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# Case Study: Evaluation of single nucleotide polymorphisms on 3 candidate genes in a population of forage-tested yearling bulls

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## ABSTRACT

*The objective of the current study was to evaluate the association of single nucleotide polymorphisms (SNP) on 3 candidate genes for growth and performance traits in bulls participating in a forage-based performance bull test. Single nucleotide polymorphisms on 3 candidate genes including calpastatin (CAST), growth hormone (GH1), and IGF-1 were used for association analysis. These SNP were genotyped on 47 purebred Angus, Braford, and Brahman bulls on a forage-based performance bull test. The measured traits included ADG, birth weight, d-0 test weight, 112-d weight, hip height, marbling, rib-eye area, and scrotal circumference. Single nucleotide polymorphism associations were reported as significant if $P < 0.05$ and were observed on the IGF-1 and CAST genes for growth and production traits. A total of 10 unique SNP (rs109022910, rs109199979, rs109327701, rs132665612, rs132951819, rs136875549, rs136939207, rs137140434, rs137601357, and rs137651874) located on both the IGF-1 and GH1 genes were associated ($P < 0.05$) with growth and performance traits. Furthermore, of the 12 unique SNP associated with growth and performance, 7 of the identified SNP (rs109022910, rs109199979, rs132951819, rs136875549, rs136939207, rs137601357, and rs137651874) were significantly associated with more than one growth or performance trait. However, the only gene with SNP significantly associated ($P < 0.05$) with carcass traits was the GH1 gene in which a single SNP (rs137651874) was associated with rib-eye area. Although multiple SNP were associated with evaluated traits, these SNP must be validated in larger and more diverse populations before implementation into selection strategies.*

**Key words:** carcass, single nucleotide polymorphism, performance bull test, growth, forage

## INTRODUCTION

Multiple tools have been developed to increase the accuracy of selection in the beef industry. Tools such as EPD and performance testing have aided in the improvement of beef traits over the past few decades. However, Collins et al. (1997) have reported that single nucleotide polymorphisms (**SNP**) are responsible for a variety of phenotypes. The rate of genetic improvement achieved by marker-assisted selection may be greater than by selection based on EPD (Davis and DeNise, 1998). Single nucleotide polymorphisms have been associated with a variety of phenotypes, including disease resistance (humans), milk production, fertility, meat quality and composition, and vulnerability (Collins et al., 1997; Baeza et al., 2011; Mullen et al., 2011). Associations can be tested between a SNP and a specific trait to potentially identify the underlying genomic component contributing to the observed variation in performance for that trait.

Three known candidate genes calpastatin (*CAST*), growth hormone (*GH1*), and *IGF-1* were chosen for SNP analysis. The *GH1* gene was included in the association study because of previous reports of the gene being associated with milk production, fertility, growth regulation, and carcass quality (Thomas et al., 2007; Mullen et al., 2010). Similar to *GH1*, *IGF-1* has been reported to exhibit an association with growth production as well as meat quality in animals (Yao et al., 1996; Machado et al., 2003; Andrade et al., 2008). In a study conducted by Pringle et al. (1997) it was

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reported that *CAST* demonstrates an association with meat tenderness. The objective of this study was to evaluate the associations between growth and production traits and the chosen SNP for each candidate gene.

### *Experimental Animals*

All animals were treated and maintained in accordance with the principles and guidelines outlined in the *Guide for the Care and Use of Agricultural Animals in Research and Teaching*. A population of 47 bulls born in 2010 and 2011 from the Angus (18), Braford (27), and Brahman (2) breeds were evaluated for 112 d on a forage-based performance bull test. The test was conducted at the Louisiana State University AgCenter Central Station's Purebred Beef Unit, and bulls were maintained on native forage and ryegrass. After weaning, bulls were maintained on pasture for 2 to 3 mo before the start of the forage-based performance bull test. After d-0 test weights were collected, weights were taken on each bull every 28 d until 112 d were completed. At the completion of the performance test, 112-d weight and ADG were calculated. Furthermore, ultrasound carcass data, including rib-eye area and backfat, were collected at the end of the testing period along with hip height and scrotal circumference. Performance bull test data from bulls participating in a forage-based performance test in 2010 and 2011 were evaluated for associations between SNP and growth and performance.

### *Blood Collection and DNA Extraction*

Blood was collected (20 mL) from all bulls on the performance bull test at the Louisiana State University AgCenter Central Research Station's Purebred Beef Unit. The blood was collected via jugular venipuncture. After collection, blood was transferred into 15-mL tubes and centrifuged at 3,889 × *g* at 4°C for 20 min. Following centrifugation, white-blood-cell buffy coats were removed and transferred to 250-μL micro centrifuge tubes. Buffy coats were lysed and digested so that DNA could be extracted using a previously described saturated salt procedure (Miller et al., 1988). After DNA extraction was completed, 200 μL of DNA working solutions was prepared with a combination of DNA and rehydration buffer. Unused buffy coat, extracted DNA, and working solutions were all stored at −4°C.

### *SNP and Genotyping*

Previously reported SNP on the candidate genes *CAST*, *GH1*, and *IGF-1* were collected from the dbSNP website (http://www.ncbi.nlm.nih.gov/projects/SNP/). Single nucleotide polymorphisms were selected that were evenly distributed across the entire candidate genes genomic sequence. Single nucleotide polymorphisms, allele substitutions, and forward and reverse primer sequences are reported in Supplemental Tables S1 to S3 (http://dx.doi.org/10.15232/pas.2014-01337). Single nucleotide polymorphism genotyping was performed using sequenome technology (Sequenome, San Diego, CA) and was conducted by NeoGene LLC (Lincoln, Nebraska).

### *Statistical Analysis*

The mixed-model procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC) was used to evaluate statistical significance of SNP associations. Models were fitted individually with each trait and a different SNP in each analysis. Dependent variables of ADG, birth weight, weaning weight, d-0 test weight, 112-d weight, hip height, backfat, rib-eye area, intramuscular fat, and scrotal circumference were fit into the model, and variables of year, breed, and individual SNP genotype were fit in the model as fixed effects to evaluate potential SNP associations. Sire was included in the model as a random effect. Single nucleotide polymorphisms that exhibited more than one genotype were incorporated into the analysis, and statistical significance was assessed at $P < 0.05$. Any SNP with only one genotype was excluded from the analysis because of the lack of marker effects.

## RESULTS AND DISCUSSION

The evaluation of 3 SNP located on 3 candidate genes indicated significant genotypic effects for growth, performance, and carcass characteristics were present on all 3 candidate genes. Although breed was incorporated into the statistical model, it was not a significant sources of variation contributing to significantly associated SNP identified in the current study. However, although breed was not a significant source of variation, it is important to note that for some SNP only one breed was represented in all 3 genotypes at the locus (Table 1). However, in this experiment any breed × genotype interactions would not be detected statistically.

Single nucleotide polymorphisms with significant genotype effects associated with growth and performance traits were identified on both the *CAST* gene and the *IGF-1* genes. However, the only candidate gene with a SNP with significant genotypic effects ($P < 0.05$) with carcass traits was the *GH1* gene. This was somewhat surprising as the *GH1* gene has been reported by previous studies as a favorable candidate gene in cattle for the improvement of growth, fertility, and meat and milk production (Thomas et al., 2007; Mullen et al., 2010). However, the current study only identified one SNP that contained significant genotype effects with ultrasound carcass traits (rib-eye area; rs137651874). Animals inheriting the heterozygous and major homozygous allele genotypes (TC and CC) had larger ($P < 0.05$) rib-eye areas than Angus animals, which were the only animals inheriting the minor homozygous allele genotype in the current study (Table 2). These results would indicate that the *GH1* gene is a valuable candidate, and in particular SNP rs137651874 when evaluating rib-eye area.

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