POLYMORPHISMS OF HISTONE DEACETYLASE 1 AND 3 GENES AND FATTY ACID BINDING PROTEIN 3 AND 4 GENES AND THEIR ASSOCIATIONS WITH ECONOMIC TRAITS IN SWINE

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INTRODUCTION

Acetylation/deacetylation of histones (chromosomal proteins) plays an important role in regulating gene transcription (Taunton *et al.*, 1996) and is mediated largely by histone deacetylases (*HDAC*s). *HDAC*s regulate different cellular functions, such as chromatin structure, gene expression and cell cycle progression (Jepsen *et al.*, 2000 ; McKinsey *et al.*, 2000). Fatty acid binding proteins (*FABP*s) are involved in regulating lipid metabolism and transport in the cytoplasm. FABP 3 has been significantly associated with intramuscular fat percentage (IMF) and body weight, but not clearly with backfat (BF) in pigs (Gerbens *et al.*, 1999 and 2000). The objectives of this study were to identify genetic polymorphisms in *HDAC* 1 and 3 and *FABP* 3 and 4 and to assess their associations with growth, carcass and meat quality traits in a resource population.

METHODS AND MATERIALS

Resource population. For QTL detection, a resource population of 429 pigs was developed from crosses of Hampshire (H), Duroc (D) and Large White (LW) breeds (H×D)×LW. Each sow was mated to two F_1 sires to contribute two litters of progeny to the project. The (H×D)×LW progeny were raised in a facility equipped for individual feed intake measurements and were slaughtered for carcass cutout at a live weight of around 110 kg.

Trait measurement. For each pig, 110 traits were measured. These traits included growth performance (e.g. body weight, ultrasonic backfat thickness and loin eye area), carcass composition (e.g. weight of loin primal, ham primal, loin fat, loin lean, ham fat, defatted ham) and meat quality (e.g. dry matter, drip loss, marbling score, colour and intramuscular fat).

DNA extraction. High quality genomic DNA was extracted from ear notch or meat samples from the F_2 progeny (Sambrook *et al*., 1989).

Primer design and identification of genetic polymorphism. For *HDAC 1*, primers (F: aacaggtggaagggatatacctaag, R: tagaaatgtaccattttattacaaa) were designed from a known sequence (GenBank access number BE236276) to produce a 333 bp PCR product, which was sequenced. An A/G substitution caused the elimination of a *Hinf I* site, producing two alleles (denoted as 1 for A and 2 for G). For *HDAC 3*, two pairs of primers were generated. The first pair of primers (F: acggtctttataagaagatgatcgt, R: ctgggaggcctggtacggcttgaag) was designed from a known sequence (GenBank access number BG609283) and gave an approximately 1400 bp PCR product. Sequencing of this product disclosed a G/A substitution, causing a *Tsp509 I* polymorphism with two alleles (denoted as 1 for G and 2 for A). The second pair of primers (F: atccatgagcttaatgggagttc, R: ctgggaggcctggtacggcttgaag) was designed from the first PCR product sequence in order to clearly distinguish the two *Tsp509 I* alleles. For *FABP 4*, a pair of

primers (F: agaattctttccttgactcaggatt and R: caatttgaaggcaatctcagtattt) was designed to amplify a region including a *Bsm I* polymorphic site based on a known sequence (GenBank access number Y16039). For *FABP 3*, a known pair of primers (Gerbens, 1999) was used to amplify a region including polymorphic loci for *Hae III* and *Msp I*. All PCR amplifications were performed in a 10 µl reaction solution. For *FABP 3* and *4*, the PCR reaction solution contained 1.0 µl 10× PCR buffer II, 0.6 µl 25 mM $MgCl_2$, 0.6 µl 10 mM each dNTP, 0.1 µl of each primer (20 pmol/µl), 0.1 µl AmpliTaq for *FABP 3* or AmpliTaq Gold for *FABP 4* and 1.3 µl 50 ng/µl DNA template. For *HDAC 1* and *3*, the PCR reaction solution contained 1.0 µl 10× PCR buffer II, 0.73 µl 25 mM $MgCl_2$, 0.6 µl 10 mM each dNTP, 0.1 µl of each primer, 0.1 µl AmpliTaq Gold and 1.3 µl 50 ng/µl DNA templates. AmpliTaq and AmpliTaq Gold were obtained from the Applied Biosystems (Foster City, CA). The PCR cycling conditions for *FABP 3* were 94 °C (3 min), then 94 °C (0.5 min)—65 °C (0.5 min)—72 °C (0.5 min) for 8 cycles, then followed by 94 °C (0.5 min)—57 °C (0.5 min)—72 °C (0.5 min) for 27 cycles and finally 72 °C (7 min). The cycling conditions for *FABP 4* were the same as for *FABP 3* except that the initial 94 °C (3 min) was replaced by 95 °C (10 min). The cycling conditions for *HDAC 1* were 95 °C (10 min), then 94 °C (0.5 min)—61.5 °C (5/6 min)—72 °C (1.1 min) for 2 cycles, followed by 94 °C (0.5 min)—59.5 °C (5/6 min)—72 °C (1.1 min) for 33 cycles, and finally 72 °C (7 min). For *HDAC 3*, the cycling conditions for the first primer pair were the same as for *FABP 4*; those for the second primer pair were the same as for *HDAC 1*.

PCR-RFLP. For each polymorphic locus, PCR products were digested with restriction enzymes in 10 µl solution including 5 µl PCR product solution and the products were separated by electrophoresis through a 2% agarose gel. The reaction buffer and temperature used were as recommended by New England BioLabs Inc (Beverly, MA). The RFLP alleles were: for the *Bsm I* locus in *FABP 4*, allele 1 with one fragment (783 bp) and allele 2 with two fragments (587 and 196 bp); for the *Hinf I* locus in HDAC 1, allele 1 with two fragments (219 and 114 bp) and allele 2 with three fragments (190, 114 and 29 bp); for the *Tsp509 I* locus in HDAC 3, allele 1 with two fragments (700 and 500 bp) and allele 2 with three fragments (500, 420 and 280 bp). RFLPs for the *Hae III* and *Msp I* loci in *FABP 3* were as reported by Gerbens (1999).

Statistical analysis. The statistical model used was :

$$y = \mu + \text{Herd-Pen-Season} + \text{Sire} + \text{Dam} + \text{Sex} + \text{Genotypes} + \text{Covariable} + \text{Residual}$$

with Sire and Dam effects assumed random and other effects assumed fixed. The covariable was body weight for backfat and loin eye area, hot carcass weight for carcass composition and meat quality or age for body weight. Analysis was performed using SAS PROC MIXED (SAS Institute, 2001). Because *FABP 3* (2 polymorphic loci) and *HDAC 1* (1 polymorphic locus) are on the same chromosome, the experiment-wise error rate (α=0.05) was divided by three to give α=0.017.

RESULTS AND DISCUSSION

Polymorphic loci and frequencies of genotypes for *HDAC* 1 and 3 and *FABP* 3 and 4 are presented in Table 1. Two alleles (1 and 2) and three genotypes (11, 12 and 22) were coded for each locus.

Table 1. Genotype frequencies for *HDAC* and *FABP* polymorphic loci

Gene	Locus	Frequencies of genotypes		
		11	12	22
FABP3	*Hae III*	0.410	0.469	0.121
FABP3	*Msp I*	0.666	0.267	0.067
FABP4	*Bsm I*	0.036	0.422	0.542
HDAC1	*Hinf I*	0.019	0.288	0.693
HDAC3	*Tsp509 I*	0.050	0.348	0.602

Tables 2 through 5 summarize the associations between candidate genes and traits that were significant or approached significance. The remainder of the 110 traits is not presented. *FABP3-Msp I* was significantly or almost significantly associated with body weight (BW) (measured 6 times over the range of 43.5 to 79.9 kg) with genotype 22 being superior in all cases and significantly associated with hock weight (Table 2). Associations between the *FABP4-Bsm I* locus and IMF and picnic fat weight were significant (Table 4). The *Hinf I* locus in *HDAC1* had a significant association with both jowl and hock weight (Table 3). There were significant associations between the *Tsp509 I* locus in *HDAC 3* and weight of butt internal fat, chub fat, and trimmed belly.

Table 2. Associations of *FABP3-Msp I* genotypes with body weight and hock weight

Trait	Mean [B] (kg)	Estimates of genotypes [A] (kg)		P > F
		11	12	
BW 1	43.489 kg	-2.957 ± 1.339 *	-0.425 ± 1.134	0.038
BW 2	49.724 kg	-3.320 ± 1.425 *	-0.196 ± 1.207	0.017
BW 3	57.166 kg	-3.388 ± 1.517 *	-0.383 ± 1.284	0.031
BW 4	65.318 kg	-3.786 ± 1.629 *	-0.686 ± 1.379	0.032
BW 5	72.119 kg	-4.404 ± 1.774 *	-0.682 ± 1.502	0.017
BW 6	79.880 kg	-4.405 ± 1.864 *	0.047 ± 1.578	0.009
Hock weight	1.131 kg	0.106 ± 0.036 **	0.004 ± 0.031	0.003

A: Effect of genotype 22 is set to 0 in SAS PROC MIXED and omitted in all tables.
B: Sample mean of a trait. */**: significant/very significant compared with genotype 22.

Table 3. Associations of *HDAC1-Hinf I* genotypes with picnic fat, jowl and hock weights

Trait	Mean (kg)	Estimates of genotypes (kg)		P > F
		11	12	
Picnic fat weight	0.185	- 0.024 ± 0.026	- 0.039 ± 0.015 **	0.027
Jowl weight	1.258	- 0.226 ± 0.075 **	- 0.060 ± 0.042	0.012
Hock weight	1.131	- 0.013 ± 0.057	0.085 ± 0.031 **	0.006

Table 4. Associations of *FABP4-Bsm I* genotypes with IMF and picnic skin weight (kg)

Trait	Mean	Estimates of genotypes		P > F
		11	12	
IMF	3.573 (%)	2.295 ± 0.945 *	1.192 ± 0.450 *	0.022
Picnic skin weight	0.164 (kg)	-0.028 ± 0.012 *	-0.001 ± 0.006	0.023

Table 5. Associations of *HDAC3-Tsp509 I* genotypes with butt internal fat, chub fat and trimmed belly weights

Traits	Mean (kg)	Estimates of genotypes (kg)		P > F
		11	12	
Butt internal fat	0.210	0.008 ± 0.014	- 0.016 ± 0.006 *	0.017
Chub fat	0.135	0.010 ± 0.008	0.009 ± 0.004 *	0.033
Trimmed belly	3.549	0.038 ± 0.088	0.104 ± 0.039 **	0.030

CONCLUSION

In this research, genotype 11 at the *Msp I* locus of *FABP 3* (equivalent to DD in Gerbens, 1999) was significantly associated with body weight at various stages of growth (Table 2). This confirms the results of Gerbens *et al.* (1999). The *FABP3-Hae III* locus did not show significant associations with IMF, BW and BF unlike the results of Gerbens *et al.* (1999 and 2000). For *FABP4-Bsm I*, the homozygote 11 was significantly associated with increased IMF and reduced picnic skin weight in contrast to homozygote 22. Previous research using a microsatellite marker in the vicinity of the *FABP4-Bsm I* locus gave inclusive results for association with IMF (Gerbens *et al.*, 1998 and 2000). This is the first report of significant associations between *HDAC 1* or *HDAC 3* and primal cut composition traits in pigs. These findings warrant further scrutiny in other populations.

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