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EFFECTS OF *TEX11* AND *AR* POLYMORHISMS ON REPRODUCTION AND GROWTH TRAITS IN AUSTRALIAN BEEF CATTLE

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SUMMARY

Previous genome-wide association analyses indicated QTL regions located in X chromosome for scrotal circumference (SC) and percentage of normal sperm (PNS). The association between SNP in two potential candidate genes (*TEX11* and *AR*) on chromosome X and observed phenotypic variation of SC and PNS were analysed. As expected from QTL findings, these SNP could explain more than 1% of the additive genetic variance for SC. Three SNP in *TEX11* and a SNP in *AR* were significant for SC measurements taken at 12, 18 and 24 months of age. SNP in exon 1 of *TEX11* gene had extremely significant effects on SC12, SC18 and SC24 with *P*-values ranging from 10^{-39} to 10^{-46}. An association between a SNP in *TEX11* and weight measurements was also identified. Associations reported herein suggest that these SNP in *TEX11* and *AR* might aid genomic selection for SC and weight if included in genotyping panels.

INTRODUCTION

Fertility has important economic impact for livestock and fertility traits are considered in breeding programs. However, fertility is a rather complex phenotype that can be described by many indicator traits, such as scrotal circumference (SC) or sperm quality (Cammack *et al*. 2009). Traits considered as indicators of fertility are expressed late in life and mostly have low heritability (Cammack *et al*. 2009). Complexity, low heritability and late expression create challenges for selective breeding. Yet, some fertility traits are of moderate heritability, such as SC (heritability ranged from 0.29 to 0.78) (Cammack et al. 2009) and percentage of normal sperm (PNS, heritability = 0.35 (Kealey et al. 2006)). Bull fertility traits including SC and PNS are commonly measured at bull breeding soundness evaluation and can be used for improvement of fertility. Bull SC is also utilized for female fertility improvement due to its correlation with heifer age at puberty (Evans *et al*.,1999). Thus, SC and PNS are selection traits for beef cattle fertility.

Previous genome-wide association analyses reported QTL regions in chromosome X as associated with SC and PNS in Brahman and Tropical composite bulls (Fortes *et al*. 2012; Fortes *et al*. 2013). These QTL regions indicate that the *TEX11* and *AR* genes are candidates for identifying putative causative mutations. This study was carried out to identify and test putative causative mutations in these genes.

MATERIALS AND METHODS

Animals and phenotype. Animal care and Use Committee approval was not required for this research since samples and data used were from existing databases. Data were obtained from 1,178 Brahman bulls, 1,360 Tropical Composite bulls and 167 crossbreds (Tropical composite vs Brahman). These animals were the progeny of sires from Beef CRC. In total, 2705 bulls were analysed together in this study. Traits analysed were: SC and weight (WT) measured at 12, 18 and 24 months and PNS measured at 18 and 24 months. Measurement details for the Beef CRC

populations were described previously (Burns *et al*. 2013; Corbet *et al*. 2013). Genomes of 16 Brahman bulls (sires of genotyped animals) were utilized to generate VCF format files with variants information. Variant Effect Predictor (VEP) was used to predict the functional consequences of detected variants.

Genotyping and Linkage. Using Taqman assays, 2,705 bulls were genotyped for 4 SNP: Tex11-r38k, Tex11-g297d, Tex11-r696h and AR-intron6. Linkage disequilibrium (LD,r^2) was estimated pair-wise for genotyped SNP, using SVS software (Release 8.3.0, Golden Helix, Inc.).

Analyses. Association of selected SNP with SC, WT and PNS was examined using SVS software (Release 8.3.0, Golden Helix, Inc.) A mixed model analysis of variance was used to estimate the SNP effect and its significance level. The mixed model can be written as an equation: $Y_i = X\beta + Z\mu + S_j a_j + e_j$, where Y represents the phenotypic measurement for the i^{th} animal, X is the incidence matrix relating fixed effects in β, Z is the incidence matrix relating to random additive polygenic effects of animal in μ, S is a vector of genotypes of each animal at SNP (j), a_j is the additive effect of the jth SNP, and e_j is the random residual effect. SNP is fitted was random and fixed effects were those of contemporary group (year, management group and breed). Age was fit as a covariant.

RESULTS AND DISCUSSION

Only 3 nsSNP discovered in *TEX11* and none in *AR* using the genome sequences available. These SNP are more likely to alter protein sequence and structure, and be beneficial or deleterious. It would however be inaccurate to state that all functional changes are based on protein coding sequence. The alteration of regulatory sites also can disrupt the expression of target genes (Knight *et al*. 2003). An intronic SNP in *AR* was also tested. From LD analysis, the SNP Tex11-r38k and Tex11-r696h were completely linked with r^2 value of 1. Therefore, it was impossible to differentiate the effects between these two SNP in all animals. Consequently, the SNP Tex11-r38k was used to represent both in the following results and discussion. For all the other pairs, the estimates of LD were lower than 0.6. The effect of these SNP could be interpreted separately.

The Tex11-g297d SNP had a slightly lower association with SC and WT measurements relative to the results obtained for Tex11-r38k, which had *P*-values in the range of 10^{-39} to 10^{-46} (Table 1). In Brito *et al*. (2002), increased SC has been related to increased sperm production but decreased semen quality. In our results, these same SNP were not associated with PNS, a measurement of semen quality. In 2003, Martínez-Velázquez reported that SC is positively correlated with growth traits. SNP that showed associations with both SC and WT could be expected. The SNP in *AR* showed an association with SC but not WT. We tested the relative relevance of Tex11-r38k to the QTL previously described (Fortes *et al*. 2012; Fortes *et al*. 2013) by fitting it as a fixed effect in the GWAS model. When Tex11-r38k was utilized as a fixed effect, the associations between common SNP in chromosome X (Illumina chip variants) and SC measurements were reduced (Figure 1). This result is consistent with expectations from causative variants that are able to explain the underpinning QTL.

Associations *TEX11* SNP with bull fertility were first studied by Lyons *et al*. (2013). The results obtained here validated that study. The substitution from G to A on *TEX11* might have negative effect on bull performance (smaller SC). Deleterious effect of Tex11-r38k was predicted according to SIFT. *TEX11* competes with estrogen receptor beta for a specific binding to HPIP protein (Yu *et al.* 2012). The *TEX11* protein region that binds to HPIP protein is between amino acids 370 and 947 (Yu *et al.* 2012), indicating that SNP Tex11-r696h that change amino acid at position 696 and are completely linked to SNP Tex11-r38k may be the best functional mutations. Further studies about biological role played by *TEX11* and its SNP in bull fertility are warranted.

Table 1.Significance and estimated effects of selected SNP on reproductive and growth traits in mixed bull population

Trait	SNP	p-value	Effect	SE	%Va
PNS18	Tex11-r38k	0.0124	-1.6716	0.6676	0.3180
	Tex11-g297d	0.1112	1.5053	0.9447	0.1291
	AR-intron6	0.1472	-1.4475	0.9982	0.1069
PNS24	Tex11-r38k	0.4681	-0.3611	0.4977	0.0215
	Tex11-g297d	0.2197	0.8396	0.6839	0.0614
	AR-intron6	0.7849	0.1848	0.6771	0.0030
SC12	Tex11-r38k	$2.39x10^{-39}$	-0.7431	0.0557	6.2944
	Tex11-g297d	0.0002	-0.2863	0.0775	0.5120
	AR-intron6	0.0105	-0.1937	0.0757	0.2467
SC18	Tex11-r38k	$6.03x10^{-46}$	-0.8270	0.0570	7.3867
	Tex11-g297d	0.0003	-0.2842	0.0794	0.4837
	AR-intron6	$1.04x10^{-05}$	-0.3430	0.0776	0.7339
SC24	Tex11-r38k	$2.44x10^{-43}$	-0.7708	0.0548	6.9741
	Tex11-g297d	0.0005	-0.2646	0.0765	0.4516
	AR-intron6	$1.94x10^{-05}$	-0.3200	0.0748	0.6900
WT12	Tex11-r38k	$7.04x10^{-08}$	-3.0673	0.5674	1.0926
	Tex11-g297d	0.0003	-2.7609	0.7704	0.4832
	AR-intron6	0.3354	-0.7250	0.7525	0.0351
WT18	Tex11-r38k	$3.97x10^{-08}$	-3.7138	0.6742	1.1329
	Tex11-g297d	0.0016	-2.8693	0.9110	0.3732
	AR-intron6	0.35401	0.8534	0.9206	0.0324
WT24	Tex11-r38k	$2.50x10^{-07}$	-3.7426	0.7237	1.0010
	Tex11-g297d	0.0007	-3.2871	0.9785	0.4249
	AR-intron6	0.1255	1.5132	0.9874	0.0887

CONCLUSION

Our results provide evidence for a key role of *TEX11* in male reproduction in beef cattle. The SNP Tex11-r38k and/or Tex11-r696h are proposed as functional mutations in *TEX11*. The *AR* gene remains as a candidate gene as its SNP was also associated with SC. As shown, SNP in these candidate genes influence SC and PNS in *Bos indicus* and their crossbreds. As a result, these associated SNP could be incorporated in low-density chips to facilitate genetic evaluation.

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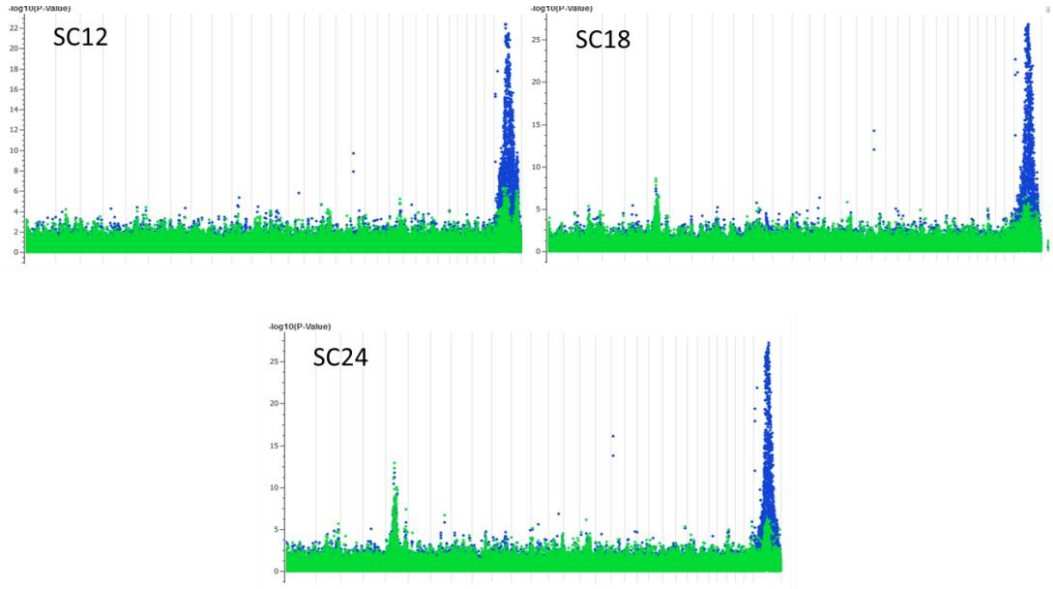

Figure 1. The association between SNP in 30 chromosome regions of beef cattle and scrotal circumference at 3 different ages. The chromosomal positions are in the x-axis and –Log (*P*-values) are in the y-axis. The blue indicates effect of all SNP on SC, the green reveals effect of SNP on SC with Tex11-r38k as a fixed effect.