Genetic characterization of coat color genes in Brazilian Crioula sheep from a conservation nucleus

Lilian Cristina Gomes Cavalcanti[1], José Carlos Ferrugem Moraes[2], Danielle Asis de Faria[3], Concepta Margaret McManus[1], Alcebiades Renato Nepomuceno[1], Carlos José Hoff de Souza[2], Alexandre Rodrigues Caetano[3] and Samuel Rezende Paiva[3]

[1]Universidade de Brasília, Faculdade de Agronomia e Medicina Veterinária, Campus Universitário Darcy Ribeiro, CEP 70910-970 Brasília, DF, Brazil. E-mail: lilian1mel@gmail.com, concepta@unb.br, alcebiades.rna@hotmail.com [2]Embrapa Pecuária Sul, Rodovia BR-153, Km 633, Vila Industrial, Caixa Postal 242, CEP 96401-970 Bagé, RS, Brazil. E-mail jose.ferrugem-moraes@embrapa.br, carlos.hoff-souza@embrapa.br [3]Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Avenida W5 Norte (Final), CEP 70770-917 Brasília, DF, Brazil. E-mail: danyafp@gmail.com, alexandre.caetano@embrapa.br, samuel.paiva@embrapa.br

Abstract – The objective of this work was to identify single nucleotide polymorphisms (SNPs) in resequencing data from *MC1R*, *ASIP*, and *TYRP1* genes derived from Crioula sheep (*Ovis aris*) with different coat colors. Polymorphisms in the *ASIP* (agouti-signaling protein), *MC1R* (melanocortin 1 receptor), and *TRYP1* (tyrosinase-related protein 1) genes were analyzed in 115 sheep from Embrapa's conservation nucleus of crioula sheep, in Brazil. A total of 7,914 bp were sequenced per animal, and 14 SNPs were identified. Two additional assays were performed to detect duplications and deletions in the *ASIP* gene. Ninety-five percent of the coat color variation was explained by epistatic interactions observed between specific alleles in the *MC1R* and *ASIP* genes. Evidence suggests an important role of *TYRP1* variants for wool color, despite their low frequencies. The marker panel was efficient enough in predicting coat color in the studied animals and, therefore, can be used to implement a marker-assisted selection program in the conservation nucleus of sheep of the crioula breed.

Index terms: *Ovis aris*, *ASIP*, genetic resources conservation, marker-assisted selection, *MC1R*, *TYRP1*.

Caracterização genética de genes para cor da pelagem do núcleo de conservação da ovelha Crioula no Brasil

Resumo – O objetivo deste trabalho foi identificar polimorfismo de nucleotídeo único (SNPs) em dados de ressequenciamento dos genes *MC1R*, *ASIP* e *TYRP1*, obtidos de ovelhas Crioulas (*Ovis aries*) com cores de pelagem distintas. Polimorfismos nos genes *ASIP* (agouti-signaling protein), *MC1R* (melanocortin 1 receptor) e *TYRP1* (tyrosinase-related protein 1) foram analisados em 115 ovinos, provenientes do núcleo de conservação da ovelha Crioula, da Embrapa. No total, 7.914 pb foram sequenciados por animal, e 14 SNPs foram identificados. Dois ensaios adicionais foram realizados para detectar duplicações e deleções no gene *ASIP*. Noventa e cinco por cento da variação de cor da pelagem foi explicada pelas interações epistáticas entre alelos específicos dos genes *MC1R* e *ASIP*. Evidências sugerem um importante papel das variantes *TYRP1* para cor da lã, apesar das suas baixas frequências. O painel de marcadores usado foi eficiente em detectar alelos associados à coloração da pelagem nos animais estudados e, portanto, pode ser usado na implementação de um programa de seleção assistida por marcadores, no núcleo de conservação de ovinos da raça Crioula.

Termos para indexação: *Ovis aris*, *ASIP*, conservação de recursos genéticos, seleção assistida por marcadores, *MC1R*, *TYRP1*.

Introduction

Crioula sheep (*Ovis aries* L.) have been locally adapted to Southern Brazil after centuries of use for lamb and wool production, during colonization of the region. Because of the presence of naturally colored medullated wool fibers in the wool produced by Crioula sheep, it is widely used for manufacturing handicrafts and in industrial tapestry in Brazil (Moreira & Silva, 2004; Arco, 2017).

Sheep wool color can naturally vary from white to black, and dark and pale brown, and grey were also seen (Gonçalves et al., 2010). The Crioula breed shows a distinct and frequent wool color called *mouro*, which can be defined as a mix of black and brown fibers. Some variations of *mouro* color are also frequent

among flocks, such as *mouro malhado*, which shows the same mix of black and brown, but with white spots along the body (Moraes & Souza, 2011).

The Crioula breed is maintained by producers and a conservation nucleus, located at Embrapa Pecuária Sul, Pelotas, RS, Brazil. Several studies have been carried out to characterize and preserve the breed (Vaz, 2000; Moraes & Souza, 2011). The identification of genetic factors responsible for the determination of skin and wool color in this breed would allow selection and directional breeding for specific colors, aiming at different niche producers, transformation industries, and sale activities. It can also assist the planning of the optimal composition of the Crioula germplasm bank, in order to preserve the greatest possible genetic diversity for these traits.

Although mammalian coat color is a polygenic trait, three well-characterized genes are responsible for determining most of the observed variations: *ASIP* (agouti-signaling protein), *MC1R or MSH* (melanocortin 1 receptor or melanocyte stimulating hormone), and *TRYP1* (tyrosinase-related protein 1) (Searle, 1968; Klungland & Vage, 2000). These genes have been studied in several species, such as cow (Hanna et al., 2014), pig (Liu et al., 2016), rabbit (Utzeri et al., 2014), goat (Fontanesi et al., 2009), water buffalo (Miao et al., 2010), yak (Chen et al., 2009), horse (Rieder, 2009), and sheep (Han et al., 2015).

Epistatic interactions between *ASIP* and *MC1R* affect sheep wool color, such as the *MC1R* loss-of-function (E^+E^+ genotypes), which triggers the production of pheomelanin and results in light-color phenotypes. Conversely, the presence of the dominant *MC1R* allele (E^DE_-) results in the constitutive activation of the receptor and, therefore, dark colors are present due to eumelanin production (Barsh et al., 2000; Girardot et al., 2005). In general, the presence of the *MC1R* wildtype allele is required for the observation of *ASIP* gene products (Vage et al., 1999; Fontanesi et al., 2011; Hepp et al., 2012). Few studies have been performed to evaluate these genes in locally adapted sheep breeds in Brazil (Hepp et al., 2012, 2016; Muniz et al., 2016).

Recent analyses, performed with wool color data from Crioula sheep progenies with different coat colors, in Embrapa's conservation nucleus, revealed a bimodal distribution between white/light (pheomelanic) colors and dark (eumelanic) colors (Moraes & Souza, 2011). In the present work, resequencing data from fragments of the *MC1R*, *ASIP* e *TYRP1* genes derived from these animals were analyzed to search for sequence variants associated with coat color, which could be used to optimize management and use of Crioula breed germplasm from conservation nucleus.

Materials and Methods

Rams, ewes, and respective progeny from 56 matings (N=115), derived from the Embrapa's conservation nucleus of Crioula sheep, were used in this study. The coat colors of 4-month-old sheared lambs were phenotyped by colorimetry (Figure 1), using the Munsell system, following procedures described in Moraes & Souza (2011). Matings were previously performed within the germplasm conservation program following criteria established considering two groups of color classification (Table 1), as follows: group 1, composed of five sires with light wool (pheomelanic) – two white, two white-masked, and one pale brown ram; and group 2, composed of three sires with dark wool (eumelanic) – two black ones, and one *mouro* ram.

Lymphocytes were used for DNA extraction, using a modified protocol from Miller et al. (1988). Extracted DNA was quantified by spectrophotometry (Nanodrop ND1000) and visualized in 1% agarose gels. PCR-amplified fragments from *MC1R*, *TYRP1*, and *ASIP* were generated using the primers listed in Table 2. PCR products were purified with ExoSAP-IT, according to manufacturer protocols, and sequenced in both directions with an ABI PRISM 3100 Genetic Analyser, using BigDye Terminator v3.1 Cycle Sequencing kit.

Sequence electropherograms were analyzed with ABI software (Sequencing Analysis v. 5.1. and SeqScape v. 2.7), in order to obtain consensus sequences between forward and reverse runs, and to identify variants between individuals. All obtained sequences were aligned with reference sequences for each gene (*MC1R*, Y13965; *ASIP*, EU420022; *TYRP1*, EF102109/DQ530058), and errors due to low-quality points on the sequence were manually edited using SeqScape. Observed single nucleotide polymorphisms (SNPs) were extracted from the alignments and compared with those previously described in literature. Arlequin 2000 v.3.5 software (Excoffier & Lischer, 2010) was used to estimate haplotypes within each gene, based on the final list of the observed SNPs.

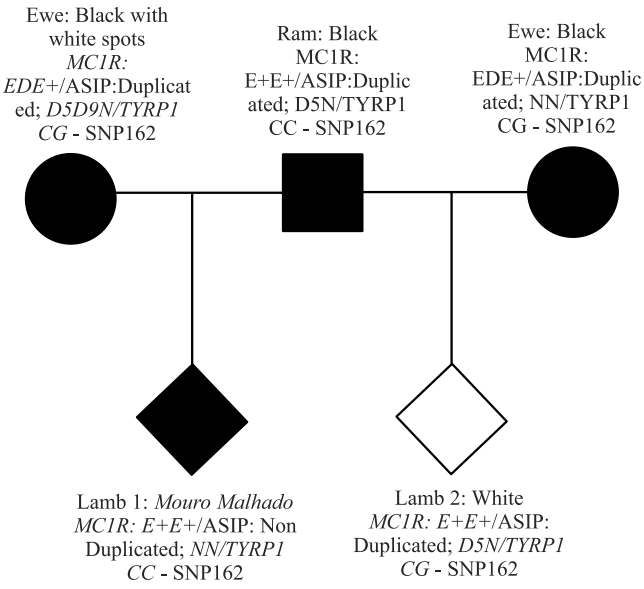

Figure 1. Heredogram showing the segregation of the MC1R, ASIP, and TYRP1 alleles in two distinct crosses of Crioula sheep (*Ovis aries*). Dark symbols and white symbols represent the groups dark wool, and white or pale wool, respectively. Progeny sex was not represented. MCIR, E+, wild type allele; and ED, dominant allele; ASIP, D5N or NN, deletion; TYRP1, CC or GG. genotypes of SNP 162.

MC1R SNP genotypes were used to check the recorded pedigree using a parentage test implemented in Cervus 3.0.7 (Kalinowski et al., 2007). All trios were checked for inconsistencies, and samples with unconfirmed parentage were eliminated from further analyses.

Fragment analysis was used to genotype D_5 and D_9 deletions observed in *ASIP* exon 2, and to genotype the *ASIP* gene duplication (Norris & Whan, 2008). The previously described primer sequences (Fontanesi et al., 2011), used for this analysis, are listed in Table 2. PCR reactions were performed as previously described, and annealing temperatures used for each primer pair are also shown in Table 2. Amplified fragments were genotyped using an ABI 3730 DNA analyzer. Genotypes were scored and sized using GeneMapper version 4.0 (AB, Foster City, CA).

Fisher's exact test was used to identify associations between coat color patterns and observed genotypes. Tested animals were divided into two groups (light and dark coat colors). Association analyses were performed using the FREQ procedure of SAS version 9.4 (SAS Institute Inc. Cary, NC, USA), including all samples.

Table 1. Number of Brazilian Crioula sheep (*Ovis aries*) matings by wool color chosen to test genes previously described, associated with sheep wool color.

Description of the herd crosses	Number of crosses		Number of progenies	
	Eliminated[(1)]	Included	Dark	White/Pale
Group 1, white or pale wool				
White x black	0	4	2	3
White x white	0	1	0	1
White x *mouro*	0	2	4	0
White x *mouro malhado*	1	0	0	0
White x white masked	0	1	1	1
White masked x black	0	2	1	1
White masked x white	0	2	0	2
White masked x *mouro*	1	0	0	0
White masked x black with white spots	0	2	2	0
White masked x *mouro malhado*	0	1	1	0
Pale brown x white	1	2	0	2
Subtotal	3	17	11	10
Group 2, dark wool				
Black x black	7	9	8	2
Black x *mouro*	0	2	1	1
Black x black with white spots	1	1	1	0
Black x *mouro malhado*	2	3	3	0
Mouro x black	1	4	2	2
Mouro x *mouro*	0	1	0	1
Mouro x black with white spots	1	3	1	2
Mouro x *mouro malhado*	0	1	1	0
Subtotal	12	24	17	8
Total	15	41	28	18

[(1)]Crosses eliminated after the contradictory results of the paternity test between the *MC1R* SNPs and pedigree records.

Results and Discussion

Brazilian Crioula sheep naturally show different wool colors, ranging from black to white, and including several tones of gray and brown, with or without spots. The most frequent phenotype observed in the studied samples was black (43.4%), and the least frequent one was pale brown (0.86%). All 115 tested animals were classified only in two groups – light wool (31%) and dark wool (79%) – because of the low number of samples, number for each existing wool color, and in order to simplify the analysis.

Partial sequencing of the three main genes previously shown to determine coat color in mammals revealed a total of 14 SNPs after the analysis of 7.914 bp per animal (*MC1R*, 953 bp; *ASIP*, 5.353 bp; and *TYRP1*, 1.609 bp). Five previously reported SNPs were observed in the *MC1R* gene. Three SNPs generated synonymous substitutions (c.429C>T, c.600T>G, and c.735T>C) (Fontanesi et al., 2010), while two SNPs (c.218T>A, and c.361G>A) (Vage et al., 1999), resulted in missense mutations p.M73K e p.D121N, respectively. A total of six different estimated haplotypes were observed in the analyzed samples. SNPs c.218T>A and c.361G>A are present in the dominant black allele (E^D) and were observed in two estimated haplotypes, H5 and H6 (Table 3). All other observed haplotypes were denominated as wild types (E^+).

Observed genotypic frequencies for $E^D\_$ and E^+E^+ were 49 and 51%, respectively. Genotypes $E^D\_$ and E^+E^+ were found to be significantly associated with dark and light wool colors, respectively (Fisher exact test, p = 1.0e-4, Table 4). This association has been widely described in different sheep breeds (Vage et al., 1999; Deng et al., 2009; Fontanesi et al., 2010, 2011). Paternity exclusion tests, performed with Cervus, using the sequence variation information from the

Table 2. Primer sequences and PCR conditions for *ASIP*, *MC1R* and *TYRP1* sequencing and fragment analysis.

Primer	Gene region	Primer sequence (5´- 3´)	PCR conditions(1)	Fragment length(2)	Source
*ASIP*_F2	*ASIP*/Ex2	acactgggctgtgggatg	62/1.5	410	Gratten et al. (2010)
*ASIP*_R2		agggacacttgattcctcca			
ASIP_F3	*ASIP*/Ex3	ctctttgctgtcagtctctgg	60/2.0	391	Gratten et al. (2010)
*ASIP*_R3		tggtagctttctgtttctctgg			
*ASIP*_F4	*ASIP*/Ex4	cagctagggtgctctgtgg	60/1.5	650	Gratten et al. (2010)
*ASIP*_R4		agcctcagggctaagcaac			
Agt_8b	*ASIP*	aacaggttcatggaagaattg			
Agt_7b	*ASIP*/Del	6FAM-ctacctgactgccttctctg	55/2.0	165 -174	Norris & Whan (2008)
Agt 1	*ASIP*	cattactggggacctatcaac			
Agt 11		tatcggcttggagagtgtttg			
Agt_16b	*ASIP*/Dupl	6FAM-agcaatgaggacgtgagttt	60/2.0	238 -242	Norris & Whan (2008)
Agt_17b		gtttctgctggacctcttgttc			
Agt_18b	*ASIP*	gtgccttgtgaggtagagatggtgt			
Font_F1	*MC1R*/Ex1	agtgcctggaggtgtccatcc	64/2.5	169	Fontanesi et al. (2010)
Font_R1		ctgacgctcaccagcaagt			
Font_F2	*MC1R*/Ex1	agccatgagttgagcaggac	62/1.0	376	Fontanesi et al. (2010)
Font_R2		caggacaccagcctccag			
Font_F3	*MC1R*/Ex1	gtgagcgtcagcaacgtg	61/1.5	366	Fontanesi et al. (2010)
Font_R3		acatagaggacggccatcag			
Font_F4	*MC1R*/Ex1	gcctggttggcttcttcata	58/2.5	456	Fontanesi et al. (2010)
Font_R4		tggtctagcgatcctctttg			
Tyrp_EX1F	*TYRP1*/Ex1	tggctgttttgtactgcctg	58/1.5	750	Deng et al. (2008)
Tyrp_EX1R		ccctcccatgtactcatctgt			
Tyrp_EX4F	*TYRP1*/Ex4	tgcaggatccttctttctcc	58/2.5	400	Gratten et al. (2007)
Tyrp_EX4R		tgaatatctgtacatccgttctct			
Tyrp_INT_F	*TYRP1*/Ex1	caagagcctgatggagaagga	60/2.0	425	Deng et al. (2008)
Tyrp_INT_R		cattaaacaggggcgttgttc			

(1)Annealing temperature (ºC)/$MgCl_2$ concentration (mmol L^{-1}). (2)Base pairs. Cycling conditions: 95°C for 5 min; 35 cycles of 95° for 30 s; annealing temp. for 30 s; and 72°C for 30 s, followed by a final step of 72°C for 5 min.

MC1R gene, confirmed 41 declared matings involving 45 different parents and 46 offsprings (Table 1).

Seven SNPs were observed in the *ASIP* studied fragments; five of these were located in intronic regions, and two in exons. The polymorphisms g.5051G>C and c.5172T>A were found in exon 4, as reported by Norris & Whan (2008). The SNP g.5051G>C is not expected to disrupt *ASIP* function, while c.5172T>A has been shown to cause functional changes to the agouti protein and, therefore, to be associated with the production of white wool (Gratten et al., 2010). The presence of a previously reported duplication in the *ASIP* gene region was observed in 61 (67%) of the studied animals (Table 3). This duplication interferes in the estimation of haplotypes for this region and, therefore, SNP g.5051G>C was not included in the association tests. SNP c.5172T>A was not seen to be associated with wool color (Table 4).

Two previously reported deletions (Fontanesi et al., 2011) in *ASIP* exon 2 (5pb - g.100-104del-AGGAA; and 9pb - g.1019del-AGCCGCCTC), known as D_5 and D_9, respectively, were also observed in Crioula sheep (Table 3). Fontanesi et al. (2011) associated the genotype D_9N in Massese sheep with the production of grey wool; however, considering the presence of *ASIP* duplication, this genotype was observed both in white and dark Crioula samples. It seems that *ASIP-MC1R* interaction might not fully explain the segregation of grey pattern. Fisher´s exact tests showed that the duplication in the *ASIP* gene was significantly associated with the coat color in the Brazilian Crioula sheep, while the deletions were not (Table 4).

Combined analysis of *ASIP* and *MC1R* variants showed a significant epistatic interaction between these genes, with few exceptions (Tables 3 and 4), as it was previously described by Fontanesi et al. (2011). Dominant *MC1R* extension alleles (E^D) determine eumelanin production, resulting in black wool, whereas recessive alleles (E^+) determine production of red/yellow/pale pigmentation due to pheomelanin synthesis. For the agouti locus, eumelanin is determined by the presence of a nonagouti allele (A^a) (Searle, 1968). In the present study, alleles A^a were found together with either deletion (D_5 and D_9), as well as with the genotype AA, for the exon 4 polymorphism g.5172T>A (Table 3). Almost all pale wool animals (26 of 27) had the *ASIP* duplication and, therefore, at least one fully functional copy of the gene (A^{wt} allele), which resulted in the production of white wool in the absence of the *MC1R* extension allele (E^D). Similar observations

Table 3. Incidence of *ASIP* and *MCR1* genotypes in Crioula sheep (*Ovis aries*) with different wool colors.

ASIP polymorphism	Genotype	Number of observations[1]							Total
		White	White masked	Pale brown	Black	Black with spots	*Mouro*	*Mouro malhado*	
					Absence of *ASIP* duplication				
Exon 2 deletions	D_5N	-	-	-	11 (7)	6 (2)	-	1 (1)	18
	D_9N	-	-	-	2 (1)	-	-	-	2
	NN	1	-	-	3 (2)	1 (1)	3 (1)	1	9
	D_5D_5	-	-	-	1 (1)	-	-	-	1
	Total	1	-	-	17 (17)	7 (3)	3 (1)	2 (1)	30
Exon 4 (g.5172T>A)	TT	-	-	-	7 (5)	1	-	1(1)	9
	TA	1	-	-	7 (5)	5 (2)	3 (1)	1	17
	AA	-	-	-	3 (1)	1 (1)	-	-	4
	Total	1	-	-	17 (17)	7 (3)	3 (1)	2 (1)	30
					Presence of *ASIP* duplication				
Exon 2 deletions	D_5D_9N	1	1	1	5 (5)	1 (1)	1 (1)	-	10
	D_5N	7	7	-	12 (10)	6 (6)	2 (2)	1 (1)	35
	D_9N	2	2	-	2 (2)	-	-	1 (1)	7
	NN	3	2	-	3 (3)	-	-	-	9
	Total	13	12	1	22 (22)	7 (7)	3 (3)	2 (2)	61
Exon 4 (g.5172T>A)	TT	4	2	-	11 (10)	5 (5)	1 (1)	1 (1)	24
	TA	8	9	1	11 (9)	2 (2)	2 (2)	1 (1)	35
	AA	1	1	-	-	-	-	-	2
	Total	13	12	1	22 (22)	7 (7)	3 (3)	2 (2)	61

[1]Numbers in parentheses indicate animals carrying at least one E^D allele at the *Extension* locus; the remaining animals carry the E^+E^+ genotype.

have been reported for Merino (Norris & Whan, 2008) and Texel, Bergamasca, Sarda, and Appenninica sheep breeds (Fontanesi et al., 2011).

Out of the 63 animals with colored wool, 5 did not fit in the described epistatic model. Two *mouro* animals and one *mouro malhado* showed the wild type allele E^+, and neither *ASIP* duplication nor A^a allele. The other two black exemptions were homozygous for E^+ and had the *ASIP* duplication and the D_5 allele. All 5 animals showed at least one H2 haplotype in the *MC1R* gene, which is the most frequently observed haplotype, suggesting that the deviation from the epistatic model is not due to observed *MC1R* variants.

Two previously described SNPs (Deng et al., 2008) were observed in Crioula sheep in the *TYRP1* exon 1 (c.192G>C and c.462C>T). The SNP c.869G>T, previously associated with coat color in Soay sheep (Gratten et al., 2007), was not observed in exon 4 of Crioula sheep. If we examine *TYRP1* genotypes, together with the variants from *ASIP* and *MC1R*, it is evident that the CC genotype at c.192G>C was present only in 5 animals, including two exemptions to the *ASIP* and *MC1R* epistatic model: one black, and one *mouro malhado*. This suggests that *TYRP1* variants can affect the interaction of *Agouti* and *Extension* loci, which will change the expected pattern of wool color. However, considering the low numbers of observations with this particular genotypic combination (Table 4), the interaction between these three genes could not be tested. Higher sample numbers will be required to test this hypothesis.

The three animals that did not fit into the proposed epistatic model were likely the result of either of the following specific situations: there are polymorphisms in the studied genes which were not detected; polymorphisms in additional genes not included in the study associated with wool color in Crioula; undetected Aa recessive alleles present on the duplicated copies of the ASIP gene. Gratten et al. (2010) proposed a model in which recessive uniform wool color patterns in Soay sheep could be caused by homozygosity of any of three separate mutations in the *ASIP* gene. Recessive mutations can either impair *Agouti* protein function or abrogate *ASIP* expression, resulting in darkly pigmented uniform wool phenotypes, like those observed in Soay sheep (dark brown).

Table 4. Fisher's exact tests for the associations between wool color group (white or pale wool vs. dark wool) and *MC1R*, *ASIP*, and *TYRP1* genotypes in Crioula sheep (*Ovis aries*).

Genotype combination	Progenies (N=46)	Parents (N=45)
MC1R $E^+E^+/ E^D\_$	p<0.0001	p<0.0001
ASIP duplication	p<0.0001	p=0.02
MC1R E^+E^+/ E^D and *ASIP* duplication	p<0.0001	p<0.0001
ASIP exon 2 deletion	p=0.4	p=0.99
ASIP c.5172T>A exon 4	p=0.04	p=0.15
MC1R $E^+E^+/ E^D\_$ and *ASIP* duplication and deletion	p<0.0001	p<0.0001
TYRP1 c.192G>C exon 1	p=0.52	p=0.12
TYRP1 c.462C>T exon 1	p=0.09	p=0.007

Conclusions

1. The *MCIR*, *ASIP* and *TYRP1* polymorphisms observed for Crioula sheep (*Ovis aries*) have already been described for other breeds.

2. The proposed epistatic model between *MC1R* and *ASIP* genotypes can explain the wool color pattern for most of the studied animals.

3. *TYRP1* variants, despite being present in low frequencies, may interact with *MC1R* and *ASIP* genotypes to affect wool color.

4. The described DNA variants and markers will will be used to assist management of the genetic conservation nucleus and to direct germplasm collection and cryopreservation.

Acknowledgments

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for the research fellowships to Concepta Margaret McManus and Alexandre Rodrigues Caetano.

References

ARCO. Associação Brasileira de Criadores de Ovinos. **Crioula**. Available at: <http://www.arcoovinos.com.br/index.php/mn-srgo/mn-padroesraciais?id=44>. Accessed on: Mar. 19 2017.

BARSH, G.; GUNN, T.; HE, L.; SCHLOSSMAN, S.; DUKE-COHAN, J. Biochemical and genetic studies of pigment-type switching. **Pigment Cell Research**, v.13, p.48-53, 2000. Supplement 8. DOI: 10.1034/j.1600-0749.13.s8.10.x.

CHEN, S.-Y.; HUANG, Y.; ZHU, Q.; FONTANESI, L.; YAO, Y.-G. ; LIU, Y.-P. Sequence characterization of the *MC1R* gene in yak (*Poephagus grunniens*) breeds with different coat colors. **Journal**

of Biomedicine and Biotechnology, v.2009, article 861046, p.1-6, 2009. DOI: 10.1155/2009/861046.

DENG, W.D.; SHU, W.; YANG, S.L.; SHI, X.W.; MAO, H.M. Pigmentation in Black-boned sheep (*Ovis aries*): association with polymorphism of the *MC1R* gene. **Molecular Biology Reports**, v.36, p.431-436, 2009. DOI: 10.1007/s11033-007-9197-9.

DENG, W.D.; XI, D.M.; GOU, X.; YANG, S.L.; SHI, X.W.; MAO, H.M. Pigmentation in Black-boned sheep (*Ovis aries*): association with polymorphism of the *Tyrosinase* gene. **Molecular Biology Reports**, v.35, p.379-385, 2008. DOI: 10.1007/s11033-007-9097-z.

EXCOFFIER, L.; LISCHER, H.E.L. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. **Molecular Ecology Resources**, v.10, p.564-567, 2010. DOI: 10.1111/j.1755-0998.2010.02847.x.

FONTANESI, L.; BERETTI, F.; RIGGIO, V.; DALL'OLIO, S.; CALASCIBETTA, V.; RUSSO, V.; PORTOLANO, B. Sequence characterization of the melanocortin 1 receptor (*MC1R*) gene in sheep with different coat colours and identification of the putative e allele at the ovine *Extension* locus. **Small Ruminant Research**, v.91, p.200-207, 2010. DOI: 10.1016/j.smallrumres.2010.03.015.

FONTANESI, L.; BERETTI, F.; RIGGIO, V.; DALL'OLIO, S.; GONZALEZ, E.G.; FINOCCHIARO, R.; DAVOLI, R.; RUSSO, V.; PORTOLANO, B. Missense and nonsense mutations in melanocortin 1 receptor (*MC1R*) gene of different goat breeds: association with red and black coat colour phenotypes but with unexpected evidences. **BMC Genetics**, v.10 p.47, 2009. DOI: 10.1186/1471-2156-10-47.

FONTANESI, L.; DALL'OLIO, S.; BERETTI, F.; PORTOLANO, B.; RUSSO, V. Coat colours in the Massese sheep breed are associated with mutations in the agouti signalling protein (*ASIP*) and melanocortin 1 receptor (*MC1R*) genes. **Animal**, v.5, p.8-17, 2011. DOI: 10.1017/S1751731110001382.

GIRARDOT, M.; MARTIN, J.; GUIBERT, S.; LEVEZIEL, H.; JULIEN, R.; OULMOUDEN, A. Widespread expression of the bovine *Agouti* gene results from at least three alternative promoters. **Pigment Cell and Melanoma Research**, v.18, p.34-41, 2005. DOI: 10.1111/j.1600-0749.2004.00195.x.

GONÇALVES, G.L.; MOREIRA, G.R.P.; FREITAS, T.R.O.; HEPP, D.; PASSOS, D.T.; WEIMER, T.A.W. Mitochondrial and nuclear DNA analyses reveal population differentiation in Brazilian Creole sheep. **Animal Genetics**, v.41 p.308-310, 2010. DOI: 10.1111/j.1365-2052.2009.01986.x.

GRATTEN, J.; BERALDI, D.; LOWDER, B.V.; MCRAE, A.F.; VISSCHER, P.M.; PEMBERTON, J.M.; SLATE, J. Compelling evidence that a single nucleotide substitution in *TYRP1* is responsible for coat-colour polymorphism in a free-living population of Soay sheep. **Proceedings of the Royal Society B**, v.274, p.619-626, 2007. DOI: 10.1098/rspb.2006.3762.

GRATTEN, J.; PILKINGTON, J.G.; BROWN, E.A.; BERALDI, D.; PEMBERTON, J.M.; SLATE, J. The genetic basis of recessive self-colour pattern in a wild sheep population. **Heredity**, v.104, p.206-214, 2010. DOI: 10.1038/hdy.2009.105.

HAN, J.L.; YANG, M.; YUE, Y.J.; GUO, T.T.; LIU, J.B.; NIU, C.E. YANG, B.H. Analysis of agouti signaling protein (ASIP) gene polymorphisms and association with coat color in Tibetan sheep (*Ovis aries*). **Genetics and Molecular Research**, v.14, p.1200-1209, 2015. DOI: 10.4238/2015.February.6.22.

HANNA, L.L.H.; SANDERS, J.O.; RILEY, D.G.; ABBEY, C.A.; GILL, C.A. Identification of a major locus interacting with *MC1R* and modifying black coat color in an F2 Nellore-Angus population. **Genetics Selection Evolution**, v.46, p.1-8, 2014. DOI: 10.1186/1297-9686-46-4.

HEPP, D.; GONÇALVES, G.L.; MOREIRA, G.R.P.; FREITAS, T.R.O. de. Epistatic interaction of the melanocortin 1 receptor and agouti signaling protein genes modulates wool color in the Brazilian creole sheep. **Journal of Heredity**, v.107, p.544-552, 2016. DOI: 10.1093/jhered/esw037.

HEPP, D.; GONÇALVES, G.L.; MOREIRA, G.R.P.; FREITAS, T.R.O.; MARTINS, C.T.D.C.; WEIMER, T.A.; PASSOS, D.T. Identification of the *e* allele at the Extension locus (*MC1R*) in Brazilian Creole sheep and its role in wool color variation. **Genetics and Molecular Research**, v.11, p.2997-3006, 2012. DOI: 10.4238/2012.May.22.5.

KALINOWSKI, S.T.; TAPER, M.L.; MARSHALL, T.C. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. **Molecular Ecology**, v.16, p.1099-1106, 2007. DOI: 10.1111/j.1365-294X.2007.03089.x.

KLUNGLAND, H.; VAGE, D.I. Molecular genetics of pigmentation in domestic animals. **Current Genomics**, v.1, p.223-242, 2000. DOI: 10.2174/1389202003351364.

LIU, R.; JIN, L.; LONG, K.; CHAI, J.; MA, J.; TANG, Q.; TIAN, S.; HU, Y.; LIN, L.; WANG, X.; JIANG, A.; LI, X.; LI, M. Detection of genetic diversity and selection at the coding region of the melanocortin receptor 1 (*MC1R*) gene in Tibetan pigs and Landrace pigs. **Gene**, v.575, p.537-542, 2016. Pt. 2. DOI: 10.1016/j.gene.2015.09.032.

MIAO, Y.; WU, G.; WANG, L.; LI, D.; TANG, S.; LIANG, J.; MAO, H.; LUO, H.; ZHANG, Y. The role of *MC1R* gene in buffalo coat color. **Science China Life Sciences**, v.53, p.267-272, 2010. DOI: 10.1007/s11427-010-0026-3.

MILLER, S.A.; DYKES, D.D.; POLESKY, H.F. A simple salting out procedure for extracting DNA from human nucleated cells. **Nucleic Acids Research** . v.16, p.1215, 1988.

MORAES, J.C.F.; SOUZA, C.J.H. de. **Descrição da cor da pelagem em um rebanho de ovelhas crioulas**. Bagé: Embrapa Pecuária Sul, 2011. 26p. (Embrapa Pecuária Sul. Documentos, 114).

MOREIRA, G.R.P.; SILVA, M.C. La retomada de la crianza de ovejas criollas en el Brasil. **La Propaganda Rural**, n.1557, p.92-95, 2004.

MUNIZ, M.M.M.; CAETANO, A.R.; MCMANUS, C.; CAVALCANTI, L.C.G.; FAÇANHA, D.A.E.; LEITE, J.H.G.M.; FACÓ, O.; PAIVA, S.R. Application of genomic data to assist a community-based breeding program: a preliminary study of coat color genetics in Morada Nova sheep. **Livestock Science**, v.190, p.89-93, 2016. DOI: 10.1016/j.livsci.2016.06.006.

NORRIS, B.J.; WHAN, V.A. A gene duplication affecting expression of the ovine ASIP gene is responsible for white and

black sheep. **Genome Research**, v.18, p.1282-1293, 2008. DOI: 10.1101/gr.072090.107.

RIEDER, S. Molecular tests for coat colours in horses. **Journal of Animal Breeding and Genetics**, v.126, p.415-424, 2009. DOI: 10.1111/j.1439-0388.2009.00832.x.

SEARLE, A.G. **Comparative genetics of coat colour in mammals**. London: Academic Press,1968. 308p.

UTZERI, V.J.; RIBANI, A.; FONTANESI, L. A premature stop codon in the *TYRP1* gene is associated with brown coat colour in the European rabbit (*Oryctolagus cuniculus*). **Animal Genetics**, v.45, p.600-603, 2014. DOI: 10.1111/age.12171.

VAGE, D.I.; KLUNGLAND, H.; LU, D.; CONE, R.D. Molecular and pharmacological characterization of dominant black coat color in sheep. **Mammalian Genome**, v.10, p.39-43, 1999. DOI: 10.1007/s003359900939.

VAZ, C.M.S.L. **Morfologia e aptidão da ovelha crioula lanada**. Bagé: Embrapa Pecuária Sul, 2000. 20p. (Embrapa Pecuária Sul. Documentos, 22).

Received on January 8, 2016 and accepted on April 7, 2016