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Relationship of polymorphisms within metabolic genes and carcass traits in crossbred beef cattle[1,2,3]

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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 (Hinney 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 (Ponsuksili 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 lipogensis 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 MgCl$_2$, 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).

Table 1. Markers used to genotype crossbred steer population at the US Meat Animal Research Center

Gene[1]	Marker	BTA	GenBank accession	Position	Allele[2]	Maf[3]	Het[3]	MAF[3,4]	n[5]
DNA-PK	rs41719435	14	NW_001493203.1	19139344	T/C		8.2	91.8	546
DNA-PK	rs41624082	14	NW_001493203.1	19142787	T/C	20.1	28.4	51.5	363
DNA-PK	rs41624080	14	NW_001493203.1	19142868	T/C	26.9	36.4	36.7	555
DNA-PK	rs41718998	14	NW_001493203.1	19144428	G/A	1.6	19.6	78.8	572
DNA-PK	rs41718977	14	NW_001493203.1	19160784	C/T	10.9	53.6	35.5	358
DNA-PK	rs41718970	14	NW_001493203.1	19161636	A/G	1.6	23.6	74.8	568
DNA-PK	rs41718956	14	NW_001493203.1	19167608	A/G	13.6	51.3	35.1	579
DNA-PK	rs41718939	14	NW_001493203.1	19168775	G/A	20.3	54.3	25.4	536
DNA-PK	rs41718942	14	NW_001493203.1	19170095	T/C	12.6	53.1	34.3	563
DNA-PK	rs29010471	14	NW_001493203.1	19174409	C/T	9.8	33.1	57.1	163
DNA-PK	rs29010469	14	NW_001493203.1	19174494	G/A	13.4	51.5	35.1	561
DNA-PK	rs41718517	14	NW_001493203.1	19182910	T/C	12	51.8	36.2	544
DNA-PK	rs41726269	14	NW_001493203.1	19206278	A/G	15.5	48.5	36	484
DNA-PK	rs41726290	14	NW_001493203.1	19209632	A/G	12.4	54.3	33.3	540
FTO	rs41636316	18	NW_001493585.2	21704510	A/G	0.2	1.6	98.2	566
FTO	rs41869453	18	NW_001493585.2	21705462	T/G	10.9	42.3	46.8	530
FTO	rs41869137	18	NW_001493585.2	21712102	G/T	8.8	37.9	53.3	546
FTO	rs41868528	18	NW_001493585.2	21716981	G/A	13.5	46.5	40	538
FTO	rs41868518	18	NW_001493585.2	21725404	A/G	1.2	20.1	78.7	497
FTO	rs41868510	18	NW_001493585.2	21731831	G/A	12.3	30.3	57.4	373
FTO	rs41868507	18	NW_001493585.2	21750933	A/T			100	486
FTO	rs41868506	18	NW_001493585.2	21766124	T/C			100	585
FTO	rs41868504	18	NW_001493585.2	21781028	C/G			100	583
FTO	rs41868500	18	NW_001493585.2	21791597	A/T			100	459
FTO	rs41868498	18	NW_001493585.2	21812707	G/T			100	557
FTO	rs41636311	18	NW_001493585.2	21839762	C/T	0.4	16.6	83	568
FTO	rs41868494	18	NW_001493585.2	21849863	A/G			100	576
FTO	rs41868497	18	NW_001493585.2	21855124	T/C			100	578
FTO	rs41868493	18	NW_001493585.2	21882628	G/C	17.7	50.9	31.4	558
FASN	rs41920005	19	NW_001493692.1	52171784	G/C	20.2	45	34.8	496
FASN	rs41920002	19	NW_001493692.1	52175723	C/G	12.9	42.9	44.2	357
FASN	rs41920001	19	NW_001493692.1	52175761	A/G	6.7	35.9	57.4	565
FASN	rs41920000	19	NW_001493692.1	52175906	A/T			100	593
FASN	rs41919996	19	NW_001493692.1	52182239	T/C		5.8	94.2	573
FASN	rs41919995	19	NW_001493692.1	52182452	G/A	20.4	48.2	31.4	554
FASN	rs41919994	19	NW_001493692.1	52183794	A/G	15.3	43.9	40.8	556
FASN	rs41919993	19	NW_001493692.1	52184049	G/A	17.6	54.5	27.9	516
FASN	rs41919992	19	NW_001493692.1	52184882	A/G	28.4	39.8	31.8	493
FASN	rs41919991	19	NW_001493692.1	52185201	A/G			100	561
FASN	rs41919984	19	NW_001493692.1	52187632	C/T	21.7	54	24.3	540
FASN	rs41919986	19	NW_001493692.1	52189386	T/C	19.2	50.7	30.1	489
CART	CART_-636[6]	20	NW_003104506.1[7]	59796	G/A			100	564
CART	CART_-521[6]	20	NW_003104506.1[7]	59681	A/G	6.5	29.5	64	535
CART	CART_-398[6]	20	NW_003104506.1[7]	59558	A/G	3.6	27.8	68.6	518
CART	CART_234[6,8]	20	NW_003104506.1[7]	58926	C/T	5.2	28	66.8	576
CART	CART_579[6]	20	NW_003104506.1[7]	58608	T/C			100	586
CART	rs43738098	20	NW_003104506.1[7]	57315	T/C	26.8	20.5	52.7	332

[1]DNA-PK = DNA-protein kinase; FTO = fat mass and obesity associated; FASN = fatty acid synthase; CART = cocaine- and amphetamine-regulated transcript.

[2]m/M – minor allele/major allele.

[3]Percentage genotyped; Maf = minor allele frequency; Het = heterozygous; MAF = major allele frequency.

[4]Noninformative markers were identified having a MAF ≥ 98.0.

[5]Number of genotypes from 621 animals sampled.

[6]Zhang et al. (2008a).

[7]Reverse strand.

[8]Valle et al. (2005).

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[1] of markers with carcass traits[2]

Gene[3]	dbSNP or marker	Final BW	ADG final	HCW	Fat thickness	YG	Marbling score	Choice	RPY	Fat yield	IMF
DNA-PK	rs41719435				0.017	0.029		0.02	0.05		
DNA-PK	rs41624082			0.005							
DNA-PK	rs41718998						0.004				
DNA-PK	rs41718977				0.039						
DNA-PK	rs41718970						0.021				
DNA-PK	rs41718942				0.018						
DNA-PK	rs29010469				0.039						
DNA-PK	rs41726269				0.013						
DNA-PK	rs41726290								0.02	0.017	
FTO	rs41868528		0.044								
FTO	rs41636311	0.005	0.009	0.005							
FASN	rs41919994	0.026	0.031	0.049							
CART	CART_-521	0.043		0.025							
CART	rs43738098	0.012	0.032	0.006							0.0326

[1]Significant P-values ($P < 0.05$) are reported for single-trait SNP analyses.

[2]YG = yield grade; Choice = percentage Choice; RPY = retail product yield; IMF = cooked LM intramuscular fat.

[3]*DNA-PK* = DNA-protein kinase; *FTO* = fat mass and obesity associated; *FASN* = fatty acid synthase; *CART* = cocaine- and amphetamine-regulated transcript.

sess their association with marbling score. Haplotype analysis of the 2 *DNA-PK* markers (rs41718998 and rs41718970, respectively) elicited significant estimated effects ($P = 0.0056$) upon marbling score (Table 3). Animals heterzygote for both markers had the greatest (GA/AG; $P = 0.0014$; 513.15 ± 6.943) estimated marbling score values, whereas those with AA/GG had the least estimated marbling score (489.82 ± 4.132). The only genotypes represented within the significant haplotype analyses included AA/AG, GA/AG, and AA/GG. Other haplotypes were tested but failed to be more significant than the individual SNP tested (data not shown).

Markers within *FTO*, *FASN*, and *CART* were associated ($P < 0.05$) with final BW and ADG final, as well as HCW. Within these 3 genes, no association was detected ($P > 0.05$) with fat thickness or marbling score. However, a single SNP within CART, rs43738098, had an association ($P = 0.0326$) with LM IMF.

DISCUSSION

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-

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

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

Haplotype contrast[1]	Frequency	Estimate	SE
AA/AG[ab]	0.06	498.86	11.473
GA/AG[a]	0.19	513.15	6.943
AA/GG[b]	0.75	489.82	4.132

[a,b]Contrasts with different superscripts differ ($P = 0.0014$).

[1]Haplotype contrasts of rs41718998 and rs41718970, respectively.

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 intermuscular 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*.

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