Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Evaluation of DNA polymorphisms involving growth hormone relative to growth and carcass characteristics in Brahman steers

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Genet. Mol. Res. 5 (3): 438-447 (2006)
Received January 9, 2006
Accepted June 12, 2006
Published July 25, 2006

ABSTRACT. Associations of DNA polymorphisms in growth hormone (GH) relative to growth and carcass characteristics in growing Brahman steers (N = 324 from 68 sires) were evaluated. Polymorphisms were an Msp-I RFLP and a leucine/valine SNP in the GH gene as well as a Hinf-I RFLP and a histidine/arginine SNP in transcriptional regulators of the GH gene, Pit-1 and Prop-1. Genotypic frequencies of the GH SNP, Pit-1 RFLP, and Prop-1 SNP were greater than 88% for one of the bi-allelic homozygous genotypes. Genotypic frequencies for the GH Msp-I RFLP genotypes were more evenly distributed with frequencies of 0.43, 0.42, and 0.15 for the genotypes of +/+, +/-, and -/-, respectively. Mixed model analyses of growth and carcass traits with genotype and contemporary group serving as fixed effects and sire fitted as a random effect suggested that sire was a significant source of variation ($P < 0.05$) in average daily gain, carcass yield, and marbling score. However, measures of growth and carcass traits were similar across GH Msp-I

genotypes as steers were slaughtered when fat thickness was estimated to be ~1.0 cm. These polymorphisms within the GH gene and/or its transcriptional regulators do not appear to be informative predictors of growth and carcass characteristics in Brahman steers. This is partly due to the high level of homozygosity of genotypes. These findings do not eliminate the potential importance of these polymorphisms as predictors of growth and carcass traits in *Bos taurus* or *Bos taurus* x *Bos indicus* composite cattle.

Key words: Brahman, Growth hormone, DNA, Genetic marker, Growth, Carcass

INTRODUCTION

Growth hormone (GH) is necessary for tissue growth, fat metabolism, and homeorhesis; thus, it has an important role in reproduction, lactation, and normal body growth (Burton et al., 1994; Ohlsson et al., 1998). Because of these important relationships, GH is a candidate gene for marker-assisted selection programs in cattle.

Bos indicus cattle, which are primarily of the Brahman breed in the United States, represent heat-tolerant germplasm and are popular in crossbreeding programs in tropical and arid climates; however, there are concerns about carcass quality in the use of *Bos indicus*-influenced cattle, as beef production systems in the United States involve grain fattening. These concerns are based on the knowledge that Brahman cattle tend to be later maturing and have lower amounts of subcutaneous and intramuscular fat when compared to British *Bos taurus* breeds on an age-constant basis (Pringle et al., 1997; Wheeler et al., 2001, 2004).

The identification of positional candidate genes revealed four DNA polymorphisms that may influence somatotrope synthesis and secretion of GH. The GH gene contains an intron 4 Msp-I RFLP (Hoj et al., 1993) and an exon V leucine to valine SNP (Ge et al., 2003). Two other polymorphisms are located in genes of GH transcription factors and are an intron 2 histidine to arginine SNP in the Profit of Pit-1 gene (Prop-1; Showalter et al., 2002) and a Hinf-I RFLP in Pit-1 (Renaville et al., 1997).

Preliminary presentation of data involving these DNA polymorphisms suggested that Brangus (i.e., 3/8 Brahman *vs* 5/8 Angus composite) bulls with a heterozygous GH Msp-I RFLP genotype had greater average daily gain and carcass ultrasound measures than homozygous genotypes (Thomas et al., 2006). Additionally, GH DNA polymorphisms were evaluated among Angus and Brahman-derivative cattle, and results revealed a difference in genotypic frequencies among these breeds as Brahman cattle had high levels of homozygosity (Thomas et al., 2004). However, these samples were collected from a small Brahman population, making it difficult to interpret influences of genotype on the variation of traits versus effects of founder or ancestors in the analyses. Objectives herein were to determine the association of DNA polymorphisms in the GH gene and transcription factors of GH with growth and carcass characteristics in a large population of Brahman steers.

MATERIAL AND METHODS

Animal care and management

Four hundred and thirty spring-born, fall-weaned, male Brahman calves were purchased based on weight and feeder calf score. These calves were of known pedigree and from 27 purebred breeders throughout the State of Louisiana in the United States and were purchased over a 5-year period beginning in 1997. Upon weaning and purchase, calves were transported to the Louisiana Agricultural Experiment Station Central Station Ben Hur Farm. After 2 weeks of acclimation, calves were vaccinated (Cattle Master 4, Pfizer, Exton, PA, USA), dewormed, castrated, and dehorned if necessary.

Post-receiving, steers were managed on pastures of re-growth common Bermuda (*Cynadon dactylon*) and dalligrass (*Paspalum dilatatum*) with access to 1.81 kg steer^{-1} day^{-1} of a high-roughage corn-based ration (12% crude protein, 65% total digestible nutrient) and native hay. In early December, ryegrass (*Lolium multiforum*) became available for grazing, and pastures were stocked at a rate of 306.5 ± 11.5 kg of calf ha^{-1}, where calves typically remained for an average of 122 days. During this backgrounding phase, steers were implanted with Synovex S in 1997 and Ralgro the following years.

When these cool season forages were diminished, steers were shipped to the King Ranch Feedyard (Kingsville, TX, USA). Steers were given growth implants at receiving and a second implant 90 days post receiving. Steers from each year were fed as a single group, and slaughtered based on their body weight and fat thickness of ~1.0 cm which defined contemporary groups in the data set. Steers were slaughtered at Sam Kane Beef Processors (Corpus Christi, TX, USA) at an average age of 547.7 ± 4.9 days. Carcass data were collected within 24 h postmortem.

Animal performance and carcass data collection

Numbers of steers evaluated were 97, 70, 73, 117, and 73 in years 1997, 1998, 1999, 2000, and 2001, respectively. Data collected were average daily gain, subcutaneous fat thickness, hot carcass weight, longissimus muscle area, marbling score, yield grade, percentage retail yield, and percentage kidney, pelvic, and heart fat (Odeh, 2003). Average daily gain was calculated as the difference between initial weight and final weight divided by the number of days. Subcutaneous fat thickness was measured at the 12th and 13th rib of the carcass and described as fat thickness. Area of the longissimus muscle was measured at the 12th rib using a grid with a compensating polar planimeter or an image analysis system. Measures inferring carcass grades were assigned according to the United States Standards for Grades of Carcass Beef (USDA, 1997). Marbling score was evaluated visually from a cross-section of the longissimus muscle and graded on a scale of 100 to 800 where a score of 700 to 800 represents abundant marbling and categorized as prime quality grade and where a score of 400 to 499 is for a small marbling degree categorized as low-choice quality grade. Yield grade was estimated as the proportion of closely trimmed, boneless retail cuts from the round, loin, rib, and chuck, based on a scale of 1 to 5 where 1 was the highest yield and 5 was the lowest yield. Percentage retail yield was defined as the closely trimmed, mostly boneless cuts with a standardized fat content, and was estimated as a percentage of carcass weight where an increased value represents a decrease in percent retail product.

Blood processing and DNA extraction

Prior to shipping cattle to the feedyard, blood samples were collected with vacuum tubes coated with ethylenediaminetetraacetic acid (#VT 6457; VWR, Pittsburgh, PA, USA). After 30 min of centrifugation at 1875 *g*, white blood cell supernatant (i.e., buffy coat) was recovered from blood samples and transferred to 1.5-mL tubes. Sterile phosphate-buffered saline was added to each sample for a total volume of 500 µL, and samples were frozen and stored at -80°C.

Extraction of DNA was performed using a QIA DNA Blood Mini Kit (#51104, Qiagen, CA, USA). Concentrations of each extracted DNA sample were quantified using a 96-well microtiter plate reader (MRX-HD, Dynex technologies, Chantilly, VA, USA). Three hundred and seventy-five blood samples were successfully collected and extracted from the 430 steers, and 324 of these samples were successfully used in genotype assays.

Growth hormone leucine to valine and Prop-1 histidine to arginine SNP

Allelic discrimination was performed using the ABI PRISM® 7700 Sequence Detection System following the protocol obtained by using the File Builder program from Applied Biosystems (Foster City, CA, USA). Taqman® Universal PCR Master Mix, 40X Taqman MGB probe/primer mix, and nuclease-free water were purchased from Assays-by-Design of Applied Biosystems and mixed at a ratio of 12.5, 0.625, and 10.37 per sample with 1.5 µL TE buffer containing 15 ng DNA. Taqman® Universal PCR Master Mix included Taq polymerase, deoxynucleotide triphosphates with deoxyuridine triphosphate, and optimizing buffer components. The probe/primer mix included TaqMan® VIC and FAM fluorescent probes assigned to allele 1 and allele 2, respectively, and designed forward and reverse primers. Sequences for forward and reverse primers for the leucine to valine GH SNP were 5'CCCTTCGGCCTCTCT GTCT3' and 3'GTCATAGGTCTGCTTGAGGATCTG5', respectively. Sequences for forward and reverse primers for the histidine to arginine SNP in Prop-1 were 5'CTCCCCCAATGACCT GCTT3' and 3'CCTGTGGAGGGCTGAGATG5', respectively. Fluorescent probes and primers with VIC and FAM reporters were designed to hybridize to DNA sequences between the forward and reverse primer sites including exon V of the GH SNP and intron 2 of Prop-1. A no template control was used in one well of the 96-well plate to serve as an indicator of reagent contamination and one known sample of each genotype was also amplified on each plate to serve as an assay control. Sequences of these polymorphisms, allele designations, and references for assay development are listed in Table 1.

GH Msp-I and Pit-1 Hinf-I RFLP

Genotypes were accomplished by PCR amplification of intron 4 in the GH gene and amplification of intron 5 and exon VI in the Pit-1 gene with reagents from PCR Core Systems (Promega, Madison, WI, USA). Sequences for forward and reverse primers for the Msp-I RFLP were 5'ATCCACACCCCCTCCACACAGT3' and 3'CATTTTCCACCCTCCCCTA CA5'. These primers allowed for amplification of region 1182 to 2052 in the GH gene. Sequences for the forward and reverse primers in the Pit-1 gene were 5'AAACCATCATCTCCC TTCTTT3' and 5'AATGTACAATGTGCCTTCTGA3', respectively. Following amplification,

Table 1. Type of DNA polymorphism, gene, DNA polymorphism or RFLP fragment length, allele designation, and reference, describing four DNA polymorphisms involving growth hormone (GH) in cattle.

Type of DNA polymorphism	Gene	Polymorphism or fragment	Allele designation	Reference
SNP				
Leucine/Valine	GH	GAGCTG	Leucine	Ge et al., 2003
		GAGGTG	Valine	
Histidine/Arginine	Prop-1	CAT	Histidine	Showalter et al., 2002
		CGT	Arginine	
RFLP				
Msp-I	GH	526	(-)	Hoj et al., 1993
		635	(+)	Zhang et al., 1993
Hinf-I	Pit-1	451	A	Renaville et al., 1997
		244/207	B	

PCR products were digested with target restriction enzyme for 2.5 h at 37°C with a 15-min inactivation period at 65°C. Restriction enzyme digestion products were separated using ethidium bromide-stained 3% NuSieve agarose gels (FMC BioProducts, Rockland, MN, USA) and electrophoresis. Gels were electronically imaged (Kodak EDAS 290 System, Rochester, NY, USA) on a UV light table for interpretation of DNA fragment length relative to a 100-bp DNA ladder. Fragment lengths of these polymorphisms, allele designations, and references for assay development are listed in Table 1.

Statistical analyses

Data were analyzed with SAS (version 8.2, SAS Inst. Inc., Cary, NC, USA). Assumptions of normality of data and of equal variances of contemporary group, sire, and genotype were tested using the functions Proc Univariate and Levene's test, respectively (Littell et al., 2002). Frequencies of genotypes in each polymorphism were determined using the function of Proc Freq (i.e., chi-square test of independence). Allelic frequencies were calculated following the procedures of Lagziel et al. (2000) by using the sum of the frequency of homozygous genotypes plus half the frequency of heterozygous genotypes. If frequencies of each genotype from a DNA polymorphism were greater than 10%, then representation of each genotype was considered appropriate for mixed model analysis of variance procedures (Abecasis et al., 2001). Independent variables in the model included contemporary group and genotype as fixed effects with slaughter age fitted as a covariate. Sire was fitted as a random effect and was evaluated as a significant source of variation using the *Z* statistic to test if Ho: $\sigma_w^2 = 0$ (Littell et al., 1996). The contemporary group was the only fixed effect-requiring model alteration for unequal variances. When this was observed, the contemporary group was fitted as the repeated term in the subject line of the model. If genotype was found to be a significant ($P < 0.05$) source of variation, then preplanned pair wise comparisons of least square means were generated with the PDIFF function of SAS.

RESULTS

Simple statistics for growth and carcass traits are presented in Table 2. Allelic and genotypic frequencies are presented in Table 3. Except for the GH SNP, which was 100% leucine, a chi-square test of independence revealed differing ($P < 0.01$) percentages among each allele type and genotype. However, note the high frequency of homozygosity in the genotypes of Prop-1 SNP and Pit-1 Hinf-I RFLP. The Msp-I RFLP was the only DNA polymorphism where all three genotypes were detected at frequencies greater than 10%, and as a consequence, mixed model analysis was performed. Slaughter age and sire were significant ($P < 0.05$) sources of variation in the analysis of each trait with the exception of percent kidney, pelvic, heart fat. Contemporary group was also a significant ($P < 0.05$) source of variation. However, genotype was not detected as a significant source of variation; nonetheless, means across genotypes are presented in Table 4.

Table 2. Simple statistics for growth and carcass traits in a population of Brahman steers (N = 324 from 68 sires).

Trait	Mean	Standard deviation
ADG (kg/day)	1.49	0.23
Fat thickness (cm)	0.87	0.42
Hot carcass weight (kg)	336.45	36.89
LM area (cm^2)	85.98	8.72
Marbling score	390.30	61.68
Yield grade	2.34	0.68
Retail yield (%)	51.33	1.58
KPH fat (%)	2.12	0.43

ADG = average daily gain; LM = longissimus muscle; KPH = kidney, pelvic, heart.

DISCUSSION

Phenotypic data collected in this study appeared congruent with previous reports involving Brahman cattle (Wheeler et al., 2001; Bidner et al., 2002). According to most pricing systems for beef in the United States, carcass value is discounted when yield grade exceeds 4 and quality grade is lower than choice (i.e., marbling score <400). Steer carcasses evaluated herein appeared to have been competitive on what would be termed a grid pricing system in the United States; however, steers were approximately 18 months of age when slaughtered whereas cattle of British *Bos taurus* ancestry are typically between 12 to 16 months of age when slaughtered using fat thickness as a live animal indicator of a finished carcass (Wheeler et al., 2004).

Genetic markers, more specifically DNA polymorphisms, are being investigated as a tool for use in genetically improving livestock (Vignal et al., 2002). In dairy cattle, associations of genotype with milk production levels have been observed in the GH gene (Lucy et al., 1993; Parmentier et al., 1999; Vukasinovic et al., 1999). A high level of homozygosity was observed for markers involving GH in this population of Brahman steers. With such a high level of homozygosity, statistical analysis was not appropriate for evaluating associations in three of the four DNA polymorphisms studied (Abecasis et al., 2001). Except for the Msp-I RFLP, it is

Table 3. Allelic and genotypic frequencies in the growth hormone (GH) gene and its transcriptional regulators in Brahman steers (N = 324).

DNA polymorphism	Allele	Allelic frequency (%)	Genotype	Genotypic frequency (%)
Prop-1 SNP	Histidine	97.8	Histidine/Histidine	95.6
	Arginine	2.2	Histidine/Arginine	4.4
			Arginine/Arginine	0.0
GH SNP	Leucine	100.0	Leucine/Leucine	100.0
	Valine	0.0	Valine/Leucine	0.0
			Valine/Valine	0.0
Pit-1 Hinf-I RFLP	A	5.9	A / A	0.6
	B	94.1	A / B	10.7
			B / B	88.8
GH Msp-I RFLP	+	36.0	+ / +	15.1
	-	64.0	+ / -	41.8
			- / -	43.1

Table 4. Means of growth and carcass traits in Brahman steers across growth hormone Msp-I RFLP genotypes.

Trait	Msp-I genotype			Pooled SE
	+/+	+/-	-/-	
N	52	136	136	
ADG (kg/day)	1.51	1.49	1.47	0.02
Fat thickness (cm)	0.85	0.90	0.92	0.04
Hot carcass weight (kg)	341.00	340.75	339.10	3.56
LM area (cm^2)	85.33	86.14	85.67	0.98
Marbling score	393.93	391.82	399.75	5.75
Yield grade	2.38	2.39	2.41	0.07
Retail yield (%)	51.21	51.21	51.15	0.16
KPH fat (%)	2.12	2.15	2.10	0.05

Within a row, means were similar ($P > 0.05$).
ADG = average daily gain; LM = longissimus muscle; KPH = kidney, pelvic, heart.

important to note that the other three DNA polymorphisms were discovered in *Bos taurus* cattle and may not be applicable to *Bos indicus* cattle. Therefore, genome mapping efforts described in Henderson et al. (2005) need to be expanded to include *Bos indicus* cattle, as this situation was also observed in other studies involving Brahman cattle (i.e., 530 SNP in µ-calpain; Casas et al., 2005).

In a global population genetics study by Lagziel et al. (2000), the Msp-I RFLP was used to differentiate humped (i.e., *Bos indicus*) from humpless (i.e., *Bos taurus*) cattle where the allelic frequency of the (-) allele was greater in the humped breeds. Specifically, the frequency

of the (-) allele in Brahman cattle was 0.65 whereas it was 0.14 in Angus cattle. The frequency of the (-) allele appeared to be similar in this study of Brahman steers. This was the only polymorphism where genotypic frequency allowed statistical modeling; however, no differences were detected among genotypes in body composition or growth traits. This was not a surprising result as the steers were slaughtered based on a constant fat thickness level. Evaluation of genetic polymorphisms may be more easily evaluated in *Bos indicus vs Bos taurus* crossbred cattle as these two species originated from two separate domestications of aurochsen (*Bos primigenius*; Bradley et al., 1996). An example of the success of these types of evaluations appears in the report of Kim et al. (2003) where reciprocal 3/4:1/4 crosses of Angus and Brahman was used to identify 35 QTL associated with growth and carcass traits.

The GH gene is located at position 65.7 cM on chromosome 19 whereas Pit-1 and Prop-1 are located on chromosomes one and seven (Yang et al., 1998; Showalter et al., 2002). The current study attempted to evaluate single-locus associations of positional candidate genes with phenotypes. Single-locus studies are examples of some of the first reports of marker associations in the literature (Short et al., 1997; Moore et al., 2003). However, predictions using haplotypes may be a more effective strategy for evaluating phenotype to genotype associations. Research by Casas et al. (2005) studied several different DNA polymorphisms in the μ-calpain gene and their associations with phenotypes. Their haplotype strategy was probably more effective for detecting these associations over single-locus procedures for several reasons: 1) markers provided greater chromosome coverage, 2) haplotypes were in linkage disequilibrium, and 3) conformation of the advantageous haplotype could have been coding for the protein with the most bioactivity (see Stephen White section in Henderson et al., 2005 and White et al., 2005).

The concept of gene structure relative to function remains an important area of study as the concept delineates how causal mutations influence phenotype. For example, in transcription studies involving Prop-1, molecular constructs coding for histidine were more bioactive than constructs coding for arginine (Showalter et al., 2002). This report stimulated interest in genotyping steers for this Prop-1 polymorphism; however, because of the homozygosity, associations of genotype to phenotype could not be tested. It is currently unknown if the intron 4 Msp-I RFLP and the exon V SNP in the GH gene alter the conformation of this translated protein hormone. Nonetheless, it would be interesting to test binding affinity of the various isoforms resulting from these polymorphisms in the GH gene with potential isoforms of the GH receptor. It is also understood that the GH receptor contains many sequence polymorphisms (Hale et al., 2000; Blott et al., 2003; Ge et al., 2003).

Data of this study were used to initiate analyses to predict expected progeny differences for carcass traits now included in the sire summary of the American Brahman Breeders Association (2004). These analyses were subsequent to the findings of pedigree/familial influences on the variation of carcass traits in other populations of Brahman cattle (Riley et al., 2002). Therefore, it was not surprising to detect the effects of sire in the mixed model prediction analyses in this study. These publications and the results herein provide evidence to suggest that quantitative assessment based on familial relationships appears to be a stronger analytical tool than the use of these four DNA polymorphisms involving GH.

Previous studies in this laboratory suggested that associations exist between markers involving GH and growth and carcass traits in Brahman-derivative cattle. High levels of homozygosity were observed in the Brahman cattle of this breeding program (Thomas et al., 2004,

2006). However, the number of Brahman cattle was small (<30). Objectives herein were to evaluate the association of DNA polymorphisms in GH and transcription factors of GH to growth and carcass characteristics in a larger population of Brahman steers. This study revealed that the frequencies observed by Lagziel et al. (2000) and the high frequencies observed by Thomas et al. (2004, 2006) for markers involving GH are probably typical in other populations of Brahman cattle.

ACKNOWLEDGMENTS

Louisiana State University Agricultural Center, New Mexico Agricultural Experiment Station, and the NIH-MBRS-RISE graduate education program.

REFERENCES

Abecasis GR, Cookson WO and Cardon LR (2001). The power to detect linkage disequilibrium with quantitative traits in selected samples. *Am. J. Hum. Genet.* 68: 1463-1474.

American Brahman Breeder Association (2004). Sire summary. Available at http://www.brahman.org.

Bidner TD, Wyatt WE, Humes PE, Franke DE, et al. (2002). Influence of Brahman-derivative breeds and Angus on carcass traits, physical composition, and palatability. *J. Anim. Sci*. 80: 2126-2133.

Blott S, Kim JJ, Moisio S, Schmidt-Küntzel A, et al. (2003). Molecular dissection of a quantitative trait locus: A phenylalanine-to-tyrosine substitution in the transmembrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition. *Genetics* 163: 253-266.

Bradley DG, MacHugh DE, Cunningham P and Loftus R (1996). Mitochondrial diversity and the origins of African and European cattle. *Proc. Natl. Acad. Sci. USA* 93: 5131-5135.

Burton JL, McBride BW, Block E, Glimm DR, et al. (1994). A review of bovine growth hormone. *Can. J. Anim. Sci*. 74: 167-201.

Casas E, White SN, Riley DG, Smith TP, et al. (2005). Assessment of single nucleotide polymorphisms in genes residing on chromosome 14 and 29 for association with carcass composition traits in *Bos indicus* cattle. *J. Anim. Sci*. 83: 13-19.

Ge W, Davis ME, Hines HC, Irvin KM, et al. (2003). Association of a single nucleotide polymorphism in the growth hormone and growth hormone receptor genes with blood serum insulin-like growth factor I concentration in Angus cattle. *J. Anim. Sci.* 81: 641-648.

Hale CS, Herring WO, Shibuya H, Lucy MC, et al. (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.

Henderson D, Thomas MG and Da Y (2005). Conference review: bovine genomics from academia to industry. *Comp. Funct. Genom*. 6: 174-180.

Hoj S, Fredholm M, Larsen N and Neilson V (1993). Growth hormone gene polymorphism associated with selection for milk fat production in lines of cattle. *Anim. Genet*. 24: 91-95.

Kim JJ, Farnir F, Savell J and Taylor JF (2003). Detection of 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.

Lagziel A, DeNise S, Hanotte O, Dhara S, et al. (2000). Geographic and breed distribution of an Msp-I PCR RFLP in the bovine growth hormone (bGH) gene. *Anim. Genet.* 31: 210-213.

Littell RC, Milliken GA, Stroup WW and Wolfinger RD (1996). SAS® system for mixed models. SAS Institute Inc., Cary, NC, USA.

Littell RC, Stroup WW and Freund RJ (2002). SAS® for linear models, 4th ed. SAS Institute Inc., Cary, NC, USA.

Lucy MC, Hauser SD, Eppard PJ, Krivi GG, et al. (1993). Variants of somatotropin in cattle: gene frequencies in major dairy breeds and associated milk production. *Domest. Anim. Endocrinol*. 10: 325-333.

Moore SS, Li C, Basarab J, Snelling WM, et al. (2003). Fine mapping of quantitative trait loci and assessment of positional candidate genes for backfat on bovine chromosome 14 in a commercial line of *Bos taurus*. *J. Anim. Sci*. 81: 1919-1925.

Odeh FM (2003). Quantitative inheritance of calpastatin activity as an assessment measure for meat tenderness in Brahman steers. Ph.D. dissertation, Louisiana State University, Baton Rouge, LA, USA.

Ohlsson C, Bengtsson B, Isaksson O, Andreassen T, et al. (1998). Growth hormone and bone. *Endocr. Rev.* 19: 55-79.

Parmentier I, Portetelle D, Gengler N, Prandi A, et al. (1999). Candidate gene markers associated with somatotropic axis and milk selection. *Domest. Anim. Endocrinol*. 17: 139-148.

Pringle TD, Williams SE, Lamb BS, Johnson DD, et al. (1997). Carcass characteristics, the calpain proteinase system, and age tenderness of Angus and Brahman crossbred steers. *J. Anim. Sci.* 75: 2955-2961.

Renaville R, Gengler N, Vrech E, Prandi A, et al. (1997). Pit-1 gene polymorphism, milk yield, and conformation traits for Italian Holstein-Friesian bulls. *J. Dairy Sci*. 80: 3431-3438.

Riley DG, Chase CC, Hammond AC, West RL, et al. (2002). Estimated genetic parameters for carcass traits of Brahman cattle. *J. Anim. Sci*. 80: 955-962.

Short TH, Rothschild MF, Southwood OI, McLaren DG, et al. (1997). Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. *J. Anim. Sci.* 75: 3138-3142.

Showalter AD, Smith TP, Bennett GL, Sloop KW, et al. (2002). Differential conservation of the transcriptional domains of mammalian Prophet of Pit-1 proteins revealed by structural studies of the bovine gene and comparative functional analysis of the protein. *Gene* 291: 211-221.

Thomas MG, Enns RM, Silver GA, Garcia MD, et al. (2004). Growth hormone (GH) gene polymorphisms appear to differentially influence pituitary responsiveness to growth hormone releasing hormone (GHRH) among *Bos taurus* and *Bos indicus*-derivative cattle. *Int. Plant and Animal Genome XII*: 229 (Abstract). Available at http://www.intl-pag.org/.

Thomas MG, Silver GA and Enns RM (2006). Relationships of DNA polymorphisms in growth hormone (GH) to growth and carcass traits observed in a population of Brangus bulls with a larger number of sires. *Int. Plant and Animal Genome XIV*: P526 (Abstract). Available at http://www.intl-pag.org/.

United States Department of Agriculture (USDA) (1997). United States standards for grades of carcass beef. Available at http://www.ams.usda.gov/lsg/stand/standards/beef-car.pdf.

Vignal A, Milan D, SanCristobal M and Eggen A (2002). A review on SNP and other types of molecular markers and their use in animal genetics. *Genet. Sel. Evol*. 34: 275-305.

Vukasinovic N, Denise SK and Freeman AE (1999). Association of growth hormone loci with milk yield traits in Holstein bulls. *J. Dairy Sci*. 82: 788-794.

Wheeler TL, Cundiff LV, Shackelford SD and Koohmaraie M (2001). Characterization of biological types of cattle (Cycle V): carcass traits and longissimus palatability. *J. Anim. Sci*. 79: 1209-1222.

Wheeler TL, Cundiff LV, Shackelford SD and Koohmaraie M (2004). Characterization of biological types of cattle (Cycle VI): carcass, yield, and longissimus palatability traits. *J. Anim. Sci*. 82: 1177-1189.

White SN, Casas E, Wheeler TL, Shackelford SD, et al. (2005). A new single nucleotide polymorphism in CAPN1 extends the current tenderness marker test to include cattle of *Bos indicus*, *Bos taurus*, and crossbred descent. *J. Anim. Sci*. 83: 2001-2008.

Yang Y, Rexroad CE III, Schläpfer J and Womack JE (1998). An integrated radiation hybrid map of bovine chromosome 19 and ordered comparative mapping with human chromosome 17. *Genomics* 48: 93-99.

Zhang HM, Brown DR, DeNise SK and Ax RL (1993). Rapid communication: polymerase chain reaction restriction fragment length polymorphism analysis of the bovine somatotropin gene. *J. Anim. Sci*. 71: 2276.