IDENTIFICATION OF A POTENTIAL MARKER FOR ABSENCE OF DARK FIBRE IN VICUGNA PACOS (ALPACA)

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SUMMARY

The *Melanocortin-1 receptor* gene was sequenced in a group of 41 Australian alpacas and seven single nucleotide polymorphisms (SNPs) were identified within the coding region (D42D, N118N, L206L, E311E, T28A, G126S and R301C). Three of these SNP (T28A, G126S and R301C) showed an association with phenotypic colour variants when both skin and fibre colour were used to segregate animals into groups. We propose the identification of a haplotype (T28A/G126S), which appears to be a marker for the absence of dark pigment in alpaca fleeces. Animals with the G82/C126 combination did not have any dark pigment. Both A82G & C901T are potentially capable of altering *MC1R* function. It's therefore possible that we have identified wild type (dominant) and loss-of-function (recessive) alleles of the alpaca *MC1R* gene.

INTRODUCTION

Alpaca fibre is renowned for its strength and softness and is a highly valuable fibre in the textile industry. Colour is an important fibre characteristic because it influences the potential applications and value of the end product. Although using phenotype as a basis for breeding selection can be of assistance to breeders an understanding of the molecular characterization of coat colour would allow more effective selection. The need for research into alpaca coat colour inheritance has been highlighted by the increased demand for specific colours in the industry. The *Melanocortin-1 Receptor* is known to be a key regulator of pigment colour in mammals (Rouzaud and Hearing 2005; Hoekstra *et al.* 2006). *MC1R* encodes a receptor on the surface of the melanocyte (pigment producing cell) that mediates the proportions of dark (black-brown, eumelanin) or light (tan-red, pheomelanin) pigment in the granules transferred to the surrounding cells; so producing the visible coloration in fibre or skin epidermis (Hearing 2005; Hoekstra *et al* 2006; Tully 2007). The MC1R receptor can direct pigment production in response to external factors (e.g. melanocyte stimulating hormone and agouti signaling protein) or can be inherently defective so that it becomes constitutively active, dominant black, or deactivated, recessive yellow (Hearing 2005; Hoekstra *et al.* 2006; Tully 2007). Alpaca flocks often contain a variety of coat colour patterns and so present an opportunity to substantiate the molecular basis of coat colour. Such advancements would be highly advantageous to the alpaca industry as it could provide breeders with the knowledge to effectively select for preferred genotypes and tailor fibre production to the current demands of the market more effectively.

MATERIALS AND METHODS

Blood samples were collected from 41 alpacas. Initial sample analysis was performed on 9 entirely white and 14 entirely black animals. A second group of animals comprising a wider range of colour phenotypes were subsequently analysed (3 black/brown, 2 grey, 2 dark brown, 9 fawn, 1 rose/grey and 1 white animal).

Alpaca *MC1R* Primers MC1R-F (GGGAGAAGGTGAGTGTGAGG) and MC1R-R (GCTCTTCCTGGAGATTCGTG) were designed to hybridise to regions flanking the alpaca *MC1R* coding sequence. All polymerase chain reactions (PCR) were performed in an Eppendorf Mastercycler, in 10µl reactions. Amplified DNA was sequenced with Big Dye Terminator Technology (Applied Biosystems) and analysed on a 3730 DNA analyser (Applied Biosystems).

Complete MC1R sequences for each animal were compiled into contigs using Vector NTI software (Invitrogen 2008), and compared with genes and proteins from other species by NCBI BLASTn and BLASTx protocols (Invitrogen 2008; NCBI 2008). Initial sequence analysis aimed to determine the relationship between the SNP genotype and phenotype, which was defined as the presence of absence of black pigment in fibres and/or skin. This identified three SNP that appeared to have an association with phenotype. A Chi^2 test for association was performed on three of the SNPs, A82G, C126T and C901T. Additional animals of a range of intermediate phenotypic fibre/skin colour variants were then analysed and the genotype data segregated the animals in the same way.

RESULTS AND DISCUSSION

Seven SNPs were identified in the alpaca *MC1R* gene (Table 2). Four of the seven SNP caused no amino acid change (D42D, N118N, L206L and E311E) while the remaining three resulted in amino acid substitutions (T28A, G126S and R301C). No correlation was observed between fibre colour alone, and MC1R genotype in the 41 animals studied. However, when the animals were assigned to groups based on the presence or absence of eumelanin in fibre and skin, three SNP appeared to be associated with phenotype variation (Tables 2 & 3). A chi-squared tested for association was performed to test the association between skin/fibre phenotype and SNP genotype. All three SNP were shown to have significant correlation at 2 degrees of freedom (Table 1).

Table 1. Results from chi-squared analysis of SNP genotype versus phenotype

SNP	Likelihood Ratio	df	Asymp. Sig (2-sided)
G82A	52.644	2	.000
C126T	52.644	2	.000
C901T	38.599	2	.000

Table 2. The phenotype and *MC1R* genotypes of the initial alpaca samples examined in this study. "*E*" denotes the proposed wild type allele and "*e*" denotes the proposed recessive alleles at *MC1R*. SNP in bold are those from which showed phenotypic correlations

SNP Genotype										
82 T28A	126 D42D	354 N118N	376 G126S	618 L206L	901 R301C	933 E311E	Fibre Colour	Eumelanin Present	Proposed MC1R alleles	n*
G/G	C/C	C/C	G/G	A/A	T/T	A/A	white	No	E^e/E^e	7
G/G	C/C	C/T	G/G	A/A	T/C	A/A	white	No	E^e/E^e	1
G/G	C/C	C/C	G/G	A/G	T/C	A/G	white	No	E^e/E^e	1
G/A	C/T	C/T	G/A	A/G	T/C	A/G	black	Yes	E^+/E^e	1
G/A	C/T	C/T	G/G	A/G	T/C	A/G	black	Yes	E^+/E^e	1
G/A	C/T	C/T	G/A	A/G	C/C	A/G	black	Yes	E^+/E^e	1
A/A	T/T	T/T	A/A	G/G	C/C	G/G	black	Yes	E^+/E^+,	4
A/A	T/T	T/T	G/A	G/G	C/C	G/G	black	Yes	E^+/E^+,	1
A/A	T/T	T/T	A/A	A/G	T/C	A/G	black	Yes	E^+/E^+,	2
A/A	T/T	T/T	A/A	A/G	C/C	G/G	black	Yes	E^+/E^+,	1
A/A	T/T	T/T	A/A	A/G	C/C	A/G	black	Yes	E^+/E^+,	3

* number of samples

Genotypes A82G and C126T were in complete concordance and hence are considered to be a haplotype. Analysis identified the G82/C126 combination as a possible marker for animals which had an absence of black pigment. These SNP were correlated with the presence or absence of eumelanin in skin and fibre. All animals with the G82/C126 combination were characterised by a lack of dark pigment, while animals that were either heterozygous or had the opposite combination, A82/T126, displayed dark pigment in skin and/or fibre. The animals expressing pheomelanin-only are proposed to have the genotype E^e/E^e representing the homozygous recessive genotype at MC1R, while the eumelanic animals are proposed to have the genotypes E^+/E^+, (homozygous wild-type) or E^+/E^e, which both allow normal eumelanin expression. It is not clear from the information we have gained so far whether these mutations are causative of a change in phenotype or merely linked to the absence of black pigment. It may be possible that these SNP are linked to a promoter mutation and don't necessarily cause the phenotypic change (Hornyak *et al.* 2001; Rouzaud and Hearing 2005; Smith *et al.* 2001). Nevertheless these polymorphisms may serve as a good predictor of pheomelanic animals for breeding purposes.

Table 3. The colour phenotype and *MC1R* genotypes of the additional alpaca samples examined in this study at the three significant polymorphisms. ? denotes that eumelanin status could not be determined

SNP Genotype						
82 T28A	126 D42D	901 R301C	Fibre colour	Eumelanin present	Proposed MC1R alleles	Number of samples
A/A	T/T	C/C	fawn	Yes	E^+/E^+,	3
A/A	T/T	C/C	silver/grey	Yes	E^+/E^+,	1
A/A	T/T	C/C	dark brown	Yes	E^+/E^+,	2
A/A	T/T	C/C	medium grey	Yes	E^+/E^+,	1
A/G	T/C	C/T	black/tan	Yes	E^+/E^e	3
A/G	T/C	C/T	fawn	Yes	E^+/E^e	2
A/G	T/C	C/T	white	Yes	E^+/E^e	1
G/G	C/C	T/T	rose/grey	No	E^e/E^e	1
G/G	C/C	T/T	fawn	No	E^e/E^e	3
G/G	C/C	C/T	fawn	?	E^e/E^e	1

C901T also appeared to be a significant candidate polymorphism for phenotype effect. Animals with the A82/T126/C901 combination were capable of producing eumelanin while animals with the G82/C126/T901 combination lack any eumelanic pigment in skin or hair fibres. The C901T polymorphism occurs in an extremely significant domain pertaining to structural integrity and function of the receptor (Strader *et al.* 1994; Tao 2006). Polymorphisms in this domain are reported to impair receptor function severely (Everts *et al.* 2000; Garcia-Borron *et al.* 2005; Sanchez-Mas *et al.* 2005). If these interactions are not properly carried out, downstream processes essential for the production of eumelanin are not initiated, resulting in the default colour, pheomelanin, being produced (Hoekstra *et al* 2006; Logan *et al.* 2003; Newton *et al.* 2000).

This study has provided new information on the possible effects of MC1R alleles in alpaca fibre pigmentation. The results have highlighted a significant haplotype that appears to be a marker for the absence of black pigment. This haplotype holds significant potential for use as a marker in breeding stock selection. While this study has provided significant new information about MC1R, the nature of pigment gene interactions means that genetic analysis of a number of

other pigment genes will be necessary before the nature of colour inheritance in this species is completely understood. Investigation and characterisation of the MC1R promoter may also yield useful information about the differences in MC1R expression in animals with identical genotypes that display varying degrees of pigmentation.

ACKNOWLEDGMENTS

This research was jointly funded by the Rural Industries Research & Development Corporation and Curtin University. Thanks to the Australian Alpaca Association for their strong in-kind support of this research. The Authors wish to thank the following alpaca breeders for supplying samples from their alpacas, Sue Leitch (Brookwood Alpacas), Carolyn Emery (Windella Alpacas), Pinjarra Alpacas, Aviana Farms, Bolero Alpacas, Mangowood Farm, and Tularosa Alpacas. Thanks also to Dr Katherine Morton (The University of Sydney) and Dr Belinda Appleton (The University of Melbourne/ Alpaca Genomics Australia Pty Ltd), who supplied DNA samples for nine of the animals.

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