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SIGNATURES OF POSITIVE SELECTION FOR SCROTAL CIRCUMFERENCE IN THREE BEEF CATTLE BREEDS

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SUMMARY

This study aimed to detect genomic regions associated with scrotal circumference in Australian Brahman, Hereford, and Wagyu beef cattle breeds. The presence of selection signatures was based on the F_{ST} test, using data on the genotype and BREEDPLAN estimated breeding values for SC of 100,990 animals. Signals of selection for scrotal circumference were identified in genomic regions on several chromosomes, especially chromosome 14 in Brahman with most candidate genes under selection associated with male fertility or growth. The findings of this study may be applicable to breeding programs using more informative markers and assigning higher weights to them to increase the accuracy of genomic predictions and improve the reproductive performance of beef cattle.

INTRODUCTION

The unique genetic patterns left on the genome by natural and artificial selection in livestock populations are known as "selection signatures" (Gouveia *et al.* 2014). Detecting these signatures may help explain the selection history, adaptation, and genetic advancement of traits that may be economically important. Scrotal circumference (SC) is commonly employed as a selection criterion for breeding bulls because it is easily measured and correlated with a number of favourable reproductive traits, such as sperm motility, morphology, and concentration (Ferreira *et al.* 2021). Identifying the genomic regions and genes associated with SC could improve future animal breeding programs by improving the genomic predictions used for selection. Various statistical tests have been developed to identify selection signatures, including the fixation index (F_{ST}), which can be used to infer genetic relationships between populations based on allele frequencies. This study used the F_{ST} index to detect selection signatures associated with the SC trait in three Australian beef cattle breeds: Brahman, Hereford, and Wagyu. These breeds represent the Indicus, Taurus, and East Asian Taurus lineages, respectively, and possess economically important traits that set them apart.

MATERIALS AND METHODS

Estimated breeding values (EBVs) for SC estimated independently in three Australian beef cattle breeds (20,312 Brahman, 27,356 Hereford, and 53,322 Wagyu) were based on a single-step genomic best linear unbiased prediction (ssGTBLUP) model that was extracted from BREEDPLAN along with their genomic data. The SC EBVs for genotyped animals were split into quartiles, and animals in the first and fourth quartiles were used to represent extremes for further analysis. The BREEDPLAN genomic pipeline quality control was applied to the genotypes (Connors *et al.* 2017), and imputation was performed using FImpute v3 (Sargolzaei *et al.* 2014). Additionally, PLINK v1.9 (http://www.cog-genomics.org/plink/1.9/) was used to remove single-nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) < 5% after imputation. The SNPs after quality control for Brahman, Hereford, and Wagyu were approximately 72K, 77K, and 67K, respectively. The GCTA v1.94.1 software (Yang *et al.* 2011) was used to calculate F_{ST} values, which indicate genetic differences between populations. A sliding window of five SNPs for F_{ST} values was applied to reduce noise and consider linkage disequilibrium between SNPs. Two distinct strategies were implemented to represent selection signals. The initial strategy utilised a standard F_{ST} value range

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(F_{ST} > 0.25 as very high differentiation, 0.15 - 0.25 as high, 0.05 - 0.15 as moderate, and < 0.05 as low). The second strategy involved considering only the top 0.1% of high windowed F_{ST} outliers as representative of selection signals. The BiomaRt R package (Durinck *et al.* 2009) was used to identify genes (ARS-UCD1.2 cow genome) within genomic regions under selection extending 250 kilobases (kb) upstream and downstream of significant SNPs. Functional enrichment analysis was also performed on the gene set using the DAVID Bioinformatics Resources (https://david.ncifcrf.gov/) to identify biological processes associated with SC.

RESULTS AND DISCUSSION

The distribution of high and low estimated breeding values (EBVs) for the trait of interest in each population (SC) is presented on the right side of Figure 1. Based on the genetic differences between the Q1 and Q4 groups, the EBVs for SC in the Brahman, Wagyu, and Hereford breeds were high, moderate, and low, respectively. In Brahman, the results of the top 0.1 % windowed F_{ST} values ($F_{ST} \geq 0.15$) showed that only chromosome 14 was under strong directional selection (Figure 1A). In agreement with our findings, some genome-wide association studies detected genomic regions for SC on chromosome 14 at 20–25 Mb for the Brahman breed (Fortes *et al.* 2012a; Fortes *et al.* 2012b; Soares *et al.* 2017). Genomic regions on chromosome 14 play an important role for both reproductive and growth traits across various cattle breeds through their pleiotropic effects. Candidate genes included *RP1*, *XKR4*, *TOX*, *PLAG1*, *PENK*, *RPS20*, *NSMAF*, *SNTG1*, and *MOS*. For example, *TOX* is a transcription factor that controls the development of puberty in tropically-adapted Brahman and Nellore beef cattle (Fortes *et al.* 2012a; de Camargo *et al*. 2015). From the set of 26 candidate genes, some significant Gene Ontology (GO) biological processes (P < 0.05) were found, including animal organ morphogenesis (GO: 0009887), protein metabolic process (GO: 0019538), establishment of spindle orientation (GO: 0051294), organic hydroxy compound catabolic process (GO: 1901616), and metabolic process (GO: 0008152).

In Wagyu, signals of selection were detected within several genomic regions distributed across seven chromosomes (BTA2, BTA3, BTA6, BTA7, BTA8, BTA14, and BTA20; Figure 1B). Chromosome 6 exhibited the signals of selection at 32 – 41 Mb, harbouring several genes involved in beef cattle growth, such as *SLIT2* and *CCSER1* (Smith *et al.* 2019). The *CATSPER3* gene on BTA7 encodes a specific ion channel in sperm and has been found to be exclusively expressed in the bovine testis. It has been related to male fertility in cattle (Johnson *et al.* 2017; Nani and Peñagaricano 2020). The most significant biological processes identified from 54 candidate genes on all chromosomes were associated with genitalia development (GO: 0048806), reproductive structure development (GO: 0048608), reproductive process (GO: 0022414), regulation of multicellular organismal development (GO: 2000026), sex differentiation (GO: 0007548), and positive regulation of nitrogen compound metabolic process (GO: 0051173).

In the Hereford cattle genome, several regions under selection pressure were detected across eight chromosomes (BTA1, BTA4, BTA5, BTA6, BTA8, BTA10, BTA11, and BTA15) that contained 92 candidate genes (Figure 1C). The region on BTA5 (105 Mb) was localized close to genes related to cattle growth (e.g. *FGF6*, *FGF23*, and *CCND2*) (Bernard *et al.* 2009; Bolormaa *et al.* 2014; Yin and König 2019; Fang *et al.* 2020). For instance, genes *FGF6* and *FGF23*, both members of the fibroblast growth factor family, play a role in various biological processes such as angiogenesis, tissue regeneration, oncogenesis, and morphogenesis (Yin and König 2019). *AKAP3* gene on BTA5 plays roles in spermatozoa, including acrosome reaction and sperm capacitation/motility (Han and Peñagaricano 2016; Selvaraju *et al.* 2018). On BTA11, there were two significant genomic regions at 71–75 Mb and 29 Mb. The 29 Mb region overlaps with regions identified by Irano *et al.* (2016), which were associated with the SC trait based on genome-wide association studies (GWAS) in Nellore. Xu *et al.* (2022) reported that the *CIB4* gene is positively associated with testis size. The following biological processes from all candidate genes related to

the SC were identified: animal organ morphogenesis (GO: 0009887), animal organ development (GO: 0048513), regulation of nitrogen compound metabolic process (GO: 0051171), regulation of muscle contraction (GO: 0006937), tissue development (GO: 0009888), and regulation of metabolic process (GO: 0019222).

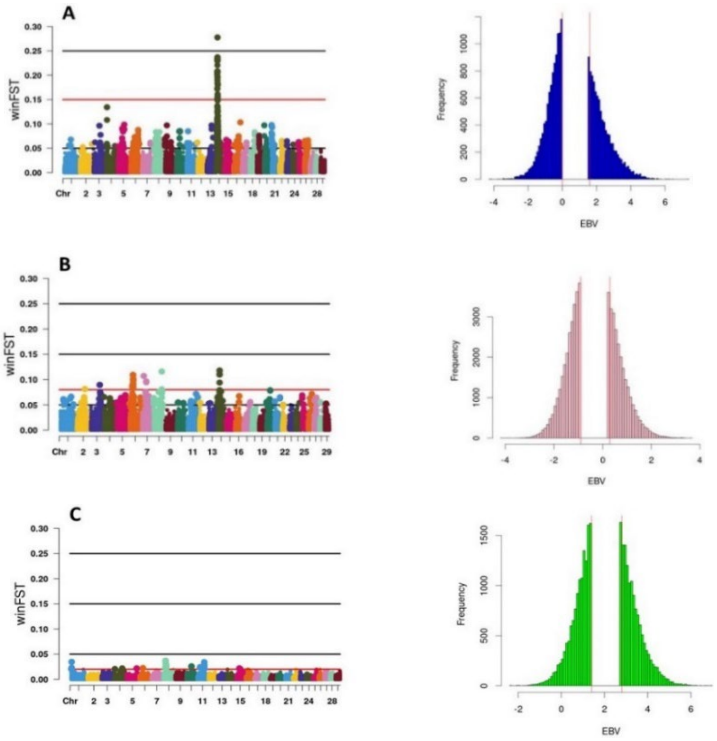

Figure 1. Manhattan plots of selection signatures for scrotal circumference using F_{ST} values with plots of the distribution of EBVs for (A) Brahman (B) Wagyu (C) Hereford. Windowed F_{ST} values are on the y-axis, chromosomal positions are on the x-axis, and the threshold lines represent the 0.1% (red) and standard F_{ST} value range (black) in the Manhattan plots

Selection signatures based on SC EBVs highlighted genes under selection in three Australian beef cattle breeds. The Brahman breed has lower reproductive rates (Reverter and Boe-Hansen 2011) than Wagyu and Hereford breeds, and improving fertility is a breeding program focus for many Brahman breeders. Putative signals of divergence within the Brahman breed had the strongest F_{ST} values compared to other breeds, suggesting higher differentiation in the breed for this trait. The Wagyu and Hereford breeds exhibited moderate and low levels of regional genetic differentiation, respectively. These results show that even though these breeds have different estimated breeding values (EBVs) for the SC in their populations, these diversities are not concentrated in certain genomic regions. GWAS can be used to confirm the relationships between the SC phenotype and genotype. Then, combining information from both selection signatures and GWAS could help in the validation of the informative SNPs. These SNPs can be used to improve the accuracy of genomic predictions for SC in the future. For example, combining these SNPs as a fixed effect or giving them greater weight in the genomic relationship matrix can potentially lead to improved accuracy in genomic predictions. In addition, the identification of informative SNPs improves our understanding of the biological mechanisms regulating the reproductive performance of beef cattle breeds.

CONCLUSIONS

Genotype data, along with EBV information, can provide insights into selection events for traits of interest in breeding programs. In this study, candidate genomic regions and genes associated with SC were detected using this method. These genomic regions could be confirmed by other validation studies, such as GWAS, to improve the genetic evaluation of animal breeding programs.

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REFERENCES

Bernard C., Cassar-Malek I., Renand G. and Hocquette J.-F. (2009) *Meat Sci.* **82:** 205.

Bolormaa S., Pryce J.E., Reverter A., Zhang Y., Barendse W., Kemper K., Tier B., Savin K., Hayes B.J. and Goddard M.E. (2014) *PLoS genetics* **10:** e1004198.

Connors N., Cook J., Girard C., Tier B., Gore K., Johnston D. and Ferdosi M. (2017) *Proc Assoc Advmt Anim Breed Genet.* **22:** 317.

de Camargo G.M., Costa R.B., Lucia G., Regitano L.C., Baldi F. and Tonhati H. (2015) *Reprod. Fert. Develop.* **27:** 523.

Durinck S., Spellman P.T., Birney E. and Huber W. (2009) *Nat Protoc* **4:** 1184.

Fang L., Cai W., Liu S., Canela-Xandri O., Gao Y., Jiang J., Rawlik K., Li B., Schroeder S.G. and Rosen B.D. (2020) *Genome Res.* **30:** 790.

Ferreira C.E., Campos G.S., Schmidt P.I., Sollero B.P., Goularte K.L., Corcini C.D., Gasperin B.G., Lucia Jr T., Boligon A.A. and Cardoso F.F. (2021) *Theriogenology* **172:** 268.

Fortes M., Lehnert S., Bolormaa S., Reich C., Fordyce G., Corbet N., Whan V., Hawken R. and Reverter A. (2012a) *Anim. Prod. Sci.* **52:** 143.

Fortes M.R., Reverter A., Hawken R.J., Bolormaa S. and Lehnert S.A. (2012b) *Biol. Reprod.* **87:** 58.

Gouveia J.J.d.S., Silva M.V.G.B.d., Paiva S.R. and Oliveira S.M.P.d. (2014) *Genet. Mol. Biol.* **37:** 330.

Han Y., Peñagaricano F. (2016) *BMC Genet* **17:** 1.

Irano N., de Camargo G.M.F., Costa R.B., Terakado A.P.N., Magalhães A.F.B., Silva R.M.d.O., Dias M.M., Bignardi A.B., Baldi F. and Carvalheiro R. (2016) *PLoS One* **11:** e0159502.

Johnson G.P., English A.-M., Cronin S., Hoey D.A., Meade K.G., Fair S. (2017) *Biol. Reprod.* **97:** 302.

Nani J.P. and Peñagaricano F. (2020) *BMC Genomics* **21:** 1.

Reverter T. and Boe-Hansen G. (2011) Meat and Livestock Australia.

Sargolzaei M., Chesnais J.P. and Schenkel F.S. (2014) *BMC Genomics* **15:** 1.

Selvaraju S., Parthipan S., Somashekar L., Binsila B.K., Kolte A.P., Arangasamy A., Ravindra J.P. and Krawetz S.A. (2018) *Syst. Biol.Reprod. Med.* **64:** 484.

Smith J.L., Wilson M.L., Nilson S.M., Rowan T.N., Oldeschulte D.L., Schnabel R.D., Decker J.E. and Seabury C.M. (2019) *BMC Genomics* **20:** 1.

Soares A., Guimarães S., Kelly M., Fortes M., e Silva F., Verardo L., Mota R. and Moore S. (2017) *J Anim. Sci.* **95:** 3331.

Xu H., Sun W., Pei S., Li W., Li F. and Yue X. (2022) *Front. Genet.* **12:** 2883.

Yang J., Lee S.H., Goddard M.E. and Visscher P.M. (2011) *Am. J. Hum. Genet.* **88:** 76.

Yin T. and König S. (2019) *Genet. Sel. Evol.* **51:** 1.