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Short communication

Genetic variation in *KRTAP11.1* gene of Suri alpaca (*Vicugna pacos*) from Puno, Peru

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Abstract

The objective of this study was to identify genetic single nucleotide polymorphisms (SNPs) in the coding region of the *KRTAP11.1* gene from the Suri alpaca population from Puno, Peru. Genomic DNA (n = 119) was used to amplify the 636 bp fragment of the coding region from the *KRTAP11.1* gene. The polymerase chain reaction products of the *KRTAP11.1* gene were sequenced by both strands and polymorphism type SNPs were identified. All SNPs (n = 3) were in Hardy-Weinberg equilibrium and had a high level of genotypic linkage disequilibrium with the presence of two haplotypes. All genetic polymorphisms generate non-synonymous amino acids changes: SNP 110 T>G (Ser>Ala), SNP 363 T>G (Phe>Cys) and SNP 375 A>C (Tyr>Ser). The results indicated the presence of moderate genetic diversity in the coding region of the *KRTAP11.1* gene from the Suri alpaca population.

Introduction

Peru has more than 3 million alpacas with its fleece being a most important economic trait (Food and Agriculture Organization , 2005). There are two different alpaca phenotypes: Huacaya and Suri, the phenotype Huacaya has highly crimped, soft, compact fibers while Suri has less crimped, silky and lustrous fibers (Presciuttini et al., 2010). These difference could be related to the proteins involved in fiber conformation. Genetic studies with neutral markers (microsatellites) support the presence of high genetic variability in Huacaya and Suri alpaca populations from Peru (Rodriguez, 2009); however limited studies on their genetic variability in gene coding fiber proteins are available.

The principal structural proteins in the mammal's hair are keratins and keratin-associated proteins (KAPs; Rogers, 2002). The KAPs are part of the matrix that surrounds the keratin intermediate filaments in cortical cells and the cuticle and these structures are part of the hair follicle, with the cuticle being responsible for fiber development (Rogers, 2006).

The KRTAP11.1 gene codes for a KAP11.1 protein, a small protein with high sulfur content, which shows constitutive expression in the hair follicle cortex and matrix (Gong et al., 2011). *KRTAP11.1* has been identified in humans (Rogers et al., 2002), mice (Huh et al, 1994), cattle (Zimin et al., 2009), sheep (Gong et al., 2011) and the Huacaya alpaca (Foppiano, 2016); however, there are no reported studies of genetic diversity in the Suri alpaca.

Materials and Methods

Genomic DNA samples were isolated from 191 Suri alpacas from Puno, Peru using a GF-1 Tissue DNA Extraction Kit (Vivantis) following the manufacturer's instructions.

PCR amplification of the *KRTAP11.1* gene-coding region was realized using the primers forward11af: 5′-TTGAAGCTGTCAACCAACCTT-3 and reverse11aR: 5′-CACAGAGCAAGACAAGTTCCC-3′ previously designed using the Primer3 software (Untergasser, 2012). The polymerase chain reaction (PCR) amplification was performed in a final volume of 20 µL containing: 5 ng of genomic DNA, 0.2 mM dNTPs

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(Thermo Scientific), 1X PCR buffer (20mM Tris-HCl pH 8.4, 50mM KCl; Thermo Scientific), 2.0 mM $MgCl_2$ (Thermo Scientific), 1.0 U HotStart Taq DNA polymerase® (Thermo Scientific) and 10 pmol of each primer (Invitrogen). The PCR conditions were: one cycle of 95ºC for 4 min, 30 cycles of 94ºC for 30 s, 60ºC for 30 s and 72ºC for 30 s, with a final extension of 72ºC for 10 min.

The PCR products were sequenced for both strands using a BigDye terminator® v3.1 kit (Life Technology) in a Genetic Analyzer ABI 3137 XL (service provided by Macrogen Inc.). Sequencing edition, alignment and single nucleotide polymorphism (SNP) identification was realized using the Mega 7 software (Kumar, 2016). Gene diversity estimators as allele frequencies, heterozygosity (H_E) and polymorphic information content (PIC) were calculated using the Cervus v3.0 software (Kalinowski, 2007).

Tests for Hardy Weinberg equilibrium and genotypic linkage disequilibrium were calculated using the Genepop v.4.0 software (Raymond and Rousset, 1995).

Results and Discussion

Three bi-allelic SNPs were identified in the 636bp fragment of the *KRTAP11.1* codon region. These were the same three SNPs that have already been reported in the Huacaya alpaca; however, there was allele frequency contrast between the samples (Foppiano, 2016). The allelic frequency and diversity estimators are detailed in Table 1.

The tree SNPs were in Hardy Weinberg equilibrium ($p > 0.05$) and complete genotype and linkage disequilibrium ($p < 0.05$). They also showed organization in two haplotypes with frequencies of 0.5262 and 0.4738, in contrast with the reported values in the Huacaya alpaca (Foppiano, 2016).

The *KRTAP11.1* genetic variation present in a coding region, contrasts with what is reported from sheep (Gong, 2011). Three non-conservative missense mutations were present in KRTAP11.1: SNP 110 T>G (Ser>Ala), SNP 363 T>G (Phe>Cys), SNP 375 A>C (Tyr>Ser) similar what has been observed in the Huacaya alpaca (Foppiano, 2016). The KAP11.1 protein is an important structural protein responsible for the chemical and physical structure of fibers also has an important function in keratin assembly (Fujimoto, 2014), and drastic non-synonymous mutation (Phe>Cys) could generate significant changes in the structure of alpaca fiber (Fujimoto, 2014), like has been detected in the Suri and Huacaya phenotypes.

This is a first report of SNP polymorphism in the codon region of the *KRTAP11.1* gene from the Suri alpaca. These results have indicated the presence of genetic polymorphisms with importance in fiber structure and suggest their importance as potential molecular genetic markers to explain the Suri and Huacaya phenotype in alpacas.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Table 1 Allelic frequency, heterozygosity and polymorphic information content of *KRTAP11.1* gene in Suri Alpaca

Locus	Allele	F.(a)	HObs	HExp	PIC
SNP110	T	0.5262	0.497	0.5	0.374
	G	0.4738			
SNP363	T	0.4738	0.497	0.5	0.374
	G	0.5262			
SNP375	A	0.5262	0.497	0.5	0.374
	C	0.4738			

F(a) = allelic frequency; Hobs = observed heterozygosity; HExp = expected heterozygosity; PIC = polymorphic index content

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