Short Communication: Association analyses of a single nucleotide polymorphism in the promoter of *OLR1* with growth, feed efficiency, fat deposition, and carcass merit traits in hybrid, Angus and Charolais beef cattle

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Received 21 September 2012, accepted 17 February 2013.

Vinsky, M., Islam, K., Chen, L. and Li, C. 2013. **Short Communication: Association analyses of a single nucleotide polymorphism in the promoter of *OLR1* with growth, feed efficiency, fat deposition, and carcass merit traits in hybrid, Angus and Charolais beef cattle**. Can. J. Anim. Sci. **93**: 193–197. A single nucleotide polymorphism (SNP) in the promoter region of oxidized low density lipoprotein (lectin-like) receptor 1 (OLR1) (c. −495 T>C) was identified and analyzed for associations with 10 traits related to growth, feed efficiency, body fat deposition and carcass merit traits in hybrid ($n=456$), Angus ($n=567$) and Charolais ($n=423$) beef cattle populations. Significant allele substitution effect ($P=0.023$) was found for residual feed intake (RFI) in the Angus population. The allele "C", which had a frequency of 0.24 in the Angus population, was associated with decreased RFI. The Angus steers with the "CC" genotype had a lower RFI value (i.e., more efficient) than the Angus steers carrying the 'TT' genotype. The SNP was also found to have significant dominance effects on final ultrasound rib-eye area (FUREA) ($P=0.0004$) and carcass rib-eye area (CREA) ($P=0.009$) in the Angus steer population. The Angus steers with the "CT" genotype had smaller rib-eye areas of both ultrasound and carcass measures than the average of the steers with the homozygous genotypes. However, the SNP did not show significant associations with the traits examined in either the hybrid or the Charolais steer population at $P<0.05$. *OLR1* plays a role in lipid metabolism, and analyses of transcript binding site based on the transcription element search system revealed that the "T" allele of the c.-495T>C SNP introduces a presumptive binding site for CCAAT/enhancer binding protein alpha (C/EBPα). However, further investigation is required to delineate the possible regulatory role of the SNP on growth and efficiency of energy utilization in relation to different biological types of beef cattle.

Key words: Beef cattle, residual feed intake, candidate gene, oxidized low density lipoprotein (lectin-like) receptor, single nucleotide polymorphism

Vinsky, M., Islam, K., Chen, L. et Li, C. 2013. **Brève Communication: Analyses d'association entre un SNP du promoteur de l'*OLR1* et la croissance, l'efficacité alimentaire, le dépôt de graisse et la qualité de la carcasse chez les bovins de boucherie Angus et Charolais et de race croisée**. Can. J. Anim. Sci. **93**: 193–197. Les auteurs ont identifié un polymorphisme mononucléotidique (SNP) sur le site promoteur du gène *oxydized low density lipoprotein (lectin-like) receptor (OLR1)* (c. −495 T>C) et étudié les associations avec dix paramètres liés à la croissance, à l'efficacité alimentaire, au dépôt de graisse et à la qualité de la carcasse chez les bovins de boucherie de race croisée ($n = 456$), Angus ($n = 567$) et Charolais ($n = 423$). Ils ont découvert un effet de substitution allélique significatif ($P = 0{,}023$) pour l'ingestion d'aliments résiduelle (IAR) chez les sujets Angus. L'allèle C, d'une fréquence de 0,24 chez les bovins Angus, a été associé à une diminution de l'IAR. Les bouvillons Angus de génotype CC se caractérisaient par une IAR plus faible (donc plus efficace) que ceux du génotype TT. Par ailleurs, chez les bouvillons Angus, le SNP a eu un effet de dominance significatif sur la surface finale du faux-filet mesurée aux ultrasons ($P = 0{,}0004$) et sur la surface du faux-filet mesurée sur les carcasses ($P = 0{,}009$). Les bouvillons Angus de génotype CT présentaient une surface de faux-filet mesurée aux ultrasons et sur la carcasse plus faible que la surface moyenne des bouvillons homozygotes. Le SNP ne présente cependant aucune association significative avec les caractères examinés chez les bouvillons de race croisée ou Charolais ($P < 0{,}05$). Le gène *OLR1* intervient dans le métabolisme des lipides et l'analyse des sites de liaison des produits de transcription au

Abbreviations: **ADG**, average daily gain; **AFAT**, average backfat thickness; **AN**, Angus; **CH**, Charolais; **CMAR**, carcass marbling score; **CREA**, carcass rib eye area; **DMI**, daily dry matter intake; **FUBF**, ultrasound backfat thickness at the end of feedlot test; **FUREA**, ultrasound rib eye area at the end of feedlot test; **HCW**, hot carcass weight; **HY**, hybrid; **LMY**, lean meat yield; **OLR1**, oxidized low density lipoprotein (lectin-like) receptor 1; **RFI**, residual feed intake; **SNP**, single nucleotide polymorphism

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moyen du système de recherche des éléments de transcription a révélé que l'allèle T du SNP c.-495T > C insère un site de liaison présomptif à la séquence CCAAT/activateur de liaison de la protéine alpha (C/EBPα). Il faudrait cependant entreprendre des recherches plus poussées pour préciser le rôle de ce SNP dans la régulation de la croissance et de l'efficacité de l'utilisation de l'énergie chez les bovins de boucherie de types biologiques différents.

Mots clés: Bovins de boucherie, ingestion des aliments résiduelle, gène candidat, oxidized low density lipoprotein (lectin-like) receptor, polymorphisme mononucléotidique

OLR1 is a cell surface receptor of oxidized low density lipoproteins on bovine vascular endothelial cells (Sawamura et al. 1997). OLR1, a protein encoded by the *OLR1* gene, contains C-type lectin family like extracellular domain at the C-terminal and supports binding, internationalization and proteolytic degradation of oxidized low density lipoproteins (Sawamura et al. 1997; Murase et al. 2000), which establishes its role in lipid metabolism (Ringseis et al. 2007; Liao et al. 2008). Additionally, OLR1 can bind to phospholipids (Dunn et al. 2008) and oxidized red blood cells as well as apoptotic cells and remove these from circulation, suggesting the in vivo physiological role of *OLR1* in metabolism (Oka et al. 1998; Murase et al. 2000).

The bovine *OLR1* gene was located in the interval of 106 to 108 cM on bovine chromosome (BTA) 5 (Khatib et al. 2006) or 106703849–106715154 bp in Btau_4.6.1 and a QTL for postnatal body weight has been identified in the vicinity of the gene in an Angus beef cattle population (McClure et al. 2010). In dairy cattle, QTLs for milk yield and milk fat percentage have also been detected near the *OLR1* gene (Heyen et al. 1999; de Koning et al. 2001; Olsen et al. 2002), and a single nucleotide polymorphism (SNP) at 3′ untranslated region of *OLR1* (NW_215807:g. 8238C > A) was found to be associated with milk fat yield and fat percentage in Holstein dairy cattle (Khatib et al. 2006; Wang et al. 2012) and Polish Holstein-Frisian bulls (Komisarek and Dorynek, 2009). In addition, intragenic haplotypes of the bovine *OLR1* gene also showed associations with milk fat yield and fat percentage in Holstein dairy cattle (Khatib et al. 2006) and in Italian Brown Swiss (Khatib et al. 2007). In this study, we identified a SNP in the promoter region of the bovine *OLR1* gene (c. −495 T > C) and analyzed the associations of the SNP with growth, feed efficiency, fat deposition and carcass merit traits in hybrid, Angus and Charolais beef cattle populations.

The hybrid (HY), Angus (AN) and Charolais (CH) beef cattle populations used in this study included 456 hybrid steers of 80 sires from the Kinsella research substation of University of Alberta, Canada, 567 purebred Angus steers of 42 sires and 423 purebred Charolais steers of 44 sires from the One-four research substation at the Lethbridge Research Centre of Agriculture and Agri-Food Canada (AAFC). The HY population was produced from crosses between Angus, Charolais, or hybrid bulls and the Kinsella ranch's hybrid dam lines, which was described previously (Nkrumah et al. 2007). The purebred AN and CH steers were produced by AI and a single sire mating system using registered bulls of the Canadian Angus and Charolais Associations, respectively, as described in Islam et al. (2009). All three cattle populations were managed according to the guidelines established by the Canadian Council on Animal Care (1993).

Growth, feed efficiency, fat deposition and carcass merit traits analyzed in this study included average daily gain (ADG), daily dry matter intake (DMI), residual feed intake (RFI), ultrasound backfat thickness at the end of feedlot test (FUBF), ultrasound rib eye area at the end of feedlot test (FUREA), hot carcass weight (HCW), average backfat thickness (AFAT), lean meat yield (LMY), carcass rib eye area (CREA) and carcass marbling score (CMAR). Phenotype data collection and calculation of ADG, DMI, RFI, AUBF and AUREA were also described previously (Nkrumah et al. 2007; Islam et al. 2009). Carcass measurements were available on 374 steers for the hybrid population.

We amplified and sequenced an 815 bp gene region between c.-744bp and c.71bp of the *OLR1* gene (NCBI reference sequence: NW_001495095.3) in a panel of eight pairs of half-sib steers using a forward primer 5′GCATCCCTACAGCAACTTTGT3′ and a reverse primer 5′CCTTTTGCTGTCTTGCCATTTG3′. A SNP in the position of −495 (c.-495T > C) was discovered through sequence comparison (ss537663520). The genotyping of the *OLR1* gene-specific SNP was carried out using an ABI Step-One-Plus™ real-time thermocycler using a 5 nuclease allele discrimination assay (Applied Biosystems, Foster City, CA), with a forward primer 5′ATGGCTAGAGAGATGAGTCATATGTGA3′ and a reverse primer (5′TGGTGTCTTTCATAAGTCTCA CATTTTCTT3′), and two TaqMan MGB fluorogenic probes targeted at the two alleles, with VIC™ reporter dye for allele "T" and FAM™ reporter dye for allele "C". The sequences of the probes for allele "T" and allele "C" detection were VIC 5'TGGTCAGCGATAGGCT3' and FAM 5'TGGTCAGCAATAGGCT'. The genotypes of the panel of eight pairs of half-sib steers by sequencing were confirmed by the genotypes obtained by the discrimination assay.

Associations between the SNP and the 10 traits related to growth, feed efficiency, fat deposition and carcass merit traits were examined separately for each population by fitting the following mixed linear regression model using ASReml (Gilmour et al. 2000):

$$y = Xb + Za + e$$

where **y** is the vector of phenotypes for the trait analyzed; **X** is the design matrix for fixed effects; **b** is the vector of fixed effects including population mean, the SNP effects and other fixed effects. For the hybrid population, other fixed effects included feedlot test batch over 3 yr (six levels) and sire breed (three levels by breed of sire as Angus, Charolais and hybrid). For the Angus and Charolais populations, other fixed effect included the feedlot test batch over 6 yr (24 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 random residuals. The three genotypes: CC, CT, and TT, were coded as 0, 1, and 2 respectively, and the SNP allele substitution effect was estimated by the regression analysis. The dominance effect was estimated by subtracting the average of solutions for homozygous genotypes from the solution of the heterozygous genotype according to Falconer and Mackay (1996). To adjust the animal's age effect, animal age at the start of the feedlot test was included in the model as a linear covariate for analyzing the SNP associations with growth, feed efficiency and ultrasound traits while animal's age at slaughter was included in the model as a linear covariate with the carcass merit traits.

The c.-495T > C SNP was found polymorphic for all three populations. The 'C' allele was the minor allele with a frequency of 15.6, 24.3 and 9.0% in the HY, AN and CH, respectively. All three populations conformed to Hardy–Weinberg equilibrium ($P > 0.05$). A significant allele substitution effect on RFI ($P = 0.023$) was found in the Angus steer population at a significance level of $P < 0.05$ with the 'C' allele associated with a lower value of RFI (Table 1). The Angus steers with the "CC" genotype had a lower RFI value or were more

Table 1. Least-square means and estimated effects of *OLR1* c.-495T > C SNP (ss537663520) on growth, feed efficiency, fat deposition and carcass merit traits in hybrid, Angus and Charolais beef cattle populations

		c. −495T > C genotypes[x]						
Trait[z]	Breed[y]	CC	CT	TT	Allele substitution effect ± SE[w]	*P* value	Dominance effect ± SE[v]	*P* value
ADG	AN	1.45 ± 0.04 (30)	1.45 ± 0.02 (216)	1.47 ± 0.02 (321)	0.01 ± 0.02	0.46	−0.01 ± 0.02	0.696
	CH	1.57 ± 0.11 (3)	1.55 ± 0.03 (70)	1.54 ± 0.02 (350)	−0.01 ± 0.03	0.728	−0.01 ± 0.06	0.923
	HY[u]	1.40 ± 0.10 (9)	1.44 ± 0.03 (124)	1.47 ± 0.02 (323)	0.03 ± 0.03	0.199	0.00 ± 0.05	0.953
DMI	AN	9.67 ± 0.17	9.65 ± 0.10	9.77 ± 0.09	0.09 ± 0.06	0.186	−0.07 ± 0.09	0.445
	CH	9.15 ± 0.50	9.23 ± 0.14	9.22 ± 0.10	−0.001 ± 0.11	0.989	0.04 ± 0.26	0.861
	HY	10.27 ± 0.41	10.42 ± 0.14	10.60 ± 0.11	0.18 ± 0.12	0.135	−0.02 ± 0.23	0.921
RFI	AN	−0.08 ± 0.11	−0.09 ± 0.06	0.04 ± 0.06	0.10 ± 0.04	**0.023***	−0.07 ± 0.06	0.233
	CH	−0.29 ± 0.27	−0.09 ± 0.08	−0.02 ± 0.06	0.08 ± 0.06	0.208	0.07 ± 0.14	0.624
	HY	0.03 ± 0.28	0.02 ± 0.08	0.06 ± 0.06	0.03 ± 0.08	0.678	−0.02 ± 0.16	0.876
FUBF	AN	14.15 ± 0.55	15.02 ± 0.30	14.83 ± 0.27	0.07 ± 0.21	0.746	0.54 ± 0.31	0.090
	CH	6.24 ± 1.10	8.15 ± 0.30	7.91 ± 0.20	−0.06 ± 0.25	0.797	1.08 ± 0.58	0.067
	HY	8.09 ± 0.97	9.47 ± 0.32	9.31 ± 0.25	0.05 ± 0.28	0.86	0.76 ± 0.54	0.16
FUREA	AN	82.17 ± 1.21	78.31 ± 0.49	79.82 ± 0.40	0.25 ± 0.48	0.596	−2.69 ± 0.76	**0.0004****
	CH	81.58 ± 3.90	87.02 ± 1.01	87.20 ± 0.66	0.60 ± 0.87	0.489	2.63 ± 2.07	0.206
	HY	86.32 ± 2.55	82.97 ± 0.74	82.85 ± 0.52	−0.54 ± 0.72	0.449	−1.61 ± 1.44	0.267
HCW	AN	338.83 ± 4.12	337.15 ± 2.30	337.12 ± 2.10	−0.43 ± 1.57	0.778	−0.82 ± 2.29	0.716
	CH	344.32 ± 14.05	349.31 ± 3.21	344.96 ± 1.79	−3.58 ± 3.04	0.242	4.67 ± 7.57	0.535
	HY	305.37 ± 10.23	310.34 ± 3.28	312.91 ± 2.62	2.86 ± 2.87	0.32	1.20 ± 5.64	0.824
AFAT	AN	16.10 ± 0.82	16.42 ± 0.45	16.87 ± 0.41	0.42 ± 0.31	0.183	−0.06 ± 0.46	0.885
	CH	6.34 ± 1.63	8.60 ± 0.39	7.87 ± 0.23	−0.48 ± 0.36	0.182	1.50 ± 0.88	0.091
	HY	12.60 ± 1.51	12.40 ± 0.48	12.57 ± 0.38	0.12 ± 0.42	0.771	−0.18 ± 0.83	0.819
LMY	AN	54.49 ± 0.72	53.47 ± 0.40	53.27 ± 0.36	−0.39 ± 0.28	0.16	−0.41 ± 0.41	0.308
	CH	61.92 ± 1.73	61.68 ± 0.43	62.28 ± 0.27	0.53 ± 0.38	0.169	−0.42 ± 0.92	0.642
	HY	58.25 ± 1.38	57.58 ± 0.45	57.46 ± 0.36	−0.19 ± 0.39	0.629	−0.28 ± 0.76	0.708
CREA	AN	83.80 ± 1.41	80.28 ± 0.70	81.12 ± 0.62	−0.18 ± 0.55	0.735	−2.18 ± 0.83	**0.009***
	CH	88.01 ± 5.07	95.59 ± 1.45	95.92 ± 1.03	0.95 ± 1.17	0.417	3.62 ± 2.66	0.177
	HY	85.95 ± 2.95	82.88 ± 0.91	83.22 ± 0.71	−0.09 ± 0.83	0.907	−1.70 ± 1.63	0.299
CMAR	AN	3.16 ± 0.09	3.19 ± 0.05	3.19 ± 0.04	0.01 ± 0.03	0.674	0.01 ± 0.05	0.833
	CH	2.13 ± 0.24	2.39 ± 0.07	2.38 ± 0.05	0.01 ± 0.06	0.819	0.13 ± 0.13	0.300
	HY	2.52 ± 0.18	2.55 ± 0.06	2.51 ± 0.05	−0.04 ± 0.05	0.494	0.04 ± 0.10	0.688

[z]ADG, average daily gain (kg d^{-1}); DMI, dry matter intake (kg d^{-1}); RFI, residual feed intake (kg d^{-1}); FUBF, final ultrasound backfat (mm); FUREA, final ultrasound rib eye area (cm^2); HCW, hot carcass weight (kg); AFAT, average backfat (mm); CMAR, carcass marbling score; CREA, carcass rib eye area (cm^2); LMY, lean meat yield (%).

[y]Angus steer population; CH, Charolais steer population; HY, hybrid steer population.

[x]Least square means ± SE for genotypes CC, CT and TT.

[w]Substitution effect of one allele in the population with the other allele (Falconer and Mackay 1996).

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

[u]For hybrid cattle, ADG, DMI, RFI, FUBF and FUREA were measured on (9) CC, (124) CT, and (323) TT cattle, while carcass traits HCW, AFAT, LMY, CREA and CMAR were measured on (7) CC, (102) CT, and (265) TT cattle.

*,**$P < 0.05$ and $P < 0.001$, respectively.

efficient than the Angus steers carrying the "TT" genotype. The SNP was also found to have significant dominance effects on final ultrasound rib-eye area ($P = 0.0004$) and carcass rib-eye area ($P = 0.009$) in the Angus steer population (Table 1). The Angus steers with the "CT" genotype had smaller rib-eye areas of both ultrasound and carcass measures than the average of the steers with the homozygous genotypes. The SNP also showed a tendency of dominance effect on FUBF and RFI in the Angus steer population with the "CT" genotype having more final ultrasound backfat thickness than the average of the steers with the homozygous genotypes at a P value of 0.09. The SNP did not show significant associations with the traits examined in both the hybrid and Charolais steer populations at $P < 0.05$. The "CT" genotype tended to have more FUBF and AFAT in the Charolais steer population. However, the associations did not reach the significance level of $P < 0.05$, and in addition the association results in the Charolais populations should be interpreted with caution due to the low number of steers ($N = 3$) with the "CC" genotype (Table 1).

A preliminary analysis of the *OLR1* gene sequence using the Neural Network Promoter Prediction program (http://www.fruitfly.org/seq_tools/promoter.html) (Reese 2001) identified the most likely promoter region being 98 bp upstream of the c. −495T > C SNP. TESS (Transcription element search system) (http://www.cbil.upenn.edu/tess) (Schug 2008) further revealed that the "T" allele of the c.-495T > C introduces a presumptive binding site (ATTGC) for CCAAT/enhancer binding protein alpha (C/EBPα). Taniguchi and Sasaki (1996) reported the presence of C/EBPα in bovine genome and found a 92.5% similarity in amino acid sequence with the rat C/EBPα protein. In animals, C/EBPα is known to have high expression in several tissues involved in lipid and carbohydrate metabolism including adipose tissue, liver, and muscle (Birkenmeier et al. 1989; Antonson and Xanthopoulos 1995), suggesting the role of *OLR1* in growth, body fat and muscle deposition and maintenance and therefore feed efficiency. In general, more energy is needed to deposit fat in animals than protein since protein synthesis is energetically more efficient than fat synthesis (McDonald et al. 1988; Archer et al. 1999). Although protein turnover leads to a higher maintenance requirement than fat (McDonald et al. 1988), energy required to deposit fat may play a major role in determining feed efficiency in growing steers. In comparison with the hybrid and Charolais breeds, Angus has, on average, a greater fat depth (Table 1), presumably due to early maturity in Angus allowing the steers to produce more fat at a younger age (Gregory et al. 1994). In the Angus population, the "CC" genotype steers that were more efficient in feed efficiency also showed a tendency for smaller amount of backfat deposition (FUBF and AFAT) but larger rib-eye or muscle areas (FUREA and CREA) in comparison with the "TT" genotype (Table 1), suggesting a role for *OLR1* in body fat deposition and thus energy utilization in growing steers. However, feed efficiency in beef cattle is a complex trait and is believed to be affected by multiple genes. Moreover, in this study we tested the association of the OLR1 SNP for 10 traits in three populations, which, if adjusted for multiple testing, would make the associations of the SNP with RFI and CREA insignificant. Therefore, further investigations are required to validate the associations and to delineate the possible regulatory role of *OLR1* DNA polymorphisms on growth, fat deposition and efficiency of energy utilization in relation to different biological types of beef cattle.

This work was supported by the AAFC Livestock Genetics & Genomics Program funds and the AAFC A-base peer-reviewed research projects (RBPI-1139 and RBPI-1752). The authors gratefully acknowledge the contributions of Dr. Denny Crews and Ronda Carlson to the management of the Angus and Charolais herds and phenotypic data collection. The collection of phenotypic data for the hybrid steers under research projects led by Dr. Stephen Moore is also gratefully acknowledged.

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