^{b}$

CD: Cashmere fineness, CT: Cashmere thickness, CY, Cashmere yield, BWC: Body weight after combed. Estimates are given as Mean±SE. Data with a different letter (a, b, c) within the same line differ significantly at $0.01<p<0.05$. SE: Standard error of means

DISCUSSION

It is known from past studies (Froesch *et al.,* 1996) that insulin-like growth factor (IGF-1) is a peptide that plays an important stimulatory role in skeletal growth, cell differentiation and metabolism. In addition, there is further demonstration that the IGF-1 gene is important in the control of hair cycles (Philpott *et al.,* 1995; Nixon *et al.,* 1997) and believed to be involved in growth of wool fiber. So this dissertation mainly specializes in the association between polymorphisms in insulin-like growth factor-1 (IGF-1) and cashmere traits data with three Xinjiang local goat breeds in China.

In the past, some researches investigated the association between polymorphisms of IGF gene and livestock production traits. For example, the study of Lan *et al.* (2007) detected for the first time the polymorphisms of goat IGFBP-3 gene by PCR-SSCP and DNA sequencing methods. The associations of the HaeIII and XspI PCR-RFLPs of goat IGFBP-3 locus with milk traits were analyzed in dairy goat, but the significant statistical results were not found between them ($p>0.05$). Other study (Kumar *et al.,* 2006) was carried out to study nucleotide sequencing and DNA polymorphism by PCR-RFLP of IGFBP-3 gene in sheep and its comparison with cattle and buffalo. There was approximately 93% similarity in the amino acid sequence of sheep with cattle and buffalo.

In this present study, the mutation was detected at IGF-1-P1 locus, a novel of SNP was revealed in exon4 (Genebank D26119: 1617 G>A, 1620 C>T) by DNA sequencing method. In addition, further analysis indicates that the two mutations belong to silent mutation. The SNP at IGF-1-P1 region may not be a causal mutation in IGF-1 protein, which maybe lead to protein with the same amino acids sequence but different structural and functional properties (Komar, 2007). In the Nanjiang Cashmere Goat populations, the cashmere fineness of AA genotype individual was significantly lower than that of AB genotype ($p<0.05$). The body weight of AC genotype individual was significantly higher than that of BB genotype ($p<0.05$). These results indicated that the polymorphism of IGF-1 gene might be relevant to cashmere production traits. The discovery is the first reported in international goat.

By the PCR-SSCP method, the result indicated that there were four genotypes (named as genotype AA, AB, AC and BB). However, among of four genotypes the Xinjiang goat populations only have genotype AA and BB, genotype CC was disappeared. There may be two reasons, first, because the Xinjiang goat populations should only have genotype AA and BB and the other is because the samples of genotype CC could not be collected. On the other hand, gene heterozygosity, effective allele numbers and PIC of IGF-1-P1 locus were lower in Xinjiang Goat population than that of Nanjiang Cashmere Goat population. This reflected that there was not a very high genetic diversity within Chinese Capra IGF-1 gene in analyzed populations, which could explain that all analyzed samples were Homozygosity individuals.

CONCLUSION

As IGF-1 associated with IGFs system, at the P1 locus, a novel of SNP was revealed in exon4 (Genebank D26119: 1617 G>A, 1620 C>T). The genetic variations at IGF-1-P1 loci may alter protein function.

In addition, in the Nanjiang Cashmere Goat population, at IGF-1-P1 loci, the polymorphism of IGF-1 gene is significantly associated with cashmere production traits. The cashmere fineness of AA genotype individual was significantly lower than that of AB genotype ($p<0.05$). The body weight of AC genotype individual was significantly higher than that of BB genotype ($p<0.05$). The IGF-1 gene can be regarded as candidate gene on cashmere production traits.

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