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Relationship of polymorphisms within metabolic genes and carcass traits in crossbred beef cattle¹,²,³

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ABSTRACT: Feed intake has been shown to alter neurological signaling related to feeding behavior and subsequent activation of adipogenic mechanisms. Fat characteristics are pivotal for carcass and meat quality, including marbling score, flavor, and tenderness. The objective of this study was to establish the association of SNP, from genes functionally related to fat metabolism and obesity, with growth, fat, and carcass traits in steers. A total of 33 informative SNP from candidate genes [cocaine- and amphetamine-regulated transcript (CART), DNA-protein kinase (DNA-PK), fatty acid synthase (FASN), and fat mass and obesity associated (FTO)] were used to genotype crossbred steers (n = 620), and associations with growth and carcass traits were assessed. Five markers within the DNA-PK gene were associated (P < 0.05) with fat thickness. One of these SNP was also associated (P < 0.05) with percent choice, yield grade, and retail product yield. Additionally, 2 unique DNA-PK SNP were associated (P < 0.05) with marbling score. Three haplotypes were observed using these SNP and were significantly (P = 0.0014) associated with marbling score. Slaughter weight, ADG, and HCW were associated (P < 0.05) with SNP from CART, FTO, and FASN. Data from this study indicate that polymorphisms within candidate genes have an indirect relationship with lipogenesis. Replication of these results within other populations will be necessary to establish if these markers will be successful as predictors of fatness components and carcass traits in cattle.

Key words: carcass trait, cattle, cocaine- and amphetamine-regulated transcript gene, DNA-protein kinase gene, fat mass and obesity-associated gene, fatty acid synthase gene

INTRODUCTION

Carcass and meat quality are influenced by fatness traits including fatty acid composition, fat depot partitioning, and activity of fat storage, accumulation, and release (Hausman et al., 2009). Several candidate genes have been identified as functional contributors to fat metabolism. Cocaine- and amphetamine-regulated transcript (CART, located on BTA20) has been linked to appetite behavior in mammalian species (Bannon et al., 2001; Vicentic and Jones, 2007). Polymorphisms within the CART gene have been associated with carcass traits such as backfat thickness and lean content in swine (Stachowiak et al., 2009); however, SNP assessed in cattle are yet to be associated with growth, performance, or carcass traits (Sherman et al., 2008). The fat mass and obesity associated (FTO; located on BTA18) gene has been associated with human obesity using genome-wide association analyses (Himney et al., 2007; Willer et al., 2009). In knockout models, FTO null mice had reduced postnatal body growth, adiposity, and lean mass vs. wild-type or heterozygote counterparts (Fischer et al., 2009). An SNP within FTO was associated with visible intermuscular fat, backfat thick-
ness, and lean cuts within a Duroc population of pigs (Fontanesi et al., 2010). Fatty acid synthase (FASN; located on BTA19) catalyzes the de novo synthesis of fatty acids in cells. Fat metabolism and obesity traits have been associated with FASN expression or polymorphisms in various species including the pig (Ponsuksil et al., 2007), human (Berndt et al., 2007; Schleinitz et al., 2010), and cattle (Abe et al., 2008). The DNA-protein kinase (DNA-PK; located on BTA14) has been identified as a DNA damage repair product that recognizes and binds double-strand DNA breaks (Meek et al., 2004). However, recent work has shown that DNA-PK plays a role in transcriptional activation of FASN (Wong et al., 2009; Wong and Sul, 2009).

Therefore, the objective of the current study was to test genetic variations within candidate genes selected for their role in lipogenesis with fat and growth traits. Our findings suggest that DNA-PK SNP are associated with fat traits, whereas SNP within FASN, FTO, and CART are predominantly associated with growth-related traits in beef cattle.

MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the US Meat Animal Research Center (USMARC) Animal Care and Use Committee.

Animals and Phenotypic Traits

Cycle VIII of the Germplasm Evaluation project (GPE8) included 620 crossbred steers that were used in this study (Wheeler et al., 2010). Approximately equal numbers of calves were produced from 127 purebred sires representing tropically adapted breeds, including Beefmaster, Brangus, Bonsmara, and Romosinuano, as well as Hereford and Angus. All dams were Angus or MARC III cows. The GPE8 animals were selected for the current study because a large portion of cattle in the southeast region of the United States have Bos indicus breeding within their populations, making them as relevant as British or Continental breeds. Furthermore, GPE8 cattle also contain Bos taurus background, allowing stratification of information gathered over diverse animal populations.

Animal care, management, and phenotypic data collection were performed as described previously for GPE8 cattle (Wheeler et al., 2010). Body weights (final BW) were recorded and ADG (ADG final) at slaughter was calculated based on BW over time. Steers were slaughtered at a commercial facility, and carcass traits were acquired by USMARC-trained personnel after a 36-h chilling period at 0°C. Carcass trait measurements including HCW, fat thickness, yield grade (YG), marbling score, percentage Choice, boneless retail product yield (RPY), and fat yield were calculated based on whole-rib dissection carcass data as described previously (Shackelford et al., 1995). Cooked LM intramuscular fat (IMF) was calculated from ether-extractable fat (wet-weight basis) determined according to AOAC (1985) methods as described by Wheeler et al. (2001).

SNP and Genotyping

Reported SNP from DNA-PK, FASN, and FTO were collected from the dbSNP website (http://www.ncbi.nlm.nih.gov/projects/SNP/). Six SNP for CART were identified from the literature (Valle et al., 2005; Zhang et al., 2008a), and 1 SNP for CART was identified from dbSNP. Of these SNP, 47 were successfully designed into assays (Table 1). These SNP were genotyped using primer extension on the Sequenom MassArray system (Sequenom Inc., San Diego, CA) as described elsewhere (Rempel et al., 2010).

Genomic DNA was isolated from whole blood using a saturated salt procedure (Miller et al., 1988). Ten-microliter PCR reactions contained 10 ng of genomic DNA, 0.5 U HotStar Taq (Qiagen, Valencia, CA), 1× of supplied buffer with 3.5 mM MgCl₂, 250 μM dNTP (Invitrogen, Carlsbad, CA), and 100 nM leading and lagging amplification primers (Integrated DNA Technologies, Coralville, IA). The primer extension reaction used 0.625 to 1.25 μM probe primer (Integrated DNA Technologies) and was performed according to the manufacturer’s recommendations for iPLEX chemistry (Sequenom). When appropriate, samples were run twice to increase the number of scoreable genotypes.

Statistical Analyses

The GPE8 population was analyzed using the Mixed Model procedure (SAS Inst. Inc., Cary, NC) with carcass traits as dependent variables using similar methods previously reported (White et al., 2005; Casas et al., 2006). The model included fixed effect for GPE8 sire line (Beefmaster, Brangus, Bonsmara, and Romosinuano and British breeds), GPE8 dam line (Angus, MARC III), year of birth (2001, 2002), slaughter group (1, 2, 3, or 4) within year, and genotype. Each SNP was evaluated independently. Weaning age was also included as a linear covariate. Sire was included in the model as a random effect nested within sire line. Reported P-values were nominal and were not corrected for multiple testing.

The markers rs41718998 and rs41718970 were incorporated into haplotypes. Haplotypes were generated using the procedure HAPLOTYPE from SAS. Haplotypes were analyzed using the MIXED procedure of SAS, with the previously described model.

RESULTS

Within the population tested, 12 of the 47 SNP assays were noninformative with a MAF ≥98% within the tested population (Table 1). Of the remaining informative markers, 14 were significant for various carcass traits (Table 2).
Significance of association between SNP and traits is presented in Table 2. Five markers within DNA-PK were associated ($P < 0.04$) with fat thickness, and 2 markers (rs41718998 and rs41718970) were associated with marbling score ($P < 0.03$). Marker rs41719435 was associated with fat thickness ($P = 0.017$), percent Choice ($P = 0.02$), YG ($P = 0.029$), and RPY ($P = 0.05$). Marker rs41624082 was associated ($P = 0.005$) with HCW, whereas another marker, rs41726290, was associated with RPY ($P = 0.02$) and fat yield ($P = 0.017$).

Markers rs41781998 and rs41718970, within the DNA-PK gene, associated with marbling score were combined in haplotypes and evaluated as such to as-
Table 2. Reported associations of markers with carcass traits

<table>
<thead>
<tr>
<th>Gene</th>
<th>dbSNP or marker</th>
<th>Final BW</th>
<th>ADG final</th>
<th>HCW</th>
<th>Fat thickness</th>
<th>YG</th>
<th>Marbling score</th>
<th>Choice</th>
<th>RPY</th>
<th>Fat yield</th>
<th>IMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA-PK</td>
<td>rs41719435</td>
<td>0.017</td>
<td>0.029</td>
<td>0.02</td>
<td>0.05</td>
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<tr>
<td>DNA-PK</td>
<td>rs41624082</td>
<td>0.005</td>
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<tr>
<td>DNA-PK</td>
<td>rs41718998</td>
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<tr>
<td>DNA-PK</td>
<td>rs41718977</td>
<td>0.039</td>
<td>0.013</td>
<td>0.004</td>
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<tr>
<td>DNA-PK</td>
<td>rs41718970</td>
<td></td>
<td></td>
<td>0.021</td>
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<td>DNA-PK</td>
<td>rs41718942</td>
<td>0.018</td>
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<td>rs29010469</td>
<td>0.039</td>
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<tr>
<td>DNA-PK</td>
<td>rs41726269</td>
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<tr>
<td>DNA-PK</td>
<td>rs41726290</td>
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<tr>
<td>FTO</td>
<td>rs41865328</td>
<td>0.044</td>
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<td></td>
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<tr>
<td>FTO</td>
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<td>0.009</td>
<td>0.005</td>
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<tr>
<td>FASN</td>
<td>rs41919994</td>
<td>0.026</td>
<td>0.031</td>
<td>0.049</td>
<td></td>
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<tr>
<td>CART</td>
<td>CART_-521</td>
<td>0.043</td>
<td>0.025</td>
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<tr>
<td>CART</td>
<td>rs43738098</td>
<td>0.012</td>
<td>0.032</td>
<td>0.006</td>
<td>0.0326</td>
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</table>

1Significant P-values (P < 0.05) are reported for single-trait SNP analyses.
2YG = yield grade; Choice = percentage Choice; RPY = retail product yield; IMF = cooked LM intramuscular fat.

A compelling find from this work was the association of markers within the gene, DNA-PK, which recently was proposed to play a key role in the transcription of FASN (Wong et al., 2009; Wong and Sul, 2009), there-

Table 3. Haplotype analysis of DNA-protein kinase (DNA-PK) markers, rs41718998 and rs41718970, and estimated effect on marbling score

<table>
<thead>
<tr>
<th>Haplotype contrast</th>
<th>Frequency</th>
<th>Estimate</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA/AG</td>
<td>0.06</td>
<td>498.86</td>
<td>11.43</td>
</tr>
<tr>
<td>GA/AG</td>
<td>0.19</td>
<td>513.15</td>
<td>6.943</td>
</tr>
<tr>
<td>AA/GG</td>
<td>0.75</td>
<td>489.82</td>
<td>4.132</td>
</tr>
</tbody>
</table>

*a,bContrasts with different superscripts differ (P = 0.0014).
1Haplotype contrasts of rs41718998 and rs41718970, respectively.

by indirectly affecting fat metabolism. Specifically, upon feeding or administration of an insulin treatment, the transcription factor for FASN, upstream stimulatory factor-1, interacts with phosphorylated DNA-PK within a complex of protein partners leading to FASN activation. In the current study, multiple SNP within DNA-PK were associated with fat thickness and marbling score. Two fat thickness QTL have been previously identified on BTA14 using genome scans within the general vicinity of DNA-PK (Casas et al., 2000, 2003). Casas et al. (2003) also detected a QTL for YG. Likewise within the current study, we found associations of a single DNA-PK SNP with fat thickness, percent Choice, YG, and RPY traits; however, genotyping frequencies may have affected this outcome. Furthermore, a marbling score QTL has been reported on BTA14 within 2 Mb of the DNA-PK location (Takasuga et al., 2007). We also found 2 DNA-PK markers that had single-trait associations with marbling score, and haplotype analysis indicated combined allelic effects on marbling score estimates. It is tempting to speculate mutations within DNA-PK may affect its expression level or its ability to appropriately phosphorylate/dephosphorylate, thereby influencing downstream events such as FASN expression. Future studies into expression of DNA-PK and FASN in relationship to SNP within DNA-PK will provide insight into this theory.

Polymorphisms within FTO have been associated with human (Hinney et al., 2007; Hubacek et al., 2009), mouse (Church et al., 2009), and pig (Fontanesi et al., 2010) obesity-related traits. In the current study, FTO SNP were associated with growth traits such as ADG final, final BW, and HCW. We are unaware of any other reports in cattle that identify associations of FTO SNP with growth traits. However, ADG on test in a Berkshire × Yorkshire pig population yielded suggestive associations with FTO SNP (Fan et al., 2009). The same FTO polymorphism had associations...
with residual feed intake and ADFI within a Yorkshire population but was not associated with ADG or carcass traits (Fan et al., 2010). It was recently reported that FTO null mice had reduced postnatal BW, body length, and bone mineral density growth in comparison with heterozygote and wild-type controls, but lean and fat mass content were similar among genotypes (Gao et al., 2010). These studies suggest our FTO associations with growth traits in cattle are plausible even in the absence of an association with fat traits.

Of the 10 informative FASN markers genotyped in this study, only a single SNP was associated with growth traits (final BW, ADG final, and HCW). There were no associations with traits reflective of lipogenesis (fat thickness and marbling score). Human studies have shown an increased expression of FASN in adipose tissue from obese subjects and an association of SNP within FASN with expression of FASN in obese cases (Berndt et al., 2007; Schleinitz et al., 2010). Similarly, obese German Landrace pigs had greater hepatic expression of FASN in contrast to lean Pietrain pigs (Ponsuksili et al., 2007). A genome-wide QTL within the region of FASN on BTA19 was reported for C14:0 content in backfat and C14:0 and C14:1 content in intramuscular fat (Abe et al., 2008). Additionally, associations of SNP identified in the 3‘ region of FASN SNP with fatty acid composition in beef cattle have been reported (Zhang et al., 2008b; Abe et al., 2009). The SNP within this study were distributed across FASN from exon 1 through intron 33, which would be proximal to SNP reported for fatty acid composition associations. Our study did not look directly at fatty acid profiles but rather the effect of FASN SNP on carcass traits related to growth and lipogenesis.

Similar to FTO and FASN, CART SNP were primarily associated with growth traits within the cattle tested in the current study. The SNP within the promoter region of CART (CART-521) that was previously identified and had associations with BW and ADG in a Nanyang cattle population (Zhang et al., 2008a) also had associations with BW and HCW within the current study. Furthermore, a second SNP within the 3‘ UTR of CART (rs43738098) was also associated with BW at slaughter and HCW, as well as ADG in the current study. Within the current study, this same SNP was associated with IMF. A previous report using a single CART SNP within the 3‘ region of the gene did not elicit any associations with growth, performance, or carcass traits in beef cattle (Sherman et al., 2008). However, it was not made clear as to the exact location of the SNP, and may not be the same SNP used in the current study that did associate with growth traits and a single fat trait.

In conclusion, evaluation of SNP from lipogenic-related candidate genes yielded associations within a crossbred cattle population with relevant growth and carcass trait information. Most significant previously reported QTL for fat and marbling score traits overlay the region in which SNP from DNA-PK were found to associate with like traits. These data lend support to the functional relationship of these candidate genes with fat and carcass trait characteristics. Future studies will include the assessment of other populations as well as further characterization of the biological components involved in the lipogenic pathway and how mutations within DNA-PK potentially alter the activation or inhibition of FASN.

**LITERATURE CITED**


