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# GENETIC MARKERS ASSOCIATED WITH MALE REPRODUCTIVE TRAITS ACROSS 2 BEEF CATTLE BREEDS: BRAHMAN AND TROPICAL COMPOSITE

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## SUMMARY

We report chromosomal regions identified as significant for bovine male fertility according to genome-wide association studies carried out in two independent populations of cattle. Reported chromosomal regions harboured single nucleotide polymorphisms (SNP) that were associated ($P < 0.01$) with inhibin, insulin growth factor 1 (IGF1), scrotal circumference (SC) or percentage of normal sperm (PNS) in both Brahman (n = 1,130) and Tropical Composite (n = 1,085) cattle. Bulls were genotyped with Illumina SNP chips (50K and 700K) and association analyses were performed using animal models. Chromosomes 2, 3, 5, 7 and 10 had SNP that were associated with inhibin in both breeds. SNP associated with IGF1 were located on chromosomes 5, 6, 10 and 14 in both breeds. SNP associated with SC mapped to chromosomes 9, 13 and X in both breeds. Only chromosome X had SNP associated to PNS in both breeds. Comparing the associations of SNP to traits measured in both Brahman and Tropical Composite cattle breeds is an important validation strategy for selecting markers that could be used for genomic selection in a multi-breed program. Markers associated with inhibin, IGF1, SC and PNS may contribute to the selection of bulls with improved reproductive performance.

## INTRODUCTION

Single nucleotide polymorphisms (SNP) are used in genomic selection. Association of SNP across beef cattle breeds provides validation for independent genome-wide association studies (GWAS) and may contribute to increase the accuracy of genomic selection. Validation of SNP is a step towards the discovery of causative mutations that could aid genomic selection and genetic gain (Weller and Ron 2011; Snelling *et al.* 2012). Causative mutations have an advantage in comparison to random SNP: they are not dependent on linkage disequilibrium (LD), and so they can be used for selection over generations, across breeds and in breeds that were not in the reference population.

Reproductive performance of bulls has an impact on the economic gain of a farm. Measuring correlated traits, such as scrotal circumference (SC) and percent normal sperm (PNS) allows for selection of bulls with improved reproductive performance (Holroyd *et al*. 2002; Moser *et al.* 1996). Hormonal levels of inhibin and insulin growth factor 1 (IGF1) correlate with reproductive traits and may also aid selection (Corbet *et al.* 2013). The aim of this study is to report validated SNP associated with inhibin, IGF1, SC and PNS, by comparing GWAS carried out in 2 independent populations of bulls: Brahman (BRAH) and Tropical Composites (TC). Only BRAH results were published previously (Fortes *et al.* 2012). Reports of validated SNP associations point to genomic regions that merit further research targeting the discovery of causative mutations for fertility in bulls.

## MATERIALS AND METHODS

**Animals, Traits and Genotypes.** Blood samples for DNA extraction were obtained from 1,130 BRAH and 1,085 TC bulls. These bulls were bred by the Cooperative Research Centre for

Beef Genetic Technologies and details concerning project design and measurement of reproductive traits have been reported (Burns *et al*. 2013; Corbet *et al*. 2013). In short, blood levels of inhibin were measured at 4 months of age, circulating IGF1 at 6 months, scrotal circumference (SC) at 12 months and percent normal sperm (PNS) at 24 months. BovineSNP50 chips (Matukumalli *et al*. 2009) were used to genotype all bulls. Some samples were replicated for quality control and Bead Studio software (Illumina Inc., San Diego, CA 2006) was used to call genotypes. SNP with call rates < 80% or minor allele frequency < 0.01 were discarded. High-density (HD) genotyping of selected TC cattle was performed. Missing 50K genotypes for BRAH and HD genotypes for TC were imputed using BEAGLE (Browning and Browning 2010). Quality control and imputation resulted in 50,354 SNP genotypes for 1,115 BRAH and 729,068 for 1,019 TC.

**Statistical Analyses.** GWAS were performed for each breed and each trait separately. SNP effects were estimated using an animal model. Solutions were estimated with Qxpak5 (Perez-Enciso and Misztal 2011), using a likelihood ratio test to compare the model with versus the model without each SNP against a chi-squared distribution with 1 degree of freedom. This test was performed for one SNP at a time.

## RESULTS AND DISCUSSION

**Table 1. SNP associated with reproductive traits in Brahman and Tropical Composite bulls***

| Chromosome | Mb (number of sig. SNP $P < 0.01$) | Brahman | | Tropical Composite | |
|---|---|---|---|---|---|
| | | max | min | max | min |
| ***Inhibin*** | | | | | |
| 2 | 104(1) | $8.1 \times 10^{-3}$ | | $4.3 \times 10^{-3}$ | |
| 3 | 60(1) | $2.1 \times 10^{-3}$ | | $7.0 \times 10^{-4}$ | |
| 5 | 108(1) | $2.8 \times 10^{-3}$ | | $5.8 \times 10^{-3}$ | |
| 7 | 73(1) | $5.1 \times 10^{-3}$ | | $3.6 \times 10^{-3}$ | |
| 10 | 17(1) | $5.0 \times 10^{-3}$ | | $3.8 \times 10^{-3}$ | |
| ***IGF1*** | | | | | |
| 5 | 34(2), 42(1) | $7.0 \times 10^{-7}$ | $6.8 \times 10^{4}$ | $2.7 \times 10^{-3}$ | $8.7 \times 10^{-3}$ |
| 6 | 105(1) | $1.4 \times 10^{-3}$ | | $7.0 \times 10^{-5}$ | |
| 10 | 47(1) | $7.8 \times 10^{-3}$ | | $9.7 \times 10^{-3}$ | |
| 14 | 21(1), 23(1), 24(2), 25(7), 26(1), 27(1), 28(4), 30(2), 32(1), 33(1) | $1.0 \times 10^{-16}$ | $5.9 \times 10^{3}$ | $3.1 \times 10^{-8}$ | $9.6 \times 10^{-3}$ |
| ***SC*** | | | | | |
| 9 | 91(1) | $4.3 \times 10^{-3}$ | | $6.4 \times 10^{-3}$ | |
| 13 | 78(1) | $6.9 \times 10^{-3}$ | | $2.0 \times 10^{-3}$ | |
| X | 54(1), 60(1), 62(1), 63(2), 65(1), 66(6), 68(2), 69(3), 70(2), 71(1), 72(2), 73(3), 75(1), 76(1), 77(1), 80(1), 81(3), 82(2), 84(2), 85(3), 86(3), 87(2), 91(2), 92(4), 93(3), 94(1), 98(1), 100(1), 102(2), 105(2), 108(2) | $4.9 \times 10^{-10}$ | $8.7 \times 10^{-3}$ | $1.1 \times 10^{-29}$ | $3.6 \times 10^{-3}$ |
| ***PNS*** | | | | | |
| X | 40(3), 41(1), 43(1),47(1), 50(1),52(1),53(1),55(1) | $6.9 \times 10^{-7}$ | $3.3 \times 10^{-13}$ | $7.9 \times 10^{-7}$ | $3.8 \times 10^{-10}$ |

*Traits: Inhibin, IGF1, scrotal circumference (SC) and percentage of normal sperm (PNS) in Brahman and Tropical Composite bulls. Mega base pairs (Mb) position, number of significant SNP within the Mb, and minimum and maximum *P*-values are reported for each breed.

The GWAS performed in 2 independent populations revealed SNP that were associated ($P < 0.01$) across 2 beef cattle breeds (Table 1). More than one chromosome had SNP associated with BRAH and TC for Inhibin, IGF1 and SC. Only chromosome X had validated SNP associated with PNS, but these were located in multiple regions. Together, BRAH and TC results are evidence for polygenic regulation of these reproductive traits.

The X chromosome harboured validated SNP for SC and PNS, spread across millions of base pairs (Figure 1). These regions with multiple associated SNP might be an indication for multiple quantitative trait loci (QTL). Within our results, SNP associations that point to QTL close to 48 and 110 Mb of the X chromosome provide supporting evidence for results that were first reported in Holstein bulls (Blaschek *et al*. 2011). Further, candidate genes underpinning these QTL on the X chromosome were proposed in the previous Brahman study (Fortes *et al.* 2012). For example, the androgen receptor gene (*AR*) localized at 88 Mb of the X chromosome is a candidate gene for SC, due to the position of associated SNP and its physiological role (Quigley 1998).

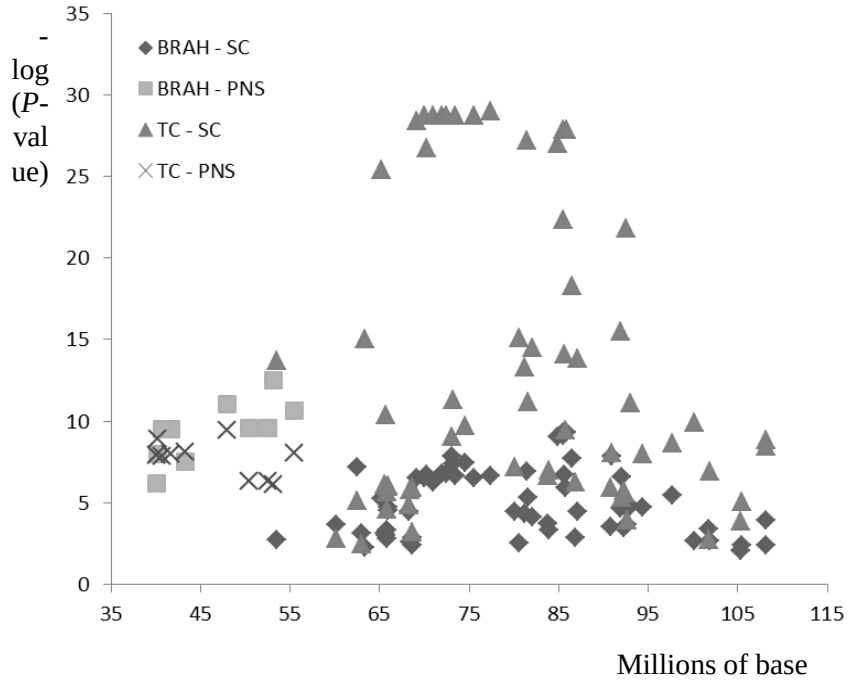


**Figure 1. Validated polymorphisms in the X chromosome.** Acronyms in figure: Brahman (BRAH), Tropical Composite (TC), Scrotal Circumference (SC), Percent Normal Sperm (PNS).

The region around 25 Mb of chromosome 14 had the highest number of validated SNP for IGF1 and it confirms a known QTL associated with IGF1 in female cattle as well as height, weight and puberty, across various breeds (Karim *et al*. 2011; Littlejohn *et al*. 2011; Hawken *et al*., 2012; Nishimura *et al*. 2012). A putative causative mutation on chromosome 14 near the pleiomorphic adenoma 1 (*PLAG1*) gene was proposed by a study on Holstein and Jersey cattle (Karim *et al*. 2011). However, a direct effect of this mutation on IGF1 levels remains to be investigated. The molecular mechanism linking *PLAG1* function to IGF1 levels is unclear. It is possible that *PLAG1* acts as a transcription factor regulating the expression of the *IGF1* gene.

## CONCLUSION

The QTL presented here for inhibin, IGF1, SC and PNS were confirmed across independent cattle populations. These validated QTL point to genomic regions that merit further research, specifically targeting the discovery of causative mutations affecting reproductive traits in bulls. A putative causative mutation for chromosome 14 was proposed and merits functional investigation. Causative mutations underpinning the other validated QTL are unknown. Targeting candidate genes that emerge from the cross-validation of significantly associated SNP between BRAH and TC could lead to the discovery of causative mutations. Knowledge on causative mutations would improve the accuracy of genomic selection and facilitate its use across cattle breeds and over multiple generations.

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