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GROWTH ENDOCRINE AXIS AND BOVINE CHROMOSOME 5: ASSOCIATION OF SNP GENOTYPES AND REPRODUCTIVE PHENOTYPES IN AN ANGUS, BRAHMAN AND ROMOSINUANO DIALLELE

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ABSTRACT: The growth endocrine axis influences reproduction. A QTL associated with enhanced ovulation exists on chromosome 5 in cattle and there are 6 genes underlying this region involved in the mechanisms of GH action. Resequencing exons, 5' and 3' untranslated regions and conserved non-coding regions of these genes in a multi-breed resource population revealed 75 SNP usable for genotype to phenotype association studies. In the current study, phenotypes included age at first calving, calving interval, days to calving, and pregnancy rate. Data were collected from developing heifers (n = 650) of a diallele composed of Angus, Brahman, and Romosinuano breeds. A SNP in the promoter of the signal transducer and activator of transcription (STAT)2 gene, which is a second messenger of GH, had minor allele frequency > 10% across the three breeds. This SNP did not deviate from Hardy-Weinberg equilibrium (X^2 = 1.00, P > 0.31), so deemed useful for genotype to phenotype association analyses. Since the remaining SNP appeared to predict breed, they were used to correct for population stratification using STRUCTURE, which revealed three distinctive ancestral clusters. No significant association was detected between the STAT2 genotype and reproductive traits in mixed effects analyses using genotype as a fixed term, sire as a random term, and coefficient of ancestry as a covariate; however, the interaction of SNP genotype and ancestral cluster was associated with the traits days to calving (P < 0.05) and calving interval (P < 0.10). Interaction plots revealed a higher estimated effect of heterozygous genotype in cluster 1 (inferred primarily from Brahman) and lower estimates in clusters 2 and 3 (inferred primarily from *Bos taurus*). The heterozygous genotype extended these trait levels ~100 d. A SNP in the promoter of the STAT2 gene was associated with fertility trait levels in admixed cows of the breeds Angus, Brahman, and Romosinuano. The effect appeared to be a non-additive genetic relationship as heterozygous genotype extended levels of traits indicative of postpartum rebreeding.

Key Words: heifer, reproduction, SNP genotype.

Introduction

Components of the GH/IGF axis play important roles in reproductive processes, such as folliculogenesis, steroidogenesis, and embryonic development. Growth hormone acts through its receptor (GHR) to increase hepatic secretion of IGF-1 as a consequence of activating cell signaling pathway proteins (Lucy, 2008).

A QTL associated with ovulation and (or) twinning in cattle exists on chromosome 5, and the IGF-1 gene underlies this region (Allan et al., 2009; Kim et al., 2009). There were other genes underlying this QTL known to be involved in the GH/IGF axis, suggesting these genes may also be regulators of fertility.

Brahman cattle are typically slower to reach sexual maturity compared to most *Bos taurus* breeds (Lopez et al., 2006). Objective herein was to evaluate associations of genotypes from SNP derived from candidate genes of the GH/IGF axis with reproductive phenotypes of beef heifers from the breeds Angus, Brahman and Romosinuano.

Materials and Methods

Genes and SNP Discovery

Genes in the GH/IGF-1 endocrine axis from a 23 Mb QTL-region of bovine chromosome 5 were identified on the GenMAPP diagram of Farber et al. (2006). These genes were IGF-1, IGFBP6, Pro-Melanin Concentrating Hormone (PMCH), Signal Transducers and Activators of Transcription 2 and 6 (STAT2,6) and Suppressor of Cytokine Signaling 2 (SOCS2). Functional regions of each gene were resequenced using DNA from 48 unrelated cattle according to the procedures described by Rincon et al. (2007) and Garrett et al. (2008). They were: 1000 bp of the 5'-untranslated region, exons, and the 500 bp of the 3'-untranslated region. If a gene contained multiple exons (> 10), then a Vista alignment (http://pipeline.lbl.gov/) was conducted to identify conserved introns and exons. Resequencing was completed at SeqWright (Houston, TX) and provided results of SNP, indel, and microsatellite. Finally, SNP were confirmed with CodonCode aligner (CodonCode® Corporation, Dedham, MA) and then tested to be synonymous or non-synonymous using Polyphen (http://genetics.bwh.harvard.edu/pph), and if they were a tag SNP (Haploview; http://www.broad.mit.edu/mpg).

Animals, Phenotype, and Genotype

Angus, Brahman, and Romosinuano heifers and their reciprocal crosses from a diallele design (n = 650) were born from 2002 to 2005. These heifers were raised in the cow-calf system of the USDA-ARS, Subtropical Agricultural Research Station, in Brooksville FL.

After weaning, heifers were exposed to bulls until determined to be pregnant by rectal palpation. After calving, heifers were exposed to bulls for ~90 d from March 20 to June 20 each year. First calving date was collected and reported as the trait, age at first calving. Second calving date was collected and used to calculate, calving interval, as the difference in days between first and second calving. Similarly, the trait, days to calving, was calculated as the difference in days between the second calving date and the first date of the last breeding season that heifers were exposed to bulls. The categorical trait, pregnancy rate, was determined after the second breeding season.

Genotyping was performed using 25 ng/μL of DNA from each heifer. Amplicons derived from PCR and the Sequenom MassArray platform were used to determine SNP genotypes (GeneSeek, Inc., Lincoln, NE). Genotypes were coded 11 for homozygous, 12 for heterozygous, and 22 for opposing homozygous.

Statistics

Analyses were conducted using SAS (Version 9.2; SAS Inst. Inc., Cary, NC), which included Genetic Analysis Tools of SAS (Saxton, 2004).

Simple Statistics and Frequencies

Simple descriptive statistics for growth traits (e.g., birth weight, 205-d weight, and 365-d weight), and continuous reproductive traits (e.g., age at first calving, calving interval, and days to calving), were calculated using PROC MEANS. Pregnancy rate was calculated using PROC FREQ. Allele and genotype frequencies, as well as deviation from Hardy-Weinberg equilibrium, were estimated with PROC ALLELE.

Population Stratification Analyses

Because an admixed population was involved in this study, STRUCTURE was used to infer population stratification (Pritchard et al., 2007). This program estimated each heifer's proportion of membership or admixture termed, Ancestry Coefficient.

Association of Genotype to Phenotype

Association analyses were conducted using PROC MIXED for continuous traits and PROC GLIMMIX for categorical traits. Only polymorphisms with genotype frequencies greater than 10% were considered appropriate to be included in association analyses. 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 SNP genotype,
B_j = fixed effect of year of birth (i.e., 2002, 2003, 2004, and 2005),
C_k = fixed effect of age of dam (i.e., 2, 3, 4, 5 to 10, or 11 yr and older; BIF, 2006);
D_l = covariate of coefficient of ancestry (i.e., admixture proportion from inferred Brahman cluster),
E_m = covariate of ordinal birth date of the heifer,
F_n= random effect of sire using the *Z* statistic to test if Ho:$\sigma_w^2 = 0$, and
e_{ijklmn} = random residual error.

The association model was also executed with either breed or cluster category and their interaction with genotype as a fixed effect, which replaced the covariate, coefficient of ancestry. Proc GPLOT was used to visualize the interaction.

If genotype term was found to be a significant ($P < 0.05$) source of variation in association analyses for continuous traits, preplanned pairwise comparisons of least squares means were generated with PDIFF. These mean separation tests were executed within LSMEANS in the mixed procedure, which included Bonferroni adjustment.

Results and Discussion

Results of resequencing and related bioinformatics revealed 75 SNP in the genes of GHR, IGF-1, IGFBP6, PMCH, SOCS2, STAT2, and STAT6 (Rincon et al., 2007; Garrett et al., 2008). Only a SNP in the promoter of the STAT2 gene had minor allele frequency > 10% across the three breeds. This SNP did not deviate from Hardy-Weinberg equilibrium ($X^2 = 1.00$, $P > 0.31$). Population stratification analysis was executed with 56 of the SNP useful as ancestral informative markers (i.e., minor allele frequency < 10%), which suggested 3 clusters of ancestral subpopulations in this study (Table 1). Genetic markers are commonly used to correct for population stratification using structured methods (Pritchard et al., 2007). In this study, the admixed population included one *Bos indicus* breed (Brahman), two *Bos taurus* breeds (Angus and Romosinuano), and their reciprocal crosses. The genetic structure observed herein suggested Brahman cattle were a unique breed-group because genetic structure arose primarily from one cluster. Structure output data from Angus and Romosinuano breeds revealed that they shared historic ancestry. Similar results were reported by McKay et al. (2007) using 2 *Bos indicus* and 8 *Bos taurus* breeds.

Table 2 includes the simple statistics of the traits evaluated in this study. Year of birth ($P < 0.01$), coefficient of ancestry ($P < 0.05$) and sire ($P < 0.05$) were significant sources of variation in prediction of reproductive traits. No significant association was detected between the STAT2 SNP genotypes with any reproductive trait using the model that included coefficient of ancestry as covariate. However, these genotypes interacted with cluster for prediction of the traits days to calving ($P < 0.05$) and calving interval ($P <$

0.10). Mixed model analyses of these traits involving the interaction of cluster and genotype revealed a higher estimated effect of heterozygous genotype (~100 d) in cluster 1 (inferred from Brahman), and lower estimates in clusters 2 and 3 (inferred from *Bos taurus* breeds). Sliced interaction plotting (Figure 1) also revealed that the heterozygous genotype appeared unfavorable, which suggested a non-additive genetic effect. This plot was only of the purebred cattle.

The STAT proteins are involved as signal transducers of interferon actions on uterine epithelium during early pregnancy, including uterine receptivity and maternal immune response (Schindler and Plumlee, 2008). The STAT proteins are also important intracellular second messengers of GH, IGF, and leptin, which are involved in puberty and postpartum rebreeding (Fruhbeck, 2006). In the current study, a SNP in the promoter of the STAT2 gene was found to be associated with the traits days to calving and calving interval. The interaction among STAT2 genotypes for these traits and ancestral cluster suggested a non-additive relationship as the heterozygous genotype appeared to extend calving interval and days to calving.

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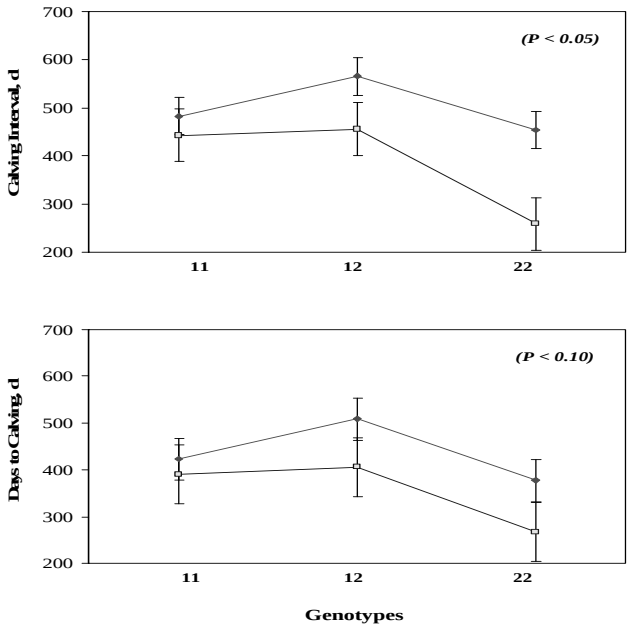

Figure 1. Graphic representation of the interaction among SNP genotypes in STAT2 (e.g., 11, 12 and 22) and ancestral clusters for the traits calving interval and days to calving. Clusters from *Bos indicus* (1; upper line) and *Bos taurus* (2 and 3; lower line) purebreds are represented.

Table 1. Ancestry proportions of Angus, Brahman and Romosinuano diallele breed groups in clusters outputted from STRUCTURE.

		Ancestry Clusters		
Breed Group	N	1	2	3
Angus	66	0.012	0.694	0.294
Brahman	59	0.878	0.029	0.082
Romosinuano	129	0.040	0.461	0.498
Angus x Brahman	116	0.462	0.334	0.204
Angus x Romosinuano	136	0.039	0.497	0.464
Brahman x Romosinuano	143	0.474	0.234	0.292

Table 2. Number of observations, and mean ± SE for birth weight, 205-d weight, 365-d weight, age at first calving, calving interval, days to calving, and pregnancy rate in a diallele of Angus, Brahman and Romosinuano heifers.

Breed Group	N	Growth Traits[1]			Reproductive Traits			
		Birth Wt (kg)	205-d Wt (kg)	365-d Wt (kg)	Age at First Calving (d)	Calving Interval (d)	Days to Calving (d)	Pregnancy Rate (%)
Angus	66	29.1±0.4	171.9±2.1	237.9±4.0	796.4±18.7	465.9±24.6	405.8±26.0	84.8
Brahman	59	30.2±0.5	200.2±2.3	252.2±2.9	934.9±27.7	546.4±24.9	470.1±26.1	69.4
Romosinuano	129	29.7±0.2	176.7±1.8	222.4±2.5	774.4±7.2	433.3±13.8	389.8±15.4	89.9
Angus x Brahman	116	33.1±0.3	208.1±2.4	281.0±2.7	763.8±13.0	421.4±11.1	357.0±11.6	91.3
Angus x Romosinuano	136	30.8±0.3	187.3±1.8	249.2±2.2	721.6±8.9	450.7±12.2	364.9±11.5	88.9
Brahman x Romosinuano	143	33.0±0.4	207.0±1.8	265.2±2.3	786.1±9.3	423.0±12.2	372.4±12.9	90.2

[1]Traits adjusted according to Beef Improvement Federation guidelines (2006).