Making Moorit: Mutations in TYRP1 are responsible for brown coat color in different United States sheep breeds

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Summary

Brown coat color, the B Locus, in sheep is known to be recessive to black; however, the potential molecular cause(s) across various breeds have not yet been discovered. Based on evidence from other species, *TYRP1* is hypothesized to carry mutations responsible for changes from black to brown pigment in sheep breeds raised for wool. In the present study, we investigated the *TYRP1* gene by Sanger sequencing the coding regions across several sheep populations within the United States that contain black and brown individuals. We identified two SNPs associated with brown coat color (chr2:80,608,128G>T and chr2:80,611,700C>T) located within exon 3 and 4, respectively. The exon 3 SNP leads to an amino acid change from cysteine to phenylalanine (C290F) and the exon 4 SNP introduces a premature stop codon in the TYRP1 protein (R356X). These SNPs segregate by breed where R356X is found in Icelandics and Shetlands, C290F is found in Natural Colored Finewool and Finnsheep, and Romeldales and Romneys carry both mutations. The results from this study provide more insight into coat color genetics in sheep and will allow breeders to make more accurate breeding decisions to meet their wool markets.

Keywords: Ovis aries; B locus; tyrosinase-related protein 1; wool; black sheep

Introduction

Shepherds have been selecting specific coat color and various patterns in sheep for hundreds of years. This has led to some breeds containing a plethora of color variation, while other breeds have predominantly white wool. In the United States, brown and black wool can be sold into niche markets like hand-spinning fleeces for a premium over white wool. Color is an important part of breed development as certain breeds have become renowned for their color or patterns, such as the California Variegated Mutants within the Romeldale breed. There are seven genetic loci thought to be involved with coat color variation in sheep (Adalsteinsson, 1983). Aside from the white pattern regulated by *ASIP* (Norris and Whan, 2008) and the dominant black color controlled by *MC1R* (Våge et al., 1999), very little is known about the DNA polymorphisms responsible for other patterns and color in U.S. sheep.

Brown, also called moorit, is a color present in several sheep breeds that are raised for wool production within and outside the United States. *Tyrosinase related protein 1 (TYRP1)* was identified as a candidate gene for the B locus in domestic sheep due to its effect on black to brown eumelanin pigmentation in dogs (Schmutz et al., 2002), Dexter cattle (Berryere et al., 2003), mice (Zdarsky et al., 1990), pigs (Ren et al., 2011), rabbits (Utzeri et al., 2014), and a wild population of Soay sheep (Gratten et al., 2007). The B locus in Icelandic sheep behaves in a recessive manner similar to mice (Adalsteinsson, 1970). Some sheep breeds such

as the Castlemilk Moorit or Manx Loughtan are assumed to be fixed at the B locus for the brown allele, while others such as the Finnsheep and Icelandic are known to be variable. However, to date no molecular studies to identify mutations responsible for brown have been conducted within the United States sheep population. The aim of this study was to investigate the *TYRP1* gene for possible polymorphisms leading to the brown coat color phenotype.

Material and methods

All sheep were sampled in accordance with the Cornell University Institutional Animal Care & Use Committee. All sheep were privately owned and owner consent was given prior to sample collection. Photos taken by the sampler or owner were used for classifying individuals as black or brown. Hair, skin, and wool color were all used to quantify an animal as black or brown as opposed to wool color alone to avoid potential misclassification due to sun bleaching of the fleece. Generally, the hair, skin, and wool are the same base pigment allowing for classification into black or brown. Examples of black and brown individuals can be seen in Figure 1.

Whole blood was collected via 10mL vacutainers containing the anti-coagulant EDTA. DNA was extracted using the Qiagen Puregene Protocol (Gentra Systems, Inc. Minneapolis, MN). Primers were designed to capture the seven coding regions of ovine *TYPR1* using Primer 3 software (Untergasser et al. 2012). The standard PCR protocol included an initial 3min at 95°C followed by 35 cycles of 95°C for 30s, annealing temperature for 30s, and 72°C for 30s, and a final 3min extension time at 72°C. PCRs for all seven products were performed on twelve individuals, four black and eight brown, across four breeds. These PCR products were Sanger sequenced at the Cornell Biological Resource Center to identify polymorphisms within the coding regions in different breeds. Sequences were visually analyzed using Sequencher® version 5.4.1 (Gene Codes Corp.). Two exonic mutations were identified for further analysis: (1) The G to T nucleotide change in exon 3 at chr2: 80,608,128bp and (2) the C to T nucleotide change in exon 4 at chr2: 80,611,700bp. The SNP in exon 3 was previously identified by Gratten *et al*. (2007) and causes a cysteine to phenylalanine amino acid change (C290F). The SNP in exon 4 was not previously published as associated with color and results in a premature stop codon (R356X) that removes the last 33% of the amino acid sequence.

PCR-RFLP tests were designed to genotype additional animals at these two SNPs. Chr2:80,608,128G>T results in a cut site recognized by the TaqαI enzyme. After a 65°C overnight digestion with TaqαI, the G allele band is not cut (583 bp band) and the T allele band is cut into fragment sizes of 314 and 269 bp, using x3_F and x3_R primers. Chr2:80,611,700C>T results in a cut site recognized by the AlwNI enzyme. This PCR product (x4_F and x4_R primers) was digested at 37°C overnight and the C allele resulted in two bands, 286 and 200 bp in length whereas the T allele generated three bands, 200, 148, and 138 bp. These PCR-RFLPs were used to genotype the remainder of the individuals in the study.

Results and Discussion

In total, 486 individuals across 9 breeds were genotyped at these two SNPs. The exon 3 SNP was present in the Finnsheep, Natural Colored Finewool, Romeldale, and Romney

breeds. The exon 4 SNP was present in the Shetland, Icelandic, Romeldale, and Romney breeds. Neither SNP was identified in the Jacob, California Red, or Tunis breeds. Genotype counts by breed and color are presented in Table 1. Associations were performed with a Fisher's exact test in R (RCoreTeam, 2014) comparing genotypes between black and brown individuals. White individuals were genotyped to determine potential carrier status and allele frequencies within breeds. White individuals genotyped were excluded from the statistical analysis because the white pattern generally results in a masking of black or brown pigment. The Natural Colored fine wool and Finnsheep only carried the exon 3 SNP which associated with brown color ($p<0.001$). The Shetland and Icelandic breeds only carried the exon 4 SNP which associated with brown color ($p<0.001$). However, the Romeldale and Romney appear to carry both polymorphisms which also associate with brown color ($p<0.001$). Regardless of breed, it appears that both copies of *TYRP1* need to be altered to result in a brown sheep. We hypothesize that brown sheep heterozygous at both sites for the brown causing variants on opposing DNA strands have similar disruption to the *TYRP1* gene as a homozygous individual at either of the variant sites. This explains the brown individuals with both heterozygous genotypes in the Romney and Romeldale breeds. None of the black individuals across all breeds ($n=212$) are heterozygous at both positions. This is similar to how the mutations in the canine *TYRP1* lead to a brown phenotype (Schmutz et al., 2002).

We tested the Jacob breed as an external control since there has been no documented moorit individuals in this breed and those tested carried neither mutation. The red color seen in the California Red and Tunis breeds does not appear to be attributable to these SNPs as none of the animals tested from these breeds carried either of these polymorphisms. However, other mutations within *TYRP1* not observed in the present study could be contributing to the red color seen in the California Red and Tunis breeds.

Despite the classical genetic research done in sheep coat color over the past century, these two mutations are the only variants in ovine *TYRP1* that are currently associated with brown color. Further study and a larger sample size are needed to determine if these two mutations differ in the specific shade of brown color within the breeds carrying both mutations.

Conclusion

We conclude that the *TYRP1* mutations associated in the present study are contributing to brown coat color in the U.S. populations of Finnsheep, Icelandic, Natural Colored Finewool, Romeldale, Romney, and Shetland breeds. These variants could be used for breeding for or against brown sheep in the U.S and could allow breeders to select for more profitable colors to fill niche markets. Further genomic study of coat color in sheep using next generation sequencing technology would allow for a greater understanding of the genes and mechanisms responsible for controlling coat color variation in sheep.

Breed	**Color**	**N**	**C290F/R356X Genotype[1]**								
			GG/CC	GG/CT	GG/TT	GT/CC	GT/CT	GT/TT	TT/CC	TT/CT	TT/T
California Red	Red	13	13	.	.	.	.	.	.	.	.
Tunis	Red	3	3	.	.	.	.	.	.	.	.
Finnsheep	Black	12	2	.	.	10	.	.	.	.	.
	Brown	4	.	.	.	.	.	.	4	.	.
	White	6	4	.	.	2	.	.	.	.	.
Icelandic	Black	8	1	7	.	.	.	.	.	.	.
	Brown	3	.	.	3	.	.	.	.	.	.
	White	1	.	.	1	.	.	.	.	.	.
Jacob	Black	28	28	.	.	.	.	.	.	.	.
NC Fine	Black	3	.	.	.	3	.	.	.	.	.
	Brown	9	.	.	.	.	.	.	9	.	.
Romeldale	Black	65	25	4	.	36	.	.	.	.	.
	Brown	18	.	.	1	.	3	.	14	.	.
	White	14	8	.	.	6	.	.	.	.	.
Romney	Black	118	112	4	.	2	.	.	.	.	.
	Brown	9	.	.	4	.	4	.	1	.	.
	White	153	153	.	.	.	.	.	.	.	.
Shetland	Black	9	1	8	.	.	.	.	.	.	.
	Brown	10	.	.	10	.	.	.	.	.	.
Total		486	350	23	19	59	7	.	28	.	.

Table 1. Genotype counts by breed and color class among sampled individuals.

[1] "." Means no observations

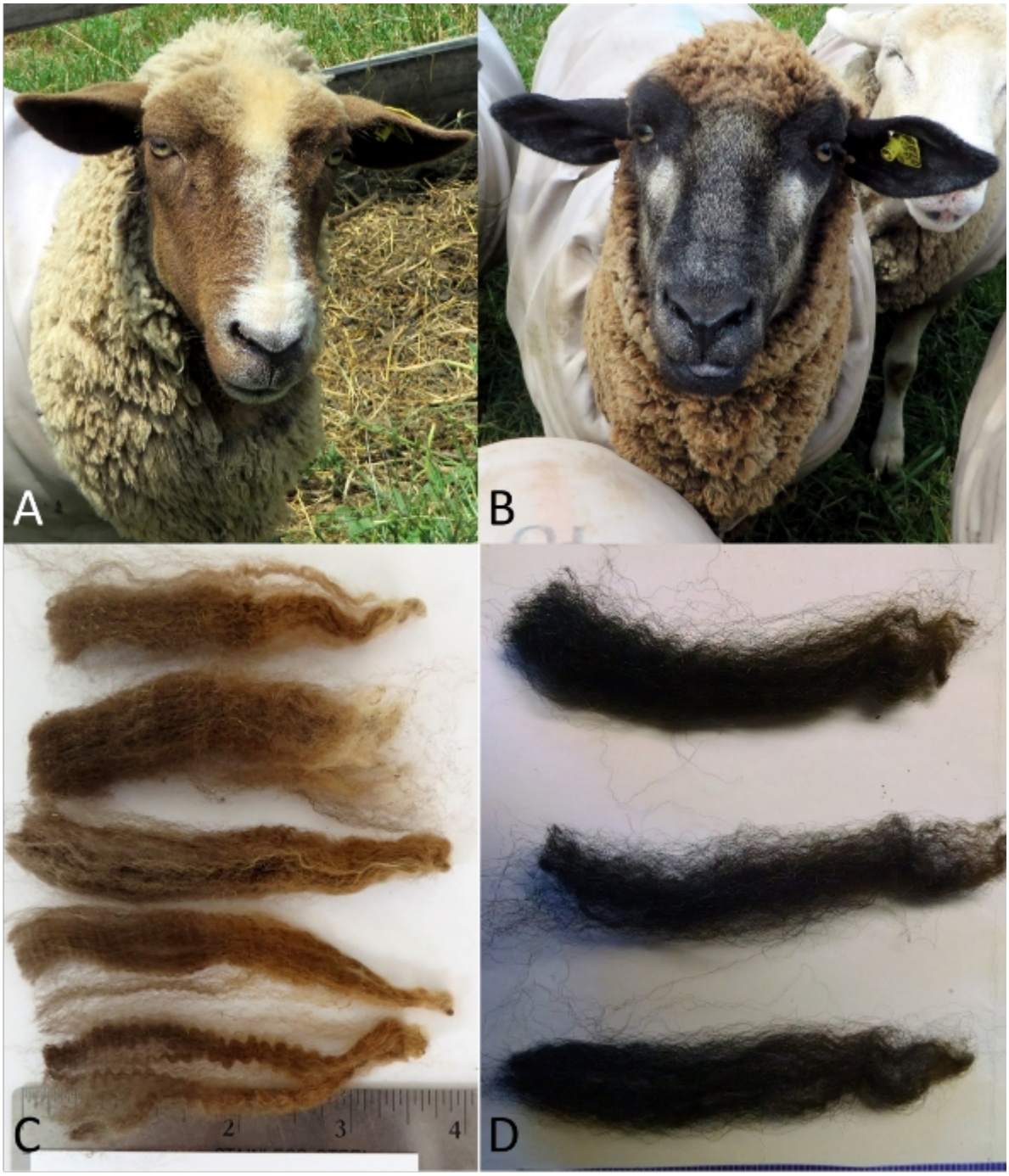

Figure 1. Color differences between black and brown individuals. A) Brown individual B) Black individual C) Brown wool color and D) Black wool color.

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