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The Polymorphism of a Mutation of IGF-1 Gene on Two Goat Breeds in China

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Abstract: Searching for effects of candidate gene polymorphisms on cashmere production traits is an important goal for goat industry. Genetic variations in IGF-1 may alter protein function. This study investigated the association between polymorphisms in insulin-like growth factor-1 (IGF-1) and cashmere traits data with two Xinjiang local goat breeds. The results showed IGF-1-P1 locus has polymorphisms. The polymorphism locus of IGF-1-P1 by PCR-RFLP and DNA sequencing methods in 530 individuals from two Xinjiang local goat breeds in China. The frequencies of genotype AA in two goat breeds (Xinjiang goat, Nanjiang cashmere goat) were 0.487 and 0.277. Genotype BB was 0.274 and 0.486. Genotype AB was 0.239 and 0.236. The polymorphisms of the IGF-1 gene were associated with cashmere yield, fiber diameter, body weight in cashmere goat. However, concern on cashmere production traits among three genotypes were shown not significantly ($p>0.05$).

Key words: PCR-RFLP, IGF-1 gene, cashmere goat, cashmere traits, genotypes, China

INTRODUCTION

In the present study, the insulin-like growth factor-1 (IGF-1), insulin-like growth factor-2 (IGF-II) and insulin, plays a central role in the regulation of development and growth by its potent metabolic and mitogenic action (Reinecke and Collet, 1998). Hence, the polymorphisms of the IGF-1 gene analyzed as a genetic marker candidate for people widely believed to be involved in carcinogenesis (Furstenberger and Hans-Jorg, 2002; Pollak *et al*., 2004). Report the Insulin-Like Growth Factor (IGF) system plays a significant role in the regulation of the metabolism and physiology of mammalian growth (LeRoith *et al*., 1992; Tatar *et al*., 2003) and in a variety of health outcomes (Rosen, 1999; Juul, 2003; Juul *et al*., 2002; Vasan *et al*., 2003). The IGF-1 protein can accelerate the proliferation and differentiation of the cells in the skin (Zhang *et al*., 2005; Philpott *et al*., 1994). There is growing evidence that the IGFs are important in the control of hair cycles (Philpottt *et al*., 1995; Nixon *et al*., 1997).

In addition, it believed to be involved in growth of wool fiber traits. In this study, we focus on associations between the IGF-1 gene and a number of cashmere production traits data. These studies could help explain experimental and influence on cashmere production traits. It suggested that the IGF-1 plays a role in cashmere production traits, also. To date, there are few polymorphisms studies within cashmere production traits of IGF-1 gene have been reported. Few molecular research in Xinjiang local goat, also. Therefore, it was a preliminary and interesting research to analyze the genetic variations of IGF-1 gene in 530 goats individual of Xinjiang in China. Herein, we are the first to identify the novel genetic variation of cashmere goat IGF-1 gene by PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymor-phism) and DNA sequencing methods, which will possibly contribute to conducting association analysis and evaluating them as genetic markers in cashmere production and other performance for animal breeding and genetics.

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MATERIALS AND METHODS

Animal source: Five hundred and thirty all unrelated animals (Xinjiang goat, n = 220; South of Xinjiang Cashmere goat, n = 310) were collected and used in this study. The Xinjiang goat were from the breeding centre of KuErLe of Xinjiang in China, the Nanjiang cashmere goat were from AkeSu Goat Research Center of Xinjiang. Many records of cashmere traits and body weight were used to statistical analysis.

DNA preparation and Primer design: Genomic DNA of 530 cashmere goat were isolated from 2% heparin-treated blood samples and stored at -80°C, following standard procedures (Sambrook and Russell, 2001). According to the sequence of IGF-1 (GenBank accession number D26119), one pair of PCR primers was designed with Primer 5.0, as follow:

Forward: 5'- CACAGCGTATTATCCCAC -3'
Reverse: 5'- GACACTATGAGCCAGAAG -3'

They were used to amplify 363 bp PCR products, containing for Capra IGF-1 gene exon 4 and partial of intron4 locus (P1 locus).

PCR amplification: One pair of PCR primers was designed using Primer 5.0 software to amplify the coding and flanking region of Capra IGF-1 gene (GenBank accession D26119), the size of PCR products was 363 bp. The 25 μL volume contained: 50 ng genomic DNA, 0.5 μM of each primer, 1× Buffer (including 1.5 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 followed by 35 cycles of 94°C for 40 sec, annealing for 35 sec and 72°C for 35 sec and a final extension at 72°C for 10 min.

PCR-RFLP and DNA sequencing: Aliquots of 10 μL PCR products of IGF-1 gene were incubated with 6 units restriction enzyme HaeIII (MBI fermentas) for 8 h at 37°C, then electorphoresed on 2% agarose gel stained with 1× TBE buffer (89 mM Tris, 89 mM boric acid and 2 mM Na_2EDTA), containing 200 ng mL^{-1} ethidium bromide. A 6 μL aliquot of PCR products was added to 1.5 uL of loading dye (0.025% bromophenol blue, 0.025% xylene cyanol, 40%, w/v sucrose) and the gels were run at a constant voltage (100 V) for 0.3-0.5 h.

The PCR fragments from different patterns in the two breeds were amplified by the pair of primers were sequenced in both directions by ABI PRIZM 363 bp DNA sequencer (PerkinElmer) and the sequences were analyzed with BioXM software (version 2.6).

Statistical methods and analysis: In these goat breeds, base on the genotypes of IGF-I-P1-HaeIII locus in two goat breeds, the genotypic frequencies, the allelic frequencies and Hardy-Weinberg equilibriums were directly. Differences genotypic frequencies at IGF-I-P1 locus among indigenous goat and cashmere goat populations in China were analyzed and using a χ^2-test, which were performed by SPSS software (version16.0) (Norusis, 2008). 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.

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

Furthermore, statistical analysis was performed for records of cashmere traits in Nanjiang cashmere goat (n = 310) and Xinjiang goat (n = 220). 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} = The trait measured on each of the ijklmth animal
μ = The overall population mean
S_i = The fixed effect associated with the ith ram
D_{ji} = The fixed effect associated with jth ewe with ram i
Ak = Fixed effect due to the kth age
G_l = The fixed effect associated with lth genotype (IGF-1/AA, AB and BB genotype)
$(SG)_{il}$ = Interaction between the ith ewe and the lth genotype
E_{jiklm} = 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*., 2009).

RESULTS AND DISCUSSION

In this study, screening for polymorphisms in all the analyzed regions revealed the presence of a polymorphic site in IGF-1 (Table 1 and 2). The mutation was detected at IGF-1-P1 locus (Fig. 1), however, it was demonstrated that there were three genotypes (named as genotype AA, BB and AB) (Fig. 2) of the exon 4 and intron 4 partial region (P1 locus) by PCR-RFLP method. DNA sequencing analysis showed, in this locus, the sequence of PCR products of P1 locus is the same to sequence (D26119) in Genebank. In intron 4 region, a novel of SNP was revealed in intron 4 (Genebank D26119 g. 5752G>C). The frequency of genotype AB is low in two breeds (Xinjiang goat, 0.236; Nanjiang Cashmere goat, 0.239). Genotypic and haplotypic frequencies of IGF-1-P1 locus in the two breeds were showed in Table 2. Frequencies of haplotype IGF-1-P1-A, B were 0.605, 0.395 in Xinjiang goat (n = 220), 0.606, 0.394 in Nanjiang Cashmere goat (n = 310). A haplotype and AA genotype were predominant in Nanjiang cashmere goat. The χ^2-test showed that the genotype distributions of IGF-1-P1 loci ere in agreement with Hardy-Weinberg equilibrium in two breeds (Table 1 and 2). Based on χ^2-test, genotypic frequencies of the various polymorphism at IGF-1-P1 loci were found to be significantly different in the two breeds ($p<0.01$), as well as allelic frequencies among the two breeds were also significantly different ($p<0.01$). Gene homozygosity, gene heterozygosity, effective allele numbers, PIC of IGF-1 gene in the two breeds, respectively (Table 2). According 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 Xinjiang goat population, Nanjiang Cashmere goat was at median polymorphic level. Gene heterozygosity, effective allele numbers and PIC of IGF-1 gene in Xinjiang goat population were higher than that of Nanjiang cashmere goat population. According to Nei and Roychoudhury (1974) and Nie and Lie (1979) methods, the population genetic indexes (namely, gene homozygosity, gene heterozygosity, effective allele Numbers (Ne) and Polymorphism Information Content (PIC)) were calculated (Table 2). Among the loci of the two populations showed low polymorphism.

The values of PIC, He of Xinjiang goat breed in the loci were higher than that of Nanjiang cashmere goat populations, which implied that the polymorphism and genetic variation of Xinjiang goat breed were higher than that of Nanjiang cashmere goat. Gene homozygosity varied from 0.522 (Xinjiang goat) to 0.523 (Nanjiang

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

	Observed genotypes and frequencies				Allelic frequencies		
Breeds	AA	AB	BB	Total	A	B	χ^2(HWE)
Xinjiang goat	61/0.277	52/0.237	107/0.486	220	0.3955	0.6045	77.473
Nanjiang cashmere goat	151/0.487	74/0.2387	85/0.274	310	0.6065	0.3935	56.252

Bold values: Genotype frequencies at the IGF-1-P1 (Exon4 and partial of intron4 region) locus; χ^2 (HWE): Hardy-Weinberg Equilibrium χ^2-value

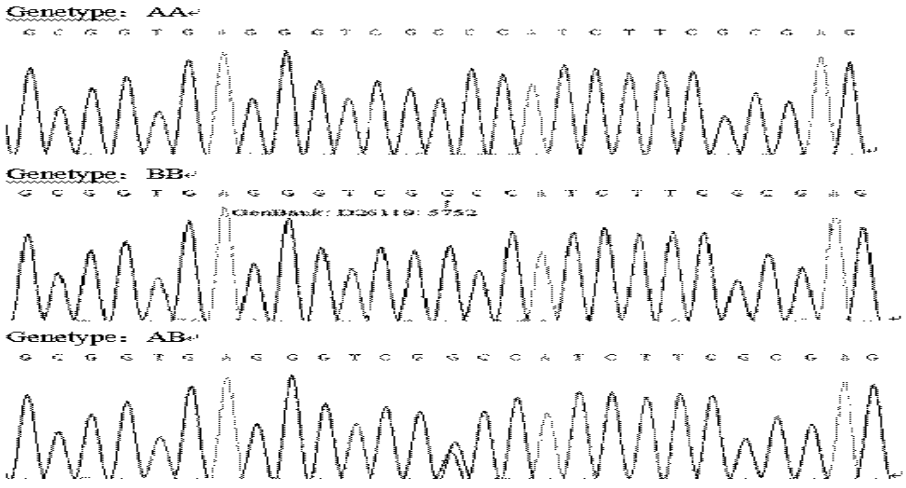

Fig. 1: Sequencing maps from different genotypes in cashmere goat IGF-1 gene

Table 2: Genetic indexes in two Xinjiang local goat in China

Breeds	Ho	He	Ne	PIC
Xinjiang goat	0.522	0.478	1.916	0.364
Nanjiang cashmere goat	0.523	0.477	1.913	0.363

Ho: Gene Homozygosity; He: Gene Heterozygosity; Ne: Effective allele numbers; PIC: Polymorphism Information Content

Table 3: Association of genotypes at the IGF-1 gene Exon4 and partial of intron4 region locus with growth traits in Nanjiang cashmere goat

	Genotypes at IGF-1 gene			
Cashmere traits	AA (Mean±SE)	AB (Mean±SE)	BB (Mean±SE)	p-value
BWC (kg)	21.563±0.222	21.216±0.317	21.788±0.296	>0.05
CD (μm)	15.748±0.070	15.621±0.099	15.794±0.093	>0.05
CT (cm)	4.712±0.057	4.615±0.082	4.702±0.076	>0.05
CY (g)	476.656±8.527	459.257±12.181	465.529±11.366	>0.05

BWC: Body Weight after Combed, CD: Cashmere Fineness, CT: Cashmere Thickness, CY: Cashmere Yield, *Values with different superscripts within the same line differ significantly at p>0.05. SE: Standard Error of Means

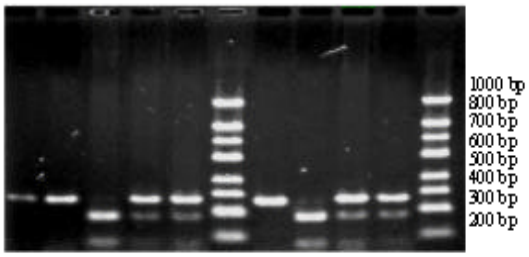

Fig. 2: PCR-RFLP patterns of the IGF-1-P2 in Cashmere goat, line: 6, 11: 100-bp ladder marker, line: 1, 2, 7 homozygous AA genotype (363 bp), line: 3, 8 homozygous BB genotype (264-99 bp), line: 4, 5, 9, 10 heterozygous AB genotype (363-264-99 bp)

cashmere goat) and Ne ranged from 1.913 (Nanjiang cashmere goat) to 1.916 (Xinjiang goat). The minimum and maximum PIC values were 0.363 and 0.364. This reflected that there was not a very high genetic diversity within Chinese cashmere goat IGF-1 gene in analyzed populations, which could explain that all analyzed samples were healthy individuals. In this study, we also revealed that the polymorphism of IGF-1 gene associated with fiber diameter, cashmere thickness, cashmere yield and body weight in Nanjiang cashmere goat, firstly. No other statistically significant differences were observed between the AA, BB and AC genotypes of the two breeds concerning growth traits ($p>0.05$) (Table 3).

The present study is the first report on polymorphism of IGF-1 gene associated with production traits data in cashmere goat in China. This research attempted to detect polymorphism at IGF-1 gene in two goat breeds of Xinjiang in China. The result showed that there was a mutation at IGF-1-P1 locus, which the first reported in Chinese goat. Just in Xinjiang goat was monomorphism at IGF-1-P1 locus, so mixture of genotypes maybe resulted in that gene heterozygosity, effective allele numbers and PIC of IGF-1-P1 locus were higher 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. Although, there were polymorphisms at IGF-1-P1 locus in these two breeds, genotypic frequencies and haplotypic frequencies of IGF-1-P1 locus were found not to be significantly different in the two breeds, which lead us more to believe that IGF-1 gene is very conservation in different breeds.

Cashmere traits were an important trait in cashmere goat. Few researchers have reported that genetic variation at IGF-1 loci might play an important role in determining various cashmere traits and associated with variation in fiber diameter, cashmere yield, the body weight after combed cashmere, cashmere thickness.

It is very interesting that among of fiber diameters, cashmere yield, cashmere thickness, body weight of Xinjiang goat, Nanjiang cashmere goat are thinner than that of others cashmere goat.

These results were could be explained that the polymorphism of IGF-1 gene might be relevant to fiber diameter, cashmere yield and cashmere thickness of cashmere. The SNP at IGF-1-P1 region may not be a causal mutation in IGF-1 protein, which may be lead to protein with the same amino acid sequence but different structural and functional properties (Komar, 2007).

We know that the genome is highly redundant in terms of tRNA species for each amino acids, but enigmatically under-represents a number of specific codons (Nei and Roychoudhury, 1974). In the synthesis of IGF-1 protein, if the change of base at this position is not represented by a corresponding codon within the nuclear tRNA, the rate of expression of the IGF-1 protein may change, which may affect fiber diameter, cashmere yield and cashmere thickness of cashmere.

CONCLUSION

This study revealed a novel SNP in cashmere goat of IGF-1-P1 locus. The SNP is association with fiber diameter, cashmere yield and body weight not significantly. It will be practical for the improvement of Chinese native goat and the breeding of genuine cashmere goat in China. The further research is warranted. However, the above described SNP (Genebank D26119: 5752G>C) of IGF-1 gene extend the spectrum of genetic variation of cashmere goat IGF-1 gene.

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