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The bovine *fatty acid binding protein 4* gene is significantly associated with marbling and subcutaneous fat depth in Wagyu x Limousin F_2 crosses

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Summary

Fatty acid binding protein 4 (FABP4), which is expressed in adipose tissue, interacts with peroxisome proliferator-activated receptors and binds to hormone-sensitive lipase and therefore, plays an important role in lipid metabolism and homeostasis in adipocytes. The objective of this study was to investigate associations of the bovine *FABP4* gene with fat deposition. Both cDNA and genomic DNA sequences of the bovine gene were retrieved from the public databases and aligned to determine its genomic organization. Primers targeting two regions of the *FABP4* gene were designed: from nucleotides 5433–6106 and from nucleotides 7417–7868 (AAFC01136716). Direct sequencing of polymerase chain reaction (PCR) products on two DNA pools from high- and low-marbling animals revealed two single nucleotide polymorphisms (SNPs): AAFC01136716.1:g.7516G>C and g.7713G>C. The former SNP, detected by PCR-restriction fragment length polymorphism using restriction enzyme *Msp*A1I, was genotyped on 246 F_2 animals in a Waygu × Limousin F_2 reference population. Statistical analysis showed that the *FABP4* genotype significantly affected marbling score ($P = 0.0398$) and subcutaneous fat depth ($P = 0.0246$). The *FABP4* gene falls into a suggestive/significant quantitative trait loci interval for beef marbling that was previously reported on bovine chromosome 14 in three other populations.

Keywords association, beef cattle, fatty acid binding protein 4, marbling, subcutaneous fat depth.

Fatty acid binding proteins (FABP4) are a family of small, highly conserved cytoplasmic proteins that bind long-chain fatty acids and other hydrophobic ligands (Kaikaus *et al.* 1990). Their major functions include fatty acid uptake, transport and metabolism. So far, nine distinct members have been identified in this gene family (Damcott *et al.* 2004), including adipocyte fatty acid binding protein or fatty acid binding protein 4. Fatty acid binding proteins play a major role in the regulation of lipid and glucose homeostasis through interaction with peroxisome proliferator-activated receptors (PPARs), which are located in the cell nucleus. Specifically, the FABP4/fatty acid complex activates the PPAR-γ isoform, which in turn regulates transcription of FABP4 (Damcott *et al.* 2004). In addition, FABP4 appears to be involved in lipid hydrolysis and intracellular fatty acid trafficking through direct interaction and binding to hormone-sensitive lipase (Shen *et al.* 1999), which is a primary enzyme involved in lipid catabolism (Tansey *et al.* 2003). Recently, *FABP4* and *FABP5* were proposed as potential candidate genes for obesity as they are located within a quantitative trait loci (QTL) region for serum leptin levels in mice (Ogino *et al.* 2003). Leptin, a 16-kDa protein secreted from white adipocytes, is involved in the regulation of food intake, energy expenditure and whole-body energy balance (Jiang & Gibson 1999). Thus, FABP4 may play an important role in lipid metabolism and homeostasis in adipocytes.

Here we report development of genetic markers in the bovine *FABP4* gene and significant association of the gene with marbling and subcutaneous fat depth (SFD) in a Wagyu × Limousin F_2 population. This reference population was developed jointly by Washington State University and the USDA, ARS Fort Keogh Livestock and Range Research Laboratory (Miles City, MT, USA). The F_1 crosses, including six F_1 bulls and 113 dams, were generated at

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Washington State University and transferred to the USDA research station in Fall, 1998. *Inter se* mating of these F_1 animals produced 71 F_2 progeny in 2000, 90 in 2001 and 109 in 2002 respectively. Growth rate, carcass and meat quality data, including marbling scores and SFD, were collected on all F_2 calves. Marbling score is a subjective measure of the amount of intramuscular fat in the longissimus muscle based on USDA standards (http://www.ams.usda.gov/). Subcutaneous fat depth was measured at the 12th–13th rib interface perpendicular to the outside surface at a point three-fourths the length of the longissimus muscle from its chine bone end. Marbling scores varied from 4 = Slight^0 to 9.5 = Moderately Abundant^{50} (SD = 1.00), and SFD measurements ranged from 0.1 to 1.3 inches (SD = 0.18) in this F_2 population. Based on the availability of both phenotypic data and DNA samples, 246 observations were used in the current study. Two DNA pools were formed for detecting associations with *FABP4* polymorphisms: one from 20 individuals with the highest marbling scores and one from 20 individuals with the lowest marbling scores.

The cDNA sequence of the bovine *FABP4* gene (X89244) was reported previously (Specht *et al.* 1996). The genomic DNA sequence of the bovine gene (AAFC01136716) was then retrieved from the 6X bovine genome sequence database (http://www.hgsc.bcm.tmc.edu/projects/bovine/). Primers were designed from these sequences: one pair (TCGTAAACTTAGATGAAGGTGCTCTGG and ACGTATCCAGCAGAAAGTCATGGAG) targeted a region from nucleotides 5433 to 6106, while the second pair (ATATAGTCCATAGGGTGGCAAAGA and AACCTCTCTTTGAATTCTCCATTCT) amplified a region between nucleotides 7417 to 7868. Direct sequencing of polymerase chain reaction (PCR) products from the two DNA pools revealed two single nucleotide polymorphisms (SNPs): AAFC01136716.1:g.7516G>C and g.7713G>C. The first SNP was genotyped by PCR-restriction fragment length polymorphism using restriction enzyme *Msp*A1I, with the *G* allele resulting in 100- and 352-bp bands of the 452-bp amplicon and the *C* allele resulting in a 452-bp band. In the 232 animals genotyped, the *C* allele frequency was 0.75.

The phenotypic data for marbling scores and SFD in the 232 animals were analysed with a mixed linear model using the PROC MIXED module in SAS v9.1 (Littell *et al.* 1996). Sources of variation included year of birth, gender, age at harvest and genotype effect of the *FABP4* gene as fixed effects and a random effect to account for polygenic background. The covariance structure of the polygenic effect was defined by a numerical relationship calculated from the pedigree using SAS macro LORG (Zhang 2004; http://people.cornell.edu/pages/zz19/research/LORG). The residual effect was assumed to have identical independent distribution with unknown variance. The additive genetic variance and residual variance components were estimated using the ridge-stabilized Newton–Raphson algorithm for restricted maximum likelihood estimation (Wolfinger *et al.* 1994). Tests of marker effects were performed using the Kenward–Roger method for calculating denominator degrees of freedom. This method uses an adjusted estimator of covariance matrix to reduce small sample bias (Kenward & Roger 1997). Pair-wise comparisons of least squares means were performed using Fisher's protected least significant difference (LSD) *t*-test procedure. The results are presented as least-squares means of genotypes in Table 1. Statistical analyses revealed that genotype significantly affected marbling scores ($P = 0.0398$) and SFD ($P = 0.0246$; Table 1). Despite marbling scores of *GC* (FABP4:g.7516) animals being higher than those of *CC* animals, neither the additive effect nor the dominance deviation was significantly different from zero. The small number of animals with the *GG* genotype may contribute to the inability to detect specific genetic effects given the overall significance of the genotypic effect on marbling score.

Several reports have demonstrated that bovine chromosome 14 (BTA14) harbours significant or suggestive QTL for marbling. Casas *et al.* (2003) reported a suggestive QTL for marbling in a *Bos indicus* × *Bos taurus* family located at 47 cM on BTA14. Taylor and Schnabel (2004 ; http://animalgenomics.missouri.edu/) recently developed a DNA repository from the semen of 1600 registered bulls representing 14 generations of the American Angus Association for an Angus Genome Project and confirmed the existence of a marbling QTL with a similar location as the QTL identified by Casas *et al.* (2003). In purebred Japanese black cattle, a QTL for marbling was found in the centromeric regions of BTA14 (Imai *et al.* 2004). Standardization of these marker locations based on the newest version of bovine linkage map (Ihara *et al.* 2004) demonstrated that these QTLs span an interval between 59 and 70 cM on BTA14. Integration of both the genetic map (Ihara *et al.* 2004) and the radiation hybrid (RH) map (Itoh *et al.* 2005)

Table 1 Associations of the bovine *fatty acid binding protein 4* gene (AAFC01136716.1:g.7516G>C) with marbling score and subcutaneous fat depth (SFD) in Waygu × Limousin F_2 crosses.

Genotype	No. of animals	Marbling score[1] Mean ± SE[3]	SFD (in inches)[2] Mean ± SE[3]
CC	139	5.791 ± 0.236^a	0.378 ± 0.035^a
GC	72	6.106 ± 0.248^b	0.428 ± 0.037^b
GG	21	6.211 ± 0.239^{ab}	0.418 ± 0.032^{ab}
F-value		3.27	3.77
P-value		0.0398	0.0246

[1]Marbling score: 4 = slight to 9.5 = moderately abundant.
[2]Subcutaneous fat depth measured at the 12th–13th rib interface perpendicular to the outside surface at a point three-fourths the length of the longissimus muscle from its chine bone end.
[3]Mean within a column that do not have common superscripts are significantly different ($P < 0.05$).

of BTA14 predicted that the *FABP4* gene should be placed somewhere between 63.2 and 63.9 cM on the linkage map of the bovine chromosome. These data indicate that the *FABP4* gene falls into a QTL interval for marbling reported in three different populations as described above.

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