4-1-2023

White coat color of a Black Angus calf attributed to an occurrence of the delR217 variant of *MITF*

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Received: 17 February 2023 | Accepted: 1 April 2023

DOI: 10.1111/age.13327

SHORT COMMUNICATION

ANIMAL GENETICS WILEY

White coat color of a Black Angus calf attributed to an occurrence of the delR217 variant of *MITF*

Abstract

A white calf, with minimal pigmented markings, was born to two registered Black Angus parents. Given the possibility of an unknown recessive or de novo dominant mutation, whole-genome sequencing was conducted on the trio of individuals. A 3-bp in-frame deletion in *MITF* was identified; this mutation was unique to the calf but identical to the delR217 variant reported in both humans and murine models of Waardenburg syndrome type 2A and Tietz syndrome. Given the coat color phenotype and identity of the mutation, our data support that this calf represents the first instance of this recurring *MITF* mutation in cattle.

Coat color is often the first phenotype of an animal observed; the genetics of coat color, therefore, has been well studied. In cattle, hide color is considered an indicator of breed-type and performance, as cattle with predominantly black hides often have higher meat quality than cattle of other colors (Parish et al., 2018; Williams et al., 2012). The Black Angus breed of cattle is known, in part, for their black hide. Due to selection for this phenotype, most Angus are homozygous for the dominant black (E^D) variant of *MC1R* (Klungland et al., 1995; Matukumalli et al., 2009). Angus cattle with white markings outside of the udder or scrotal region are not allowed in the registry (American Angus Association).

The appearance of novel white patterning in an animal's coat is often attributed to variants in one of a suite of genes affecting melanocyte function. Bovid examples include variation of *KIT* causative of the Hereford pattern (Grosz & MacNeil, 1999) and color-sidedness (Durkin et al., 2012), *MGF* associated with roan in Shorthorn and Belgian Blue cattle (Seitz et al., 1999), and *MITF* determinant of white spotting in Brown Swiss (Hofstetter et al., 2019), Holstein, and Italian Simmental (Fontanesi et al., 2012). The impetus for this study was the report of a nearly all white bull calf born to two registered Black Angus parents (Figure 1). Parentage of the calf was confirmed, and the unexpected phenotype hypothesized to be due to a previously undocumented recessive, or a de novo dominant mutation. Whole-genome sequencing was therefore employed to identify variation potentially causative of this unexpected coat color.

EDTA blood was obtained from the calf and its dam and a straw of semen from the sire. DNA was isolated using previously described methods (Sieck et al., 2020) and sent for library preparation (KAPA HyperPrep; Roche) and 150-bp paired-end whole-genome sequencing at Admera Health. Sequencing was conducted on an Illumina NovaSeq.

The raw sequence was pre-processed using TrimGalore (doi:10.5281/zenodo.5127899) prior to mapping to the ARS-UCD1.2 reference genome using BWA MEM (Li, 2013). Variants were called using the GATK Haplotype Caller (Van der Auwera & O'Connor, 2020) after marking duplicates (Danecek et al., 2021) and realigning indels. In addition to the targeted trio, data from 144 other cattle sequenced in the lab were included, which represented other Angus and closely related cattle (Angus ($N=9$), Angus-cross ($N=23$), Brangus ($N=9$), Red Angus ($N=4$)), as well as Hereford, Charolais, and Red Angus/Simmental/Gelbvieh composites. Whole-genome sequence of the calf, sire, and dam averaged 12.9× coverage (11.3–15.5). Both parents and the calf were confirmed homozygous for the dominant black (E^D) allele (g.14705671T>C; Klungland et al., 1995).

Considering a possible recessive, a query of the data using VCFTOOLS (Danecek et al., 2011) identified 1798 autosomal loci for which both the sire and dam were heterozygous and the calf homozygous that were not present in the homozygous state in any other individual, and for which no more than 6 alternative alleles were present in the other 144 individuals; 88 of these loci were unique to the trio (not identified in any of the other individuals). None of the 1798 variants were in genes associated with white coloration (Baxter et al., 2019); these variants were not further considered.

The calf had 342 variants that were absent in all other individuals (hypothesized de novo dominant) with a predicted 'high' or 'moderate' impact on gene function

(Ensembl Variant Effect Predictor; McLaren et al., 2016) in one of 160 annotated genes. Three of the genes, *PSENEN*, *SLC29A3*, and *MITF*, were previously associated with pigmentation (Baxter et al., 2019). Variants in both *PSENEN* and *SLC29A3* were followed-up with Sanger sequencing of the trio, which failed to find evidence of their presence in any of the three individuals (Method S1). These variants were considered erroneous genotype calls from whole-genome sequencing and removed from consideration. The third candidate was a 3-bp in-frame deletion in *MITF* (chr22.g.31628133-31628135del), detected in two of nine sequencing reads of the calf (Figure S1). The deletion was predicted to result in the loss of an arginine residue at position 224 of isoform ENSBTAT00000080989.1. Sanger sequencing confirmed the mutation was present in the calf but absent in both the sire and dam (Figure 2). No evidence of structural variation in the regions surrounding candidate loci was observed via visual inspection of the reads using Integrative Genomics Viewer (Robinson et al., 2011).

Variation in *MITF* is often associated with the auditory-pigmentation syndromes Waardenburg syndrome type 2A (WS2A) and Tietz syndrome (TS). First described in 1963, TS is characterized by a generalized loss of pigment and bilateral deafness (Tietz, 1963). Often considered less severe than TS, characteristics of WS2A include varying degrees of patchy depigmentation, heterochromia irides, and in some individuals, hearing loss (Read & Newton, 1997; Tassabehji et al., 1995). Due to their overlapping clinical features and similar genetic etiology including non-truncating variation in the basic domain of *MITF*, WS2A and TS are sometimes considered jointly (Cortes-Gonzalez et al., 2016; Leger et al., 2012).

FIGURE 1 Image of the white Angus calf. The calf had pigmented spots around his eyes, on top of his head including his ears, and a patch of black on the dorsal side of his neck. Eyes, not formally evaluated, appeared to have normal pigment.

The delR217 mutation, and that found in the white calf result in the deletion of one of a string of four arginine residues in the basic domain of the protein. This domain is responsible for DNA binding and is highly conserved (Hallsson et al., 2007). Multiple, independent instances of the delR217 mutation have been identified in humans. Patients with this mutation (all heterozygous) were classified with either TS or WS2A. All were reported to have hearing loss, but variability was observed in other features such as the color of irides and depigmentation of skin and/or hair (Chen et al., 2010; Tassabehji et al., 1995; Yang et al., 2013 and reviewed in Leger et al., 2012). In murine models, the semi-dominant delR217 variant ($MITF^{Mi}$) can be observed in a homozygous state. Although $MITF^{Mi/+}$ mice have reduced eye pigment and only small patches of white on the belly and/or head, $MITF^{Mi/Mi}$ mice have a solid white coat, micropthalmia, ostepetrosis, mast cell deficiency, and inner ear defects (Steingrimsson et al., 2003, 2004). The breeders of this Angus calf reported no indication that his hearing or eyesight was impaired; other than his hide coloration, the calf appeared normal. However, no formal clinical evaluation of auditory or ocular systems was conducted and deficits in these senses cannot be ruled out. Given the *MITF* variant identified and deficit in skin pigmentation, we postulate that the mutation in this calf represents a novel bovid instance of WS2A/TS.

This report joins two other instances of *MITF*-driven auditory–pigmentation disorders reported in cattle. The entirety of *MITF* was deleted in a Holstein with a lack of pigment and micropthalmia (Wiedemar & Drogemuller, 2014). Proximal to the variant identified

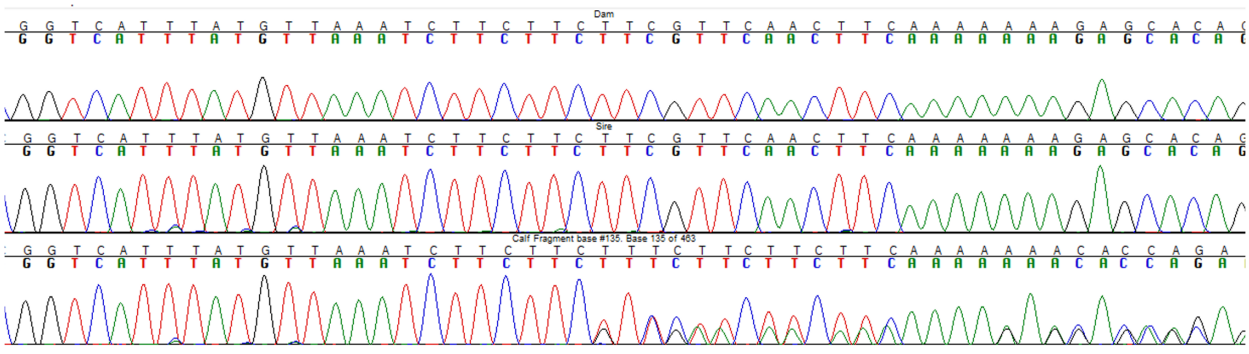

FIGURE 2 Sanger sequencing chromatogram of the dam (top), sire (middle), and calf (bottom) across the *MITF* candidate locus. The 3-bp deletion is evident in the calf but is not present in the parents. The forward strand is shown; *MITF* is coded for on the reverse strand.

in this study, a missense variant in German Fleckvieh resulted in TS; affected Fleckvieh were completely white with pink skin, bilateral deafness, and heterochromatic irides (Philipp et al., 2011). The variant identified in Fleckvieh (22.g.31628131C>A) was also within the basic domain of the protein, two amino acids prior to the in-frame deletion present in the white Angus calf.

The impact of this bovine mutation on ocular, ophthalmologic, and orthopedic function remain unexplored. It is also unclear what background variation or other factors are responsible for the variability in phenotype observed among species and among individuals. Given the wealth of information on the orthologous mutations in other species, it is likely that homozygosity for this variant would result in an animal that is not viable for production. The identification of this variant represents another instance of the recurrence of the delR217 variant of *MITF*, the presence of which rejects the possibility that this calf's unique coat color might be attributed to a novel recessive trait circulating in the breed.

KEYWORDS

Bos taurus, *MITF*^{Mi}, pigmentation, Tietz syndrome, Waardenburg syndrome type 2A

ACKNOWLEDGMENTS

Anna Fuller and Sophie Korytowski provided laboratory support for this project., The American Angus Association partially funded the investigation.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Whole-genome sequence data are available as BioProject PRJNA936134 in the NCBI Sequence Read Archive.

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