Session 32
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Asip AND *MC1R* IN COAT COLOUR VARIATION IN ALPACA

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Importance of Alpaca

Coat colour is an important objective in the selection of alpaca, especially in animals reared for fine fibre production. White, black and different shades of brown are the phenotypes preferred for textile production (Fig 1). Naturally coloured fibre is especially appreciated by the textile industry for manufacturing of ecologically sustainable and organic products. An estimated 20% of coloured wool is presently produced in Peru and very limited information is available about the genetic basis of coat colour. The ability to determine the mechanisms of coat colour inheritance in alpacas would be highly valuable to the industry allowing precise breeding programmes for desired colours. So far Powell *et al. (Small Rum Res., 2008)* characterised the Melanocortin 1 Receptor (*MC1R*) gene in multi coloured USA alpaca population and up to now no study has been carried out on Agouti signaling protein (*Asip*) gene.

Fig 1. Alpacas at the Experimental Station of Quimsachata, Peru.
(Phtos courteously supplied by Dr. G. Lebboroni)

Objectives

To characterize polymorphisms at *Asip* and *MC1R* loci in a Peruvian alpaca population and to verify potential association with coat colour.
(In collaboration with the "Instituto Nacional de Innovación Agraria" (INIA) at Experimental Station of Quimsachata, Peru) .

Why *Asip* and *MC1R*?

Pigmentation is a complex process known to be influenced by more than 130 genes. The primary regulation of pigment type is controlled by two key genes known as *MC1R* gene and *Asip* gene by regulating the type, amount and distribution pattern of the pigments eumelanin and pheomelanin. In general, eumelanins and pheomelanins coexist in the same melanocyte and the pigmented phenotype is based on the mixture of the two pigments. The interaction between *Asip* and *MC1R* determines which pigment is present (eumelanin or pheomelanin) by controlling the amount of *MSH* bound to its *MC1R* receptor. If *MSH* is bound to *MC1R* the melanocyte produces eumelanins and the colour can be black or brown or related colour (Fig 2A). If *Asip* is bound to *MC1R*, *MSH* is unable to bind and pheomelanins are produced (Fig 2B). The epistatic relationship between *Asip* and *MC1R* genes makes it especially difficult to make prediction on the effect of such *MC1R* polymorphisms without knowledge of the *Asip* genotype.

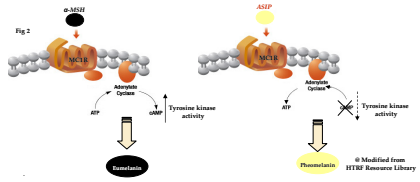

Fig 2. Diagram of melanins switching by inter play between the receptor MC1R an its ligands ASIP and MSH

Experimental design

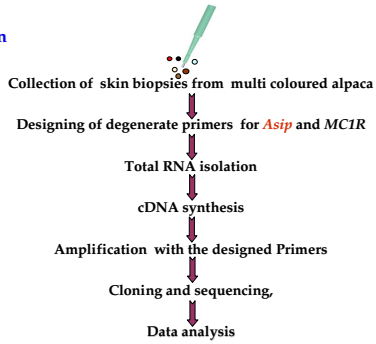

Table 1 . Nucleotide position and amino acid changes for 5 variants SNPs of alpaca Agouti signaling protein gene

Nucleotide Position	Mutation	Amino acid change	Effect on protein due to amino acid change
11	C/G	T/S	Polar
18	A/C	No change	N/S
290	C/A	No change	N/S
291	T/C	C/R	Slightly polar to polar
352	G/A	R/H	Polar

Preliminary Results

Up to now, we characterized the whole coding sequences of both *Asip* and *MC1R* in 15 multi coloured Peruvian alpacas and the results are summarised below.

- The complete coding region of *Asip* comprises of 402 bp and it codes for a protein of 133 aa and the sequence identity of *Asip* cDNA was observed to be 89 % , 88 % & 85 % with Cattle, Pig and Monkey respectively.
- Comparison between *Asip* sequences shows 5 mutations (SNPs), in particular 2 of them are synonymous mutations whereas, the remaining 3 resulted in non synonymous mutations producing amino acids substitutions as listed in Table 1.
- The complete coding region for *MC1R* comprises of 954 bp and it codes for a protein of 317 aa and sequence identity of *MC1R* cDNA was observed to be 88%, 87% & 86%, with pig, goat and cow respectively.
- Comparison between *MC1R* sequences shows 9 mutations (SNPs) of which 7 of them have already been described by Powell *et al.(2008)* in USA alpaca population whereas the remaining 2 mutations represent novel polymorphisms observed for the first time within our Peruvian population. In particular, 4 of the described mutations are synonymous mutations and 5 are non synonymous mutations as listed in Table 2.

Tabel 2, Nucleotide position and amino acid changes for 9 variants SNPs of alpaca Melanocortin 1 receptor gene

Nucleotide Position	Mutation	Amino acid change	Effect on protein due to amino acid change
82	A/G	T/A	Polar to nonpolar
92*	C/T	T/M	Polar to nonpolar
126	C/T	No change	N/S
259*	A/G	M/V	Nonpolar to polar
354	T/C	No change	N/S
376	A/G	S/G	Polar to nonpolar
618	G/A	No change	N/S
901	C/A	R/C	Polar to slightly polar
933	G/A	No change	N/S

* Novel SNPs identified in this study

ASIP Structure

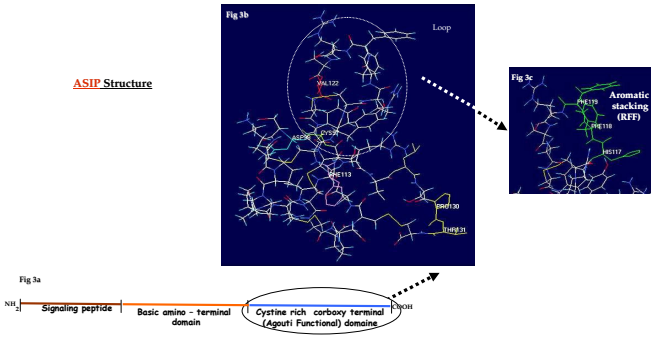

Fig 3a. Schematic representation of the main domains of Agouti signaling protein , Fig 3b. 3D Structure of the Agouti functional domain in which the region of loop is encircled; Fig 3c Enlargement of loop structure presenting the R117H change at RFF motif, "aromatic stacking" and alteration in structure (structures obtained by modelling with SPViewer from 1Y7J Human).

MC1R Structure

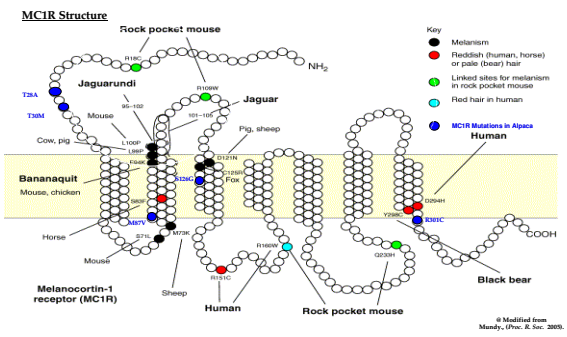

Fig4, Melanocortin-1 receptor structure showing some of the mutation presemptively associated to coat colour variation in mammals. Blue colour indicates variants identified in Peruviana alpaca population

Discussion

This is the first study reporting the *Asip* gene structure and its polymorphism in alpaca. Regarding the 3 non synonymous mutations that leads to amino acid substitution, two of them (C98R and R108H) are located in the Agouti functional domain (Fig 3a) and one (T4S) is located in the signaling peptide (Fig 3a), which may affect the efficiency binding of ASIP to MC1R. The molecular modelling of Agouti domain shows no appreciable change in the structure with C98R substitution (Fig 3b). On the contrary substitution at the position R117H in the region of the loop (fig 3b & 3c) within RFF residues can determine an alteration in the ASIP structure known as "aromatic stacking" (Fig 3c), which in turn can significantly affect the interaction between ASIP and MC1R as already reported by McNulty *et al.* (*J.Mol.Biol. 2008*).

In the melanocortin 1 receptor we observed 5 mutations, which are located in signal peptide recognition site (T28A and T30M), second (M87V), third (S126G) and seventh transmembrane regions (R301C) respectively. These sites have been reported in earlier as hot spots for functional mutation (Fig 3), which may leads to deactivation or activation of the MC1R. At the moment, molecular docking study of ASIP and MC1R that may shed light on the mechanism of their interaction, is hampered by the lack of NMR structure for MC1R. Each one of the identified mutation could possibly be involved with coat colour determination.

Future goals

1.To sample more *Asip* and *MC1R* alleles and possibly to correlate their polymorphism with coat colour variation in the Peruvian alpaca population; 2.To analyze the relative levels of *Asip* and *MC1R* gene expression in white and coloured animals by real time quantitative PCR. 3.To establish a genotyping assay to assist breeding programmes for coat colour in alpaca by means of TaqMan approach .