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Association analyses of a SNP in the promoter of *IGF1* with fat deposition and carcass merit traits in hybrid, Angus and Charolais beef cattle

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Summary

A SNP in the promoter region of *insulin like growth factor-1* (*IGF1*) (c.−512C>T) was analysed for associations with 10 fat deposition and carcass merit traits in hybrid (n = 455), Angus (n = 204) and Charolais (n = 186) beef cattle populations. Significant associations of the SNP were found for ultrasound backfat thickness (P = 0.030), carcass average backfat (P = 0.015) and carcass lean meat yield (LMY) (P = 0.023) in the Angus beef population, with the 'CC' genotype showing higher fat depth and lower LMY than the 'TT' genotype. Analyses of transcription factor binding sites based on transcription element search system prediction revealed that the 'C' allele introduces a binding site for nuclear factor I, which has an adipose tissue-specific regulatory role and thus may contribute to the SNP effect on fat deposition in the population of pure Angus cattle, a breed with greater fat depth than the hybrid and Charolais breeds.

Keywords candidate gene, cattle, *insulin like growth factor-1*, single nucleotide polymorphism.

Insulin like growth factor-1 (IGF1) is one of the insulin-like growth factors that have an essential role in regulating animal growth and metabolism (Hossner *et al.* 1997). In beef cattle, serum IGF1 concentration has been found to have significant correlations with fat deposition and carcass merit traits (Anderson *et al.* 1988; Davis & Simmen 2000). The *IGF1* gene was mapped on bovine chromosome (BTA) 5 at 73.5 cM (Grosse *et al.* 1999) and several studies have identified quantitative trait loci regions associated with fat level and carcass traits in the vicinity of *IGF1* in beef cattle (Casas *et al.* 2000; Li *et al.* 2004a). Ge *et al.* (1997) reported a single nucleotide polymorphism (SNP) (C>T) in the promoter region of *IGF1* (Gene Bank Accession no. AF017143) 512 bp upstream from the start codon (c.−512C>T). This SNP was later evaluated for its association with growth traits in beef cattle, with significant associations for weight gain during the first 20 days after weaning and on-test weight in Angus (Ge *et al.* 2001), and a small dominance effect on birth weight in commercial lines of *Bos taurus* (Li *et al.* 2004b). In this study, we further investigated the association of the *IGF1* SNP with fat deposition and carcass merit traits in three unrelated cattle populations including hybrid, Angus and Charolais populations.

The hybrid population consisted of 455 steers of 28 sires from the University of Alberta Kinsella ranch, and the population has been described by Nkrumah *et al.* (2007). The two purebred populations include 204 Angus steers of 18 sires and 186 Charolais steers of 19 sires from the Onefour Research Substation of the Agriculture and Agri-Food Canada Research Centre at Lethbridge. The purebred Angus and Charolais steers were born in 2004 and 2005, and linked to the pedigree databases maintained by the Canadian Angus and Charolais Associations respectively. All animals were managed according to the guidelines established by the Canadian Council of Animal Care (CCAC, 1993). Ultrasound measurements [backfat thickness and rib-eye area (REA)] were taken every 28 days during the feedlot tests. Final ultrasound backfat, ultrasound rib-eye area as well as average daily gain of ultrasound backfat and average daily gain of ultrasound REA, which were obtained by regression analyses, were analysed in this study. Carcass merit traits, which include carcass weight, carcass REA, average backfat (AVBF), lean meat yield (LMY), carcass

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marbling score and slaughter weight were collected as previously described (Nkrumah *et al.* 2007). For the hybrid population, the carcass measurements were available on 381 animals.

DNA samples were prepared using a QuickGene DNA whole blood kit S (DB-S; Intermedico, Markham, Canada) from blood samples collected by venipuncture from each animal. For the hybrid population, parentage identification was performed using a panel of bovine microsatellite markers as described previously (Nkrumah *et al.* 2007). The genotyping of the *IGF1* c.−512C>T SNP marker was performed as described by Ge *et al.* (2001). First, DNA templates were amplified using primers IGF677F (5′-ATTACAAAGCTGCCTGCCCC-3′) and IGF897R (5′-ACCTTACCCGTATGAAAGGAATATACGT-3′). The PCR products were then digested using SnaBI (TAC/GTA) (New England Biolabs, Pickering, Canada) by incubation at 37 °C for 2 h. The fragments were separated on 1.5% agarose gels by electrophoresis with 0.5× TBE buffer and stained using ethidium bromide. The genotype of each animal was determined based on the fragment profile, with allele 'C' uncut (250 bp) and allele 'T' cut (224 + 26 bp).

Associations between the SNP and the 10 ultrasound and carcass traits were examined separately for each population by fitting the following mixed linear regression model using ASReml (Gilmour *et al.* 2000):

$$y = \mathrm{Xb} + \mathrm{Za} + e$$

Where y is the vector of phenotypes for the trait analysed; X is the design matrix for fixed effects; and b is the vector of coefficients of the regression on the fixed effects including the SNP effects. For the hybrid population, other fixed effects included feedlot test batch over 3 years (six levels) and breed (three levels by breed of sire as Angus, Charolais and Hybrid). For the Angus and Charolais populations, other fixed effects included the feedlot test batch over 2 years (eight levels). Z is the incidence matrix for the random animal effects; a is the vector of the polygenic effects, and e is the vector of residuals. The three SNP genotypes, CC, CT and TT, were coded as 0, 1 and 2 respectively, and the SNP allele substitution effect was estimated via the regression analysis. The dominance effect was estimated by subtracting the average of solutions for homozygous genotypes from that of heterozygous genotypes. To adjust the animal's age effect, animal age at slaughter was included in the model as a linear covariate.

The counts of the three SNP genotypes in the three populations are shown in Table 1; the intralocus SNP genotypic frequencies conformed to Hardy–Weinberg equilibrium proportions for all three populations ($P > 0.05$). Among the 10 fat deposition and carcass merit traits examined in the three cattle populations, the alleles of the SNP were found to have significant associations with ultrasound backfat, AVBF and LMY in the Angus population ($P < 0.05$) (Table 1), with an allele substitution effect of −0.57 mm, −1.09 mm and 0.90 % on the three fat-related traits respectively. The 'C' allele, which has a frequency of 0.56 in the Angus population, is associated with significantly higher ultrasound backfat thickness, higher carcass average backfat thickness and lower carcass LMY in comparison to the 'T' allele. Animals with the 'CC' genotype have about 13% more carcass average fat and 3.3% less LMY than animals carrying the 'TT' in the Angus population. However, the SNP was not associated with fat related traits in the hybrid or Charolais populations examined. No associations between the SNP and any other traits were detected in the three beef populations and no significant dominance effects were found on the traits examined for the three beef populations.

A preliminary analysis of the *IGF1* gene sequence using Transcription element search system (TESS, http://www.cbil.upenn.edu/tess) (Schug 2003) revealed that the 'C' allele of the *IGF1* SNP introduces a putative binding site (TCCA) for nuclear factor I (NFI) (Nagata *et al.* 1983). This NFI is a family of multifunctional transcription factors occurring in four isoforms in vertebrates and acting as transcriptional activators or repressors (Gronostajski 2000). In bovine, three NFI transcription factors (NFIA, NFIB and NFIC) have been reported in the databases (http://www.ncbi.nlm.nih.gov/sites/entrez). In general, NFI can act as an activator or repressor for many genes that are ubiquitously expressed as well as hormonally, nutritionally and developmentally regulated (Gronostajski 2000). The adipocyte-specific NFI regulation over gene expression was demonstrated using the 3T3-F442A cell line (Graves *et al.* 1991). Miura *et al.* (2004) also reported the regulatory role of NFI on white adipose tissue-specific gene expression in transgenic mice. In addition, NFI controls the expression of *stearoyl CoA desaturase gene 1* during preadipocyte differentiation in the mouse 3T3 cell line (Singh & Ntambi 1998). This stage of differentiation of precursor cells into mature fat cells is accompanied by enhanced expression of *IGF1* in transgenic mice (Rajkumar *et al.* 1999), which indicates the role of *IGF1* in fat cell developmental processes. In Angus beef cattle, Davis & Simmen (2000) reported that bulls with lower IGF1 concentration had higher backfat thickness. Similarly, circulating IGF1 was found to correlate negatively with carcass fat percentage, fat accretion rate and fat thickness in Simmental crossbred bulls (Anderson *et al.* 1988). In this study, the promoter SNP of *IGF1* was found to be significantly associated with ultrasound and carcass backfat thickness in Angus steers but not in the hybrid and Charolais populations. The three unrelated populations used in this study represent different biological types. In comparison to the hybrid and Charolais breeds, Angus has greater fat depth on average (Table 1), presumably due to the early maturity in Angus, which allows the steers to produce more fat at a younger age (Gregory *et al.* 1994). It remains

Table 1 Least-square means of fat deposition and carcass merit traits and estimated effects of the *IGF1* c.–512C>T SNP in hybrid, Angus and Charolais beef cattle populations.

Trait[1]	Population	*IGF1* genotypes[2]			Allele substitution effect[3]	*P*-value	Dominance effect[4]	*P*-value
		CC	CT	TT				
UBF	Hybrid	8.99 ± 0.42 (58)	9.18 ± 0.25 (232)	9.25 ± 0.28 (165)	0.11 ± 0.22	0.652	0.31 ± 0.28	0.894
	Angus	15.98 ± 0.40 (60)	15.73 ± 0.32 (106)	14.70 ± 0.49 (38)	−0.57 ± 0.27	0.030*	0.24 ± 0.34	0.400
	Charolais	8.08 ± 0.45 (33)	8.41 ± 0.29 (97)	8.20 ± 0.34 (56)	0.96 ± 0.24	0.903	0.25 ± 0.30	0.351
UREA	Hybrid	83.87 ± 1.03	82.77 ± 0.54	83.74 ± 0.64	0.22 ± 0.57	0.711	−1.01 ± 0.70	0.173
	Angus	80.67 ± 0.97	80.77 ± 0.81	81.50 ± 1.19	0.36 ± 0.66	0.633	−0.21 ± 0.80	0.779
	Charolais	81.37 ± 1.25	84.40 ± 0.75	83.94 ± 0.92	0.97 ± 0.72	0.104	1.37 ± 0.92	0.188
AUBF	Hybrid	0.03 ± 0.002	0.03 ± 0.001	0.03 ± 0.001	0.24 ± 0.12	0.858	0.27 ± 0.15	0.786
	Angus	0.07 ± 0.004	0.07 ± 0.003	0.06 ± 0.005	−0.30 ± 0.28	0.286	0.51 ± 0.37	0.875
	Charolais	0.03 ± 0.004	0.03 ± 0.002	0.03 ± 0.003	0.30 ± 0.22	0.679	0.26 ± 0.29	0.395
AUREA	Hybrid	0.16 ± 0.007	0.16 ± 0.003	0.16 ± 0.004	0.10 ± 0.36	0.926	−0.21 ± 0.45	0.563
	Angus	0.21 ± 0.011	0.20 ± 0.008	0.20 ± 0.014	−0.36 ± 0.84	0.711	−0.13 ± 0.11	0.200
	Charolais	0.21 ± 0.014	0.22 ± 0.008	0.22 ± 0.010	0.50 ± 0.84	0.354	−0.40 ± 0.11	0.608
SWT	Hybrid	547.8 ± 7.58	542.4 ± 5.03	544.3 ± 5.60	−0.95 ± 3.80	0.824	−2.86 ± 4.61	0.656
	Angus	561.7 ± 4.43	566.1 ± 3.63	566.0 ± 5.51	2.53 ± 3.09	0.585	2.79 ± 3.84	0.227
	Charolais	567.1 ± 6.87	567.5 ± 4.18	561.0 ± 5.10	−3.75 ± 3.86	0.289	4.42 ± 4.86	0.336
CWT	Hybrid	317.4 ± 4.52	312.5 ± 3.02	314.1 ± 3.35	−0.56 ± 2.26	0.823	−2.55 ± 2.74	0.442
	Angus	327.8 ± 2.95	330.0 ± 2.42	328.4 ± 3.64	0.60 ± 2.03	0.994	2.02 ± 2.50	0.185
	Charolais	332.6 ± 4.68	336.2 ± 2.72	333.7 ± 3.42	−0.95 ± 2.75	0.994	2.95 ± 3.58	0.413
AVBF	Hybrid	11.50 ± 0.58	12.14 ± 0.31	12.15 ± 0.37	0.17 ± 0.32	0.611	0.167 ± 0.40	0.607
	Angus	18.20 ± 0.60	16.90 ± 0.49	16.09 ± 0.76	−1.09 ± 0.44	0.015*	−0.48 ± 0.56	0.387
	Charolais	7.25 ± 0.64	8.26 ± 0.37	7.69 ± 0.45	0.92 ± 0.38	0.869	0.73 ± 0.49	0.135
LMY	Hybrid	58.49 ± 0.56	57.93 ± 0.34	57.85 ± 0.39	−0.24 ± 0.30	0.430	−0.89 ± 0.36	0.729
	Angus	52.88 ± 0.53	53.90 ± 0.42	54.65 ± 0.67	0.90 ± 0.39	0.023*	0.33 ± 0.51	0.520
	Charolais	62.45 ± 0.63	62.15 ± 0.37	62.56 ± 0.46	0.11 ± 0.38	0.692	−0.37 ± 0.51	0.435
CREA	Hybrid	85.14 ± 1.23	83.83 ± 0.77	83.60 ± 0.87	−0.73 ± 0.64	0.257	−0.20 ± 0.78	0.806
	Angus	81.30 ± 1.00	82.45 ± 0.78	83.14 ± 1.29	0.95 ± 0.77	0.252	0.44 ± 1.05	0.586
	Charolais	91.78 ± 1.81	94.51 ± 1.07	94.26 ± 1.05	0.98 ± 1.05	0.291	1.14 ± 1.35	0.447
CMAR	Hybrid	2.44 ± 0.08	2.48 ± 0.05	2.50 ± 0.05	0.16 ± 0.40	0.690	−0.15 ± 0.49	0.962
	Angus	3.39 ± 0.07	3.29 ± 0.06	3.37 ± 0.09	−0.20 ± 0.51	0.722	−0.88 ± 0.62	0.144
	Charolais	2.44 ± 0.11	2.45 ± 0.07	2.49 ± 0.08	0.26 ± 0.59	0.797	−0.24 ± 0.73	0.828

[1]UBF, ultrasound backfat, mm; UREA, ultrasound rib eye area, cm^2; AUBF, average daily gain of ultrasound backfat, mm; AUREA, average daily gain of ultrasound rib eye area, cm^2; SWT, slaughter weight, kg; CWT, carcass weight, kg; AVBF, average backfat, mm; LMY, lean meat yield, %; CREA, carcass rib eye area, cm^2; CMAR, carcass marbling score.

[2]Least square mean and SE for genotypes CC, CT and TT. The counts of the genotypes CC, CT and TT are in parentheses. Intralocus SNP genotypic frequencies conformed to Hardy–Weinberg equilibrium proportions for all the three populations at $P > 0.05$.

[3]Substitution of one allele in the population with the other allele (Falconer & Mackay 1996).

[4]Estimated by subtracting the average of solutions for homozygous genotypes from that for heterozygous genotype (Falconer & Mackay 1996).

*$P < 0.05$.

undetermined, however, whether the significant *IGF1* SNP association in the Angus population is due to the linkage phase change between the SNP and the causative SNP or SNPs across the populations, or whether it is due to an adipose tissue related regulatory role of the *IGF1* promoter SNP on fat deposition.

Recently, Helgeson & Schmutz (2008) reported that an A>T SNP in *pro-melanin-concentrating hormone* (*PMCH*), located in close proximity to *IGF1*, was significantly associated with average fat and grade fat in two crossbred populations of *Bos taurus*. The SNP, which is located in the regulatory region of *PMCH*, has been proposed to introduce a binding site for transcriptional repressor, adenovirus E4 promoter binding protein and consequently affects fat deposition in beef cattle (Helgeson & Schmutz 2008). Therefore, further validation of the SNP associations in different cattle populations and functionality analyses of the *IGF1* SNP as well as the *PMCH* SNP will likely provide insight into the genetic mechanisms regulating the deposition of backfat in beef cattle.

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