

Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle¹

F. S. Schenkel^{*2}, S. P. Miller^{*}, X. Ye^{*}, S. S. Moore^{†3}, J. D. Nkrumah[†], C. Li[†], J. Yu[†], I. B. Mandell^{*}, J. W. Wilton^{*}, and J. L. Williams[‡]

^{*}University of Guelph, Guelph, Canada N1G-2W1; [†]University of Alberta, Edmonton, Canada T6G-2P5; and [‡]Roslin Institute, Roslin, United Kingdom EH25 9PS

ABSTRACT: Studies with different populations are required to properly characterize the robustness of associations of polymorphisms in candidate genes with economically important traits across beef cattle populations before this sort of genetic information can be used efficiently in breeding and management decisions. The objective of this study was to evaluate the association of previously reported SNP in the bovine leptin gene with carcass and meat quality traits from a large sample of crossbred beef cattle. Five SNP (UASMS1, UASMS2, UASMS3, E2JW, and E2FB) were genotyped on 1,111 crossbred bulls, heifers, and steers. The measured traits included fat, lean, and bone yield (%) by partial rib dissection, grade fat, LM area, HCW, quality grade, LM i.m. fat, and tenderness evaluation of LM and semitendinosus muscle. Only four SNP were analyzed (UASMS1, UASMS2, E2JW, and E2FB), because UASMS1 and UASMS3 were completely linked. A univariate mixed-inheritance animal model was used to evaluate the association of either genotypes or haplo-

types with the traits. The two leptin exon 2 SNP were associated with fat and lean yield and grade fat (E2JW, $P < 0.01$; E2FB, $P < 0.05$), and they interacted in their effect on LM tenderness ($P < 0.01$). The leptin promoter SNP were either not associated with any of the traits (UASMS2) or with fat yield only (UASMS1). Three haplotypes (TCAC, CCAT, TTAC) were at high frequency in the population (88%) and had similar effects on all the traits. Compared with the common haplotypes, one haplotype (CCTT) showed a significantly different effect on fat and lean yield and grade fat ($P < 0.01$), and one haplotype (TTTT) had a different effect on LM tenderness ($P < 0.03$). Therefore, important associations between SNP within the leptin gene with lean yield, fatness (fat yield and subcutaneous fat), and tenderness were detected. Results confirm some of the previously reported associations, but diverge with respect to others, showing that further efforts are required to validate some prospective associations.

Key Words: Beef Cattle, Carcass Traits, Haplotype Analysis, Leptin Gene, Meat Quality, Single Nucleotide Polymorphism

©2005 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 2005. 83:2009–2020

Introduction

Leptin, the hormone product of the obese (leptin) gene located on BTA 4 (Stone et al., 1996; Pomp et al., 1997),

is considered to play a role in the regulation of appetite, energy partition, and body composition (Houseknecht et al., 1998; Baile et al., 2000). Leptin is synthesized and expressed predominantly by adipocytes (Houseknecht et al., 1998) and relates to the feedback system that regulates long-term body fat weight and composition (Hossner, 1998). Studies have found associations of serum leptin concentration with carcass adipose depots and carcass characteristics of beef cattle (Minton et al., 1998; Geary et al., 2003).

¹Researchers from Univ. of Guelph acknowledge Beef Improvement Ontario (BIO) and Ontario Cattlemen's Association for providing data; BIO, Natural Sciences and Engineering Research Council of Canada, and Ontario Ministry of Agriculture and Food for financial support; and the Canadian Foundation for Innovation and Ontario Innovation Trust for support of required computing infrastructure. Researchers from Univ. of Alberta acknowledge the financial support from the Canada/Alberta Beef Industry Development Fund through Grant No. 2000AB364 awarded to S. S. Moore. We thank P. J. Boettcher for his contributions to the haplotype analysis.

²Correspondence: Dept. of Anim. and Poultry Sci., Room 018 (phone: 519-824-4120, ext. 58650; fax: 519-767-0573; e-mail: schenkel@uoguelph.ca).

³The author holds a U.S. patent application (currently under review) on the association of the three SNP in the leptin gene promoter (UASMS1, UASMS2, and UASMS3) used in this study with fatness traits in cattle.

Received January 5, 2005.

Accepted June 3, 2005.

Leptin has been considered a candidate gene for performance, carcass, and meat quality traits in beef (Fitzsimmons et al., 1998; Buchanan et al., 2002; Lagonigro et al., 2003). Several SNP have been reported in the leptin gene (Buchanan et al., 2002; Lagonigro et al., 2003; Nkrumah et al., 2005). Associations of molecular polymorphisms within exon 2 (Buchanan et al., 2002; Nkrumah et al., 2004) or the promoter region (Crews et al., 2004; Nkrumah et al., 2005) of the leptin gene with carcass and meat quality traits recently were reported in beef cattle, with some associations not being consistently verified across studies.

Before this sort of genetic information can be used efficiently in breeding and management decisions, studies with different populations are required to properly characterize the robustness of the associations of leptin polymorphisms with economically important traits across beef cattle populations. The objective of this study was to evaluate the association of five previously reported SNP in the bovine leptin gene with carcass and meat quality traits from a large sample of crossbred beef cattle.

Materials and Methods

Cattle

The animals were commercially fed heifers (165), steers (231), and bulls (61) from industry sires, heifers (40), steers (375), and bulls (48) from the University of Guelph breeding project, and steers (191) from a University of Guelph feeding trial carried out at a feedlot in Rockwood, Ontario, Canada. The three sources of cattle were identified as Commercial, Elora, and Rockwood, respectively. Animals were crossbred, with breed composition formed by several breeds. The major contributing breeds were Angus (**AN**), Charolais (**CH**), Limousin (**LI**), and Simmental (**SM**). The average contribution of these four breeds to the breed composition of animals having any fraction of the mentioned breeds were 0.46, 0.50, 0.50, and 0.50 for AN, CH, LI, and SM, respectively, for Commercial cattle; 0.24, 0.36, 0.38, and 0.41 for Elora cattle; and 0.51, 0.53, 0.59, and 0.41 for Rockwood cattle. Thus, with the primary use of purebred sires, the Elora cattle represented more advanced crosses in dams, whereas the Commercial and Rockwood cattle were more representative of first-generation crossbreds.

Rockwood and Commercial cattle represented AI breeding, as well as some natural service. In both cases, sires were known and had extended pedigree information. Elora and Rockwood animals were a result of ongoing research at the University of Guelph. Elora cattle originated from three breeding herds with exclusive AI breeding and coordinated sire use across herds and extended pedigree information. Breeding herds were located at the University's Elora Beef Cattle Research Centre, New Liskeard Agricultural Research Station, and the Agriculture and Agri-food Canada Research

Station in Kapuskasing. Cattle from the three herds were fed postweaning at the University of Guelph Elora Beef Cattle Research Centre feedlot and were involved in various postweaning trials. These trials and treatments were accommodated into the statistical analyses. Animals were slaughtered on the basis of a target commercial finishing endpoint of 8 mm of backfat thickness.

DNA Isolation, Polymorphism Detection, and Genotyping

The sources of DNA were frozen steaks, with the exception of 48 bulls from Elora, from which venous blood was taken. The DNA was isolated using the standard phenol/chloroform method (Sambrook et al., 1989; Hoelzel, 1992). The cell lysis buffer was modified as a 0.8 M concentration of urea, including 0.8 M urea, 2% SDS, 100 mM Tris·HCl, 200 mM NaCl, and 2 mM EDTA, pH 7.5.

Five SNP (E2FB, E2JW, UASMS1, UASMS2, and UASMS3) in the leptin gene were investigated. Two SNP, E2FB (Buchanan et al., 2002) and E2JW (Lagonigro et al., 2003, originally referred to as 252-SNP), were located within the leptin exon 2, whereas UASMS1, UASMS2, and UASMS3 (Nkrumah et al., 2005) were within the leptin promoter region.

The genotyping of each SNP was carried out using the 5' nuclease allelic discrimination assay on an ABI Prism 7700 sequence detector (Applied Biosystems, Inc., Foster City, CA). Details of the procedures were described by Nkrumah et al. (2005). The DNA from a subset of the genotyped animals was sequenced across each polymorphism, and the sequence results were used to confirm the genotypes obtained by discrimination assays.

Phenotypic Information

Information on LM tenderness at 2 (**SFL2**), 7 (**SFL7**), 14 (**SFL14**), and 21 d (**SFL21**) postmortem and of semitendinosus muscle tenderness at 7 d (**SFS7**) postmortem, chemical fat (**CF**), grade fat (**GFAT**), quality grade (**QG**), LM area (**LMA**), lean (**LEANYL**), fat (**FATYL**), and bone (**BONEYL**) yield and HCW were available on most of the 1,111 genotyped animals (Table 1).

Warner-Bratzler shear force measurements (kg) were used as an objective method of assessing tenderness (Shackelford et al., 1999). Grade fat is the backfat thickness measurement taken at the 12th- and 13th-rib interface. Longissimus muscle area is the measure of the LM area at the 12th- and 13th-rib interface using a tracing of the muscle. Chemical fat was determined by the chemical analysis of a core meat sample that determined the percentage of i.m. fat. Lean, fat, and bone yield were determined by dissection of a four-bone rib section. Quality grade is the marbling grade used for grading in Canada, with most carcasses falling in one of three grades (A, AA, AAA). Because only a few

Table 1. Number of phenotypic records on the carcass and meat quality traits with corresponding means, standard deviations, and coefficients of variation

Trait ^a	No. of records	Mean	SD	CV, %
FATYL, %	914	24.5	5.12	20.9
LEANYL, %	914	56.1	5.03	9.0
BONEYL, %	914	19.4	2.62	13.5
GFAT, mm	923	9.3	3.37	36.2
LMA, cm ²	901	86.7	13.64	15.7
HCW, kg	920	336.0	49.23	14.7
SFL2, kg	720	5.3	1.71	32.3
SFL7, kg	885	4.8	1.40	29.2
SFL14, kg	884	4.3	1.25	29.1
SFL21, kg	878	3.8	0.96	25.3
SFLavg, kg	716	4.5	1.03	22.9
SFS7, kg	877	5.3	1.10	20.7
CF, %	920	4.0	1.58	39.5
QG A, % ^b	142	15.4		
QG AA, % ^b	546	59.1		
QG AAA, % ^b	236	25.5		

^aLean (LEANYL), fat (FATYL), and bone (BONEYL) yield; grade fat (GFAT); LM area (LMA); LM shear force (SFL) at 2, 7, 14, and 21 d postmortem; average shear force across aging times (SFLavg); semitendinosus muscle shear force (SFS) at 7 d postmortem; LM chemical fat (CF); and quality grade (QG).

^bObserved frequency of the quality grades.

carcasses were classified as Prime, those animals were combined with AAA carcasses for the analyses. A complete description of carcass measures available and methods used are given in Laborde et al. (2001).

Commercial and Rockwood cattle were slaughtered at Better Beef Ltd. slaughter plant in Guelph, whereas the Elora cattle were slaughtered at the University of Guelph Meat Science Laboratory. All standardized carcass and meat quality measures were made at the University of Guelph Meat Science Laboratory, except for GFAT, HCW, LMA, and QG for Commercial and Rockwood cattle, which had these measures taken at Better Beef by University of Guelph meat scientists.

A total of 1,104, 1,111, 1,106, 1,068, and 1,109 animals had genotypes available for UASMS1, UASMS2, UASMS3, E2FB, and E2JW SNP, respectively. The genotypes for UASMS1 and UASMS3 were almost perfectly linked. Only three out of 1,104 genotypes for UASMS1 and UASMS3 did not match each other (that is, the C and T alleles in UASMS1 were not associated with the C and G alleles in UASMS3, respectively, in only three animals). Thus, UASMS3 was dropped from the analyses, and allele frequencies and association with the traits for UASMS1 would be extended to UASMS3.

The genotypes of all animals were used to determine the allelic frequencies. For the study of association between SNP in the leptin gene and carcass and meat quality traits, only animals with required phenotypic information and with genotypes available for all four SNP (UASMS1, UASMS2, E2JW, and E2FB) were used. The resulting number of records ranged from 720

for SFL2 to 924 for QG. Table 1 gives the number of records, mean, SD, and CV of the analyzed traits.

Statistical Analyses

All analyses were performed using the statistical software SAS (SAS Inst., Inc., Cary, NC) and ASREML (Gilmour et al., 2000). Descriptive characteristics of quantitative traits were obtained using SAS PROC MEANS. Allele frequencies were tabulated and compared by χ^2 analysis using SAS PROC FREQ.

Genotype Analyses. Association of the genotypes with the traits was evaluated by genetic analysis using ASREML, fitting a mixed inheritance model (SNP genotypes plus polygenic effects). The model included SNP genotypes as fixed effects:

$$Y_{ijklm} = u + \sum_{i=1}^4 \text{Gen}_{i(j)} + \text{Sex}_k + \text{Slg}_l + \beta_1 \text{AN} \quad [1] \\ + \beta_2 \text{LI} + \beta_3 \text{CH} + \beta_4 \text{SM} + \text{Pol}_m + e_{ijklm}$$

where Y_{ijklm} is the trait measured in the m th animal of k th sex and l th slaughter group; u is the overall mean for the trait; $\text{Gen}_{i(j)}$ is the effect of the i th genotype for j th SNP (UASMS1, UASMS2, E2JW, and E2FB) in the leptin gene; Sex_k is the fixed effect of the k th sex (bull, heifer, and steer); Slg_l is the fixed effect of the l th slaughter group (94 levels); β_1 , β_2 , β_3 , and β_4 are the regression coefficients on breed composition of AN, CH, LI, and SM, respectively; Pol_m is the random additive genetic (polygenic) effect of the m th animal; and e_{ijklm} is the residual random effect associated with the m th animal.

Following Fernando et al. (1998), as genotypes were known, the mixed-model equations of Henderson (1984) for Model [1] were used in the analyses. The additive relationship matrix based on the general pedigree was used to model the covariances among polygenic effects. Animals originated from 125 sires and all sires were known. With respect to the dams, 43% of the animals had dams identified. Average size of paternal half-sib families was 8.9. Percentages of sires with fewer than five, 6 to 10, 11 to 15, and more than 15 offspring were 36, 26.4, 27.2, and 10.4%, respectively.

Slaughter groups were defined as animals from the same source (Commercial or Rockwood), and with the same slaughter date or animals from Elora coming from the same trial and feed treatment, and killed in the same season (December to February, March to May, June to August, and September to November).

The repeated shear force measurements of LM across postmortem periods were analyzed individually within each period, as the average shear force over periods (**SFLavg**), and as the intercept and slope of the individual linear regression of shear force measurements on postmortem days. The effect of the four SNP in the leptin gene on quality grade was analyzed by χ^2 analysis (PROC FREQ), as well as a linear trait using AS-

REML, applying Model [1]. In this case, scores of 1, 2, and 3 were assigned to quality grades A, AA, and AAA, respectively.

To keep reasonable probability values for Type I error, two levels of tests were performed. For initial assessment of the results, an overall value of $P < 0.05$ (α) was used. For a more detailed review of the results, a modified Bonferroni correction was used (α/\sqrt{n} ; Mantel, 1980) to account for the number of tests. The value of n was determined using a SNP-wise approach combined with grouping traits according to type (Ye, 2003). Traits were grouped into two groups as follows: carcass yield traits (LEANYL, FATYL, BONEYL, GFAT, LMA, and HCW) and meat quality traits (CF, QG, SFL2, SFL7, SFL14, SFL21, SFLavg, and SFS7). Because there were four SNP, n was equal to 24 (4×6) and 32 (4×8) for carcass and meat quality traits, respectively, with the corresponding modified Bonferroni-corrected significance levels of 0.010 and 0.009.

Initially, two-way interactions between SNP were fit into the model, but there was a significant interaction only between E2JW and E2FB SNP for shear force of LM. For all other traits, the interactions were dropped from the model. For LM shear force, the joint genotype effect of E2JW and E2FB were included in the model.

Variations were estimated from the data and assumed known for estimation and testing purposes. Probabilities associated with the Wald F -statistics output by ASREML were obtained using error degrees of freedom that accounted for the estimated fixed effects, but ignored the fact that variances were estimated. This, however, should not be a problem, because the number of records on all traits was relatively high and variances were estimated by translation invariant functions of the data by REML (Henderson, 1984).

Haplotype Analyses. Association of the haplotypes for the SNP in the leptin gene and carcass and meat quality traits was evaluated by genetic analysis using ASREML, applying Model [1] replacing genotype effects by regressions on haplotype probabilities. The haplotype probabilities were reconstructed using the algorithm and HAPROB software developed by Boettcher et al. (2004). This software estimates probabilities of haplotype combinations for members of half-sib families, given that genotypes are known for all siblings, but unknown for all parents. The accuracy of reconstruction of the halfsibs' haplotypes by the HAPROB software is high. For instance, the accuracy varies from 64 to 94% for reconstruction of haplotypes of individuals from halfsib families of two to 10 offspring, when three loci with three alleles are considered (Boettcher et al., 2004).

Table 2 shows the 16 possible haplotypes and their corresponding probabilities. The three most frequent haplotypes had a summed probability of 0.88. Therefore, there were many rare haplotypes, and the least probable ones were joined into one group, which was referred to as haplotype 10.

Results

Allele Frequencies

The χ^2 test for differences in allele frequency among breeds (animals with breed composition $\geq 5/8$ for a given breed) was not significant for any SNP in the leptin gene (Table 3). However, for UASMS1, Simmental tended to have a lower frequency of the C allele than the other breeds ($P = 0.11$) and, for E2FB, Angus tended to have a lower frequency of the C allele than the other breeds ($P = 0.15$). The latter result is in line with that reported by Buchanan et al. (2002), where Angus had a lower frequency of the C allele than Charolais and Simmental, and with that reported by Nkrumah et al. (2004), where cattle from an Angus foundation line had a lower frequency of the C allele than cattle from other lines with different foundation breeds.

Considering all genotyped cattle, the T allele was predominant over the C allele for UASMS1 and E2FB (57.6 vs. 42.4%, and 61.1 vs. 38.9%, respectively), whereas for UASMS2, the C allele was much more common than T (73.8 vs. 26.1%). The E2JW SNP showed the largest difference in allele frequencies in the population. For this SNP, the T allele was rare compared with the A allele (4.0 vs. 96.0%). These overall frequencies are in agreement to those reported by Nkrumah et al. (2005) for UASMS1, Crews et al. (2004) and Nkrumah et al. (2005) for UASMS2, Buchanan et al. (2002) and Nkrumah et al. (2004) for E2FB, and Lagonigro et al. (2003) for E2JW.

The frequencies of genotypes were in agreement with Hardy-Weinberg equilibrium (Falconer and Mackay, 1996) within all SNP (the probabilities of the χ^2 tests for deviation from the equilibrium were equal to 0.75, 0.17, 0.98, and 1.00 for UASMS1, UASMS2, E2JW, and E2FB, respectively).

Equilibrium in genotypic frequencies when considering jointly two SNP was tested by a χ^2 test of expected and observed frequencies of gametic types (Falconer and Mackay, 1996). The only two SNP whose genotypes were jointly in equilibrium were UASMS1 and E2JW. All other pairwise tests showed a significant disequilibrium ($P < 0.01$).

Genotype Analyses

Genotypes did not significantly influence LMA, BONEYL, CF, HCW, SFS7, and QG (Table 4). Genotypes for E2JW and E2FB significantly influenced LEANYL, FATYL, and GFAT, whereas genotypes for UASMS1 (or, alternatively, UASMS3) significantly influenced FATYL. The analyses of LM shear force in each particular postmortem day, and as an average shear force over the postmortem days, showed a significant effect of the E2JW.E2FB genotypes (the interaction E2JW by E2FB was significant; Table 4).

Table 5 shows the least squares means for UASMS1, E2JW, and E2FB genotypes and breeds with the corres-

Table 2. Haplotype probabilities in the beef population

Haplotypes				Prob 1 ^a	Prob 2	Code ^b
UASMS1	UASMS2	E2JW	E2FB			
T	C	A	C	0.34241	0.35177	1
C	C	A	T	0.33621	0.33133	2
T	T	A	C	0.20399	0.20407	3
C	C	A	C	0.02217	0.02116	4
T	C	A	T	0.02037	0.02084	5
T	T	A	T	0.01862	0.01532	6
C	C	T	T	0.01757	0.01717	7
T	T	T	T	0.01619	0.01215	8
C	T	A	T	0.01550	0.01465	9
C	T	A	C	0.00379	0.00362	10
T	C	T	T	0.00272	0.00212	10
T	C	T	C	0.00201	0.00237	10
T	T	T	C	0.00166	0.00166	10
C	T	T	T	0.00098	0.00104	10
C	C	T	C	0.00038	0.00031	10
C	T	T	C	0.00036	0.00047	10

^aProb 1 = probability of the haplotype in all genotyped animals; Prob 2 = probability of the haplotype in animals genotyped for all four SNP and with phenotypic records.

^bHaplotype code for the analyses.

ponding significance levels of the Wald F -tests for LEANYL, FATYL, and GFAT. The same table also presents the estimated polygenic heritability for the traits. The heritabilities for FATYL, LEANYL, and GFAT were moderate to high (0.62, 0.52, and 0.45, respectively), which agree with expected values from literature (Mrode et al., 1990; Burrow et al., 2001; Utrera et al., 2004).

For E2JW, there were only two animals with genotype TT, which were excluded from the analyses. Therefore, only solutions for genotypes AA and AT were obtained. The T allele was associated with less FATYL and GFAT and more LEANYL compared with the A allele. The estimated differences between the heterozy-

gous and homozygous genotypes were -1.5% , -1.2 mm, and 1.9% for FATYL, GFAT, and LEANYL, respectively ($P < 0.05$ for all differences), corresponding to 0.29, 0.36, and 0.38 phenotypic SD of the corresponding traits, respectively.

For E2FB, the C allele was associated with less FATYL and GFAT and more LEANYL than the T allele. The estimated differences between the homozygous genotypes CC and TT were -1.9% , -1.0 mm, and 2.3% for FATYL, GFAT, and LEANYL ($P = 0.09, 0.19, \text{ and } 0.05$, respectively). The heterozygous genotype had, however, similar FATYL, LEANYL, and GFAT to the homozygous TT genotype, indicating a large degree of dominance of T over C. Differences of the CC genotype and

Table 3. Allele frequencies within breeds and in the entire beef population for the UASMS1, UASMS2, E2JW, and E2FB SNP in the leptin gene

SNP ^b	Alleles	Breed ^a					Total
		Angus	Limousin	Charolais	Simmental	Other	
UASMS1	C, %	48.8	48.3	45.4	34.6	38.4	38.9
	T, %	51.2	51.7	54.6	65.4	61.6	61.1
	No. ^c	43	30	11	68	952	1,104
UASMS2	C, %	73.3	65.5	77.3	69.8	74.4	73.8
	T, %	26.7	34.5	22.7	30.2	25.6	26.1
	No. ^c	43	30	11	68	959	1,111
E2FB	C, %	45.4	51.7	54.6	58.8	58.3	57.6
	T, %	54.6	48.3	45.4	41.2	41.7	42.4
	No. ^c	43	30	11	68	916	1,068
E2JW	A, %	95.4	95.0	90.9	97.8	96.0	96.0
	T, %	4.6	5.0	9.1	2.2	4.0	4.0
	No. ^c	43	30	11	68	957	1,109

^aAnimals with breed composition $\geq 5/8$ for a given breed. Other includes animals with breed composition $< 5/8$ for all breeds.

^bNo significant differences in allele frequencies among breeds ($P = 0.11, 0.46, 0.15, \text{ and } 0.47$ by χ^2 test for UASMS1, UASMS2, E2FB, and E2JW, respectively).

^cNumber of animals.

Table 4. Tests for the association of single nucleotide polymorphisms in leptin gene with lean (LEANYL), fat (FATYL), and bone (BONEYL) yield; grade fat (GFAT); chemical fat (CF), LM area (LMA); HCW; LM shear force (SFL) at 2, 7, 14 and 21 d postmortem; average shear force (SFLavg); semitendinosus muscle shear force at 7 d postmortem (SFS7); and quality grade (QG) in the beef cattle population

Trait	SNP in leptin gene				
	UASMS1	UASMS2	E2JW	E2FB	E2JW × E2FB
<i>P</i> > <i>F</i> ^a					
LEANYL	0.11	0.28	0.003	0.038	
FATYL	0.012	0.39	0.010	0.013	
BONEYL	0.66	0.10	0.28	1.00	
GFAT	0.08	0.45	0.006	0.016	
LMA	0.77	0.11	0.22	0.80	
HCW	0.69	0.63	0.76	0.79	
CF	0.86	0.93	0.60	0.71	
SFL2	0.78	0.82			0.005 ^b
SFL7	0.40	0.80			0.05 ^b
SFL14	0.10	0.36			0.009 ^b
SFL21	0.73	0.35			0.09 ^b
SFLavg	0.57	0.42			0.001 ^b
SFS7	0.89	0.07	0.11	0.50	
QG	0.49	0.97	0.78	0.62	
QG, <i>P</i> > χ^2	0.14	0.73	0.30	0.47	

^aSignificance level of the Wald *F*-test for the effect of genotypes on carcass and meat quality traits. For QG, a χ^2 test for the effects of genotypes also was performed.

^bFor tenderness of LM, a significant interaction between E2JW and E2FB was found. The genotypes for these two SNP were analyzed jointly.

Table 5. Association of single nucleotide polymorphisms in the leptin gene with lean yield (LEANYL), fat yield (FATYL), and grade fat (GFAT) in the beef cattle population

Item	Trait						
	FATYL, %	No. ^a	GFAT, mm	No.	LEANYL, %	No.	
Polygenic <i>h</i> ²	0.62 ± 0.14		0.45 ± 0.15		0.52 ± 0.14		
SNP least squares means							
E2JW	AT	22.4 ± 0.87 ^y	69	8.9 ± 0.59 ^y	70	58.6 ± 0.90 ^y	69
	AA	23.9 ± 0.69 ^z	845	10.1 ± 0.46 ^z	853	56.8 ± 0.71 ^z	845
E2FB	CC	21.8 ± 1.00 ^y	309	8.7 ± 0.69 ^y	313	59.1 ± 1.04 ^y	309
	CT	24.0 ± 0.78 ^z	449	10.2 ± 0.54 ^z	454	57.1 ± 0.81 ^z	449
	TT	23.7 ± 0.88 ^{yz}	156	9.7 ± 0.61 ^{yz}	156	56.9 ± 0.91 ^z	156
UASMS1	CC	22.9 ± 1.05 ^{yz}	133	9.5 ± 0.73 ^y	133	58.1 ± 1.10 ^y	133
	CT	22.3 ± 0.84 ^y	449	9.1 ± 0.57 ^y	453	58.3 ± 0.87 ^y	449
	TT	24.4 ± 0.81 ^z	332	10.1 ± 0.56 ^y	337	56.7 ± 0.85 ^y	332
Breed least squares means							
Simmental	20.1 ± 1.12		8.2 ± 0.77		59.1 ± 1.16		
Limousin	22.4 ± 1.33		9.4 ± 0.87		59.9 ± 1.34		
Charolais	23.3 ± 1.26		9.4 ± 0.86		58.3 ± 1.30		
Angus	26.9 ± 1.02		11.2 ± 0.68		53.5 ± 1.04		
<i>P</i> -value for SNP and fixed effects							
E2JW	0.010*		0.006*		0.003*		
E2FB	0.013		0.016		0.038		
UASMS1	0.012		0.08		0.11		
Breed ^b	0.011		0.006		0.009		
Sex	0.27		0.70		0.61		
Slgr ^c	0.000		0.000		0.000		

*Significant effect of genotype after modified Bonferroni correction for multiple tests, *P* = 0.05.

^aNumber of animals.

^bTest for the regression coefficients on the breed composition. Only the probability for the largest Wald *F*-test is shown.

^cSlgr is the effect of slaughter group.

^{y,z}Within a column, for each SNP, means without a common superscript letter differ, *P* < 0.05, for multiple comparisons.

Table 6. Association of single nucleotide polymorphisms in the leptin gene with tenderness (LM shear force; SFL) in the beef cattle population measured at different postmortem days (2, 7, 14, and 21 d)

Item	Trait							
	SFL2, kg	No. ^a	SFL7, kg	No.	SFL14, kg	No.	SFL21, kg	No.
Polygenic h ²	0.37 ± 0.14		0.09 ± 0.10		0.39 ± 0.14		0.14 ± 0.10	
E2JW.E2FB SNP least squares means								
AA.CC	5.03 ± 0.46 ^x	240	4.48 ± 0.28 ^x	299	3.91 ± 0.26 ^{xy}	298	3.56 ± 0.20 ^x	296
AA.CT	5.20 ± 0.39 ^x	328	4.57 ± 0.23 ^x	394	3.81 ± 0.21 ^x	394	3.60 ± 0.17 ^x	390
AA.TT	5.56 ± 0.45 ^x	105	5.03 ± 0.28 ^x	126	4.46 ± 0.26 ^{yz}	126	3.62 ± 0.20 ^x	126
AT.CC	—	0	—	1	—	1	—	1
AT.CT	5.00 ± 0.45 ^x	31	4.41 ± 0.27 ^x	41	3.92 ± 0.26 ^{xy}	41	3.42 ± 0.21 ^x	41
AT.TT	6.98 ± 0.53 ^y	16	5.53 ± 0.34 ^x	24	4.97 ± 0.31 ^z	24	4.11 ± 0.25 ^x	24
<i>P</i> -value for SNP and fixed effects								
E2JW.E2FB	0.005*		0.06		0.009*		0.09	
Breed ^b	0.031		0.04		0.18		0.13	
Sex	0.25		0.86		0.76		0.17	
Slgr ^c	0.000		0.000		0.000		0.000	

*Significant effect of genotype after modified Bonferroni correction for multiple tests, $P = 0.05$.

^aNumber of animals.

^bTest for the regression coefficients on the breed composition. Only the probability for the largest Wald F -test is shown.

^cSlgr is the effect of slaughter group.

^{x,y,z}Within a column, means without a common superscript letter differ, $P < 0.05$, for multiple comparisons.

the heterozygous genotype were all significant ($P < 0.05$) and correspond to 0.43, 0.44, and 0.40 phenotypic SD of the corresponding traits, respectively.

For UASMS1, the C allele was associated with less FATYL, with the estimated difference between the homozygous genotypes CC and TT equal to -1.5% ($P < 0.05$). The heterozygous genotype had similar FATYL as the homozygous CC genotype, indicating a large degree of dominance of C over T. There was a trend ($P < 0.15$) that the C allele might be associated with less GFAT and more LEANYL. The estimated differences between the homozygous genotypes CC and TT were -0.6 mm and 1.4% for GFAT and LEANYL, respectively.

Differences between genotypes for E2JW were also significant considering the modified Bonferroni correction for multiple tests, which was not the case for E2FB and UASMS1, indicating stronger evidence for the association of E2JW genotypes with FATYL, GFAT, and LEANYL than for E2FB and UASMS1.

Table 5 shows that there was a significant effect of breed on FATYL, GFAT, and LEANYL. As expected (Burrow et al., 2001), Angus was the fattest breed with the least LEANYL. Simmental had the least FATYL and GFAT, followed by Limousin and Charolais; however, Limousin showed the greatest LEANYL. There was no significant effect of sex on FATYL, GFAT, and LEANYL, likely because this effect was partially confounded with slaughter group, which had a highly significant effect (Table 5).

The analyses of LM shear force in each particular postmortem day showed that the joint E2JW.E2FB genotypes had a significant effect on tenderness. Table 6 presents the least squares means for the E2JW.E2FB

genotypes, with the corresponding significance levels for the Wald F -tests.

Genotype AT.TT was significantly associated with tougher LM. Differences in shear force between genotypes AT.TT and AT.CT were substantial (1.98, 1.12, 1.05, and 0.69 kg for SFL2, SFL7, SFL14, and SFL21, respectively). Estimates for genotype AT.CC were not obtained because there was only one animal with this genotype. Differences in shear force between genotypes AA.TT and AA.CT were smaller and mostly nonsignificant. The magnitude of the differences between AT.TT vs. AT.CT and AA.TT vs. AA.CT illustrates the interaction between the E2JW and E2FB SNP, where a larger difference exists for the AT E2JW SNP genotype. Estimates for genotypes AA.CT and AA.CC were not significantly different.

Table 6 also gives the estimated polygenic heritabilities, which for SFL7 and SFL21 were lower than for other postmortem days, but were within the expected literature range (Burrow et al., 2001).

Results for average shear force over the four postmortem measures (SFLavg) are shown in Table 7. In addition to the genotypes' least squares means, the means for breeds also are presented. Results for E2JW.E2FB genotypes were in line with those found within the different postmortem days, with the genotype AT.TT having the toughest LM over the entire postmortem period.

To further investigate the effects of SNP genotypes on tenderness, a linear regression of the repeated measures of shear force on postmortem days for each animal was estimated and the individual intercepts (**SFLin**) and slopes (**SFLsl**) were analyzed by Model [1]. Results presented in Table 7 show a significant effect of E2J-

Table 7. Association of single nucleotide polymorphisms in the leptin gene with average tenderness of LM across different postmortem days (SFLavg) and with the intercept (SFLin) and slope (SFLsl) of the regression of tenderness measurements on days postmortem

Item	Trait					
	SFLavg, kg	No. ^a	SFLin, kg	No.	SFLsl, kg	No.
Polygenic h ²	0.42 ± 0.15		0.30 ± 0.13		0.08 ± 0.10	
E2JW.E2FB SNP least squares means						
AA.CC	4.19 ± 0.27 ^x	239	4.91 ± 0.36 ^x	299	-0.064 ± 0.017 ^x	299
AA.CT	4.28 ± 0.23 ^x	325	5.09 ± 0.29 ^x	394	-0.078 ± 0.014 ^x	394
AA.TT	4.53 ± 0.26 ^x	105	5.85 ± 0.35 ^y	126	-0.106 ± 0.017 ^x	126
AT.CC	—	0	—	1	—	1
AT.CT	4.04 ± 0.26 ^x	31	5.00 ± 0.36 ^{xy}	41	-0.076 ± 0.018 ^x	41
AT.TT	5.36 ± 0.31 ^y	16	6.59 ± 0.43 ^z	24	-0.123 ± 0.021 ^x	24
Breed least squares means						
Simmental	5.43 ± 0.34		7.28 ± 0.50		-0.16 ± 0.02	
Limousin	4.60 ± 0.37		5.82 ± 0.54		-0.10 ± 0.03	
Charolais	4.50 ± 0.36		5.45 ± 0.54		-0.10 ± 0.03	
Angus	4.84 ± 0.32		6.12 ± 0.46		-0.12 ± 0.02	
<i>P</i> -value for SNP and fixed effects						
E2JW.E2FB	0.001*		0.007*		0.20	
Breed ^b	0.11		0.046		0.13	
Sex	0.10		0.76		0.94	
Slgr ^c	0.000		0.000		0.000	

*Significant effect of genotype after modified Bonferroni correction for multiple tests, $P = 0.05$.

^aNumber of animals.

^bTest for the regression coefficients on the breed composition. Only the probability for the largest Wald F -test is shown.

^cSlgr is the effect of slaughter group.

^{x,y,z}Within a column, means without a common superscript letter differ, $P < 0.05$, for multiple comparisons.

W.E2FB genotypes on the intercept, but not on the slope, indicating that E2JW.E2FB genotypes did not influence the effect of aging on beef tenderization. The heritabilities of the slope and intercept of LM tenderness on aging times (Table 7) indicate that the intercept is moderately heritable, whereas the slope has low heritability.

There was a trend for a breed effect on tenderness (Table 7), with Simmental having the toughest LM. Slaughter group had a highly significant effect on tenderness, and sex was not significant, likely due to the partial confounding of sex with slaughter group.

Assuming the estimated allele frequencies for E2FB, E2JW, and UASMS1, and using the estimated additive ($a = \frac{1}{2}$ homozygous genotype 1 - $\frac{1}{2}$ homozygous genotype 2) and dominance deviation ($d = \text{heterozygous genotype} - [\frac{1}{2} \text{ homozygous genotype 1} + \frac{1}{2} \text{ homozygous genotype 2}]$) effects for the alleles, the percentage of phenotypic variation explained by each polymorphism was calculated using standard formula (Falconer and Mackay, 1996): $\%V = 100 \times (2pq [a + d(q - p)]^2 + [2pqd]^2) / \sigma_p^2$, where $\%V$ is the percentage of phenotypic variation explained by the polymorphism, and σ_p^2 is the phenotypic variance of the trait. For E2JW, as the TT genotype effect was not estimated, it was assumed either that the T allele shows complete dominance over C, or that the T allele has an additive effect only.

The $\%V$ for tenderness explained by genotypes E2JW and E2FB was determined considering SNP main ef-

fects only. The least squares means for E2JW genotypes (AA and AT) were 4.33 ± 0.21 and 4.70 ± 0.24 kg, respectively, and for E2FB genotypes (CC, CT, and TT) were 4.19 ± 0.27 , 4.16 ± 0.23 , and 4.95 ± 0.26 kg, respectively.

Genotypes for E2FB explained 3.8, 3.9, 3.7, and 8.4% of the phenotypic variance for FATYL, GFAT, LEANYL, and SFLavg, respectively. Genotypes for E2JW explained either 0.7, 1.0, 1.0, and 1.0%, or 0.6, 0.9, 0.9, and 0.9%, when either additive or complete dominance effects of the T allele were assumed. The UASMS1 SNP explained 3.6% of phenotypic variance for FATYL. Thus, the expected percentage of the phenotypic variation explained by E2JW was much smaller than that explained by E2FB for all traits, but this was mainly because the T allele in E2JW is rare. If, however, frequency of the T allele increased, for instance by selection, to 0.50 and assuming an additive effect and no change in phenotypic variance, then E2JW genotypes would explain 4.3, 6.3, 6.4, and 6.5% of the phenotypic variation for FATYL, GFAT, LEANYL, and SFLavg, respectively.

Haplotype Analyses

The linear effect of each of 10 haplotypes was estimated. There were three highly frequent haplotypes in the beef population (88% of all haplotypes) whose effects did not differ for any trait analyzed, even though they differ with respect to the alleles in all SNP, except

Table 8. Association of haplotypes for single nucleotide polymorphisms in the leptin gene with lean yield (LEANYL), fat yield (FATYL), and grade fat (GFAT) in the beef cattle population

Item	Trait		
	FATYL, %	GFAT, mm	LEANYL, %
Polygenic h^2	0.61 ± 0.14	0.43 ± 0.14	0.54 ± 0.14
Least squares means of the most frequent haplotypes ^{ab}			
Haplotype 1	25.19 ± 1.03	10.79 ± 0.71	55.79 ± 1.07
Haplotype 2	25.05 ± 1.03	10.52 ± 0.71	56.16 ± 1.07
Haplotype 3	25.30 ± 1.05	10.83 ± 0.72	55.77 ± 1.09
Significant estimated haplotype contrast			
7 - (1 + 2 + 3)/3 ^b	-2.26 ± 0.84	-1.84 ± 0.58	2.42 ± 0.87
<i>P</i> -value ^c	0.007*	0.002*	0.006*
SNP allele contrasts			
E2JW A - T, [7 + 8 - (2 + 6)]/2 ^b	2.07 ± 0.81	1.41 ± 0.56	-2.87 ± 0.85
<i>P</i> -value ^c	0.011	0.012	0.001*
E2FB C - T, [4 + 3 + 1 - (2 + 6 + 5)]/3 ^b	-1.77 ± 0.85	-1.02 ± 0.59	1.71 ± 0.89
<i>P</i> -value ^c	0.036	0.09	0.05
UASMS1 C - T, [4 + 9 + 2 - (1 + 6 + 5)]/3 ^b	-1.29 ± 0.99	-0.91 ± 0.69	0.89 ± 1.03
<i>P</i> -value ^c	0.19	0.19	0.39
UASMS2 C - T, [5 + 1 + 2 - (6 + 3 + 9)]/3 ^b	0.12 ± 0.73	0.47 ± 0.51	0.63 ± 0.76
<i>P</i> -value ^c	0.87	0.36	0.41

*Significant effect after modified Bonferroni correction for multiple tests, $P = 0.05$.

^aDifferences among the least squares means of the most frequent haplotypes were all nonsignificant.

^bSee Table 2 for haplotype codes.

^cSignificance level of the *t*-test.

E2JW. This may indicate an effect of another SNP linked to the four SNP or some degree of epistasis among the SNP within the same chromosome. The average effect of the three common haplotypes was used as a control and all other haplotypes were contrasted against this average.

Differences in allele effects within each SNP were obtained through a linear contrast of haplotype solutions, which differed by only one allele at a given SNP. Haplotype 10 was not used in these contrasts because it comprised the joint effect of several rare haplotypes.

None of the other haplotypes significantly differed from the three most frequent haplotypes in their effect on LMA, BONEYL, CF, HCW, SFS7, and QG (data not shown). For FATYL, GFAT, and LEANYL, the effect of Haplotype 7 (CCTT) was significantly different from the three most frequent haplotypes in the population as shown in Table 8. Replacing the three most frequent haplotypes by Haplotype 7 would significantly decrease FATYL and GFAT by -2.26% and -1.84 mm, respectively, and increase LEANYL by +2.42%, corresponding to 0.44, 0.55, and 0.48 phenotypic SD of the corresponding traits, respectively.

Table 8 also shows the estimates of differences in the effects of the alleles within each SNP. In agreement with the genotype analyses, the T allele for E2JW decreased FATYL and GFAT and increased LEANYL compared with the A allele. With respect to E2FB, the results also agreed with the genotype analyses, where the C allele decreased FATYL and GFAT and increased LEANYL compared with the T allele.

Differences in the effects of UASMS1 alleles on FATYL, GFAT, and LEANYL were all nonsignificant. Nevertheless, the genotype analyses showed that UASMS1 genotypes significantly influenced FATYL. The contrast between haplotype effects is, however, estimating only the additive linear effect of the alleles.

For LM shear force at 2 and 14 d postmortem and for the average shear force over the 21 d postmortem, the effect of Haplotype 8 (TTTT) was significantly different from the three most frequent haplotypes in the population as shown in Table 9. Replacing the three most frequent haplotypes with Haplotype 8 would significantly decrease tenderness, increasing SFL2, SFL14, and SFLavg by 1.06, 0.58, and 0.55 kg, corresponding to 0.62, 0.46, and 0.53 phenotypic SD of the corresponding measurements, respectively.

Table 9 also shows the estimates of differences in the effects of the alleles within each SNP. In agreement with the genotype analyses, the T allele for E2JW increased toughness compared with the A allele. There were no significant differences between alleles for E2FB. The genotype analysis showed a significant interaction between the E2JW and E2FB SNP, which is not accounted for when estimating allele effects by contrasts between haplotype effects. With respect to the other two SNP, the allele differences also were nonsignificant.

Analysis of the intercept and slope of individual regressions of shear force measurements on days postmortem (data not shown) revealed that the effect of Haplotype 8 (TTTT) was significantly different from the

Table 9. Association of haplotypes for single nucleotide polymorphisms in the leptin gene with LM shear force in the beef cattle population

Item	Trait ^a		
	SFL2, kg	SFL14, kg	SFLavg, kg
Polygenic h ²	0.37 ± 0.14	0.37 ± 0.13	0.39 ± 0.15
Least squares means of the most frequent haplotypes ^{bc}			
Haplotype 1	4.87 ± 0.52	4.06 ± 0.28	3.84 ± 0.31
Haplotype 2	4.72 ± 0.51	4.02 ± 0.28	3.90 ± 0.30
Haplotype 3	4.77 ± 0.52	3.93 ± 0.28	3.93 ± 0.30
Significant estimated haplotype contrast			
8 - (1 + 2 + 3)/3 ^c	1.06 ± 0.41	0.58 ± 0.28	0.55 ± 0.24
<i>P</i> -value ^d	0.009*	0.037	0.021
SNP allele contrasts			
E2JW A - T, [7 + 8 - (2+6)]/2 ^c	-0.72 ± 0.33	-0.36 ± 0.22	-0.40 ± 0.20
<i>P</i> -value ^d	0.031	0.10	0.041
E2FB C - T, [4 + 3 + 1 - (2 + 6 + 5)]/3 ^c	0.24 ± 0.42	-0.15 ± 0.23	0.09 ± 0.25
<i>P</i> -value ^d	0.56	0.50	0.72
UASMS1 C - T, [4 + 9 + 2 - (1 + 6 + 5)]/3 ^c	0.30 ± 0.47	-0.13 ± 0.27	0.10 ± 0.28
<i>P</i> -value ^d	0.53	0.63	0.71
UASMS2 C - T, [5 + 1 + 2 - (6 + 3 + 9)]/3 ^c	0.21 ± 0.32	0.02 ± 0.20	0.10 ± 0.19
<i>P</i> -value ^d	0.51	0.93	0.59

*Significant effect after modified Bonferroni correction for multiple tests, $P = 0.05$.

^aSFL2, SFL14, and SFLavg = LM shear force at 2 and 14 d postmortem, and average shear force over a 21-d postmortem period, respectively.

^bDifferences among the least squares means of the most frequent haplotypes were all nonsignificant.

^cSee Table 2 for haplotype codes.

^dSignificance level of the *t*-test.

effect of the most frequent haplotypes for the intercept (0.96 ± 0.39 kg), and that there was a significant difference between the alleles T and A (0.57 ± 0.30 kg) for E2JW, but not for the other SNP. There were no significant differences in the haplotype effects on the slope of the regressions.

Adjustment to Different End Points

Analyses also were carried out adjusting records for either a common HCW or a common slaughter age through the inclusion of a fixed linear regression on either HCW or slaughter age in Model [1] (data not shown). Results were, however, very similar to those from the analyses without adjustment (Tables 4 to 7). For instance, the significance levels for the Wald *F*-tests for the effects of E2JW, E2FB, and UASMS1 genotypes on FATYL were equal to 0.010, 0.012, and 0.014 adjusting for HCW, and equal to 0.009, 0.019, and 0.020 adjusting for slaughter age. The least squares means for E2JW (AT and TT), E2FB (CC, CT, and TT), and UASMS1 (CC, CT, and TT) genotypes were 22.4 and 23.9%; 21.8, 24.0, and 23.7%; and 22.8, 22.3, and 24.4% respectively, adjusting for HCW. Adjusting for slaughter age, the same features were 22.9 and 24.4%; 22.4, 24.5, and 24.2%; and 23.4, 22.8, and 24.8%, respectively. Therefore, differences between genotypes were similar when two alternative endpoints were considered.

Discussion

Results reported are statistical associations whose biological and genetic validity cannot be proven within

the scope of this study. Although two different analyses (genotype and haplotype) were carried out, strengthening the validity of some associations found, the nature of the quantitative data analyzed, and the intrinsic difficulties of this type of analyses preclude strong inferences on the biological and genetic meaning of the associations. Nonetheless, the findings constitute important evidence that, together with other literature reports, could lead to new investigations to better understand these associations, which would include the exclusion of possible effects of other nearby polymorphisms in linkage disequilibrium with the SNP investigated in this study.

The association of E2FB with FATYL, GFAT, and LEANYL found in the current study corroborates the results reported by Nkrumah et al. (2004), where E2FB was genotyped in 144 commercial cattle from five genetic lines with different foundation breeds. These authors concluded that animals carrying the T allele vs. the C allele produce carcasses with poorer grades and lower lean meat yields, but which do not differ in carcass marbling. Buchanan et al. (2002) also reported a significant E2FB genotype effect on grade fat and average fat (mean value of three measures of backfat thickness along the 12th rib), with the T allele associated with higher fat, but with no significant association with carcass marbling score. Crews et al. (2004), however, did not find association of E2FB with carcass traits of 433 Charolais and Charolais-cross steers, which included backfat thickness and carcass marbling.

As in the current study, Crews et al. (2004) did not find a significant association of E2FB with HCW and

LMA. These authors also did not find an association of E2FB with either DMI or residual feed intake, which agrees with the results of Nkrumah et al. (2004), who reported only a trend ($P > 0.10$) of TT animals having positive residual feed intake.

Strong evidence of an association of E2JW with FATYL, GFAT, and LEANYL was found in the current study. In the original study that described this SNP, however, Lagonigro et al. (2003) reported a nonsignificant association of E2JW with the percentage of s.c. and ultrasound backfat thickness (at 10 mo of age) from 169 Holstein-Charolais F₂ bull calves. The same authors also reported no significant association of E2JW with carcass i.m. fat and marbling score, which agrees with the results of the present study, showing no association of E2JW with either CF or QG.

Lagonigro et al. (2003) reported a significant association of E2JW with the average feed intake of bull calves from 6 to 12 mo of age, with the AT genotype having higher daily intake than the AA genotype. The findings of the current investigation showed, however, that AT animals had lower FATYL and GFAT and higher LEANYL than AA animals, which might create the expectation that AT animals would have lower feed intake, if growth rate was unchanged, which is contrary to the findings of Lagonigro et al. (2003).

A significant association of E2FB and E2JW with tenderness of LM across aging times was found in the current investigation. In fact, these two SNP interacted in their effect on tenderness. No other results were found in the available literature on the effect of these two SNP on beef tenderness for comparison.

Phenotypic and genetic relationships between marbling and tenderness (measured by either shear force evaluation or taste panel) are not especially high, but show favorable direction (Bertrand et al., 2001), indicating that higher marbling is slightly associated with higher tenderness. Analyses of LM tenderness also were carried out adjusting records for either CF or slaughter age through the inclusion of a fixed linear regression on either CF or slaughter age in Model [1] (data not shown). Results were, however, very similar to those from the analyses without adjustment. For instance, the probabilities for Wald F -tests for the effects of the joint E2JW.E2FB genotypes on SFLav were equal to 0.001, adjusting for either CF or slaughter age. The least squares means for E2JW.E2FB genotypes (AA.CC, AA.CT, AATT, AT.CT, and AT.TT) were 4.12, 4.23, 4.50, 3.99, and 5.28 kg, adjusting for CF, respectively. The same features adjusting for slaughter age were 4.14, 4.23, 4.49, 3.99, and 5.31 kg, respectively.

Results indicate that the estimated genotype effects on tenderness were not due to differences in marbling or age of the animals. An association of the leptin receptor gene with pork meat tenderness was reported by Choi et al. (2003). These authors found an association between microsatellite polymorphisms within the leptin receptor gene and LM shear force of 354 Korean Native × Landrace F₂ boars at 12 wk of age.

In the present study, UASMS2 was not significantly associated with any of the carcass and meat quality traits considered. Nevertheless, Crews et al. (2004) reported a significant association of UASMS2 with carcass marbling score, LM area, and HCW of 433 Charolais and Charolais-cross steers, and Nkrumah et al. (2005) reported significant association of UASMS2 with ultrasound backfat thickness and marbling score of 150 crossbred animals (131 steers and 19 bulls). Contrasting results, such as these across studies, illustrate the need to validate associations across different populations before adoption is practical in widespread industry breeding programs. Crews et al. (2004) and Nkrumah et al. (2005) also reported a significant association of UASMS2 with daily DM intake and residual feed intake, indicating that UASMS2 might be associated with feed efficiency.

The UASMS1 SNP (or, alternatively, UASMS3) was significantly associated with FATYL and tended to have relationships with GFAT and LEANYL. In agreement with the present results, Nkrumah et al. (2005) reported a significant association of UASMS3 SNP with ultrasound backfat thickness. The same authors also reported an association of UASMS3 with daily DMI and BW.

Our findings confirm the association previously reported in the literature of the E2FB leptin exon 2 SNP with carcass lean meat yield and fatness (fat yield and grade fat). This SNP alone explains approximately 4% of the phenotypic variation for these traits. The T allele is associated with lower lean meat yield and higher fatness; however, the increased fatness does not translate into higher i.m. fat (marbling).

The other SNP in the leptin exon 2, E2JW, also is associated with carcass lean meat yield and fatness (fat yield and grade fat), with no association with i.m. fat. The T allele is implicated in higher lean meat yield and lower fatness. This SNP alone explains approximately 1% of the phenotypic variation for these traits. This allele is, however, quite rare, and the variance associated with this SNP increases to 4 to 6% of phenotypic variance if the frequency of the T allele was to increase to 50%, with an assumed additive effect.

The E2JW and E2FB polymorphisms are associated with tenderness of LM, and they interact in their effect. Individually, these two SNP explain approximately 1 and 8% of the phenotypic variation on tenderness, respectively. As mentioned before, the E2JW T allele is quite rare. The variance associated with this SNP would increase substantially as the T allele moves to a more intermediate frequency. Nevertheless, no other studies on the effect of these SNP on tenderness of beef cattle have been published, and no QTL for tenderness in BTA 4 have been reported. Clearly, more investigation is needed to confirm the results for tenderness found in this investigation to exclude, for instance, possible effects of other genes in linkage disequilibrium with the leptin SNP.

Two SNP in the leptin promoter, UASMS1 and UASMS3, are completely linked in the population and are significantly associated with fat yield. Another leptin promoter polymorphism, UASMS2, is not significantly associated in this population with any carcass and meat quality traits analyzed, which disagrees with two previously reported studies on this polymorphism.

Three particular haplotypes (TCAC, CCAT, and TTAC) within the leptin gene are highly frequent in the population and do not differ in their effects on carcass and meat quality traits, even though they carry different alleles. This might indicate the effect of other SNP linked to the four SNP considered in this study or some degree of epistasis among the SNP within the same chromosome.

Implications

Important associations between two single nucleotide polymorphisms within the leptin exon 2 and lean yield were detected. Current genetic improvement schemes that use estimated breeding values from ultrasound carcass data could potentially benefit through the use of these associations. The significant associations of those polymorphisms with tenderness are even more interesting, as current improvement programs for this potentially important trait are quite limited. Results confirm some of the previously reported associations, but diverge with respect to others, showing that further efforts are required to validate some prospective associations.

Literature Cited

- Baile, C. A., M. A. Della-Fera, and R. J. Martin. 2000. Regulation of metabolism and body fat mass by leptin. *Annu. Rev. Nutr.* 20:105–127.
- Bertrand, J. K., R. D. Green, W. O. Herring, and D. W. Moser. 2001. Genetic evaluation of beef carcass traits. *J. Anim. Sci.* 79(E. Suppl.):E190–E200.
- Boettcher, P. J., G. Pagnacco, and A. Stella. 2004. A Monte Carlo approach for estimation of haplotype probabilities in half-sib families. *J. Dairy Sci.* 87:4303–4310.
- Buchanan, F. C., C. J. Fitzsimmons, A. G. Van Kessel, T. D. Thue, D. C. W. Sim, and S. M. Schmutz. 2002. Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. *Genet. Sel. Evol.* 34:105–116.
- Burrow, H. M., S. S. Moore, D. J. Johnston, W. Barendse, and B. M. Bindon. 2001. Quantitative and molecular genetic influences on properties of beef: A review. *Aust. J. Exp. Agric.* 41:893–919.
- Choi, B. H., T. H. Kim, Y. M. Cho, H. Y. Lee, J. T. Jeon, and I. C. Cheong. 2003. Association study between porcine LEPR-derived microsatellite polymorphisms and economic traits. *J. Anim. Sci. Tech.* 45:679–688.
- Crews, D. H., J. D. Nkrumah, J. Yu, and S. S. Moore. 2004. Association of single nucleotide polymorphisms in the bovine leptin gene with feedlot and carcass characteristics of crossbred steers. *Can. J. Anim. Sci.* 84:749–750. (Abstr.)
- Falconer, D. S., and T. F. C. Mackay. 1996. *Introduction to Quantitative Genetics*. Longman Group Ltd., Essex, U.K.
- Fernando, R. L., C. Stricker, and T. Wang. 1998. Detection and utilization of single genes without DNA assays. *J. Dairy Sci.* 81:64–75.
- Fitzsimmons, C. J., S. M. Schmutz, R. D. Bergen, and J. J. McKinnon. 1998. A potential association between the BM1500 microsatellite and fat deposition in beef cattle. *Mamm. Genome* 9:432–434.
- Geary, T. W., E. L. McFadin, D. M. MacNeil, E. E. Grings, R. E. Short, R. N. Funston, and D. H. Keisler. 2003. Leptin as a predictor of carcass composition in beef cattle. *J. Anim. Sci.* 81:1–8.
- Gilmour, A. R., B. R. Cullis, S. J. Welham, and R. Thompson. 2000. *ASREML Reference Manual*. IACR-Rothamsted Experimental Station, Harpenden, U.K.
- Henderson, C. 1984. *Applications of Linear Models in Animal Breeding*. Univ. of Guelph, Guelph, Ontario, Canada.
- Hoelzel, A. R. 1992. *Molecular Genetic Analysis of Populations: A Practical Approach*. Oxford Univ. Press, Oxford, U.K.
- Hossner, K. L. 1998. Cellular, molecular and physiological aspects of leptin: Potential applications in animal production. *Can. J. Anim. Sci.* 78:463–472.
- Houseknecht, K. L., C. A. Baile, R. L. Matteri, and M. E. Spurlock. 1998. The biology of leptin: A review. *J. Anim. Sci.* 76:1405–1420.
- Laborde, F. L., I. B. Mandell, J. J. Tosh, J. W. Wilton, and J. G. Buchanan-Smith. 2001. Breed effects on growth performance, carcass characteristics, fatty acid composition, and palatability attributes in finishing steers. *J. Anim. Sci.* 79: 355–365.
- Lagonigro, R., P. Wiener, F. Pilla, J. A. Woolliams, and J. L. Williams. 2003. A new mutation in the coding region of the bovine leptin gene associated with feed intake. *Anim. Genet.* 34:371–374.
- Mantel, N. 1980. Assessing laboratory evidence for neoplastic activity. *Biometrics* 36:381–399.
- Minton, J. E., D. J. Bindel, J. S. Drouillard, E. C. Titgemeyer, D. M. Grieger, and C. M. Hill. 1998. Serum leptin is associated with carcass traits in finishing cattle. *J. Anim. Sci.* 76(Suppl.):231. (Abstr.)
- Mrode, R. A., C. Smith, and R. Thompson. 1990. Selection for rate and efficiency of lean gain in Hereford cattle. II. Evaluation of correlated responses. *Anim. Prod.* 51:35–46.
- Nkrumah, J. D., C. Li, J. B. Basarab, S. Guercio, Y. Meng, B. Murdoch, C. Hansen, and S. S. Moore. 2004. Association of a single nucleotide polymorphism in the bovine leptin gene with feed intake, feed efficiency, growth, feed behaviour, carcass quality and body composition. *Can. J. Anim. Sci.* 84:211–219.
- Nkrumah, J. D., C. Li, J. Yu, C. Hansen, D. H. Keisler, and S. S. Moore. 2005. Polymorphisms in the bovine leptin promoter associated with serum leptin concentration, growth, feed intake, feeding behavior, and measures of carcass merit. *J. Anim. Sci.* 83:20–28.
- Pomp, D., T. Zou, A. C. Clutter, and W. Barendse. 1997. Rapid communication: mapping of leptin to bovine chromosome 4 by linkage analysis of a PCR-based polymorphism. *J. Anim. Sci.* 75:1427.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning, a Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Shackelford, S. D., T. L. Wheeler, and M. Koohmaraie. 1999. Evaluation of slice shear force as an objective method of assessing beef longissimus tenderness. *J. Anim. Sci.* 77:2693–2699.
- Stone, R. T., S. M. Kappes, and C. W. Beattie. 1996. The bovine homologue of the obese gene maps to chromosome 4. *Mamm. Genome* 7:399–400.
- Utrera, A. R., and L. D. Van Vleck. 2004. Heritability estimates for carcass traits of cattle: A review. *Genet. Mol. Res.* 3:380–394.
- Ye, X. 2003. Identification of quantitative trait loci in swine using a candidate gene approach. Ph.D. Diss., Univ. of Guelph, Guelph, Ontario, Canada.