

ASSOCIATION OF SNPs IN THE *LEPTIN* AND *LEPTIN RECEPTOR* GENES WITH DIFFERENT FAT DEPOTS IN BEEF CATTLE

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INTRODUCTION

The *leptin receptor* gene (*LEPR*) produces a high affinity receptor that mediates the regulation of the *leptin* gene. Leptin, the hormone product of the *leptin* gene, is considered to play a role in the regulation of appetite, energy partition, and body composition of mammals (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 the long term body fat weight and composition (Hossner, 1998). Associations of molecular polymorphisms within exon 2 (Buchanan et al., 2002; Nkrumah et al., 2004; Schenkel et al. 2005) and the promoter region (Crews et al., 2004; Nkrumah et al., 2005) of the *leptin* gene with carcass and meat quality traits were reported in beef cattle. Schenkel et al. (2005) reported significant association of two single nucleotide polymorphisms (SNPs) in the *leptin* exon 2 with subcutaneous fat (grade fat) and fat yield (from 4-bone rib dissection). The objective of this study was to evaluate the association of four previously reported SNPs in the *leptin* gene and one SNP in the *leptin receptor* gene with fat deposition in crossbred beef cattle.

MATERIAL AND METHODS

Cattle. The animals were commercially fed heifers (157), steers (183) and bulls (59) from industry sires and heifers (30), steers (323), and bulls (48) from the University of Guelph breeding project. Animals were crossbred with breed composition formed mainly by Angus, Charolais, Limousin, and Simmental. Commercial cattle represented AI breeding as well as some herd bulls. Animals from the University of Guelph breeding program originated from exclusive AI breeding and were fed post-weaning to a fat constant endpoint at the University of Guelph's Elora Beef Cattle Research Centre. Commercial animals were marketed based on the feedlot operators' definition of finish, which was a visual appraisal of fat cover and weight.

DNA isolation and genotyping. The DNA from frozen steaks (or venous blood from 48 Elora bulls) was isolated using the standard phenol/chloroform method (Hoelzel, 1992). Four SNPs in the *leptin* gene were investigated. Two SNPs, *E2FB* (Buchanan et al., 2002) and *E2JW* (Lagonigro et al., 2003, originally referred to as 252-SNP), were located within the *leptin* exon 2, while *UASMS1* and *UASMS2*, (Nkrumah et al., 2005) were within the *leptin* promoter region. One SNP in exon 20 of *LEPR* (Liefers et al., 2004) was also investigated. 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.). Details of procedures were described by Nkrumah et al. (2005).

Phenotypic information. Measures of grade fat (GFAT, n=794), chemical fat (CFAT, n=789), subcutaneous fat (SFAT, n=773), inter-muscular fat (IFAT, n=773), body fat (BFAT, n=773) and fat yield (FATYL, n=786) were available on most of the genotyped animals. Grade fat was the backfat thickness measurement taken at the 12th and 13th rib interface. Chemical fat was the chemical analysis on a core meat sample from the longissimus muscle that determined the percentage of intra-muscular fat. Subcutaneous fat, inter-muscular fat, body fat, and fat yield were determined by dissection of a 4-bone rib section. The mean and SD of the traits were 9.3

$\pm 3.3\text{mm}$, $4.0 \pm 1.6\%$, $9.7 \pm 2.4\%$, $11.6 \pm 3.1\%$, $2.8 \pm 1.1\%$, and $24.0 \pm 5.0\%$ for GFAT, CFAT, SFAT, IFAT, BFAT, and FATYL, respectively.

Statistical analyses. Descriptive statistics of the analyzed traits and allele frequencies were obtained using SAS (SAS Institute, Inc., Cary, NC). Association of the genotypes with the traits was evaluated using ASREML (Gilmour et al., 2000), fitting the following model:

$$Y_{ijklm} = u + \sum_{j=1}^5 \text{Gen}_{i(j)} + \text{Sex}_k + \text{Slg}_l + \beta_1 \text{AN} + \beta_2 \text{LM} + \beta_3 \text{CH} + \beta_4 \text{SM} + \text{Pol}_m + e_{ijklm}, \text{ where:}$$

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 fixed effect of the i -th genotype for the j -th SNP;

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 (82 levels);

$\beta_1, \beta_2, \beta_3, \beta_4$ are the regression coefficients on percentage of Angus, Charolais, Limousin, and Simmental breed;

Pol_m is the random additive genetic (polygenic) effect of the m -th animal;

e_{ijklm} is the residual random effect associated with the m -th animal.

The additive relationship matrix based on the general pedigree (1,966 animals) was used for modeling the covariances among polygenic effects. Slaughter groups were defined as either animals from the Commercial group with the same slaughter date or animals from Elora coming from the same trial and feed treatment, and killed in the same season (Dec. to Feb., Mar. to May, Jun. to Aug., and Sep. to Nov.) The slaughter date accounted for most of the feedlot of origin variation in the commercial cattle, because the majority of the slaughter dates (90%) had cattle from a single feedlot. Initially two-way interactions between SNPs were fit into the model. As all interactions were not significant they were dropped from the model.

RESULTS AND DISCUSSION

Allele frequencies. Allele frequencies for the *leptin* SNPs were similar to those previously reported and discussed by Schenkel et al. (2005). For *LEPR* SNP, the T allele was quite rare in the population (4.1%) compared to C and only two TT genotypes were observed, which were excluded from the analyses.

Genotype effects. As similarly reported by Schenkel et al. (2005), using data that included the genotyped animals in the current study, *E2JW* and *E2FB* had significant association with GFAT and FATYL and no association with CFAT (Table 1). When looking at the contribution of different fat depots to FATYL by dissection, *E2JW* and *E2FB* were only significantly associated with SFAT ($P=0.007$ and $P=0.06$, respectively). The *E2JW* AT genotype decreased GFAT, SFAT and FATYL (-1.20 mm, -0.84% and -1.41%, respectively) compared to AA. The *E2FB* CC genotype decreased GFAT, SFAT and FATYL (-1.64 mm, -1.30% and -2.19%, respectively) compared to TT. Buchanan et al. (2002) also reported significant association of *E2FB* genotypes with grade fat and average backfat thickness along the 12th rib in beef cattle, but not with marbling fat. Lagonigro et al. (2003) reported non-significant association of *E2JW* with percentage of subcutaneous and ultrasound backfat thickness (at 10 mo of age) from 169 Holstein-Charolais F₂ bull calves. The same authors also reported non-significant association of *E2JW* with carcass intra-muscular fat.

Table 1. Genotype contrasts for grade fat (GFAT), longissimus muscle chemical fat (CFAT), and 4-bone rib dissected inter-muscular fat (IFAT), subcutaneous fat (SFAT), body fat (BFAT) and fat yield (FATYL) for SNPs in the *leptin* gene (*UASMS1*, *UASMS2*, *E2JW*, and *E2FB*) and *leptin receptor* gene (*LEPR*)

SNP	Genotype Contrast	Traits					
		GFAT(mm)	CFAT(%)	IFAT(%)	SFAT(%)	BFAT(%)	FATYL(%)
<i>UASMS1</i>	CC-TT	-1.12 ± 0.82	-0.04 ± 0.43	-0.64 ± 0.79	-0.70 ± 0.59	-0.06 ± 0.25	-1.70 ± 0.78
	CT-TT	-0.82 ± 0.53	0.00 ± 0.27	-0.67 ± 0.51	-0.73 ± 0.40	-0.05 ± 0.16	-1.57 ± 1.23
	P^A	0.28	0.99	0.42	0.16	0.95	0.09
<i>UASMS2</i>	CC-TT	-0.38 ± 0.49	0.01 ± 0.25	0.24 ± 0.47	-0.10 ± 0.35	-0.05 ± 0.15	0.03 ± 0.72
	CT-TT	-0.21 ± 0.46	0.04 ± 0.24	0.18 ± 0.44	0.14 ± 0.33	0.01 ± 0.14	0.27 ± 0.68
	P	0.65	0.98	0.88	0.36	0.76	0.75
<i>E2JW</i>	AT-AA	-1.20 ± 0.43	-0.13 ± 0.23	-0.60 ± 0.41	-0.84 ± 0.31	0.05 ± 0.13	-1.41 ± 0.64
	P	0.006	0.58	0.14	0.007	0.71	0.028
<i>E2FB</i>	CT-TT	-0.39 ± 0.58	0.02 ± 0.30	-0.07 ± 0.55	-0.55 ± 0.41	0.09 ± 0.17	-0.37 ± 0.86
	CC-TT	-1.64 ± 0.78	0.01 ± 0.40	-0.75 ± 0.74	-1.30 ± 0.55	0.05 ± 0.23	-2.19 ± 1.15
	P	0.049	1.00	0.41	0.06	0.84	0.06
<i>LEPR</i>	CT-CC	-1.08 ± 0.38	-0.12 ± 0.20	-0.67 ± 0.37	-0.65 ± 0.28	-0.05 ± 0.11	-1.22 ± 0.56
	P	0.005	0.53	0.07	0.019	0.68	0.034

^AProbability of the F-test for the SNP genotype effect.

The promoter *UASMS1* SNP tended to show association with FATYL ($P=0.09$). The *UASMS1* CC genotype tended to decrease FATYL (-1.70%). Schenkel et al. (2005) reported significant association of *UASMS1* with FATYL in a larger sample of cattle. The promoter *UASMS2* SNP was not significantly associated with any of the traits analyzed. The *LEPR* SNP was associated with GFAT ($P=0.005$), SFAT ($P=0.019$) and FATYL ($P=0.034$), and tended to show association with IFAT ($P=0.07$). As for the *Leptin* SNP, there was no association of *LEPR* SNP with CFAT. The heterozygous genotype CT for *LEPR* SNP decreased GFAT, SFAT, and FATYL (-1.08 mm, -0.65%, and -1.22%, respectively) and tended to decrease IFAT (-0.67%) compared to CC.

CONCLUSION

In the crossbred beef cattle population studied, *E2JW* and *E2FB* *leptin* SNPs and *LEPR* SNP were associated with subcutaneous fat deposition and fat yield, while *UASMS1* SNP tended to show association with fat yield. These four SNPs then may have merit in a selection program to improve yield grade. The studied *leptin* SNPs and the *LEPR* SNP were however not significantly associated with longissimus muscle intra-muscular fat measured by chemical extraction.

ACKNOWLEDGMENT

Researchers acknowledge BIO and OMAFRA for providing data; BIO, NSERC, OCA and OMAFRA for financial support and CFI and OIT for computing infrastructure support. The financial support from the Canada/Alberta BID Fund through grant No. 2000AB364 is also acknowledged.

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