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Short Communication

Diversity of *TNF-α* region in Chinese domestic goats

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ABSTRACT. Tumor necrosis factor-α is a cytokine with a wide range of effects on both lymphoid and non-lymphoid cells. In this study, we identified polymorphisms in major histocompatibility complex class III gene in the 4th exon and the 3' untranslated region of tumor necrosis factor-α to evaluate the immunogenetic diversity of Chinese south indigenous goat. Three single-nucleotide polymorphisms were identified and showed similar frequencies in different except MI loci. These data suggest that the high immunodiversity of the tumor necrosis factor-α region within these breeds can be used for strengthening variety improvement and promoting animal husbandry development in Chinese indigenous goats.

Key words: Chinese indigenous goat; Immunological diversity; Major histocompatibility complex class III; Tumor necrosis factor-alpha

INTRODUCTION

Tumor necrosis factor-alpha (TNF-α) is an immunomodulatory and proinflammatory cytokine with a wide range of effects in both lymphoid and non-lymphoid cell types and belongs to major histocompatibility complex (MHC) class III genes. Relative to other parts of the MHC, the MHCIII region has the highest gene density and the lowest number of pseudogenes (Kulski et al., 2002). Class III genes with a clear role in immunobiology include members of the complement cascade (C4A, C4B, C2, and Bf) and genes such as TNF-α, lymphotoxin alpha, and lymphotoxin beta. C4, C2, and Bf are genes for complement proteins (Campbell et al., 1986). A recent study investigated allelic variation in the Ovar-TNF-α locus in sheep (Alvarez-Busto et al., 2004) and mountain goat (Shafer et al., 2012), which is part of the 4th exon and the 3' untranslated region (UTR) of the gene. In sheep, single-strand conformation polymorphism and sequence analysis of a 273-bp fragment revealed 3 different alleles including 1 deletion and 1 single-nucleotide polymorphism (Alvarez-Busto et al., 2004), but no variances were observed in goat (Shafer et al., 2012). In this study, we examined the diversity in the class III Ch-*TNF*-α exon 4 and 3'UTR of native Chinese domestic goats and compared the results to those in sheep and other goat ecotypes. Our results are useful for increasing the understanding of the specific immunodiversity level of the *TNF*-α gene and accessing the breeding potential of Chinese south goats.

MATERIAL AND METHODS

DNA samples from 100 individuals of 5 indigenous goat breeds were obtained in large range in southern China; the geographic information is shown in Table 1. The 4th exon and the 3' UTR of the class III MHC gene of *TNF*-α were amplified using primers ovTNF-C1 (5'-CTGCCGGAATACCTGGACTA-3') and ovTNF-C2 (5'-TCCAGT CCTTGGTGATGGTT-3') as described by Alvarez-Busto et al. (2004). The polymerase chain reaction (PCR) protocol was conducted as described by Shafer et al (2012). Screening for polymorphisms was performed by directly sequencing the PCR products. Each 50-μL PCR contained 2 ng template DNA, 5 μL 10X PCR buffer, 1.25 U TransStart Taq DNA polymerase (Transgen, Beijing, China), 4 μL 2.5 μM dNTPs (including 25 mM $MgCl_2$), 1.5 μL 10 μM of each primer, and ddH_2O to a volume of 50 μL. The PCR procedure consisted of an initial denaturing step at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 55.2°C for 30 s, and 72°C for 1 min, and completed by an incubation at 72°C for 7 min. Amplified DNA products were electrophoresed on 1-2% agarose gels and sequenced in both directions on an ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were aligned using MEGA6 (http://www.megasoftware.net/) (Tamura et al., 2013) in accordance with the DNA peak files in Chromas2.01 (http://www.technelysium.com.au/chromas_lite.html). Linkage disequilibrium between the loci in in all individuals was analyzed using Genepop (Rousset, 2008). Observed heterozygosity (H_O) and expected heterozygosity (H_E) as well as polymorphism information content (PIC) were estimated using the Microsatellite Toolkit.

Table 1. Complete information of animals and polymorphisms in TNF-α in southern Chinese domestic goats.

Population	Code	Sample size	Breed Type	Longitude	Latitude	MI					MII						MIII				
						A	G	H_O	H_E	PIC	A	T	G	H_O	H_E	PIC	A	G	H_O	H_E	PIC
DaZu Black	DZ	22	Indigenous	29°24N#	105°27E	81.82%	18.18%	0.3636	0.3044	0.2533	-	52.3%	47.7%	0.9545	0.5106	0.3745	52.27%	47.73%	0.9545	0.5106	0.3745
GuangFeng	GF	20	Indigenous	28°21N	118°15E	100%	-	0.0000	0.0000	0.0000	-	27.5%	72.5%	0.4500	0.4090	0.3192	27.50%	72.50%	0.45	0.409	0.3192
GanXi	GX	20	Indigenous	28°02N	114°08'E	97.50%	2.50%	0.0500	0.0500	0.0476	2.5%	47.5%	50%	0.2500	0.5372	0.4103	55.00%	45.00%	0.1	0.5077	0.3725
NanJiang Yellow	NJ	18	Cultivation	32°20N#	106°49'E	100%	-	0.0000	0.0000	0.0000	16.7%	50%	33.3%	0.6667	0.6286	0.5355	50.00%	50.00%	0.4444	0.5143	0.375
BanGe	BF	20	Indigenous	31°12N	90°06'E	100%	-	0.0000	0.0000	0.0000	5%	67.5%	27.5%	0.4000	0.4782	0.3947	52.27%	47.73%	0.35	0.45	0.3425
All								0.0827	0.0709	0.0602				0.5442	0.5127	0.4068			0.4598	0.4783	0.3567

In location option: #sampling location; without '#' reflects the main productive location based on the China National Commission of Animal Genetic Resources (2011).

RESULTS

The total length of the aligned sequences was 272 bp, including 3 single-nucleotide polymorphism in the 3'UTR, MI: A>G at 1884 bp, MII: MII: A>T>G at 1888 bp as well as MIII: A>G at 1923 bp relative to the location of EF446377; however high conservation of the *TNF*-α 4th exon was observed in all individuals. Compared to the polymorphism from H_E (0.5127), H_O (0.5442), and PIC (0.4068) at the MII site and MIII H_E (0.4783), H_O (0.4598), and PIC (0.3567), extreme low diversity was observed in the MI site (H_O: 0.0827, H_E: 0.0709, and PIC: 0.0602) among all populations (Table 1). A strong linkage relationship was observed between MII and MIII in all breeds (Table 2).

Table 2. Genotypic linkage disequilibrium for each locus pair across all populations (Fisher's method, P value).

Locus pair	Chi2	df	P value
MI & MII	0.000000	4	1.000000
MI & MIII	1.381101	4	0.847473
MII & MIII	Infinity	10	Highly sign

DISCUSSION

Comparative analysis of the results obtained in our experiments revealed no indel variants in the goat TNF-α region, which agrees with the results of Alvarez-Busto et al. (2004) in sheep. A study of immunodiversity in North American goat revealed that 272 bp of *TNF*-α were monomorphic (Shafer et al., 2012). However, in current study, 3 single-nucleotide polymorphisms were identified in Chinese south indigenous goats. This indicates the potential of strengthening variety improvement and promoting animal husbandry development in Chinese south domestic goats. In addition, similar results were observed for the class III *TNF*-α gene, and preliminary studies have identified variations in sheep (Alvarez-Busto et al., 2004); however, no difference was found in their frequencies between breeds. To date, the importance of these variations remains unclear. Therefore, an increased understanding of *TNF*-α variation is important for determining the pattern of immunodiversity in goats.

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