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Promoter region of the bovine growth hormone receptor gene: Single nucleotide polymorphism discovery in cattle and association with performance in Brangus bulls[1]

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ABSTRACT: Expression of the GH receptor (GHR) gene and its binding with GH is essential for growth and fat metabolism. A GT microsatellite exists in the promoter of bovine GHR segregating short (11 bp) and long (16 to 20 bp) allele sequences. To detect SNP and complete an association study of genotype to phenotype, we resequenced a 1,195-bp fragment of DNA including the GT microsatellite and exon 1A. Resequencing was completed in 48 familialy unrelated Holstein, Jersey, Brown Swiss, Simmental, Angus, Brahman, and Brangus cattle. Nine SNP were identified. Phylogeny analyses revealed minor distance (i.e., <5%) in DNA sequence among the 5 *Bos taurus* breeds; however, sequence from Brahman cattle averaged 27.4 ± 0.07% divergence from the *Bos taurus* breeds, whereas divergence of Brangus was intermediate. An association study of genotype to phenotype was completed with data from growing Brangus bulls (n = 553 from 96 sires) and data from 4 of the SNP flanking the GT microsatellite. These SNP were found to be in Hardy-Weinberg equilibrium and in phase based on linkage disequilibrium analyses (r^2 = 0.84 and D'= 0.92). An A/G tag SNP was identified (ss86273136) and was located in exon 1A, which began 88 bp downstream from the GT microsatellite. Minor allele frequency of the tag SNP was greater than 10%, and Mendelian segregation was verified in 3 generation pedigrees. The A allele was derived from Brahman, and the G allele was derived from Angus. This tag SNP genotype was a significant effect in analyses of rib fat data collected with ultrasound when bulls were ~365 d of age. Specifically, bulls of the GG genotype had 6.1% more ($P = 0.0204$) rib fat than bulls of the AA and AG genotypes, respectively. Tag SNP (ss86273136), located in the promoter of GHR, appears to be associated with a measure of corporal fat in *Bos taurus* × *Bos indicus* composite cattle.

Key words: bovine, Brangus, deoxyribonucleic acid, growth hormone receptor, single nucleotide polymorphism

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INTRODUCTION

Growth hormone is involved in several metabolic and physiological processes (Etherton, 2004; Chagas et al., 2007). To elicit its effects, GH must bind its receptor (**GHR**; Edens and Talamantes, 1998; Zhu et al., 2001). Thus, DNA sequence variations in GHR may be beneficial for understanding how genotype(s) influence phenotype(s) for growth and carcass composition in cattle.

Bos indicus cattle, which are primarily of the Brahman breed in the United States, mature later, have less concentrations of adiposity, and greater concentrations of serum concentrations of GH when compared with Angus cattle on an age constant basis (Carroll, 1996; Thrift and Thrift, 2003; Lopez et al., 2006). Admixed populations of *Bos indicus* × *Bos taurus* cattle have proven useful for identifying QTL associated with growth and body composition (Casas et al., 2003, 2004; Kim et al., 2003).

The GHR gene underlies QTL for milk yield and composition, and percentage of body fat on chromosome 20 in cattle (Georges et al., 1995; Arranz et al., 1998; Zhang et al., 1998). Ge et al. (2003) suggested that polymorphisms in the GHR gene could be potential markers for enhanced serum concentrations of

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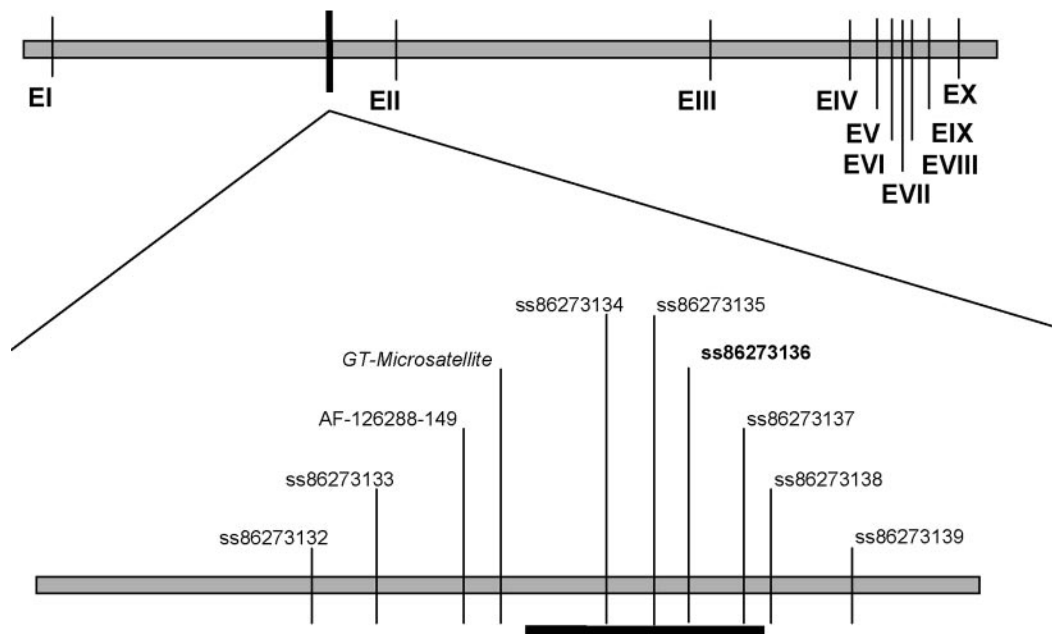

Figure 1. Schematic of 365,186 bp of DNA that constitute the bovine GH receptor gene. Exons (E) are designated with roman numerals with the transcription beginning site (ATG) located in exon II. The resequenced 1,195-bp region is shown with a bold vertical bar in the upper panel and expanded in the lower panel with details of the detected SNP [identified by their dbSNP (http://www.ncbi.nlm.nih.gov/SNP/; accessed January 15, 2008) ss number]. The GT microsatellite and Tag SNP ss86273136 are shown with italics and bold letters, respectively, with exon 1A indicated with a bold horizontal line.

IGF-I in Angus cattle. Lucy et al. (1998) described a GT microsatellite in the promoter of the GHR gene. *Bos indicus* cattle contained a tandem repeat of 11 bp, whereas *Bos taurus* cattle typically possessed a 16- to 20-bp repeat. The short allele, which was identified in *Bos taurus*, as well as *Bos indicus* cattle, was associated with less weaning weight, carcass weight, and marbling score in Angus steers (Hale et al., 2000).

Because most commercial genotyping panels in cattle involve SNP, the first objective herein was to identify SNP variation among breeds in 1,195 bp of DNA sequence in the GHR promoter. This fragment included the GT microsatellite and exon 1A, which processes different RNA spliced variants of GHR (Jiang and Lucy, 2001). The second objective was to examine linkage disequilibrium (**LD**) among SNP in this gene region and then associate SNP genotype data with growth and ultrasound carcass trait measures in Brangus bulls.

MATERIALS AND METHODS

Animals were handled and managed according to Institutional Animal Care and Use Committee Guidelines.

SNP Discovery

Animals and DNA. The PUREGENE kit (D-5500, Gentra Systems, Minneapolis, MN) was used to extract DNA from semen samples of 48 bulls. Eight bulls were from each of the dairy breeds Brown Swiss, Holstein, and Jersey and 6 each from the beef breeds Angus, Simmental, Brahman, and Brangus. Animals within each breed group shared no common ancestors in a 3-generation pedigree.

Resequencing and SNP Detection. The bovine GHR sequence was obtained from the Ensembl database (http://www.ensembl.org/index.html; accessed February 15, 2006) using the Btau_3.1 assembly (accession no. ENSBTAG00000001335). This sequence and the sequence published by Hale et al. (2000) were aligned to target 1,000 bp of DNA sequence flanking the GT microsatellite (i.e., 500 bp upstream and 500 bp downstream relative to the microsatellite).

Targeting 1,000 bp flanking the GT microsatellite yielded a fragment of 1,195 bp in size. Figure 1 describes the location of the fragment within the GHR gene and the sequence polymorphisms. The target region was located on chromosome 20 from position 34,085,318 to 34,086,512 (UCSC Genome Bioinformatics, 2008). The region was sequenced in forward and reverse segments consisting of a 460-bp amplicon 5′ to the GT microsatellite and 515- and 735-bp amplicons 3′ to the GT microsatellite. This fragment was ~25 kb downstream from exon I. The GT microsatellite began at bp 480 from the first nucleotide of the 1,195-bp fragment. In the resequenced region, an alternative exon (i.e., 1A) existed. The sequence for this exon was 88 bp downstream from the GT microsatellite. Sequencing was performed at SeqWright DNA Technology Service Lab (Houston, TX; ABI Prism) using DNA from the 48 bull samples.

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To improve sequence quality of the Brahman samples, additional resequencing was performed at the New Mexico State University (**NMSU**) Molecular Biology Core Lab. In brief, a 778-bp PCR product was amplified with procedures modified from Beauchemin et al. (2006). The forward primer was 5′-GCT CTT CCC TCT TCT GGC TT-3′, and the reverse primer was 5′-AAG CCA CAA GAG GGA AGA GC-3′. The PCR product was cleaned using ExoSAP-IT (USB, 78200, Cleveland, OH) and then sequenced using the ABI PRISM 3100 Genetic Analyzer (Foster City, CA). For the sequencing reactions, a separate set of primers was used: forward primer was 5′-GGG TTC TTG GGA TCC TTC AT-3′, and reverse primer was 5′-AAG CAA CAA GAG GGA AGA GC-3′.

Sequence traces were assembled with CodonCode software (CodonCode Corporation, Dedham, MA), and SNP were detected using the algorithm included in the mutation detection tool. The SNP detected in this study were queried for their presence in the National Center for Biotechnology Information dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/, accessed January 15, 2008; build 128). Those SNP not present in dbSNP were submitted to the database. Submitted SNP (ss) numbers were obtained and used to describe the SNP in this paper.

Phylogenetics. PopGen32 software was used to analyze unbiased genetic distance and genetic identity within the GHR promoter among cattle breeds (Nei, 1972). Sequence variation in the 1,195-bp fragment and genotypic frequencies from the 48 bulls were inputted to execute the procedure of unweighted pair-group method arithmetic average (Yeh et al., 2000).

Association of Genotype to Phenotype

Animals. Data were collected from a population of growing Brangus bulls (3/8 Brahman × 5/8 Angus) described previously by Thomas et al. (2002, 2007). In brief, 552 growing bulls from 96 sires composed the Chihuahuan Desert Rangeland Research Center (**CDRRC**) Expanded population. Animals were spring-born from 2003 to 2005 in calving seasons of approximately 60 d in length. Calves were weighed at birth and weaning. After weaning, bull calves from at least 2 reference sires were transported to NMSU Campus Farm (Las Cruces, NM) from Mound Creek Ranch (Leona, TX) and vice versa each year, so progeny of reference sires were present in both performance bull tests. Bulls were acclimated to each feeding facility for 4 wk and then performance tested for 112 d. Gain test rations were designed to achieve 1.5 kg·head^{-1}·d^{-1} gain from high-roughage and corn-supplemented diets. Nutritional values on a DM basis of the diets fed at each location were 14.9% CP and 75% TDN at the NMSU Campus Farm and 12.0% CP and 70.7% TDN at Mound Creek Ranch, respectively. Bulls were fed in a single pen or pasture at each location and had ad libitum access to feed. At the NMSU Campus Farm, feed was delivered at 0700 h each day, whereas at Mound Creek Ranch, the ration was supplied in large feeders, and the bulls had access to grazing of Bermuda (*Cynodon dactylon*) grass.

Bulls were weighed at the beginning and end of the gain tests, ADG was estimated, with the end BW adjusted to 365 d of age. Also, at the end of the tests, carcass traits were evaluated with real-time ultrasound using procedures of Perkins et al. (1992) and the requirements of the International Brangus Breeds Association (San Antonio, TX). In brief, ultrasound scanning of each bull was completed by a Centralized Ultrasound Processing Lab certified technician (Donnie Robertson, Carcass Ultrasound and AI Services, Marquez, TX). His effort included measures of fat thickness at the 12th and 13th rib (i.e., rib fat), LM area, and intramuscular fat percentage of LM. Data collection for these carcass traits began in the CDRRC population in 1998 and in the Mound Creek population in 1996. Breeding soundness exams were administered to the bulls at NMSU Campus Farm upon completion of the gain test. Scrotal circumference was measured on each bull at Mound Creek Ranch upon completion of this gain test, but the breeding soundness exam was not administered until the following autumn.

Blood Collection, DNA Extraction, and SNP Genotyping. Procedures described by Beauchemin et al. (2006) and Thomas et al. (2007) were used to collect blood from each bull, extract DNA, and conduct SNP genotyping. Three upstream SNP (ss86273132, ss86273133, and AF126288-149) and 3 downstream SNP (ss86273134, ss86273135, and ss86273136) from the GT microsatellite were chosen for genotyping based on their flanking proximity to the microsatellite. Genotyping for SNP AF126288-149 was not completed, because the reverse primer designed for this assay annealed within the GT microsatellite providing unreliable genotype data. For the years of 2003 and 2004, the 5 remaining genotype assays were completed in 340 bulls from the CDRRC Expanded population. However, for the year 2005, only SNP ss86273136, which was a tag SNP in complete LD with the other SNP, was genotyped for each bull from the CDRRC Expanded population for a total of 552 bulls with this genotype data.

Statistics

Allele, Genotype, and Haplotype Frequencies, LD, and Tag SNP. Data were analyzed with SAS (SAS Inst. Inc., Cary, NC), which included genetic analysis tools (Saxton et al., 2004), and Haploview software (Barrett et al., 2005). Frequencies of SNP alleles and genotypes at each locus were determined using PROC ALLELE. These analyses involved χ^2 distribution tests for each biallelic locus. This procedure also outputted tests of Hardy-Weinberg equilibrium, which was a result of Pearson's χ^2 statistic. PROC HAPLOTYPE was used to determine haplotype frequencies with tests that assumed Hardy-Weinberg equilibrium (Balding, 2006). Haplotypes were also visualized in Haploview. The or-

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der of SNP within haplotypes throughout the manuscript was ss86273132, ss86273133, ss86273135, and ss86273136. Linkage disequilibrium between SNP was estimated with 2 outputs from PROC HAPLOTYPE, r^2 and Lewontin's D' (Balding 2006). These values were also observed using Haploview software using the estimates described by De Bakker et al. (2005). The tagger option within Haploview was used to identify tag SNP, which by definition was a representative SNP for a region of the genome with high LD among several SNP (i.e., nonrandom association of alleles at 2 or more loci; Qin et al., 2002). If a tag SNP was identified, Mendelian segregation was confirmed from genotyping within 5 bovine families consisting of an individual, its parents, and both sets of grandparents (i.e., 4-2-1 method of Gagnon et al., 2005). These procedures included 3 families of purebred Brangus and 2 families with Angus and Brahman ancestors.

Association of Genotype to Phenotype. Mixed model association analyses were conducted with PROC MIXED (SAS Inst. Inc.). Genotype and haplotype data were considered appropriate for association analyses with phenotypes when the minor frequency of a genotype or haplotype was greater than 10% (Abecasis et al., 2001). Sire connectivity across years was also confirmed before these analyses were completed. The genotype to phenotype association model was:

$$y_{ijklmn} = \mu + A_i + B_j + C_k + D_l + E_m + F_n + e_{ijklmn},$$

where y_{ijklmn} = phenotypic value of trait; μ = population mean; A_i = fixed effect of genotype (i.e., tag SNP; ss86273136; AA, AG, or GG), or probability of haplotype, or haplotype category (i.e., Angus or Brahman); B_j = fixed effect of bull test contemporary group (i.e., Mound Creek Ranch or NMSU Campus Farm); C_k = fixed effect of year (i.e., 2003, 2004, or 2005); E_m = fixed effect of age of dam categories from the Guidelines of Beef Improvement Federation (BIF, 2006; i.e., 2, 3, 4, 5 to 10, or 11 yr and older); F_n = random effect of sire (i.e., mean = zero, variance = σ_s^2; *Z* statistic used to test if Ho:$\sigma_s^2 = 0$); and e_{ijklmn} = random residual error (mean = zero, variance = σ_e^2).

Traits analyzed with this model were birth weight, bull test ADG, scrotal circumference, LM area, LM area per kilogram of BW, percentage of intramuscular fat, and rib fat. Age of bull in days was included in a preliminary version of this model, but it was eliminated from the model for these traits, because it was not significant. Adjusted 205- and 365-d BW were computed using formulas of BIF (2006). These 2 traits were used to calculate ADG between 205 and 365 d. The interaction of genotype and year was also tested in the preliminary version of this model, but it was omitted due to lack of significance.

Association analyses using the described model with haplotype terms rather than genotype used haplotype data generated by 2 procedures. The first procedure modified from Zhang et al. (2006) computed the probability that a bull possessed a specific haplotype (i.e., Brahman haplotype of TTCG). Thus, the haplotype term was 1 if a bull could only possess the Brahman haplotype, 0.5 if he had a 50% chance of possessing this haplotype, and 0.25, 0.125, and 0.0625 if he had 25, 12.5, or 6.25% chance of possessing the Brahman haplotype, respectively. In the second procedure, a bull was assigned a haplotype category of 1 if he possessed only Angus-derived haplotypes (CCCG or CCTG) or 2 if he possessed a Brahman-derived haplotype (TTCA). If genotype or haplotype terms were found to be important ($P < 0.05$) sources of variation in association analyses, preplanned pairwise comparisons of least squares means generated with PDIFF were conducted. These mean separation tests were executed using the within option LSMEANS of the mixed procedure, which also included Bonferroni's adjustment (Weir, 2001).

RESULTS

Resequencing and SNP Discovery

A diagram of the bovine GHR gene and the identified SNP is shown in Figure 1. Nine SNP, including 3 described previously by Hale et al. (2000), were detected through resequencing 1,195 bp of DNA within the GHR promoter. Only SNP AF126288-149 was present in dbSNP (rs41639262), and it was polymorphic in Brown Swiss, Holstein, Angus, and Brangus. Six SNP (ss86273132, ss86273133, ss86273136, ss86273137, ss86273138, and ss86273139) were polymorphic between Brahman and the other breeds. The ss86273134 SNP was polymorphic only in Brahman, and the ss86273135 SNP was polymorphic only in Angus. Four SNP (i.e., ss86273134, ss86273135, ss86273136, and ss86273137) were within exon 1A.

Phylogenetics

Table 1 presents genetic distance and genetic identity information among 7 breeds of cattle for 1,195 bp of DNA in the GHR promoter. Specifically, minor distance (i.e., <5%) was observed in DNA sequence among *Bos taurus* breeds; however, sequence from Brahman cattle diverged by 27.4 ± 0.07% from the *Bos taurus* breeds, whereas divergence with Brangus was 10.7 ± 0.1%.

Allele, Genotype, and Haplotype Frequencies, LD, and Tag SNP

Allele and genotype frequency percentages for the target region of the GHR gene in the CDRRC Expanded population of Brangus bulls are presented in Table 2. Polymorphism ss86273134, which was only detected in Brahman, was omitted from further analyses because it did not appear to segregate in this population. For the remaining SNP, tests for deviation from Hardy-Weinberg equilibrium were not significant. Even though minor allele frequency of SNP ss86273135 was

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Table 1. Nei's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) among breeds in a 1,195-bp region in the promoter of the GH receptor gene

	Breed						
Breed	BS	H	J	A	S	BH	BR
Brown Swiss (BS)	****	1.0000	0.9465	0.9844	0.9987	0.2835	0.9028
Holstein (H)	0.0000	****	0.9465	0.9844	0.2835	0.2835	0.9028
Jersey (J)	0.0550	0.0550	****	0.9870	0.9615	0.1986	0.8485
Angus (A)	0.0157	0.0157	0.0131	****	0.9918	0.2349	0.8822
Simmental (S)	0.0013	0.0013	0.0392	0.0082	****	0.2723	0.9007
Brahman (BH)	1.2605	1.2605	1.6163	1.4485	1.3009	****	0.6501
Brangus (BR)	0.1023	0.1023	0.1643	0.1253	0.1046	0.4307	****

less than 10%, this SNP was included in association analyses, because it provided haplotype information from Angus. These decisions allowed the use of 4 loci for generating haplotypes and subsequent LD analyses. The order of SNP in haplotypes was ss86273132, ss86273133, ss86273135, and ss86273136, and the frequency percentages of these haplotypes are presented in Table 3. These 4 SNP appeared to be in LD ($r^2 = 0.84$ and D' = 0.92), and SNP ss86273136, which was an A to G mutation, was determined to be a tag SNP. The A was derived from Brahman, and the G was derived from Angus. Segregation of this SNP was verified in 5 families of cattle, which included Angus and Brahman ancestors. Segregation of the tag SNP occurred in 3 distinct haplotypes. Two of these haplotypes originated in Angus (CCCG and CCTG), and 1 haplotype originated in Brahman (TTCA).

Association of Genotype to Phenotype

Year and contemporary group were important factors ($P < 0.05$), and the variance due to sires differed from zero ($P < 0.05$) in 83.3% of the traits analyzed from the Brangus bulls of the CDRRC Expanded population. Also, age of dam affected ($P < 0.05$) birth weight and adjusted 205-d BW. The tag SNP (ss86273136) genotype influenced rib fat ($P = 0.0204$; Table 4). Bulls of the GG genotype had 6.1% more ($P = 0.0161$) rib fat than bulls of the AA or AG genotypes, respectively. The terms probability of haplotype or categorical assignment of an Angus- or Brahman-derived haplotype were not detected as significant sources of variation in association analyses.

DISCUSSION

The bovine GHR gene is composed of 10 exons and 9 introns spanning 365,186 bp of DNA sequence. The ATG transcription start site of this gene is in exon II. Sequence polymorphisms in 1,195 bp in the promoter of GHR in this project consisted of 9 SNP and a microsatellite. Three of these SNP were described previously by Hale et al. (2000), and 1 SNP was described by Sherman et al. (2008). Resequencing revealed SNP differences in the promoter of GHR among Brahman cattle, which are *Bos indicus*, and *Bos taurus* breeds. Morsci et al. (2006) used a similar resequencing strategy among 3 lineages of Angus cattle to identify SNP variation in adiponectin and somatostatin genes.

Phylogenetic analyses revealed divergence of sequence among Brahman cattle, which are *Bos indicus*, and 5 *Bos taurus* breeds. Brangus cattle were intermediate in these results, as would be expected, because the breed is 3/8 Brahman and 5/8 Angus. These types of relationships in admixed populations of cattle have been described by Freeman et al. (2004), Curi et al. (2005, 2006), and Edwards et al. (2007).

In the current study, allele and genotypic frequencies from the bulls of the CDRRC Expanded population

Table 2. Allele and genotype frequency percents of SNP in a 1,195-bp region in the promoter of the GH receptor gene in Brangus bulls (n = 340 from 80 sires for SNP ss86273132, ss86273133, and ss86273135 and n = 553 from 96 sires for SNP ss86273136)

	Allele frequency, %		Genotype frequency, %		
Polymorphism	C[1]	T[2]	CC	CT	TT
ss86273132	57.7	42.4	30.9	53.5	15.6
ss86273133	58.1	41.9	30.3	55.6	14.1
ss86273135	96.2	3.8	92.4	7.6	
	A[2]	G[1]	AA	AG	GG
s86273136	41.6	58.4	15.8	51.5	32.7

[1]Nucleotide derived from Angus.
[2]Nucleotide derived from Brahman.

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Table 3. Haplotype frequency percents of SNP in 1,195 bp of the promoter region of the GH receptor (GHR) gene in Brangus bulls (n = 340 from 80 sires)[1]

Haplotype	Frequency
CCCA	0.4
CCCG	51.1
CCTA	0.0
CCTG	4.0
CTCA	0.3
CTCG	2.1
CTTA	0.0
CTTG	0.0
TCCA	2.1
TCCG	0.6
TCTA	0.0
TCTG	0.0
TTCA	39.3
TTCG	0.1
TTTA	0.0
TTTG	0.0

[1]Order of SNP in these haplotypes was ss86273132, ss86273133, ss86273135, and ss86273136.

appeared comparable to these frequencies estimated in AI sires from the Semen/DNA Repository of the International Brangus Breeders Association (Thomas et al., 2008). This was expected, because the CDRRC Expanded population uses AI in its breeding program, which included several of the sires in this breed association resource. Also, Angus-derived GHR genotypes inherited in these Brangus bulls appeared to be associated with greater concentrations of rib fat. This result was also expected, because there are several reports indicating that Brahman-influenced cattle with a greater percentage of Angus have greater concentrations of body fat than those with less concentrations of Angus, when comparing the animals on an age-constant basis (Pringle et al., 1997; Bidner et al., 2002; Riley et al., 2002, 2007).

Hale et al. (2000) used the GT microsatellite in a genotype to phenotype association study in cattle. The short allele of the sequence variation was originally discovered in Brahman cattle but was later detected in Angus cattle. Angus steers possessing the short version of the microsatellite had less weaning weight, carcass weight, and marbling scores relative to steers with the longer allele. Similar results were observed by Curi et al. (2005) in *Bos indicus*-influenced composite cattle and Ohkubo et al. (2006) in Japanese Black cattle. The SNP evaluated in the current study that flanked this microsatellite were not significant predictors of these traits in growing Brangus bulls. Nonetheless, the tag SNP genotype appeared to be associated with measures of rib fat. Commercial genotyping platforms are more amenable for high-throughput analysis using SNP (Van Eenennaam et al., 2007); thus, results of the current study could provide genetic marker information for developing SNP genotyping strategies involving GHR.

Sequence variations can be classified as functional and nonfunctional. Functional SNP affect the gene product, whereas nonfunctional SNP are linked with functional regions of the gene (Khan et al., 2006; Ron and Weller, 2007; Sellner et al., 2007). Polymorphisms in 5´ and 3´ untranslated regions of a gene may affect a gene product by altering transcription factor binding or RNA stability (Crawford and Nickerson, 2005). However, additional research is needed to determine if the tag SNP (ss86273136) of this study is a quantitative trait nucleotide or causal mutation that was associated with rib fat. This type of investigation requires molecular assays that determine gene functionality. The GHR mRNA transcript length can be altered through various forms of the 5´ untranslated region. Eight variants of this transcript have been isolated in cattle (Jiang and Lucy, 2001). Exon 1A is one of these variants and is expressed primarily in the liver; however, transient transfection assays involving promoter-reporter constructs and the GT microsatellite did not suggest that this region of the GHR gene influenced level of transcription in hepatocytes (Zhou and Jiang, 2005). Therefore, the promoter of bovine GHR is very complex and requires additional study to determine its

Table 4. Least squares means for growth and ultrasound carcass traits among tag SNP ss86273136 genotypes in the GH receptor gene in Brangus bulls

	Genotypes			Pooled	
	AA	AG	GG		
Item	(n = 87)	(n = 283)	(n = 180)	SE	$P > F$
Birth weight, kg	36.60	36.51	36.84	0.51	0.2358
205-d BW, kg	271.85	267.44	273.39	4.37	0.2114
365-d BW, kg	510.34	500.00	499.14	6.07	0.1855
205-d to 365-d ADG, kg/d	1.46	1.44	1.43	0.03	0.3967
Test ADG, kg/d	1.57	1.52	1.51	0.04	0.1558
Scrotal circumference, cm	35.42	35.14	35.08	0.35	0.3525
Intramuscular fat, %	3.56	3.55	3.64	0.06	0.1202
LM area/BW, cm^2/kg	0.17	0.17	0.17	0.002	0.8178
LM area, cm^2	81.81	81.27	82.01	1.18	0.6062
Rib fat, cm	0.62	0.62	0.66	0.015	0.0204

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utility in developing tools with application to marker-assisted selection strategies.

Animals with excessive endogenous secretion of GH, or exogenously treated with bovine ST, have increased carcass meatiness (i.e., less body fat and more muscle; Early et al., 1990; Schlegel et al., 2006). Herein, investigation focused on GHR; thus, somatic tissue responsiveness to GH is most likely the functional aspect of the genetic information derived from this study, whether the effect be within a specific tissue or ubiquitous. There are literature citations to support both of these responses (Thomas et al., 2002; Govoni et al., 2004; Riley et al., 2007).

Inbred mouse models have been used to characterize complex traits such as skeletal and muscle growth (Adamo et al., 2006; Delahunty et al., 2006). Outcrossing these inbred lines revealed heterosis among genotypes for serum concentrations of IGF-I, which is a component of the growth endocrine axis. Thomas et al. (2007) described similar effects in Brangus cattle in which heterozygous genotypes in the GH gene appeared to be advantageous for weight gain, scrotal circumference, and body fat traits. However, Van Eenennaam et al. (2007) described additive effects of alleles in various genes, which now are part of commercially available genetic marker tests. Sherman et al. (2008) detected these types of allele substitution effects with SNP in the promoter and exons of GHR. These relationships were not apparent in the current study. Nonetheless, as SNP are discovered, their additive or gene interaction effects, or both, will need to be delineated, as well as their role within haplotype(s), particularly for application in breeding strategies to be applied to composite populations of cattle such as Brangus. Furthermore, selection history of the resource populations must also be considered when determining the associative effects of SNP. This could have affected the current study, because the breeding programs that compose the CDRRC Expanded population have conducted selection for carcass traits, particularly intramuscular fat, with ultrasound for almost a decade.

In summary, 9 SNP flanking a GT microsatellite in a 1,195-bp region of the bovine GHR promoter were identified from resequencing within a multibreed resource involving *Bos indicus* and *Bos taurus* cattle. Phylogenetic analysis revealed divergence among Brahman cattle and *Bos taurus* breeds. Four SNP were genotyped using DNA from Brangus bulls and were found to be in haplotype phase. A segregating A/G tag SNP (ss86273136) was identified. The A allele of this genotype was derived from Brahman and the G allele from Angus, which appeared to be related to increased rib fat.

LITERATURE CITED

Abecasis, G. R., S. S. Cherny, and L. R. Cardon. 2001. The impact of genotyping error on family-based analysis of quantitative traits. Eur. J. Hum. Genet. 9:130–134.

Adamo, M. L., X. Ma, C. L. Ackert-Bicknell, L. R. Donahue, W. G. Beamer, and C. J. Rosen. 2006. Genetic increase in serum insulin-like growth factor-I (IGF-I) in C3H/HeJ compared with C57BL/6J mice is associated with increased transcription from the IGF-I exon 2 promoter. Endocrinology 147:2944–2955.

Arranz, J.-J., W. Coppieters, P. Berzi, N. Cambisano, B. Grisart, L. Karim, F. Marcq, L. Moreau, C. Mezer, J. Riquet, P. Simon, P. Vanmanshoven, D. Wagenaar, and M. Georges. 1998. A QTL affecting milk yield and composition maps to bovine chromosome 20: A confirmation. Anim. Genet. 29:107–115.

Balding, D. J. 2006. A tutorial on statistical methods for population association studies. Nat. Genet. 7:781–791.

Barrett, J. C., B. Fry, J. Maller, and M. J. Daly. 2005. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265.

Beauchemin, V. R., M. G. Thomas, D. E. Franke, and G. A. Silver. 2006. Evaluation of DNA polymorphisms involving growth hormone relative to growth and carcass characteristics in Brahman steers. Genet. Mol. Res. 5:438–447.

BIF. 2006. Guidelines of Beef Improvement Federation. http://www.beefimprovement.org/library/06guidelines.PDF Accessed Jan. 24, 2006.

Bidner, T. D., W. E. Wyatt, P. E. Humes, D. E. Franke, and D. C. Blouin. 2002. Influence of Brahman-derivative breeds and Angus on carcass traits, physical composition, and palatability. J. Anim. Sci. 80:2126–2133.

Carroll, J. A. 1996. Pituitary gland development and somatotroph function in domestic livestock. Texas A&M University PhD Diss., College Station.

Casas, E., J. W. Keele, S. D. Shackelford, M. Koohmaraie, and R. T. Stone. 2004. Identification of quantitative trait loci for growth and carcass composition in cattle. Anim. Genet. 35:2–6.

Casas, E., S. D. Shackleford, J. W. Keele, M. Koohmaraie, T. P. L. Smith, and R. T. Stone. 2003. Detection of quantitative trait loci for growth and carcass composition in cattle. J. Anim. Sci. 81:2976–2983.

Chagas, L. M., J. J. Bass, D. Blanche, C. R. Burke, J. K. Kay, D. R. Lindsay, M. C. Lucy, G. B. Martin, S. Meier, F. M. Rhodes, J. R. Roche, W. W. Thatcher, and R. Webb. 2007. Invited review: New perspectives on the roles of nutrition and metabolic priorities in the subfertility of high-producing dairy cows. J. Dairy Sci. 90:4022–4032.

Crawford, D. C., and D. A. Nickerson. 2005. Definition and clinical importance of haplotypes. Annu. Rev. Med. 56:303–320.

Curi, R. A., H. N. de Oliveria, A. C. Silveria, and C. R. Lopes. 2005. Effects of polymorphic microsatellites in the regulatory region of IGF1 and GHR on growth and carcass traits in beef cattle. Anim. Genet. 36:58–62.

Curi, R. A., D. A. Palmeri, L. Suguisawa, H. N. de Oliveria, A. C. Silveria, and C. R. Lopes. 2006. Growth and carcass traits associated with GH1/Alu I and POUF1F1/Hinf I gene polymorphisms in zebu and crossbred beef cattle. Genet. Mol. Biol. 29:56–61.

De Bakker, P. I. W., R. Yelensky, I. Pe'er, S. B. Gabriel, M. J. Daly, and D. Altshuler. 2005. Efficiency and power in genetic association studies. Nat. Genet. 37:1217–1223.

Delahunty, K. M., K. L. Schultz, G. A. Gronowicz, B. Koczon-Jaremko, M. L. Adamo, L. G. Horton, J. Lorenzo, L. R. Donahue, C. Ackert-Bicknell, B. E. Kream, W. G. Beamer, and C. J. Rosen. 2006. Congenic mice provide in vivo evidence for a genetic locus that modulates serum insulin-like growth factor-I and bone acquisition. Endocrinology 14:3915–3923.

Early, R. J., B. W. McBride, and R. O. Ball. 1990. Growth and metabolism in somatotropin-treated steers: I. Growth, serum chemistry and carcass weights. J. Anim. Sci. 68:4134–4143.

Edens, A., and F. Talamantes. 1998. Alternative processing of growth hormone receptor transcripts. Endocr. Rev. 19:559–582.

Edwards, C. J., J. F. Baird, and D. E. MacHugh. 2007. Taurine and zebu admixture in Near Eastern cattle: A comparison of mitochondrial, autosomal, and Y-chromosomal data. Anim. Genet. 38:520–524.

Downloaded from jas.fass.org at New Mexico State University on November 26, 2008.

Etherton, T. D. 2004. Somatotropic function: The somatomedin hypothesis revisited. J. Anim. Sci. 82(E. Suppl.):E239–E244.

Freeman, A. R., C. M. Meghan, D. E. Machugh, R. T. Loftus, M. D. Achukwi, A. Bado, B. Sauveroche, and D. G. Bradley. 2004. Admixture and diversity in West African cattle populations. Mol. Ecol. 13:3477–3487.

Gagnon, A., J. Beise, and J. W. Vaupel. 2005. Genome-wide identity-by-descent sharing among CEPH siblings. Genet. Epidemiol. 29:215–224.

Ge, W., M. E. Davis, H. C. Hines, K. M. Irvin, and R. C. Simmen. 2003. Association of single nucleotide polymorphisms in the growth hormone and growth hormone receptor genes with blood serum insulin-like growth factor I concentration and growth traits in Angus cattle. J. Anim. Sci. 81:641–648.

Georges, M., D. Nielsen, M. Mackinnon, A. Mishra, R. Okimoto, A. T. Pasquino, L. S. Sargeant, A. Sorensen, M. R. Steele, X. Zhao, J. E. Womack, and I. Hoeschele. 1995. Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. Genetics 139:907–920.

Govoni, K. E., T. A. Hoagland, and S. A. Zinn. 2004. The ontogeny of the somatotropic axis in Hereford calves from birth to one year of age and its response to administration of exogenous bovine somatotropin. J. Anim. Sci. 82:1646–1655.

Hale, C. S., W. O. Herring, H. Shibuya, M. C. Lucy, D. B. Lubahn, D. H. Keisler, and G. S. Johnson. 2000. Decreased growth in Angus steers with a short TG-microsatellite allele in the P1 promoter of the growth hormone receptor gene. J. Anim. Sci. 78:2099–2104.

Jiang, H., and M. C. Lucy. 2001. Variants of the 5´-untranslated region of the bovine growth hormone receptor mRNA: Isolation and effects on translational efficiency. Gene 265:45–53.

Khan, I. A., M. Mort, P. R. Buckland, M. C. O'Donovan, D. N. Cooper, and N. A. Chuzhanova. 2006. In silico discrimination of single nucleotide polymorphisms and pathological mutations in human gene promoter regions by means of local DNA sequence context and regularity. In Silico Biol. 6:23–34.

Kim, J. J., F. Farnir, J. Savell, and J. F. Taylor. 2003. Detection of a quantitative trait loci for growth and beef carcass fatness traits in a cross between *Bos taurus* (Angus) and *Bos indicus* (Brahman) cattle. J. Anim. Sci. 81:1933–1942.

Lopez, R., M. G. Thomas, D. M. Hallford, D. H. Keisler, G. A. Silver, B. S. Obeidat, M. D. Garcia, and C. R. Krehbiel. 2006. Case study: Metabolic hormone profiles and evaluation of associations of metabolic hormones with body fat and reproductive characteristics of Angus, Brangus, and Brahman heifers. Prof. Anim. Sci. 22:273–282.

Lucy, M. C., G. S. Johnson, H. Shibuya, C. K. Boyd, and W. O. Herring. 1998. Rapid communication: Polymorphic $(GT)_n$ microsatellite in the bovine somatotropin receptor gene promoter. J. Anim. Sci. 76:2209–2210.

Morsci, N. S., R. D. Schnabel, and J. F. Taylor. 2006. Association analysis of adiponectin and somatostatin polymorphisms in BTA1 with growth and carcass traits in Angus cattle. Anim. Genet. 37:554–562.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106:283–292.

Ohkubo, T., H. Yano, S. Takahashi, N. Kimura, and M. Tanaka. 2006. *Bos indicus* type of growth hormone receptor gene is retained in Japanese Black cattle. J. Anim. Breed. Genet. 123:410–430.

Perkins, T. L., R. D. Green, and K. E. Hamlin. 1992. Evaluation of ultrasonic estimates of carcass fat thickness and longissimus muscle area in beef cattle. J. Anim. Sci. 70:1002–1010.

Pringle, T. D., S. E. Williams, B. S. Lamb, D. D. Johnson, and R. L. West. 1997. Carcass characteristics, the calpain proteinase system, and aged tenderness of Angus and Brahman crossbred steers. J. Anim. Sci. 75:2955–2961.

Qin, Z. S., T. Niu, and J. S. Liu. 2002. Partition-ligation-expectation-maximization algorithm for haplotype inference with singly-nucleotide polymorphisms. Am. J. Hum. Genet. 70:157–169.

Riley, D. G., C. C. Chase Jr, A. C. Hammond, R. L. West, D. D. Johnson, T. A. Olson, and S. W. Coleman. 2002. Estimated genetic parameters for carcass traits of Brahman cattle. J. Anim. Sci. 80:955–962.

Riley, D. G., S. W. Coleman, C. C. Chase Jr, T. A. Olson, and A. C. Hammond. 2007. Genetic parameters for body weight, hip height, and the ratio of weight to hip height from random regression analyses of Brahman feedlot cattle. J. Anim. Sci. 85:42–52.

Ron, M., and J. I. Weller. 2007. From QTL to QTN identification in livestock – Winning by points rather than knock-out: A review. Anim. Genet. 38:429–439.

Saxton, A. M., M. G. Balzarini, A. Cappio-Borlino, W. Czika, J. D. Fry, G. Gibson, J. L. L. Guerra, M. S. Kang, N. P. P. Macciotta, G. Pulina, G. J. M. Rosa, K. J. Stalder, R. J. Tempelman, R. Wolfinger, C. Xu, S. Xu, and X. Yu. 2004. Genetic analysis of complex traits using SAS. SAS Institute Inc., Cary, NC.

Schlegel, M. L., W. G. Bergen, A. L. Schroeder, M. J. VandeHaar, and S. R. Rust. 2006. Use of bovine somatotropin for increased skeletal and lean tissue growth of Holstein steers. J. Anim. Sci. 84:1176–1187.

Sellner, E. M., J. W. Kim, M. C. McClure, K. H. Taylor, R. D. Schnabel, and J. F. Taylor. 2007. Board-invited review: Applications of genomic information in livestock. J. Anim. Sci. 85:3148–3158.

Sherman, E. L., J. D. Nkrumah, B. M. Murdoch, C. Li, Z. Wang, A. Fu, and S. S. Moore. 2008. Polymorphisms and haplotypes in the bovine neuropeptide Y, growth hormone receptor, ghrelin, insulin-like growth factor 2, and uncoupling proteins 2 and 3 genes and their associations with measures of growth, performance, feed efficiency, and carcass merit in beef cattle. J. Anim. Sci. 86:1–16.

Thomas, M. G., R. M. Enns, D. M. Hallford, D. A. Keisler, B. S. Obeidat, C. D. Morrison, J. A. Hernandez, W. D. Bryant, R. Flores, R. Lopez, and L. Narro. 2002. Relationship of metabolic hormones and serum glucose to growth and reproductive development in performance-tested Angus, Brangus, and Brahman bulls. J. Anim. Sci. 80:757–767.

Thomas, M. G., R. M. Enns, K. L. Shirley, M. D. Garcia, A. J. Garrett, and G. A. Silver. 2007. Associations of DNA polymorphisms in growth hormone and its transcriptional regulators with growth and carcass traits in two populations of Brangus bulls. Genet. Mol. Res. 6:222–237.

Thomas, M. G., G. Rincon, J. F. Medrano, M. A. Elzo, and G. A. Silver. 2008. Bovine growth hormone receptor (bGHR) gene: 5´UTR resequencing, SNP detection, and association with performance in Brangus bulls. Proc. Plant and Anim. Genome XVI:251 (Abstr. P530).

Thrift, F. A., and T. A. Thrift. 2003. Review: Longevity attributes of *Bos indicus* × *Bos taurus* crossbred cows. Prof. Anim. Sci. 19:329–341.

UCSC Genome Bioinformatics. 2008. Cow (*Bos taurus*) Genome Browser Gateway. Version 4.0. http://genome.ucsc.edu/cgi-bin/hgGateway. Accessed May 15, 2008

Van Eenennaam, A. L., J. Li, R. M. Thallman, R. L. Quaas, M. E. Dikeman, C. A. Gill, D. E. Franke, and M. G. Thomas. 2007. Validation of commercial DNA tests for quantitative beef quality traits. J. Anim. Sci. 85:891–900.

Weir, B. S. 2001. Forensics. Page 725 in Handbook of Statistical Genetics. D. J. Balding, M. Bishop, and C. Cannings ed. John Wiley and Sons Ltd., New York, NY.

Yeh, F. C., R. Yang, T. J. Boyle, Z. Ye, and J. M. Xiyan. 2000. PopGene32, Microsoft Windows-based freeware for population genetic analysis, version 1.32. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta, Canada. http://cc.oulu.fi/∼jaspi/popgen/popdown.htm. Accessed January 1, 2008.

Zhang, Q., D. Boichard, I. Hoeschele, C. Ernst, A. Eggen, B. Murkve, M. Pfister-Genskow, L. A. Witte, F. E. Grignola, P. Uimari, G. Thaller, and M. D. Bishop. 1998. Mapping quantitative trait

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loci for milk production and health of dairy cattle in a large outbred pedigree. Genetics 149:1959–1973.

Zhang, Q., Y. Zhap, G. Chen, and Y. Xu. 2006. Estimate haplotype frequencies in pedigrees. BMC Bioinformatics 7(Suppl. 4):S5.

Zhou, Y., and H. Jiang. 2005. Trait-associated sequence variation in the bovine growth hormone receptor 1A promoter does not affect promoter activity in vitro. Anim. Genet. 36:156–159.

Zhu, T., E. L. K. Goh, R. Graichen, L. Ling, and P. E. Lobie. 2001. Signal transduction via the growth hormone receptor. Cell. Signal. 13:599–616.

Downloaded from jas.fass.org at New Mexico State University on November 26, 2008.

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