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Short Communication

Single nucleotide polymorphisms in candidate genes associated with gastrointestinal nematode infection in goats

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ABSTRACT. Cytokines are small cell-signaling proteins that play an important role in the immune system, participating in intracellular communication. Four candidate genes of the cytokine family (*IL2*, *IL4*, *IL13*, and *IFNG*) were selected to identify Single Nucleotide Polymorphisms (SNPs) that might be associated with resistance to gastrointestinal endoparasites in goats. A population of 229 goats, F2

offspring from an F1 intercross was produced by crossing pure Saanen goats, considered as susceptible to gastrointestinal endoparasites, with pure Anglo-Nubian goats, considered resistant. Blood was collected for DNA extraction and fecal samples were also collected for parasite egg count. Polymorphisms were prospected by sequencing animals with extreme phenotype for fecal egg count (FEC) distribution. The association between SNPs and phenotype was determined by using the Fisher exact test with correction for multiple tests. Three of the 10 SNPs were identified as significant ($P \leq 0.03$). They were found in intron 1 of IL2 (ENSBTA00000020883), intron 3 of IL13 (ENSBTA00000015953) and exon 3 of IFNG (ENSBTA00000012529), suggesting an association between them and gastrointestinal endoparasite resistance. Further studies will help describe the effects of these markers accurately before implementing them in marker assisted selection. This study is the pioneer in describing such associations in goats.

Key words: Single nucleotide polymorphism; *Capra hircus*; Cytokine; Gastrointestinal infection

INTRODUCTION

Goats are produced worldwide mainly because of their potential for milk and meat production, but they also can be used to provide leather, hair, and manure. Goats contribute to the development of rural areas and the well-being of their inhabitants (Horcada et al., 2012).

Gastrointestinal nematode infection is the most worrisome disease for producers of cattle, sheep, and goats because they cause major losses, reducing economic return due to death of the animals and negative effects on the performance of the herd (Zaros et al., 2010a; Bishop, 2012; Silva et al., 2012). The susceptibility to nematode infections seems to be related to genetic factors, and resistance may vary among breeds (Pralomkarn et al., 1997; Baker, 1998; Mandonnet et al., 2001).

The control of nematode infection relies on the administration of anthelmintics. However, long-term therapy may lead to the parasites developing resistance to the drug, reducing the efficacy of treatment. In addition, the indiscriminate use of anthelmintics is associated with the persistence of residues in milk, beef, and the environment, resulting in adverse human health and environmental effects (Corwin, 1997). One alternative to reduce the use of anthelmintics is the artificial selection of animals that may have a favorable genetic background for resistance to gastrointestinal nematode infection. Developments in molecular genetics have provided an opportunity to identify genes and pathways associated with gastrointestinal nematode infection and the host immune response. The identification of genes that influence the biological response to diseases would provide a better understanding of the physiological processes of the infection susceptibility and immune response. The identification of resistance-associated genetic markers may contribute to the implementation of marker-assisted selection in animal breeding programs.

Within this context, the aim of this study was to identify single nucleotide polymorphisms (SNPs) in candidate genes for gastrointestinal nematode infection resistance in goats to develop tools for the selection of naturally resistant animals. Four functional candidate

genes were chosen for the identification of these markers: interleukin 2 (*IL2*), interleukin 4 (*IL4*), interleukin 13 (*IL13*), and interferon gamma (*IFNG*), which belong to the cytokine family and are signaling molecules of the immune system. This is the first study that describes these associations in goats.

MATERIAL AND METHODS

Experimental animals and phenotype

The experimental animals were produced from animals of the Saanen breed, which is susceptible to gastrointestinal endoparasites, and of the Anglo-Nubian breed, which is resistant to these parasites. Two generations of crosses produced 229 F2 animals at Embrapa Caprinos e Ovinos (Sobral, Ceará, Brazil). Samples were obtained to determine the fecal egg count per gram feces according to a previously method described (Oliveira et al., 2009). The data were transformed into $\log_{10}$ (n + 1), where *n* is the number of eggs per gram feces, and analyzed using the mixed model procedure of the SAS program (2002/2003). The model included sex, sampling, and age at sampling as fixed effects and animal as a random effect. From the statistical analysis, 44 animals with extreme residual values were selected.

Amplification of DNA

Genomic DNA was extracted from blood samples as described by Tizioto et al. (2012). The fragments of interest were amplified by polymerase chain reaction (PCR). The reaction mixture contained 80 ng template DNA, 0.165 µM forward and reverse primers, 0.2 mM dNTPs, 1X buffer, 1.5 mM $MgCl_2$, and 0.13 U Platinum® Taq DNA polymerase (Invitrogen, USA) in a final volume of 15 µL. The PCR conditions were as follows: initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 94°C for 45 s, primer annealing for 45 s, and extension at 72°C for 45 s; a final extension step at 60°C for 4 min; and cooling at 4°C.

DNA sequencing

The PCR products were purified with ExoSap-IT® (Biotium, USA) for subsequent sequencing. The fragments were sequenced using the BigDye® Terminator kit in an automated ABI 3100 Avant sequencer (Applied Biosystems, USA). The sequencing steps consisted of an initial denaturation at 94°C for 2 min, followed by 25 cycles at 96°C for 20 s and primer annealing for 10 s, and a final extension step at 60°C for 4 min. The samples were cooled at 4°C.

Two regions of the *IL2* gene, 1 region of the *IL4* gene, 2 regions of the *IL13* gene, 3 regions of the *IFNG* gene, and 4 regions of the *TNFA* gene were sequenced. The primers, amplified regions, and size of the fragments are shown in Table 1.

Statistical analysis

To evaluate the influence of candidate genes on gastrointestinal nematode infection in goats, the Fisher exact test with Holm's correction for multiple tests (Holm, 1979) was used to compare allele frequencies between groups.

Table 1. Primers used for polymerase chain reaction and sequencing of the *IL2*, *IL4*, *IL13*, and *IFNG* genes.

Gene	Primers	Sequence		Fragment (bp)	Ta (°C)	SNPs
IL2	1F	5'-GAACTTCCTATCTGCTTCTCTAA-3'	Exons 1 and 2	468	55	T/A
	1R	5'-TGAAGTTACTATTTCTTCTCCTC-3'				
	2F	5'-CGGGCAGCTAAACTCTAATTTTT-3'	Exon 3	406	55	G/A
	2R	5'-CAATGTTAAAATGCCCTTCCAT-3'				
IL4	1F	5'-CCCCCTGGAAAGAAGAAATC-3'	Exon 3	393	56	A/C
	1R	5'-TCCCCAGTGCCTAGAACAGT-3'				
IL13	1F	5'-CACTCAGCTCGCTGTCCTTT-3'	Exons 2 and 3	467	64	T/C
	1R	5'-AAGTGGGTGCCAGGTCAAA-3'				
	2F	5'-TGCACTCTGTCCTCACAAGC-3'	Exon 4	595	53	-
	2R	5'-CCCTCCCTAACCCTCCTTTA-3'				
IFNG	1F	5'-CACCAAAATGCCACAAAACC-3'	Exon 1	483	57	C/T, A/C, and G/A
	1R	5'-AGAGATGAGAGCCCATCATAGA-3'				
	2F	5'-AAATAGTGCCAGCATCCAAGTT-3'	Exons 2 and 3	486	55	T/C and T/C
	2R	5'-TGCAATGATACCAAAGAAAGCA-3'				
	3F	5'-TGTGATGTGCTCCATCCTGT-3'	Exon 4 Partial	651	66	G/T
	3R	5'-TGCACAACCCACAGATTCTT-3'				

SNPs = single nucleotide polymorphisms.

RESULTS AND DISCUSSION

Functional candidate genes, such as *IL2*, *IL13*, *IL4*, and *IFNG*, are related to host resistance in different species (Zaros et al., 2010b; Ibelli et al., 2012). This study provides the first view of the effects of polymorphisms in these genes on gastrointestinal nematode infection in goats. By sequencing target regions in 44 extreme phenotype animals, it was possible to identify 2 SNPs in *IL2* (intron 1 and intron 2), 1 SNP in *IL4* (intron 3), 1 SNP in *IL13* (intron 1), and 6 SNPs in *IFNG* (2 in exon 1, 1 in intron 1, 1 in intron 2, 1 in exon 3, and 1 in exon 4) (Figure 1). All SNPs identified presented a minor allele frequency that was higher than 0.04 as shown in Table 2.

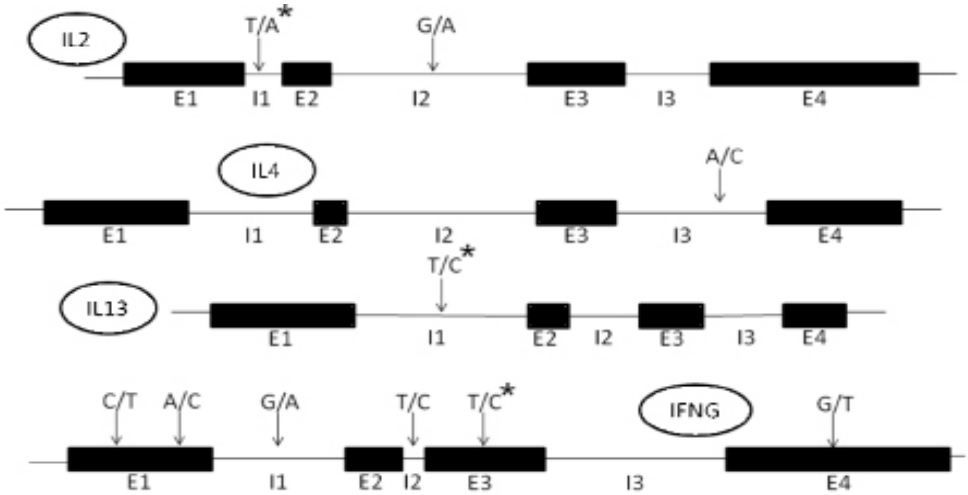

Figure 1. Schematic drawing illustrating the position of the 10 SNPs identified and their location in the genes studied. Two SNPs were identified in the *IL2* gene, one SNP in the *IL4* gene, one SNP in the *IL13* gene, and 6 SNPs in the *IFNG* gene. *SNPs potentially associated with resistance to gastrointestinal nematode infection.

Table 2. Genotypic and allelic frequencies of the *IL2*, *IL4*, *IL13*, and *IFNG* genes.

SNP, gene, and position	Genotype	Extreme animals (%)	Resistant animals (%)	Susceptible animals (%)	Allele	Extreme animals (%)	Resistant animals (%)	Susceptible animals (%)	P value
SNP (T/A),	TT	70.45	86.36	54.55	T	84.09	93.18	75.00	0.0148*
IL2, intron 1	TA	27.27	13.64	40.91	A	15.91	6.82	25.00	
	AA	2.27	0	4.55					
SNP (G/A),	GG	81.82	93.75	70.59	G	90.91	96.88	85.29	0.0894
IL2, intron 2	GA	18.18	6.25	29.41	A	9.09	3.13	14.71	
	AA	0	0	0					
SNP (A/C),	AA	65.12	59.09	71.43	A	80.23	75.00	85.71	0.0611
IL4, intron 3	AC	30.23	31.82	28.57	C	19.77	25.00	14.29	
	CC	4.65	9.09	0					
SNP (T/C),	CC	58.14	68.18	47.62	T	32.56	27.27	38.10	0.0219*
IL13, intron 1	CT	18.60	9.09	28.57	C	67.44	72.73	61.90	
	TT	23.26	22.73	23.81					
SNP (C/T),	CC	90.91	95.45	86.36	C	95.45	97.73	93.18	0.2496
IFNG, exon 1	CT	9.09	4.55	13.64	T	4.54	2.27	6.82	
	TT	0	0	0					
SNP (A/C),	AA	22.50	28.57	15.79	A	50.00	57.14	42.11	0.0348
IFNG, exon 1	AC	55.00	57.14	52.63	C	50.00	42.86	57.89	
	CC	22.50	14.29	31.58					
SNP (G/A),	AA	60.00	50.00	70.00	G	22.50	30.00	15.00	0.0427
IFNG, intron 1	AG	35.00	40.00	30.00	A	77.50	70.00	85.00	
	GG	5.00	10.00	0					
SNP (T/C),	TT	45.45	50.00	40.91	T	63.64	68.18	59.09	0.0575
IFNG, intron 2	TC	36.36	36.36	36.36	C	36.36	31.82	40.91	
	CC	18.18	13.64	22.73					
SNP (T/C),	TT	6.98	13.64	0	T	27.91	36.36	19.05	0.0207*
IFNG, exon 3	TC	41.86	45.45	38.10	C	72.09	63.64	80.95	
	CC	51.16	40.91	61.90					
SNP (G/T),	GG	44.19	50.00	38.10	G	65.12	70.45	59.52	0.0524
IFNG, exon 4	GT	41.86	40.91	42.86	T	34.88	29.54	40.88	
	TT	13.95	9.09	19.05					

SNP = single nucleotide polymorphism; *P value < 0.03 (critical value after correction for multiple tests).

Only 1 SNP found in the open reading frame of the *IFNG* gene (exon 1) was a non-synonymous mutation. This SNP causes the substitution of an asparagine (allele A) to a threonine amino acid (allele C).

The Fisher exact test was used to compare frequencies in resistant and susceptible goats and revealed a P value that was ≤0.03 for 3 of the 10 SNPs identified (Figure 1), which was the threshold value determined by correction for multiple tests. These SNPs may be potential markers for resistance to gastrointestinal nematode infection because they are likely to be associated with the phenotype studied.

Livestock genetic research aims to better understand the basis of genetic resistance to diseases. Although positional and functional candidate genes related to host resistance and immune response have been identified in different species, they have not been explored in goats. The identification of genes that influence the biological response to diseases would provide a better understanding of the physiological processes of the infection susceptibility and immune response and could contribute to the development of genetic tools to fight diseases. Genetic variants have been explored to explain differences in host resistance, but the underlying mechanisms affecting them are still poorly understood, especially in goats. This investigation contributes to the study of goat molecular markers. To our knowledge, this is the first report of SNPs associated with this disease in goats, and these SNPs have a potential application in genetic improvement efforts. Because the strategy of using animals from the extremes of the populational distribution of fecal egg count may overestimate the effects of allele substitution on the phenotype, the magnitude of these effects were not estimated in this research.

Before being efficiently used within breeding programs, the genetic information presented here should be extended to whole populations and other breeds to accurately describe these marker associations, additive effects, and their marker-quantitative trait locus phase relationships.

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