Proceedings, 10th World Congress of Genetics Applied to Livestock Production

ASIP and *MC1R* mutations causing black coat colour in five Swedish sheep breeds

C. M Rochus.[1,2,3], S. Mikko[1], A. Näsholm[1] and A. M. Johansson[1].
[1]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden,
[2]AgroParisTech, Paris, France, [3]GenPhySE, INRA, Castanet-Tolosan, France

ABSTRACT: Black coat colour in sheep can be determined by the recessive black allele (A^a) in Agouti signaling protein (*ASIP*) and the dominant black allele (E^D) in Melanocortin receptor 1 (*MC1R*). A^a is a 5 bp deletion (D_5) or a mutation (g.5172T>A), and E^D is attributed to two mutations; c.218T>A and c.361G>A. *ASIP* and *MC1R* coding regions were sequenced in 26 black and 21 white Swedish sheep from Gute, Finewool, Klövsjö, Roslags and Värmlands breeds. Three combinations of D_5 and g.5172T>A, where one or both of the mutations were homozygous, were only found in black sheep. The only wildtype animal was white with the remaining sheep, 10 black and 20 white, heterozygous in one or both of the mutations. Animals homozygous or heterozygous for mutations c.218T>A and c.361G>A were black Finewool sheep. Seven black individuals were not black due to A^a or E^D.
Keywords: *ASIP*; *MC1R*; sheep

Introduction

Consistency of pigmentation is central for wool and pelt production: there is a demand for white wool (Raadsma et al. (2013)) and naturally coloured wool and pelts uniform in colour (Adalsteinsson (1983); Näsholm, (2005)).

Adalsteinsson (1983) summarized the inheritance of colours in North European sheep breeds from research performed in the 1970s. It was hypothesized that there are seven coat colour and pattern loci and one of these loci, Agouti, now known as Agouti signaling protein (*ASIP*), is a ligand to the Melatonin receptor 1 (MC1R), and plays an important role in coat colour (Adalsteinsson (1983)). The ovine *ASIP* has been mapped to chromosome 13 (Parsons et al. (1999)) and further characterized by (Norris and Whan (2008)). Variations described in *ASIP* include a nine base pair deletion (D_9) g.10-19delAGCCGCCTC, a 5 base pair deletion (D_5) g.100-105delAGGAA, and point mutations g.5051G>C, and g.5172T>A (Norris and Whan (2008); Royo et al. (2008); Gratten et al. (2010)). Both D_5 and g.5172T>A change the function of *ASIP* and when an animal is homozygous for at least one of these mutations it possesses the recessive black allele, A^a, described by Adalsteinsson (1970).

Another locus involved in the inheritance of coat colour is the Extension locus which has 2 alleles, wildtype (E^+) and dominant black (E^D) and is dominant over the Agouti locus (Adalsteinsson (1983)). The locus is located on sheep chromosome 14 and sequenced by Våge et al., in 1999 and contains the Melanocortin receptor 1 (*MC1R*) gene. Mutations found in *MC1R* include c.199C>T, c.218T>A, c.361G>A, c.429C>T, c.600T>G and c.735C>T (Våge et al. (1999); Våge et al. (2003); Royo et al. (2008); Fontanesi et al. (2010)). Three of these mutations change the function of *MC1R*; c.199C>T; c.218T>A and c.361G>A, and the latter two are E^D.

While there have been numerous studies of *ASIP* and *MC1R* in sheep, Swedish breeds have not previously been included. The many rare and old breeds in Sweden belong to the North European short tailed breed of sheep which are characterized by their wide variation in coat colour (Dýrmundsson and Niżnikowski (2009)). The objective of this study was to analyze variants in sequenced coding regions of *ASIP* and *MC1R* in Swedish breeds that have both black and white phenotypes.

Materials and Methods

Data. Blood samples were collected from Swedish sheep breeds. The coat colour was recorded for all sheep at the time of blood collection. DNA was extracted from 1.7 mL of blood using the QIAsymphony® robot and the DNA Midi Kit (Qiagen®, Hilden, Germany). DNA samples were selected from five breeds with both black and white phenotypes. Where possible, sheep in the same breed were selected from different producers. The 47 sheep in this study were Gute (4 black, 2 white), Swedish Finewool (6, 6), Roslags (4, 8), Värmlands (6, 3) or Klövsjö (6, 2). Selected DNA samples were diluted to a concentration of 4 ng/µL.

Sequencing. BigDye© Direct Cycle Sequencing Kit and protocol (Applied Biosystems, Foster City, CA, USA) was used for sequencing both *ASIP* and *MC1R*. Primer sequences for *ASIP* and *MC1R* were from the literature. The *ASIP* primers used were ASIP_F2 and ASIP_R2 for exon 2, ASIP_F3 and ASIP_R3 for exon 3 and ASIP_F4 and ASIP_R4 for exon 4 (Gratten et al. (2010)). The *MC1R*, primers used were 2, 3 and 4 for sequencing exon 2 and 3 (Fontanesi et al. (2010)). In addition, these forward and reverse primers had M13-21 and M13-29 5' tails respectively. Sequencing products were purified using a BigDye XTerminator® Purification Kit (Applied Biosystems, Foster City, CA, USA) and capillary electrophoresis was performed with Applied Biosystems® 3500xL Dx Genetic Analyzer (Life Technologies, Foster City, CA, USA).

Data analysis. Sequences were visually inspected and aligned using CodonCode Aligner (CodonCode Corporation, Dedham, MA, USA). The reference sequences for both *ASIP* and *MC1R* were from NCBI (2013), accession numbers NM_001134303 and NM_001282528.

Results and Discussion

Table 1 reports the frequencies of variants in *ASIP* and *MC1R* in this study. In *ASIP*, three out of four mutations were polymorphic; D_5, g.5051G>C and g.5172T>A. D_5 and g.5172T>A, which are responsible for the recessive black allele, A^a, are shown in Table 2. All sheep homozygous for both D_5 and g.5172A were black but this genotype was only found in Klövsjö sheep. All sheep homozygous for either D_5 or g.5172A were black and all sheep homozygous for the wildtype alleles, N_5 and g.5172T in this study were white. Three combinations of D_5 and g.5172T>A genotypes resulted in either sheep with white fleece or sheep with black fleece.

Table 1. Allele frequencies of known mutations in Agouti signaling protein (*ASIP*) and Melanocortin receptor 1 (*MC1R*) in five Swedish sheep breeds

Gene	Mutation	Frequency
ASIP	D_5	0.57
	D_9	0
	g.5172T>A	0.29
	g.5051G>C	0.26
MC1R	c.218T>A	0.06
	c.361G>A	0.06
	c.199C>T	0
	c.429C>T	0.28
	c.600T>G	0.28
	c.735C>T	0.28

Table 2. Detected genotypes of D_5 and g.5172T>A in Agouti Signaling Protein (*ASIP*) by coat colour and breed

Coat colour	D_5 - g.5172T>A	Breed				
		F	G	K	R	V
Black	D_5D_5 - AA			3		
	D_5D_5 - TT	2	2		4	2
	N_5D_5 - AA			3		
Black	N_5D_5 - TA	3				2
White		1		2		2
Black	N_5D_5 - TT	1				2
White		2	1		8	1
Black	N_5N_5 - TA	2	2			
White			1			
White	N_5N_5 - TT	1				

F: Swedish Finewool
G: Gute
K: Klövsjö
R: Roslags
V: Värmlands

Of the six published polymorphic positions in MC1R, five were variable in this study: c.218T>A, c.361G>A, c.429C>T, c.600T>G and c.735C>T. Table 3 shows the detected combinations of E^D, c.218T>A and c.361G>A. Finewool sheep were the only breed to have variation in c.218T>A and c.361G>A. White Finewool sheep were homozygous wildtype (E^+) while five out of six black sheep were either homozygous for c.218A and c.361A, or heterozygous for both mutations. One black individual was homozygous E^+ and ASIP N_5D_5 -TA for D_5 and g.5172T>A.

Table 3. Detected genotypes of c.218T>A and c.361G>A in Melanocortin receptor 1 (*MC1R*) by coat colour and breed

Coat colour	c.218T>A - c.361G>A	Breed				
		F	G	K	R	V
Black	AA - AA	1				
	TA - GA	4				
Black	TT - GG	1	4	6	4	6
White		6	2	2	8	3

F: Swedish Finewool
G: Gute
K: Klövsjö
R: Roslags
V: Värmlands

This study demonstrates that A^a and E^D are not the only alleles that contribute to a black coat in Swedish sheep. Of the breeds studied, Gute, Klövsjö, Roslags and Värmlands are considered to be old and rare (Dýrmundsson and Niżnikowski (2009)) whereas Finewool, an improved breed, has seen the inclusion of genetic material from Germany, Britain and Spain and more recently from the Finnish Finewool breed (Svenska Finullsföreningen (2013)). In this study Roslags breed, which was estimated to have a purebred breeding population of 675 (Dýrmundsson and Niżnikowski (2009)), was homozygous wildtype for all variants except D_5. Coat colour was associated perfectly with D_5; individuals homozygous D_5 were black while heterozygous individuals were white. Klövsjö sheep, with an estimated purebred breeding population of 97 (Dýrmundsson and Niżnikowski (2009) was also interesting as they were the only sheep in this study to be homozygous g.5172A. All individuals with both copies of g.5172A were black while Klövsjö sheep with only one copy were white. Finally, Finewool sheep were the only sheep in this study to have the E^D allele. This allele has been found in many other black sheep of other breeds including Norweigian Dala (Våge et al. (1999)), Damara, Merino, Corridale (Våge et al. (2003)) and Massese (Fontanesi et al. (2010)). Our results suggest that the *E* is not from the Swedish genetic background of the Finewool but from some other source. The results in this study also suggest that black phenotype in Finewool sheep is not only the result of E^D.

The *MC1R* missense mutation, c.199C>T, was not found in this study. Previously this variant has been reported in only one Sicilian breed, Valle del Belice (Fontanesi et al. (2010)). A nine base pair deletion, D^9, first described in Australian Merino (Norris and Whan (2008)) but not seen in Soay sheep (Gratten et al. (2010)) was not present in this population.

There were three combinations of D_5 and g.5172T>A found in both black and white individuals which demonstrates that there are possibly also other alleles and genes responsible for coat colour in these Swedish breeds.

Conclusion

Mutations previously found in *ASIP* and *MCIR* were found in Swedish breeds of sheep. These mutations are associated with black coat colour but not all variation in coat colour could be explained in this study. Future work on black and white coat colours in this population should focus on other coat colour candidate genes.

Acknowledgements

CMR benefited from a joint grant from the European Commission and SLU, within the framework of the Erasmus-Mundus joint doctorate "EGS-ABG". AMJ received funding from the Royal Swedish Academy of Agriculture and Forestry (KSLA) to support collection of Swedish sheep breed samples

Literature Cited

Adalsteinsson, S. (1983). Livest. Prod. Sci., 10: 555-567.
Adalsteinsson, S. (1970). J. Agr. Res. Icel., 2:3-135.
Dýrmundsson, Ó.R., and Niżnikowski, R. (2010). Animal, 4:1275-1282.
Fontanesi, L., Beretti, F., Riggio, V., et al. (2010). Small Ruminant Res., 91:200-207.
Gratten, J., Pilkington, J.G., Brown, E.A., et al. (2010). Heredity, 104:206-214.
Gratten, J., Beraldi, D., Lowder, B.V., et al. (2007). P. Roy. Soc. Biol. Sci., 274:619-626.
Näsholm, A. (2005). Acta Agr. Scand., 55:57-65.
NCBI (2013). http://ncbi.nlm.nih.gov/ Accessed on Feb. 2013.
Norris, B.J. and Whan, V.A. (2008). Genome Res., 18:1282-1293.
Parsons, Y.M., Fleet, M.R. and Cooper, D.W. (1999). J. of Agr. Res., 50:1099-1103.
Raadsma, H.W., Jonas, E., Fleet M.R., et al. (2013). Anim. Genet., 44:547-559.
Royo, L.J., Álvarez, I., Arranz, J.J., et al. (2008). Anim. Genet., 39:290-293.
Svenska Finullsföreningen (2013). http://www.finullsforeningen.com/ Accessed on Feb. 2013.
Våge, D.I., Klungland, H., Lu, D., et al. (1999). Mamm. Genome 10:39-43.
Våge, D.I., Fleet, M.R., Ponz, R., et al. (2003). Pigm. Cell Res. 16:693-697.