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Genome-wide association study identifies variants associated with hair length in Brangus cattle

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Summary

Thermal stress limits beef cattle production and a shorter hair coat is a key thermoregulative adaptation that allows cattle to lose heat more efficiently. The objective of this study was to identify genetic variants associated with the length of the undercoat and topcoat of cattle utilizing 1456 Brangus heifers genotyped with the Bovine GGP F250 array. Seven SNPs in the *PCCA* gene were significantly associated with undercoat length. *PCCA* belongs to the biotin transport and metabolism pathway. Biotin deficiency has been reported to cause hair loss. Four SNPs in an 110 kb including a missense mutation in the *PRLR* gene were significantly associated with topcoat length. Whereas the association of this polymorphism with hair length is novel, the *SLICK* mutation in *PRLR* has previously been demonstrated to significantly impact hair length in cattle. These newly detected genetic variants may contribute to a shorter hair coat and more thermotolerant animals.

Keywords biotin, prolactin, thermotolerance

Thermal stress limits beef cattle production owing to decreased animal performance including lower pregnancy rates and a reduction in feed intake and growth (Amundson *et al.* 2006; Renaudeau *et al.* 2012). In the USA alone this results in an economic loss of $369 million annually (St-Pierre *et al.* 2003). However, this is a global issue with over 65% of the world's cattle (beef and dairy) residing in (sub) tropical areas known for hot, humid conditions (Burrow 2012). Hair coat is a key thermoregulatory adaptation. Cattle with a shorter-haired coat are able to lose heat more effectively through conductive, convective and radiative cooling at the hair–skin interface (Hansen 2004). Hair length has previously been demonstrated to have a major impact on body temperature (Hamblen *et al.* 2018) and has a high heritability (0.67 and 0.42 for undercoat and topcoat respectively) (Sarlo Davila *et al.* 2019), indicating that this trait is largely influenced by genetics. The objective of this study was to identify genetic variants associated with the length of the undercoat and topcoat of cattle.

The University of Florida Institutional Animal Care and Use Committee approved the research protocol used in this study (approval no. 201203578). This study utilized 1456 commercial Brangus heifers from the Seminole Tribe of Florida Inc. accumulated over two summers in 2016 and 2017. Hair samples were collected from the shoulder, 4 inches down from the spine and half-way along the horizontal axis of each animal. Hair samples were collected from eight groups of 200 animals: four groups over four consecutive weeks in 2016 (August 15–September 12) and four groups over four consecutive weeks in 2017 (July 31–August 28). Heifers within a year were from the same cohort and approximately the same age (about 2 years old). Hair samples were spread on white paper and photographed alongside a ruler to serve as a scale by which pixels could be converted in millimeters. IMAGEJ software (Schindelin *et al.* 2012) was used to measure hair length. The lengths of the undercoat (shorter coat closer to the body of the animal) and topcoat (longer coat that covers the undercoat) were evaluated for each individual by averaging the lengths of five short hairs and five long hairs respectively. DNA was extracted from blood samples and genotyped with the Bovine GGP F250 array (Illumina Inc.). Chromosomal assignments and positions of SNP were based on the ARS-UCD 1.2 *B. taurus* sequence assembly. Genotypes were filtered in PLINK (Purcell *et al.* 2007) to remove SNP with call rates <90% and MAFs <0.05. After quality control, 109 538 SNP were available for association analyses. Both hair length phenotypes were pre-adjusted for the fixed effect

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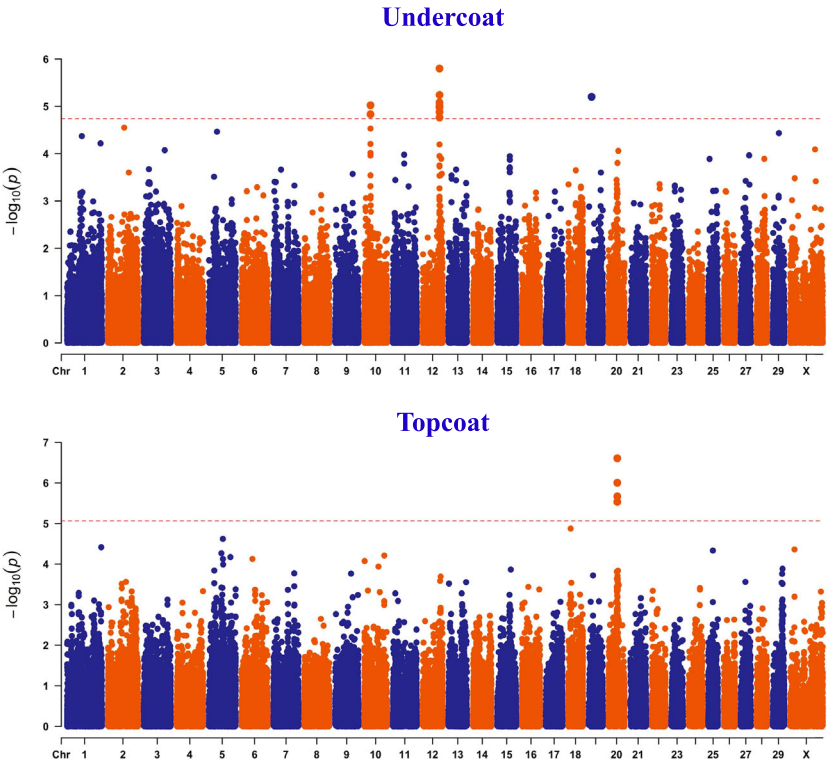

Figure 1 Manhattan plots for undercoat and topcoat length. Each dot represents an SNP. Genomic coordinates are displayed along the *x*-axis and −log10 (*P* values) are displayed along the *y*-axis. The dotted lines represent the genome-wide significance line.

collection group using linear model procedures in R. The residuals from these models were used in GWASs using the univariate procedures of GEMMA (Zhou & Stephens 2012) that fitted a single, standardized, genomic relationship matrix to account for the genetic covariance among animals. The genomic relationship matrix was constructed in GEMMA using the 109 538 SNPs that remained after quality control. To correct for multiple tests, the Benjamini–Hochberg false discovery rate was constrained to 0.2. This false discovery threshold was utilized to reduce type II error.

Manhattan plots of the GWAS results for both hair phenotypes are shown in Fig. 1. The estimated genomic heritability for undercoat length in this population was 0.30 with a standard error of 0.05. We identified eight SNPs significantly associated with undercoat length within a 300 kb region on BTA 12. This region contains the gene *PCCA*. Seven of the significant SNPs were located within *PCCA* and one of the significant SNPs was an upstream variant (rs133787763). Of the seven intragenic SNPs there were two missense mutations (rs133791585, rs210751638), four intron variants (rs383191895, rs110703144, rs134127983, rs210217784) and one splice region variant (rs41679981). *Propionyl-CoA carboxylase subunit alpha* belongs to the biotin transport and metabolism pathway (Dakshinamurti 1988). PCCA can be measured in hair roots and low PCCA levels can be used to diagnose biotin deficiencies in human medicine (Wolf & Raetz 1983). Biotin deficiencies have been widely reported in multiple species to affect hair length, growth and texture and even to result in hair loss (Rauch 1952; Bryant *et al.* 1985; Dakshinamurti 1988; Campeau *et al.* 2001; Boccaletti *et al.* 2007). Much of the literature concerning the relationship between biotin and hair addresses nutritional deficiencies and strategies for supplementing biotin in the diet (Rauch 1952; Bryant *et al.* 1985). However, heritable genetic disorders in the biotin pathway that result in changes to the hair have been reported, such as familiar uncombable hair syndrome in humans (Boccaletti *et al.* 2007), where those affected have short, coarse hair. Whereas the association of *PCCA* with hair length in cattle is novel, it is biologically relevant and a candidate for future selection programs. Two SNPs (rs444824868, rs445292907) within a 46 kb region on BTA 10 were also significantly associated with undercoat length. This region contains the gene *OR4F3*, an olfactory receptor gene.

The estimated genomic heritability for topcoat length was 0.34 with a standard error of 0.05. We identified four SNPs significantly associated with topcoat length within a 110 kb region on BTA 20. This region contains the genes *PRLR*, *AGXT2* and *DNAJC21*. Two of the significant SNPs (rs377826688, rs383738120) were intergenic variants. Of the remaining SNPs, one was a splice region variant (rs134538752) in *DNAJC21* and the other was a missense mutation (rs135164815) in *PRLR*. Each of the four significant SNPs was fitted one at a time as a fixed effect to determine if the same association signal was captured by these SNPs. The majority of the association signals was captured by the missense SNP in *PRLR*, rs135164815,

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which explained 4.0% of the variation in topcoat length. Previously identified mutations in *PRLR* have been shown to have a major effect on hair length in cattle (Littlejohn *et al.* 2014; Porto-Neto *et al.* 2018). Mice with *PRLR* KO had significantly longer and coarser hair than wt mice, indicating that prolactin inhibits hair growth (Craven *et al.* 2001). The *SLICK* mutation in *PRLR*, originally identified in Senepol cattle, is a frameshift mutation that results in a truncated protein and a dramatically shorter hair coat (Olson *et al.* 2003; Littlejohn *et al.* 2014). Subsequently, two other mutations in *PRLR* have been associated with a slick hair coat in Limonero and Carora cattle that did not carry the previously identified Senepol mutation (Porto-Neto *et al.* 2018). All three of these previously identified *SLICK* mutations are truncation mutations in exons 10 or 11 of *PRLR*. In contrast, the *PRLR* SNP identified in this study is a missense mutation (A>G) in exon 2. This missense mutation identified in this study has not been previously associated with the slick phenotype but has been found to be associated with milk production in heat-stressed Holstein cattle (Hernández-Hernandez-Corde *et al.* 2017). The prolactin hormone has been demonstrated to regulate both milk production and hair length (Horseman & Gregerson 2013; Littlejohn *et al.* 2014). This association is of great interest as *PRLR* has not previously been associated with hair length in Brangus cattle. These results indicate that the prolactin pathway may regulate hair length across multiple breeds of cattle.

These genetic variants identified for both undercoat and topcoat are of great biological relevance. The variants have the potential to be used for selection in order to breed for animals with shorter hair, leading to greater thermotolerance and potentially increasing production in hot, humid climates. The current study is limited to the potential functional variants available on the GGP F250 array and should be followed up with WGS data to refine putative functional mutations.

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Data availability statement

Genotype data is available through OSF and can be accessed at https://osf.io/u3r6m/?view_only=1a1b43cbc1b8473594d89eee8977eb8c

References

Amundson J.L., Mader T.L., Rasby R.J. & Hu Q.S. (2006) Environmental effects on pregnancy rate in beef cattle. *Journal of Animal Science* **84**, 3415–20.

Boccaletti V., Zendri E., Giordano G., Gnetti L. & De Panfilis G. (2007) Familial uncombable hair syndrome: ultrastructural hair study and response to biotin. *Pediatric Dermatology* **24**, 14–6.

Bryant K.L., Kornegay E.T., Knight J.W., Webb K.E. & Notter D.R. (1985) Supplemental biotin for swine. II. Influence of supplementation to corn- and wheat-based diets on reproductive performance and various biochemical criteria of sows during four parities. *Journal of Animal Science* **60**, 145–53.

Burrow H.M. (2012) Importance of adaptation and genotype × environment interactions in tropical beef breeding systems. *Animal* **6**, 729–40.

Campeau E., Desviat L.R., Leclerc D., Wu X., Pérez B., Ugarte M. & Gravel R.A. (2001) Structure of the PCCA gene and distribution of mutations causing propionic acidemia. *Molecular Genetics and Metabolism* **74**, 238–47.

Craven A.J., Ormandy C.J., Robertson F.G., Wilkins R.J., Kelly P.A., Nixon A.J., Pearson A.J. & Craven T. (2001) Prolactin signaling influences the timing mechanism of the hair follicle: analysis of hair growth cycles in prolactin receptor knockout mice. *Endocrinology* **142**, 2533–9.

Dakshinamurti K. (1988) Regulation of biotin enzymes. *Annual Review of Nutrition* **8**, 211–33.

Hamblen H., Hansen P.J., Zolini A.M., Oltenacu P.A. & Mateescu R.G. (2018) Thermoregulatory response of Brangus heifers to naturally occurring heat exposure on pasture. *Journal of Animal Science* **96**, 3131–7.

Hansen P.J. (2004) Physiological and cellular adaptations of zebu cattle to thermal stress. *Animal Reproduction Science* **82–83**, 349–60.

Hernandez-Cordero A.I., Castro M. A. S., Algandar R. Z., Nevarez P. L., Rincon G., Medrano J. F., Speidel S. E., Enns R. M. & Thomas M. G. (2017) Genotypes within the prolactin and growth hormone insulin-like growth factor-I pathways associated with milk production in heat stressed holstein cattle: genotypes and milk yield in heat stressed holstein cows. *Genetics and Molecular Research* **16**(4), https://doi.org/10.4238/gmr16039821

Horseman ND, Gregerson KA (2013) Prolactin actions. *Journal of Molecular Endocrinology* **52**, R95–106. https://doi.org/10.1530/JME-13-0220

Littlejohn M.D., Henty K.M., Tiplady K. *et al.* (2014) Functionally reciprocal mutations of the prolactin signalling pathway define hairy and slick cattle. *Nature Communications* **5**, 1–8.

Olson T.A., Lucena C., Chase C.C. Jr & Hammond A.C. (2003) Evidence of a major gene influencing hair length and heat tolerance in *Bos taurus* cattle1,2,3. *Journal of Animal Science* **81**, 80–90.

Porto-Neto L.R., Bickhart D.M., Landaeta-Hernandez A.J. *et al.* (2018) Convergent evolution of slick coat in cattle through truncation mutations in the prolactin receptor. *Frontiers in Genetics* **9**, 1–8.

Purcell S., Neale B., Todd-Brown K. *et al.* (2007) PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics* **81**, 559–75.

Rauch H. (1952) The effects of biotin deficiency on hair development and pigmentation. *Physiological Zoology* **25**, 145–9.

Renaudeau D., Collin A., Yahav S., de Basilio V., Gourdine J.L. & Collier R.J. (2012) Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal* **6**, 707–28.

Sarlo Davila K.M., Hamblen H., Hansen P.J., Dikmen S., Oltenacu P.A. & Mateescu R.G. (2019) Genetic parameters for hair

characteristics and core body temperature in a multibreed Brahman-Angus herd1. *Journal of Animal Science* **97**, 3246–52.

Schindelin J., Arganda-Carreras I., Frise E. *et al*. (2012) Fiji: an open-source platform for biological-image analysis. *Nature Methods* **9**, 676–82.

St-Pierre N.R., Cobanov B. & Schnitkey G. (2003) Economic losses from heat stress by US livestock industries. *Journal of Dairy Science* **86**, E52–77.

Wolf B. & Raetz H. (1983) The measurement of propionyl-CoA carboxylase and pyruvate carboxylase activity in hair roots: its use in the diagnosis of inherited biotin-dependent enzyme deficiencies. *Clinica Chimica Acta* **130**, 25–30.

Zhou X. & Stephens M. (2012) Genome-wide efficient mixed-model analysis for association studies. *Nature Genetics* **44**, 821–4.