



## Interaction between the *melanocortin 1 receptor (MC1R)* and *agouti signalling protein genes (ASIP)*, and their association with black and brown coat colour phenotypes in peruvian alpaca

Stefano Pallotti, Bathrachalam Chandramohan, Dario Pediconi, Cristina Nocelli, Antonietta La Terza & Carlo Renieri

To cite this article: Stefano Pallotti, Bathrachalam Chandramohan, Dario Pediconi, Cristina Nocelli, Antonietta La Terza & Carlo Renieri (2020) Interaction between the *melanocortin 1 receptor (MC1R)* and *agouti signalling protein genes (ASIP)*, and their association with black and brown coat colour phenotypes in peruvian alpaca, Italian Journal of Animal Science, 19:1, 1518-1522, DOI: [10.1080/1828051X.2020.1850216](https://doi.org/10.1080/1828051X.2020.1850216)

To link to this article: <https://doi.org/10.1080/1828051X.2020.1850216>



© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.



Published online: 08 Dec 2020.



[Submit your article to this journal](#)



Article views: 1471



[View related articles](#)









[View Crossmark data](#)



Citing articles: 7 [View citing articles](#)

## Interaction between the *melanocortin 1 receptor (MC1R)* and *agouti signalling protein genes (ASIP)*, and their association with black and brown coat colour phenotypes in peruvian alpaca

Stefano Pallotti<sup>a\*</sup> , Bathrachalam Chandramohan<sup>b\*</sup> , Dario Pediconi<sup>a</sup> , Cristina Nocelli<sup>a</sup> , Antonietta La Terza<sup>c</sup>  and Carlo Renieri<sup>a</sup> 

<sup>a</sup>Scuola di Scienze del Farmaco e dei Prodotti della salute, University of Camerino, Camerino, Italy; <sup>b</sup>National Institute of Science Education and Research (NISER) Bhubaneswar P.O. Jatni, Khurda, India; <sup>c</sup>Scuola di Bioscienze e Medicina Veterinaria, University of Camerino, Camerino, Italy

### ABSTRACT

The aim of this work was to assess the interaction between *MC1R* and *ASIP* genes in determining coat colour variation in black and brown Peruvian alpacas. Skin biopsies were collected from 25 black alpacas and 15 brown alpacas. Genomic DNA was isolated, amplified and sequenced. Black alpacas were dominant homozygous (*E/E*) or heterozygous (*E/e*) at *MC1R* locus while brown alpacas were heterozygous at the locus. All the black animals were heterozygous for the non-functional alleles at *ASIP* locus ( $a^H - a^{\Delta 57} - a^{ht}$ ). On the contrary, brown alpacas were dominant homozygous (*A/A*) or heterozygous (*A/a<sup>ht</sup>*) at this locus. When the combined genotypes at *MC1R* and *ASIP* were considered together, alpacas with black coats had four possible genotype combinations namely  $a^H/a^{\Delta 57} - E/E$ ,  $a^H/a^{ht} - E/E$ ,  $a^H/a^{\Delta 57} - E/e$  and  $a^H/a^{ht} - E/e$ . The brown alpacas were found to have the  $A/a^{ht} - E/e$  and the  $A/A - E/e$  genotype. Our results show that the two genes interact to synergically modulate the amount of melanin synthesised in alpaca fibre. In black coat alpacas in fact, the expression of non-functional *ASIP* results in the failure of pheomelanin production while the functional *MC1R* allows the synthesis almost exclusively of eumelanin. On the other hand, the brown coat phenotype is produced when the animal possesses at least one functional copy of the gene at both *ASIP* and *MC1R* loci. In fact, *MC1R* binds both *ASIP* and  $\alpha$ -MSH allowing a mixed melanogenesis characterised by the synthesis of eumelanin and pheomelanin granules in a similar ratio.

### HIGHLIGHTS

- The income of thousands of Peruvian alpaca farmers depends mainly on the sale of fibre in high demand on the global textile market.
- A wide variety of colours exists, therefore this fibre is preferred by the ecologically sustainable industry for organic textile products.
- Our results provide information for selection breeding program of coloured alpaca helpful to improve the condition of welfare of Andean populations.

### ARTICLE HISTORY

Received 11 August 2020  
Revised 21 October 2020  
Accepted 6 November 2020

### KEYWORDS

*ASIP*; *MC1R*; Alpaca; coat colour; genes interaction

## Introduction

Coat colour and pattern in mammals is a complex trait controlled by over 300 genetic loci at over 150 genes (Rochus et al. 2019). The relative amounts of melanin pigments in mammals are mainly controlled by the melanocortin-1-receptor (*MC1R*), encoded by the *Extension* (*E*) locus, and its peptide antagonist agouti-signalling-protein (*ASIP*), encoded by the *Agouti* (*A*)

locus (Lu et al. 1994). In the hair-bulb melanocytes, the *MC1R* is mutually bound by *ASIP* and the  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH) which lead to the synthesis of pheomelanin (yellow-red pigments) and eumelanin (dark pigments), respectively. In other words, *ASIP* acts as an antagonist of *MC1R* by nullifying the action of  $\alpha$ -MSH, therefore, loss-of-function of *MC1R* results in pheomelanin whereas loss-of-function

**CONTACT** Dr Stefano Pallotti  [stefano.pallotti@unicam.it](mailto:stefano.pallotti@unicam.it)  School of Pharmacy, University of Camerino, Camerino, MC 62032, Italy; Dr Bathrachalam Chandramohan  [chandramohan@niser.ac.in](mailto:chandramohan@niser.ac.in)  National Institute of Science Education and Research (NISER) Bhubaneswar P.O. Jatni, Khurda, Odisha 752050, India; Dr Antonietta La Terza  [antonietta.laterza@unicam.it](mailto:antonietta.laterza@unicam.it)  School of Bioscience and Veterinary Medicine, University of Camerino, Camerino 62032, Italy

\*These authors contributed equally to this work.

© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

of ASIP leads to the production of the black eumelanin (Barsh 1996). The effect of two genes upon hair colour and skin pigmentation has been extensively drawn from scientific literature dealing with genetic of coat colour. Actually, several *ASIP* and *MC1R* mutations responsible for coat colour variation have been identified in wild mammals (Liu et al. 2016; Reissmann et al. 2020) as well as in domestic animals including equids (Rieder et al. 2001; Shang et al. 2019), ruminants (Renieri et al. 2008; Henkel et al. 2019; Matsumoto et al. 2020), companion animals (Dreger et al. 2013), rabbits and rodents (Lai et al. 2019; Letko et al. 2020). To date, the two genes were addressed separately although there is a low number of paper focussed on the interaction between *MC1R* and *ASIP* in modulating the hair pigmentation. So far, this interaction has been explored in camel (Alshanbari et al. 2019), dromedary (Almathen et al. 2018), llama (Daverio et al. 2016), sheep (Fontanesi et al. 2011; Rochus et al. 2019), pig (Shi et al. 2006), horse (Rieder et al. 2001; Shang et al. 2019) and dog (Oguro-Okano et al. 2011).

The alpaca is a South American camelid which produce a soft and lustrous fibre in high demand on the global textile market (McGregor et al. 2012). A wide variety of colours exists, therefore, this fibre is preferred by the ecologically sustainable industry for organic textile products (Chandramohan et al. 2013). It is known that the differences between black and brown hair shaft colour are due to both melanogenesis intensity variation and the presence of distinctive melanosomes displaying specific morphological features (Renieri et al. 1991; Cecchi et al. 2004, 2011).

Candidate polymorphisms at *MC1R* and *ASIP* genes which may impair the structure and functionality of both proteins have been already shown to affect coat colour in this species (Powell et al. 2008; Feeley and Munyard 2009; Bathrachalam et al. 2011; Feeley et al. 2011; Guridi et al. 2011; Chandramohan et al. 2013, 2015). In fact, the Agouti locus in white and coloured (black and brown) alpacas has

been found to be affected by three different mutations consisting in two independent SNPs and one deletion of 57 bp (Feeley et al. 2011; Chandramohan et al. 2013). On the other hand, a total of eight mutations were genotyped at *MC1R* locus in white and coloured alpacas, of which seven were independent SNPs and one was a 4 bp deletion (Powell et al. 2008; Feeley and Munyard 2009; Chandramohan et al. 2015). Moreover, other tree mutations (SNPs) have been describe in this locus which are associated with fibre lightness although without any correlation with colour (Guridi et al. 2011).

Recently both genes have been mapped in alpaca. Through the use of FISH-mapping in fact, *MC1R* was assigned to chr21 and *ASIP* to chr19 (Alshanbari et al. 2019).

Although evidences suggest an interaction between the two genes, none of these paper has shed light on this. Here, we assessed the interaction between *MC1R* and *ASIP* genes in determining coat colour variation in black and brown Peruvian alpacas.

## Materials and methods

All the extended protocols and methodology were described in detail in Chandramohan et al. (2013, 2015). Briefly, skin biopsies from 25 black alpacas and 15 uniform brown alpacas (without any particular coat colour patterns such as black and tan or black face and extremities) were collected by disposable biopsy punch (8 mm diameter) and stored in RNAlater (SIGMA, Neustadt, Germany). Genomic DNA was isolated using a DNAeasy tissue kit (Qiagen S.A., Courtaboeuf, France). The extended protocols used for the amplification of genomic DNA, sequencing and sequence analysis are fully described in Chandramohan et al. (2013, 2015).

## Results

Four different alleles were found at *ASIP* locus while only two alleles were characterised at *MC1R* locus

**Table 1.** ASIP and MC1R alleles in Peruvian alpacas.

Allele	ASIP			
	g3836C > T pR98C	g3866-3923del57 C109_R127del	g3896G > A pR118H	
A	C	–	G	
<i>a<sup>H</sup></i>	C	–	A	
<i>a<sup>ht</sup></i>	T	–	G	
<i>a<sup>Δ57</sup></i>	C	Yes		
Allele	MC1R			
	g82A > G pT28A	g259A > G pV87M	g376A > G pG126S	g901C > T pR301C
E	A	A	A	C
e	G	G	G	T

ASIP: agouti signalling protein; MC1R: melanocortin 1 receptor.

**Table 2.** Alpaca *ASIP*-*MC1R* genotype combinations in the two coat colours.

Colour	<i>ASIP</i>	<i>MC1R</i>
Black	$a^H/a^{\Delta 57}$	$E/E$
	$a^H/a^{ht}$	$E/E$
Black	$a^H/a^{\Delta 57}$	$E/e$
	$a^H/a^{ht}$	$E/e$
Brown	$A/a^{ht}$	$E/e$
	$A/A$	$E/e$

*ASIP*: agouti signalling protein; *MC1R*: melanocortin 1 receptor.

(Table 1). These alleles have been already described by Chandramohan et al. (2015, 2013).

As shown in Table 2, both black and brown alpacas were heterozygous at *MC1R* locus. On the contrary, the dominant homozygous genotype was only in black animals while none of the brown alpacas were homozygote for the dominant or recessive alleles. All the black animals were heterozygous for non-functional alleles at *ASIP* locus. On the other hand, the dominant wild allele was observed only in brown alpacas for which the genotypes found were  $A/A$  and  $A/a^h$ . It should be noted that black genotypes are characterised by a high recessive allele heterogeneity ( $a^H$ ,  $a^{\Delta 57}$ ,  $a^{ht}$ ). Conversely, a single recessive allele was found to be responsible for the brown phenotype ( $a^{ht}$ ). Therefore, we proceed the analysis in order to understand how the *Asip* alleles segregate in a full black population (Table 3).

When the combined genotypes at *MC1R* and *ASIP* were considered together, alpacas with black coat had four possible genotype combinations viz.  $a^H/a^{\Delta 57} - E/E$ ,  $a^H/a^{ht} - E/E$ ,  $a^H/a^{\Delta 57} - E/e$  and  $a^H/a^{ht} - E/e$  (Tables 2 and 3) For brown alpacas, the animals were found to have the  $A/a^{ht} - E/e$  and the  $A/A - E/e$  genotype.

## Discussion

The aim of this paper was to investigate the interaction between *ASIP* and *MC1R* in determining coat colour in Peruvian alpacas. It is clear that in mammal the black and brown coat pigmentation is the result of the interaction between these two loci. Eumelanin and pheomelanin synthesis, is determined by the *MC1R* which acts as a molecular switch in melanin production. If this receptor is bounded by  $\alpha$ -MSH, eumelanin synthesis is triggered resulting in black hair. On the other hand, pigment production switch to pheomelanine if the receptor is bounded by *ASIP*

**Table 3.** Crosses involving 5 full black sires and 10 dams.

Black ram genotype	Black dam genotype	Black offspring genotype
$a^H/a^{hT} - EE$	$a^H/a^{hT} - E/e$ $a^H/a^{hT} - E/E$	$a^H/a^{hT} - E/E$ $a^H/a^{hT} - E/E$
$a^H/a^{\Delta 57} - EE$	$a^H/a^{\Delta 57} - E/E$ $a^H/a^{\Delta 57} - E/e$	$a^H/a^{\Delta 57} - E/E$ $a^H/a^{\Delta 57} - E/E$
$a^H/a^{hT} - EE$	$a^H/a^{\Delta 57} - E/E$ $a^H/a^{\Delta 57} - E/e$	$a^H/a^{\Delta 57} - E/E$ $a^H/a^{\Delta 57} - E/E$
$a^H/a^{hT} - E/e$	$a^H/a^{\Delta 57} - E/E$	$a^H/a^{\Delta 57} - E/E$
$a^H/a^{\Delta 57} - EE$	$a^H/a^{\Delta 57} - E/e$ $a^H/a^{\Delta 57} - E/e$ $a^H/a^{\Delta 57} - E/e$	$a^H/a^{\Delta 57} - E/E$ $a^H/a^{\Delta 57} - E/E$ $a^H/a^{\Delta 57} - E/E$

resulting in the brown hair phenotype (Klungland et al. 1995).

In our study, all the animals genotyped from the ten black families were heterozygous for the mutated *ASIP* allele (Table 3), suggesting that these mutations lead to a complete loss-of-function of the translated protein, and possessed at least one functional copy of *MC1R* ( $E/E$  or  $E/e$  genotype). In black coat alpacas, the non-functional *ASIP* results in the failure of the protein to properly bind to the *MC1R* receptor preventing the initiation of the pheomelanin synthesis in the melanocytes. At the same time, the functional *MC1R* binds the  $\alpha$ -MSH allowing the synthesis of the round and oval-shaped mature eumelanosomes (black pigment granules) which display transverse striations that confer the black colour to the hair shaft (Renieri et al. 1991). It is known that the eumelanosomes production in alpaca increases as the colour deepens from white to black. This implies that black fleece is characterised by the almost exclusive presence of eumelanin pigment while a very small amount of pheomelanin is produced in the black phenotype (Renieri et al. 1991; Cecchi et al. 2011) due to the lack of functional *ASIP* alleles.

The interaction between the two loci we describe here has been already observed to lead to the black phenotype in others species. Studies conducted on several equine breeds found that all the black animals were homozygous for the mutated *ASIP* alleles and had at least one functional *MC1R* alleles. Black coat horses, in fact, possess the  $a/a$  genotype at the *ASIP* locus and  $E/E$  or  $E/e$  genotype at the *MC1R* locus (Rieder et al. 2001; Shang et al. 2019). Similarly, the black coat colour in the Massese sheep breed are mainly derived by combining *ASIP* and *MC1R* mutations being determined by non-functional *ASIP* alleles (recessive) and by the dominant *MC1R* allele (Fontanesi et al. 2011). Recently, the interaction

between the two loci were also studied in some camelid species. In dromedaries a deletion and a SNP in *ASIP* have been found to be associated with the black/dark brown colour (Almathen et al. 2018; Alshanbari et al. 2019). Polymorphisms in *ASIP* and *MC1R* were identified also in llama, however none of which were completely associated to a particular colour phenotype (Daverio et al. 2016).

From our study, brown coat alpacas were found heterozygous or homozygous for the dominant *ASIP* alleles and heterozygous at *MC1R* locus (Table 2). This result clearly shows that the brown coat phenotype is produced when the animal possesses at least one functional copy of the gene at both *ASIP* and *MC1R* loci. In brown coat alpaca melanocytes in fact, *MC1R* binds both *ASIP* and  $\alpha$ -MSH allowing a mixed melanogenesis characterised by the synthesis of eumelanin and pheomelanin granules in a similar ratio. The brown hair shaft, in fact, is characterised by the presence of a mixed melanosome population at different maturation stages leading to the expression of the brown phenotype (Renieri et al. 1991; Cecchi et al. 2011). Similarly, the *E/E* – *A/a* genotype has been found to be the advantage genotype for brown coat colour in horse (Shang et al. 2019). In the same species, the dark bay coat colour, classified as sub-phenotype of brown coat colour, has been found to be expressed by the *E/E* – *A/A*, *E/e* – *A/a* or *E/e* – *A/A* genotypes (Rieder et al. 2001; Shang et al. 2019).

## Conclusions

In this study, we investigated the interaction between the *MC1R* and *ASIP*, and their association with black and brown coat colour phenotypes in Peruvian alpaca. Taken together, *MC1R* and *ASIP* genotypes show that the two genes interact to synergically modulate the amount of melanin synthesised in alpaca fibre. The black coat colour is the result of the synthesis almost exclusively of eumelanin due to lack of *ASIP* functional allele. On the other hand, the brown coat phenotype is due to the expression of both genes which leads to the synthesis of the same proportion of eumelanin and pheomelanin. In light of this, both genes have to be considered in the development of marker assisted breeding program for coat colours in alpaca.

## Ethical approval

All experiments were approved and performed in 2008 according to the guidelines of the Animal Ethics Committee of the University of Camerino.

## Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

## Funding

The authors would like to thank the Alpaca Research Foundation (ARF) for their financial support.

## ORCID

Stefano Pallotti  <http://orcid.org/0000-0003-3196-151X>  
 Bathrachalam Chandramohan  <http://orcid.org/0000-0002-3464-7553>  
 Dario Pediconi  <http://orcid.org/0000-0001-8734-3070>  
 Cristina Nocelli  <http://orcid.org/0000-0003-1376-0538>  
 Antonietta La Terza  <http://orcid.org/0000-0002-1244-1503>  
 Carlo Renieri  <http://orcid.org/0000-0003-4816-228X>

## References

- Almathen F, Elbir H, Bahbahani H, Mwacharo J, Hanotte O. 2018. Polymorphisms in *MC1R* and *ASIP* genes are associated with coat color variation in the Arabian camel. *J Hered.* 109(6):700–706.
- Alshanbari F, Castaneda C, Juras R, Hillhouse A, Mendoza MN, Gutiérrez GA, Ponce de León FA, Raudsepp T. 2019. Comparative FISH-mapping of *MC1R*, *ASIP*, and *TYRP1* in new and old world camelids and association analysis with coat color phenotypes in the dromedary (*Camelus dromedarius*). *Front Genet.* 10:340.
- Barsh GS. 1996. The genetics of pigmentation: from fancy genes to complex traits. *Trends Genet.* 12(8):299–305.
- Bathrachalam C, La Manna V, Renieri C, La Terza A. 2011. *Asip* and *MC1R* cDNA polymorphism in alpaca. In: *Fibre production in South American camelids and other fibre animals*. Wageningen: Wageningen Academic Publishers; p. 93–96.
- Cecchi T, Cozzali C, Passamonti P, Ceccarelli P, Pucciarelli F, Gargiulo AM, Frank EN, Renieri C. 2004. Melanins and melanosomes from llama (*Lama glama* L.). *Pigment Cell Res.* 17(3):307–311.
- Cecchi T, Valbonesi A, Passamonti P, Gonzales M, Antonini M, Renieri C. 2011. Quantitative variation of melanins in alpaca (*Lama pacos* L.). *Ital J Anim Sci.* 10(3):e30.
- Chandramohan B, Renieri C, La Manna V, La Terza A. 2013. The alpaca agouti gene: genomic locus, transcripts and causative mutations of eumelanin and pheomelanin coat color. *Gene.* 521(2):303–310.
- Chandramohan B, Renieri C, La Manna V, La Terza A. 2015. The alpaca melanocortin 1 receptor: gene mutations, transcripts, and relative levels of expression in ventral skin biopsies. *Sci World J.* 2015:1–9.
- Daverio MS, Rigalt F, Romero S, Vidal-Rioja L, Di Rocco F. 2016. Polymorphisms in *MC1R* and *ASIP* genes and their association with coat color phenotypes in llamas (*Lama glama*). *Small Rumin Res.* 144:83–89.

- Dreger DL, Parker HG, Ostrander EA, Schmutz SM. 2013. Identification of a mutation that is associated with the saddle tan and black-and-tan phenotypes in Basset Hounds and Pembroke Welsh Corgis. *J Hered.* 104(3):399–406.
- Feeley NL, Munyard KA. 2009. Characterisation of the melanocortin-1 receptor gene in alpaca and identification of possible markers associated with phenotypic variations in colour. *Anim Prod Sci.* 49(8):675–681.
- Feeley N, Bottomley S, Munyard K. 2011. Three novel mutations in ASIP associated with black fibre in alpacas (*Vicugna pacos*). *J Agric Sci.* 4:529–538.
- Fontanesi L, Dall'Olio S, Beretti F, Portolano B, Russo V. 2011. Coat colours in the Massese sheep breed are associated with mutations in the agouti signalling protein (ASIP) and melanocortin 1 receptor (MC1R) genes. *Animal.* 5(1):8–17.
- Guridi M, Soret B, Alfonso L, Arana A. 2011. Single nucleotide polymorphisms in the Melanocortin 1 Receptor gene are linked with lightness of fibre colour in Peruvian Alpaca (*Vicugna pacos*). *Anim Genet.* 42(6):679–682.
- Henkel J, Saif R, Jagannathan V, Schmocker C, Zeindler F, Bangerter E, Herren U, Posantzis D, Bulut Z, Ammann P, et al. 2019. Selection signatures in goats reveal copy number variants underlying breed-defining coat color phenotypes. *PLoS Genet.* 15(12):e1008536.
- Klungland H, Vage DI, Gomez-Raya L, Adalsteinnsson S, Lien S. 1995. The role of melanocyte-stimulating hormone (MSH) receptor in bovine coat color determination. *Mamm Genome.* 6(9):636–639.
- Lai W, Hu M, Zhu W, Yu F, Bai C, Zhang J, Yan S. 2019. A 4-bp deletion in the ASIP gene is associated with the recessive black coat colour in domestic guinea pigs (*Cavia porcellus*). *Anim Genet.* 50(2):190–191.
- Letko A, Ammann B, Jagannathan V, Henkel J, Leuthard F, Schelling C, Carneiro M, Drögemüller C, Leeb T. 2020. A deletion spanning the promoter and first exon of the hair cycle-specific ASIP transcript isoform in black and tan rabbits. *Anim Genet.* 51(1):137–140.
- Liu Z, Gong Y, Feng M, Duan L, Li Y, Li X. 2016. Genetic variations of the coding region of the melanocortin receptor 1 (MC1R) gene in the fox. *Vet Dermatol.* 27(3):135–e136.
- Lu D, Willard D, Patel IR, Kadwell S, Overton L, Kost T, Luther M, Chen W, Woychik RP, Wilkison WO. 1994. Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. *Nature.* 371(6500):799–802.
- Matsumoto H, Kojya M, Takamuku H, Kimura S, Kashimura A, Imai S, Yamauchi K, Ito S. 2020. MC1R c.310G>- and c.871G>A determine the coat color of Kumamoto sub-breed of Japanese Brown cattle. *Anim Sci J.* 91(1):e13367.
- McGregor BA, Ramos HE, Peña EQ. 2012. Variation of fibre characteristics among sampling sites for Huacaya alpaca fleeces from the High Andes. *Small Rumin Res.* 102(2–3):191–196.
- Oguro-Okano M, Honda M, Yamazaki K, Okano K. 2011. Mutations in the melanocortin 1 receptor,  $\beta$ -defensin103 and agouti signaling protein genes, and their association with coat color phenotypes in Akita-inu dogs. *J Vet Med Sci.* 73:1102030453–1102030453.
- Powell AJ, Moss MJ, Tree LT, Roeder BL, Carleton CL, Campbell E, Kooyman DL. 2008. Characterization of the effect of Melanocortin 1 Receptor, a member of the hair color genetic locus, in alpaca (*Lama pacos*) fleece color differentiation. *Small Rumin Res.* 79(2–3):183–187.
- Reissmann M, Lutz W, Lieckfeldt D, Sandoval-Castellanos E, Ludwig A. 2020. An Agouti-signaling-protein mutation is strongly associated with melanism in European Roe Deer (*Capreolus capreolus*). *Genes.* 11(6):647.
- Renieri C, Trabalza Marinucci M, Martino G, Giordano G. 1991. Indagine preliminari sulla qualità del pelo e sul colore del mantello in soggetti alpaca (*Lama glama pacos* PF) pigmentati. 9th Nat. Congr. ASPA, Roma, Italy, p. 905–914.
- Renieri C, Valbonesi A, La Manna V, Antonini M, Lauvergne JJ. 2008. Inheritance of coat colour in Merino sheep. *Small Rumin Res.* 74(1–3):23–29.
- Rieder S, Taourit S, Mariat D, Langlois B, Guérin G. 2001. Mutations in the agouti (ASIP), the extension (MC1R), and the brown (TYRP1) loci and their association to coat color phenotypes in horses (*Equus caballus*). *Mamm Genome.* 12(6):450–455.
- Rochus CM, Westberg Sunesson K, Jonas E, Mikko S, Johansson AM. 2019. Mutations in ASIP and MC1R: dominant black and recessive black alleles segregate in native Swedish sheep populations. *Anim Genet.* 50(6):712–717.
- Shang S, Yu Y, Zhao Y, Dang W, Zhang J, Qin X, Irwin DM, Wang Q, Liu F, Wang Z, et al. 2019. Synergy between MC1R and ASIP for coat color in horses (*Equus caballus*). *J Anim Sci.* 97(4):1578–1585.
- Shi KR, Wang AG, Yuan XF, Deng XM, Li N. 2006. Analysis of the MC1R, KIT and ASIP loci in Chinese and European pigs. *Anim Genet.* 37(3):300–302.