Asip and *MC1R* genes in Alpaca

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Introduction

Pigmentation in mammals is known to be influenced by more than 300 genes (Montoliu et al. (2009)). Among these, we find two major genes, namely agouti signalling protein (*Asip*) and melanocortin 1 receptor *(MC1R)*, which act by regulating the type, amount and distribution pattern of the pigments eumelanin and pheomelanin. Molecular genetics and pharmacological studies have shown that mutually exclusive binding (Ollmann et al. (1998)) of MC1R by the agouti protein or by α-melanocyte stimulating hormone (α-MSH) signals hair-bulb melanocytes to synthesize either pheomelanin or eumelanin, respectively. In alpaca, a wide variety of colors exists. Phenotypes, genetics of fiber traits (Frank et al. (2006)) and quantitative variation of melanin in alpaca and llamas has been studied in the context of chemical properties of melanins and morphology of melanosomes (Cecchi et al. (2007)). Only very limited information is available about the molecular basis of coat colour in alpaca. Therefore further knowledge of the molecular mechanism behind coat colour variation is needed to assist the breeder in fibre production. In this paper, we investigated and reported for the first time *Asip* and *MC1R* cDNA polymorphism in Peruvian alpaca population.

Material and Methods

Collection and storage of skin biopsies. Skin biopsies from coloured (Black, White and Brown) alpacas were collected in RNAlater (SIGMA, Germany) from Quimsachata Experimental Station, Peru. The biopsies were stored at -196°C for further analysis.

Primer designing. Orthologous sequences of *Asip* and *MC1R* from mammals were retrieved from NCBI GenBank (http://www.ncbi.nlm.nih.gov/) and aligned with EMBL ClustalW (http://www.ebi.ac.uk//Tools/clustalw/) to identify conserved regions to be used for the design of degenerate primers for the amplification of both coding regions.

RNA isolation, cDNA synthesis, cloning and sequencing. The skin biopsies were chopped into small pieces and then subjected to RNA extraction using the RNAeasy fibrous tissue mini kit (Qiagen S.A., Courtaboeuf, France), according to the manufacturer's instructions. cDNA was synthesised with reverse transcriptase enzyme (TAKARA BIO INC, Japan) followed by RT-PCR. 5' & 3'RACE experiments were carried out according to the method reported in Xianzong and Donald (2006) by using Expand long range, dNTPack (Roche, Germany). Then the desired amplicons were gel purified by using NucleoSpin Extract II

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(Macherey-Nagel, Germany), cloned into pGEM-T vector (Promega, USA) and sequenced (BMR genomics, Padova, Italy and StarSeq, Germany).

Results and discussion

At present, we have characterized the whole coding region (CDS) of *Asip* and *MC1R* cDNA in 35 multi colored Peruvian alpacas. The complete CDS of *Asip* comprises of 402 bp, which is 89%, 88% and 85% identical to cow, sheep and horse respectively. It encodes a putative protein of 133 amino acid (aa). The complete CDS of *MC1R* comprises of 954 bp, which is 97%, 90% and 88% identical to camel, dolphin and pig respectively. It encodes a putative protein of 317 aa.

Here, we described for the first time, seven single point mutations in the CDS of alpaca *Asip* cDNA, of which three were found to be silent mutations and four were missense mutations (Table 1). *MC1R* analysis unveiled a total of ten mutations in the CDS among those one was 4bp frameshift mutation, four were silent mutations and five were missense mutations (Table 2). Within the observed *MC1R* mutation, eight of them were already described by Powell et al. (2008) and Feely and Munyard. (2009) in American and Australian alpaca populations whereas the remaining two mutation represent novel polymorphisms observed for the first time within the Peruvian population. The analysis of the *Asip* 3'UTR (223 nt) showed three mutations. Among these one was a transversion and two were transition mutations (Table 1) located at 10, 38 and 77 nt downstream from the stop codon, respectively. Similarly, three transition mutations (Table 2) were also identified in the 3'UTR (602 nt) of *MC1R* and were located at 5, 166 and 398 nt downstream from the stop codon. Furthermore, two different 5'UTRs were characterized for *MC1R* (294 and 153 nt). Each one of the identified missense mutations could possibly be involved in coat colour determination.

Table 1: SNPs located in CDS, 5'and 3'UTR of *Asip*

Base position	SNPs	Amino acid change	Effect on protein
11	C/G	T/S	Polar
18	A/C	No change	--
102	G/A	No change	--
152	C/A	Y/S	Polar
290	C/A	No change	--
291	T/C	C/T	Slightly polar to polar
352	G/A	H/R	Polar
10 nt downstream to the stop codon	C/A	--	--
38 nt downstream to the stop codon	A/G	--	--
77 nt downstream to the stop codon	T/C	--	--

Table 2: SNPs located in CDS, 5'and 3'UTR of *MC1R*

Base position	SNPs	Amino acid change	Effect on protein
82	A/G	T/A	Polar to nonpolar
92*	C/T	T/M	Polar to nonpolar
126	C/T	No change	--
224-227	ACTT	Frame shift	Frame shift
259*	A/G	M/V	Nonpolar to polar
354	T/C	No change	--
376	A/G	S/G	Polar to nonpolar
618	G/A	No change	--
901	C/A	R/C	Polar to slightly polar
933	G/A	No change	--
5 nt downstream to the stop codon	T/C	--	--
166 nt downstream to the stop codon	C/T	--	--
398 nt downstream to the stop codon	G/A	--	--

* novel SNP observed in the Peruvian population

Conclusion

These findings will be helpful in understanding the regulatory mechanism of *Asip* and *MC1R* gene in alpaca coat color variation. Genotyping assays and semi quantitative RT-PCR analysis of *Asip* and *MC1R* transcripts to evaluate the association between alleles and coat color and expression dependent coat color variation are currently in progress.

References

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