Detection of single nucleotide polymorphisms associated with ultrasonic backfat depth in a segregating Meishan × White Composite population[1,2]

L. A. Kuehn, G. A. Rohrer, D. J. Nonneman, R. M. Thallman, and K. A. Leymaster[3]

USDA, ARS, US Meat Animal Research Center, Clay Center, NE 68933-0166

ABSTRACT: Multiple genomic scans have identified QTL for backfat deposition across the porcine genome. The objective of this study was to detect SNP and genomic regions associated with ultrasonic backfat. A total of 74 SNP across 5 chromosomes (SSC 1, 3, 7, 8, and 10) were selected based on their proximity to backfat QTL or to QTL for other traits of interest in the experimental population. Gilts were also genotyped for a SNP thought to influence backfat in the thyroxine-binding globulin gene (TBG) on SSC X. Genotypic data were collected on 298 gilts, divided between the F_8 and F_{10} generations of the US Meat Animal Research Center Meishan resource population (composition, one-quarter Meishan). Backfat depths were recorded by ultrasound from 3 locations along the back at approximately 210 and 235 d of age in the F_8 and F_{10} generations, respectively. Ultrasound measures were averaged for association analyses. Regressors for additive, dominant, and parent-of-origin effects of each SNP were calculated using genotypic probabilities computed by allelic peeling algorithms in GenoProb. The association model included the fixed effects of scan date and TBG genotype, the covariates of weight and SNP regressors, and random additive polygenic effects to account for genetic similarities between animals not explained by known genotypes. Variance components for polygenic effects and error were estimated using MTDFREML. Initially, each SNP was fitted (once with and once without parent-of-origin effects) separately due to potential multicollinearity between regressions of closely linked markers. To form a final model, all significant SNP across chromosomes were included in a common model and were individually removed in successive iterations based on their significance. Across all analyses, TBG was significant, with an additive effect of approximately 1.2 to 1.6 mm of backfat. Three SNP on SSC3 remained in the final model even though few studies have identified QTL for backfat on this chromosome. Two of these SNP exhibited irregular parent-of-origin effects and may not have been detected in other genome scans. One significant SNP on SSC7 remained in the final, backward-selected model; the estimated effect of this marker was similar in magnitude and direction to previously identified QTL. This SNP can potentially be used to introgress the leaner Meishan allele into commercial swine populations.

Key words: association analysis, backfat depth, single nucleotide polymorphism, swine

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INTRODUCTION

In general, gene association analyses in domesticated livestock species have been conducted using candidate gene methodology (e.g., Jokubka et al., 2006; Óvilo et al., 2006; Vidal et al., 2006). In this approach, researchers identify a polymorphism in a gene with a known function and test the association of these polymorphisms with changes in the recorded phenotypes (Tabor et al., 2002). Although attractive relative to statistical power and determining physiological causation, this approach relies on a priori knowledge of gene function. Detected associations may be due to linkage rather than causation. Unclassified genes and nontranslated genomic regions would likely be ignored.

Alternatively, genomic regions identified through QTL mapping, rather than genes with identified functions, can be considered as positional candidates for association analyses. By developing a moderately dense map of markers (e.g., SNP) within these QTL regions, one can narrow the position of the mutations responsi-

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[3]Corresponding author: Kreg.Leymaster@ars.usda.gov

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ble for the phenotypic differences, ultimately leading to fine mapping. Markers highly associated with phenotypic differences may be in linkage disequilibrium with a causative mutation and can potentially be used for marker-assisted selection after validation in other populations (Dekkers, 2004).

Backfat depth is economically relevant in swine production because of its implications for carcass composition and growth efficiency. A Meishan-derived population at the US Meat Animal Research Center has been scanned for QTL related to backfat depth (Rohrer and Keele, 1998; Rohrer, 2000). Both studies identified QTL regions for backfat on SSC 1 and 7, based on microsatellite markers. This population has since been managed as a segregating, randomly mated population, making it useful for association analysis of markers within the QTL regions identified.

The objective of this study was to detect SNP and genomic regions associated with ultrasonic backfat in this population.

MATERIALS AND METHODS

Experimental procedures involving animals were approved and performed in accordance with the US Meat Animal Research Center Animal Care Guidelines and the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Population and Phenotypes

The population examined was descended from the QTL resource population described in detail by Rohrer et al. (1999). Briefly, the original resource population was formed by reciprocally mating Meishan and a White Composite (¼ Chester White, ¼ British Landrace, ¼ Yorkshire, ¼ British Large White) line to form an F_1 generation, followed by the formation of both backcross lines. Backcross lines were mated together to form an F_3 population (50% of each breed), which was mated inter se for an additional generation.

After data collection for QTL scans (Rohrer and Keele, 1998; Rohrer et al., 1999; Rohrer, 2000), this resource population was mated to a different, lean White Composite (½ Yorkshire, ½ American Landrace). Thereafter, the line (¾ White Composite, ¼ Meishan) was mated inter se for 5 additional generations. Line development is summarized in Figure 1. Phenotypic records for this study were collected from the F_8 and F_{10} generations born in 2001 and 2003, respectively. The number of sires and dams used to produce these generations is presented in Table 1.

Ultrasonic backfat depths were recorded at an age of approximately 30 wk for gilts born in 2001 and 34 wk for gilts born in 2003. The gilts were raised under standard commercial conditions and fed corn and soybean meal-based diets with 3.90 Mcal of DE/kg on a DM basis (18% CP from 8 to 12 wk, 16% CP from 12 to 16 wk, and 15% CP thereafter). All females born in 2001 were retained and scanned for backfat (n = 159), whereas only a random sample of gilts that were potential breeding candidates was scanned in 2003 (n = 139). The gilts were scanned when the population reached a target mean weight of 113 kg of BW. Backfat measurements for each gilt were taken at 3 locations along the back using an A-mode Renco Lean-Meter (Renco Corp., Minneapolis, MN) and were averaged for data analysis. Age and backfat data for the 298 gilts are summarized in Table 1.

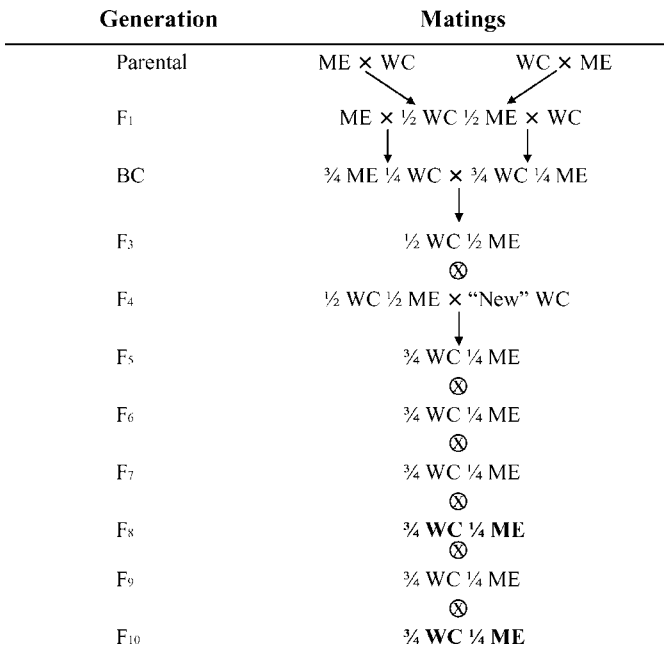

Figure 1. Diagram of development of the US Meat Animal Research Center ¾ White Composite (WC), ¼ Meishan (ME) F_8 and F_{10} line from QTL populations (BC generations).

Marker Data

Previous studies with this population had identified QTL for backfat on SSC 1 and 7 (Rohrer and Keele, 1998; Rohrer, 2000). The QTL regions identified in these studies were targeted for SNP discovery. Candidate SNP were identified using methods described by Fahrenkrug et al. (2002). Sequenced genes were selected using the random targeting approach (Fahrenkrug et al., 2002) or from comparative mapping of SSC1 (Nonneman et al., 2005b) and SSC7. In total, 6 SNP from SSC1 and 13 SNP from SSC7 were selected.

In addition to being measured for backfat depth, each gilt's age at puberty, ovulation rate, and nipple number were recorded. Based on QTL regions for these traits (Rohrer et al., 1999; Rohrer, 2000), genes were also selected from 3 regions on SSC3 (Mousel et al., 2006), from 2 regions on SSC8 (Campbell et al., 2003), and from SSC10 (Nonneman and Rohrer, 2004; Nonneman et al., 2006). An additional 16, 8, and 31 SNP were selected on SSC 3, 8, and 10, respectively, bringing the

Table 1. Backfat depth and pedigree data on 2001 and 2003 gilts

Year	No. of gilts	Backfat, mm		Age, d		BW, kg		No. of parents	
		Mean	SD	Mean	SD	Mean	SD	Sires	Dams
2001	159	27.1	4.63	211.0	2.83	116.9	15.08	16	58
2003	139	26.2	4.24	236.7	5.47	116.4	12.60	17	49

total number of SNP to 74. Although no significant associations with backfat were expected from these additional SNP, associations of these regions with backfat depth were analyzed to reveal possible antagonistic effects of SNP that may be significant for reproductive traits.

Assays for SNP genotyping were designed using a SpectroTyper (Sequenom, San Diego, CA). Assays used hME chemistry on a MassArray system (Sequenom). Briefly, 10-μL PCR reactions contained 5 ng of genomic DNA, 0.25 U of AmpliTaq Gold *Taq* (Applied Biosystems, Foster City, CA), 1× of supplied buffer, 1.5 m*M* $MgCl_2$, 200 μ*M* dNTP, and 0.4 μ*M* forward and reverse-tailed primers. The primer extension reaction used 0.6 μ*M* of probe primer according to the manufacturer's protocol.

Gilts were genotyped for the 74 SNP (see Supplemental Table, available online at http://jas.fass.org) and also for a polymorphism in the thyroxine-binding globulin gene (**TBG**) on the porcine X chromosome (Nonneman et al., 2005a). This polymorphism had shown an association with backfat depth in this population in previous work (Rohrer et al., 2004). The TBG gene lies within a backfat QTL region identified by Rohrer and Keele (1998) and Rohrer (2000). The allelic frequency of the TBG polymorphism in this population was 0.258 C and 0.742 A.

Statistical Analysis

A multilocus version of GenoProb (Thallman, 2002) was used to check the genotyping data for errors, to calculate genotypic probabilities for gilts that were unsuccessfully genotyped (for the number of animals successfully genotyped per SNP, see the Supplemental Table, available online at http://jas.fass.org), and to determine parental origin of alleles for heterozygous markers. Only 64 SNP had robust genotypic calls, as defined by GenoProb; these SNP were analyzed for associations. After investigation of the marker data, nonrobust calls were attributed to poor assay performance (a low number of animals successfully genotyped or null alleles detected).

The resulting GenoProb probabilities for each animal were used to derive regressors for regressions of backfat on additive, dominance, and parent-of-origin effects of each SNP. For animal j, the regressors (row vector $\mathbf{x}_j$) were calculated as

$$\mathbf{x}_j' = \begin{bmatrix} \mathbf{x}_A \\ \mathbf{x}_D \\ \mathbf{x}_P \end{bmatrix}_j = \begin{bmatrix} 0 & 1 & 1 & 2 \\ 0 & 1 & 1 & 0 \\ 0 & 1 & -1 & 0 \end{bmatrix} \begin{bmatrix} p_{aa} \\ p_{aA} \\ p_{Aa} \\ p_{AA} \end{bmatrix}_j,$$

where p_{aa} is the probability that j is homozygous for allele a, p_{AA} is the probability that j is homozygous for allele A, and p_{aA} and p_{Aa} are the probabilities that j is heterozygous and inherited allele a from its dam or from its sire, respectively. Values x_A, x_D, and x_P are then the additive, dominance, and parent-of-origin (via contrast of heterozygotic phase) regressors for animal j. Probabilities of heterozygous phases (p_{aA} and p_{Aa}) were generally quite high; the average phase probability was over 0.95 for all but 6 SNP. No parent-of-origin vectors (x_P) were predicted for these 6 SNP. This high average probability can be attributed to 2 factors: the pedigree structure of the phenotyped animals (8 to 10 offspring per sire, approximately 3 offspring per dam) and GenoProb accounting for multilocus inheritance.

Initially, each SNP was fitted individually using the model

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \begin{bmatrix} \mathbf{x}_1 \\ \vdots \\ \mathbf{x}_j \\ \vdots \\ \mathbf{x}_n \end{bmatrix} \begin{bmatrix} b_A \\ b_D \\ b_P \end{bmatrix} + \mathbf{e},$$

with

$$\text{var}\begin{bmatrix} \mathbf{a} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{A}\sigma_a^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{I}\sigma_e^2 \end{bmatrix},$$

where $\mathbf{y}$ is a vector of backfat data; $\mathbf{b}$ is a vector of fixed effects, including contemporary group (farrowing group and scan date), TBG genotype, and BW as a covariate; $\mathbf{a}$ is a vector of random polygenic breeding values; b_A, b_D, and b_P are the regression coefficients for the additive, dominance, and parent-of-origin effects of the SNP, respectively; and $\mathbf{e}$ is a vector of random residuals. Incidence matrices $\mathbf{X}$ and $\mathbf{Z}$ relate phenotypes to combinations of fixed or random effects. In the variance structure of $\mathbf{a}$ and $\mathbf{e}$, $\mathbf{A}$ is the numerator relationship matrix, σ_a^2 is the remaining additive variance (after accounting for TBG and the SNP fitted), and σ_e^2 is the residual

Table 2. Markers with *P*-values of less than 0.10 for average backfat (mm) using a 3-degree of freedom *F*-test (additive, dominance, and parent-of-origin effects) when fitted as a single marker

SSC	Position, cM	Marker	*P*-value	Alleles[1]	Component,[2] $P < 0.10$	Genotypic mean[3] aA	Aa	AA
3	0.0	17465.1h	0.034	A/G (0.051)	b_A[4]	0.23	–0.23	2.16
3	11.5	6559.2h	0.038	T/C (0.135)	b_P	–1.02	1.25	0.91
3	32.7	16963.2h	0.013	A/G (0.378)	b_P	1.14	–0.76	–0.63
3	32.7	27514.1h	0.015	G/A (0.489)	b_P	0.25	–1.60	–0.94
7	50.1	13438.1h	0.081	T/G (0.434)	b_A	1.04	1.57	1.75
7	60.5	45804.685h	0.061	G/T (0.269)	b_A, b_P	0.20	1.76	1.45
7	63.0	11807.1h	0.088	C/T (0.301)	b_A	1.17	1.22	2.00
7	63.0	17281.1h	0.015	G/A (0.324)	b_A	0.55	1.47	2.02
7	63.0	45796.161h	0.014	T/C (0.292)	b_A, b_P	–0.37	1.63	1.19
7	63.0	45796.385h	0.018	G/A (0.294)	b_A, b_P	–0.35	1.47	1.19
10	79.5	16865.1h	0.069	G/A (0.362)	b_P	0.98	–0.44	1.21
10	79.5	16865.2h2	0.084	A/T (0.364)	b_A, b_P	1.01	–0.25	1.33

[1]Minor/major allele; minor allele frequency of phenotyped animals is in parentheses.
[2]Components contributing to significance: b_A = additive, and b_P = parent-of-origin.
[3]Minor allele homozygote = aa = 0; and aA = minor allele from the dam.
[4]Dominance regression not fitted due to confounding; the additive effect (b_A) is the difference between the major homozygote mean and the average of the heterozygous means.

variance. Pedigree records (3,897 animals) dating to the initial foundation of the line were included in the calculation of $\mathbf{A}^{-1}$. All analyses were performed using MTDFREML (Boldman et al., 1995). In cases where the additive and dominance regressions were partially confounded due to low or no observations of the minor allele homozygous class, only the additive and parent-of-origin regressions (if parent-of-origin vectors were calculated) were fitted. Each SNP was also analyzed in a model in which the parent-of-origin regression was not fitted to add power for detection of additive and dominance effects. The power of the genotypic regressions was dependent on the genotypic frequencies for each SNP; SNP effects had to be large to detect significance when the frequency of one allele was low. Furthermore, the inclusion of the polygenic effect in the evaluation model reduces the effect of family structure for these SNP with low frequency alleles.

Variances were estimated from a model with no SNP fitted except TBG by allowing MTDFREML to iterate until a convergence criterion of 10^{-10} was obtained. These variance component estimates were consistent with other literature values for composite populations (e.g., Cassady et al., 2002; Klindt et al., 2006) and, because of erratic behavior of variance components in this small data set, were fixed for all subsequent SNP evaluation models. Effects of SNP were tested for significance using an *F*-test with 3 numerator df when all 3 SNP regressions were fitted and 2 numerator df when only the additive and dominance regressions were fitted. Individual regressions were also tested for significance using an individual *t*-test. No adjustments were made for multiple comparisons or multiple tests.

All SNP with significant *F*-tests or significant components were fitted simultaneously in a single model. To determine a final model, the least significant SNP was removed one at a time in a step-down manner until all remaining SNP were significant at a level of $P = 0.10$ or less. This backward selection procedure was performed with an initial model that included parent-of-origin (**3DF**) and a model in which parent-of-origin was not fitted for any SNP. In the 3DF case, parent-of-origin effects were only included for SNP markers when the individual parent-of-origin regression approached significance ($P < 0.10$).

RESULTS

Heritability was estimated at 0.46 ± 0.151 with a phenotypic variance of 13.84 mm^2 when no SNP (other than TBG) were included in the model. Heritability estimates were erratic when individual SNP were included in the model. No noticeable pattern was observed based on whether the SNP were significant; therefore, these changes in the estimate were attributed to the low number of records and variance components were fixed for subsequent models.

Results from fitting individual markers with $P < 0.10$ are shown in Table 2 (parent-of-origin effects included) and in Table 3 (no parent-of-origin effect fitted). Associations were detected for 12 SNP markers when parent-of-origin was fitted in the model. In these models, additive effects (b_A; generally ½ of AA value) ranged from 0.6 to 1.0 mm of backfat except for SNP 17465.1h, which had an additive effect of 2.22 mm. Parent-of-origin effects were detected for several markers based on the heterozygous phase contrast. When only additive and dominance effects were fitted for individual SNP, only 5 associations were detected. Additive effects were of similar magnitudes to the 3DF analyses.

When a reduced model was formed using backward selection, the number of SNP with significant effects

Table 3. Markers with *P*-values of less than 0.10 for average backfat (mm) using a 2-degree of freedom *F*-test (additive and dominance effects) when fitted as a single marker

SSC	Position, cM	Marker	*P*-value	Allele[1]	Component,[2] $P < 0.10$	Genotypic mean[3] Het	Genotypic mean[3] AA
3	0.0	17465.1h	0.010	A/G (0.051)	b_A[4]	0.00	2.09
7	50.1	13438.1h	0.047	T/G (0.434)	b_A	1.35	1.79
7	63.0	11807.1h	0.038	C/T (0.301)	b_A	1.20	2.00
7	63.0	17281.1h	0.012	G/A (0.324)	b_A	1.03	2.12
10	127.5	49431_198.2	0.079	C/T (0.364)	b_D	1.13	0.29

[1]Minor/major allele; minor allele frequency of phenotyped animals is in parenthesis.
[2]Components contributing to significance: b_A = additive, and b_D = dominance.
[3]Minor allele homozygote = aa = 0; and Het = heterozygotic phase average.
[4]Dominance regression not fitted due to confounding; the additive effect (b_A) is the difference between the major homozygote mean and the heterozygote mean.

dropped from 12 to 4 (not including TBG) when parent-of-origin effects were allowed in the model (Table 4). With no parent-of-origin effects, only 2 SNP remained in the model (Table 5).

DISCUSSION

Linkage disequilibrium markers are useful in genetic selection because of their consistent association with a functional mutation (Dekkers, 2004). However, results from research studies used to detect these associations are specific to the inference population. One must consider the source of linkage disequilibrium when extrapolating results to other populations. In this study, phenotypes were measured on a ¼ Meishan Composite population. Linkage disequilibrium from the initial cross of the Meishan founders to the White Composite lines likely still remains in this population, given the diverse backgrounds of these breeds. Resulting associations therefore may represent regions of interest rather than individual SNP that are in general linkage disequilibrium across diverse swine populations. Unfortunately, tissue samples were not available on all foundation lines to estimate SNP frequency differences in this population, which may have given rise to this source of linkage disequilibrium. The regions identified in this study should be scanned at a higher density in outbred populations to discover markers with higher levels of associations. Significant individual SNP should be validated in commercial populations to determine their effects before broad use.

Identification of SNP beneath QTL peaks described in Rohrer and Keele (1998) and Rohrer (2000) in the ancestors of this population facilitated the discovery of polymorphisms with significant associations with ultrasonic backfat depth (Tables 2 and 3) on SSC7. Associated SNP were located between 50 and 63 cM. This region has been identified as having a QTL for backfat depth in several studies involving Meishan germplasm (Wang et al., 1998; Rattink et al., 2000; Wada et al., 2000; Bidanel et al., 2001; Milan et al., 2002; Geldermann et al., 2003; Sato et al., 2003). There is general agreement that the QTL on SSC7 of Meishan origin results in lower backfat with an additive effect (b_A) of approximately 1 to 2 mm. The current population was a segregating 25% Meishan Composite. Three SSC7 SNP (13438.1h, 11807.1h, and 17281.1h) showed an additive effect of similar, though smaller, magnitude (1.75 to 2.12 mm difference between homozygous classes; b_A = 0.87 to 1.06 mm) with the minor allele having less depth of fat. When all of the SNP were fitted simultaneously (Tables 4 and 5), marker 17281.1h remained in the final, backward-selected model regardless of whether markers showing evidence of parent-

Table 4. Markers remaining in the average backfat (mm) model after backward selection when parent-of-origin effects were allowed in the model

SSC	Position, cM	Marker	*P*-value	Component,[1] $P < 0.10$	Genotypic mean[2] aA	Genotypic mean[2] Aa	Genotypic mean[2] AA
3	0.0	17465.1h	0.016	b_A[3]	0.00	—	2.00
3	11.5	6559.2h	0.045	b_P	–1.37	0.77	0.58
3	32.7	27514.1h	0.071	b_P	0.51	–1.19	–0.10
7	63.0	17281.1h	0.013	b_A	1.10	—	2.17
X	60.0	TBG	0.018	b_A, b_D	–2.58	—	–2.99

[1]Components contributing to significance: b_A = additive, b_D = dominance, and b_P = parent-of-origin.
[2]Minor allele homozygote = aa = 0; and aA = minor allele from the dam (also used as the heterozygous average when there was no breed-of-origin effect).
[3]Dominance regression not fitted due to confounding; the additive effect (b_A) is the difference between the major homozygote mean and the heterozygote mean.

Table 5. Markers remaining in average backfat (mm) model after backward selection when parent-of-origin effects were not allowed in the model

SSC	Position, cM	Marker	*P*-value	Component,[1] $P < 0.10$	Genotypic mean[2] Het	Genotypic mean[2] AA
3	0.0	17465.1h	0.011	b_A[3]	0.00	2.07
7	63.0	17281.1h	0.013	b_A	0.91	2.03
X	60.0	TBG	0.067	b_A	–2.10	–2.42

[1]Components contributing to significance: b_A = additive, and b_D = dominance.
[2]Minor allele homozygote = aa = 0; and Het = heterozygotic phase average.
[3]Dominance regression not fitted due to confounding; the additive effect (b_A) is the difference between the major homozygote mean and the heterozygote mean.

of-origin effects were included. This marker is the most favorable candidate for the QTL: its effect is of similar magnitude to previously reported QTL, the sample minor allele frequency (0.324) is not far removed from the 25% Meishan germplasm in this population, and the minor allele is favorable for reduced fat depth. Marker 17281.1h is in intron 7 of the zinc finger protein 76 (ZNF76) gene, located at 35 Mb on human chromosome 6. This gene is very close to peroxisome proliferative activated receptor delta (**PPARD**) at 35 Mb, which is a positional and physiological candidate for affecting backfat thickness. Markers 45796.161h and 45796.385h in PPARD were associated with backfat when fitted as a single marker (Table 2), but dropped out in subsequent analyses. If the causative mutation does lie within PPARD, 17281.1h is certainly close enough to be in linkage disequilibrium, yet other genes in the region should not be overlooked as candidates. The impact of the associated SNP on SSC7 and particularly the causative mutation of the previously reported QTL may be minute given the limited penetration of Meishan germplasm in the US swine industry. However, other studies (Nagamine et al., 2003; Vidal et al., 2005) have reported QTL segregating for backfat in this region of SSC7 in commercial swine populations, suggesting that this marker or marker area may be useful for selecting for leanness even without Meishan influence. At a minimum, identification of this SNP may allow introgression of this Meishan mutation for reduced backfat depth. If this marker is segregating in nonMeishan populations, it may be incorporated into marker assisted selection programs. Further testing of SSC7 should be conducted, especially in other populations, in order to validate the effects of this marker or to find other markers in LD with the causative mutation that are robust across different populations.

Marker SNP from SSC 3, 8, and 10 had been identified to test for mutations in association with QTL for age at puberty, ovulation rate, and number of nipples (Rohrer et al., 1999; Rohrer 2000). These same gilts had been genotyped for these SNP, and therefore, the SNP were analyzed for an association with average backfat. Resulting associations on 2 regions of SSC3 and on SSC10 (Tables 2 and 3) were not expected. Few studies have reported putative QTL for backfat measures in either of these chromosomal linkage groups. Knott et al. (1998) reported suggestive QTL for slaughter backfat on SSC3 at 113 cM (males) and 169 cM (females) in a segregating F_2 Wild Boar, Large White population. In an F_2 Pietrain × Wild Boar cross, Beeckmann et al. (2003) and Geldermann et al. (2003) discovered QTL for backfat at about 70 cM on SSC3. Putative QTL from both of these studies are likely too distal from the associated SNP in this study to be in linkage disequilibrium. Similarly, suggestive QTL for backfat on SSC10 have only been identified in a few studies: at 27 cM in females (Quintanilla et al., 2002), at 67 cM (Rohrer et al., 2005), and at 86 cM (Rohrer and Keele, 1998; first rib backfat only). In this case, the QTL peaks from Rohrer et al. (2005) and from Rohrer and Keele (1998) are close to the suggestive associations of SNP on SSC10 observed in this study at 79.5 cM (Table 2).

Ancestors of this population had been scanned for QTL for backfat at slaughter (Rohrer and Keele, 1998) and for ultrasonic backfat (Rohrer, 2000) with QTL (suggestive or significant at the whole genome level) identified on SSC 1, 7, and X. None of the SNP on SSC1 were associated with backfat in the present research, implying further SNP testing on SSC 1 may be warranted. Lack of map density may have contributed to the lack of significant associations on SSC1. The SNP associations on 2 regions of SSC 3 and on 10 in the present research may be more questionable because no QTL had been identified on these chromosomes; the SNP on SSC10 are especially questionable because none of them remained in the model after backward selection. However, there are several possible reasons for significant SNP without QTL being discovered in previous studies in this population or in other QTL scans. The SNP on SSC3 were significant primarily because of parent-of-origin effects rather than traditional additive or dominance effects; with some exceptions (e.g., Rattink et al., 2000; Rohrer et al., 2005) models with parent-of-origin effects were not used to detect QTL. Most QTL methodology relies on lines being nearly fixed for opposite alleles. In this case, if the SNP were already segregating in both the Meishan and early White Composite, or it originated from the new White Composite, its effect may not have been detected in the QTL scan. The association model used to analyze these

data would have detected a QTL if either of these scenarios existed.

Some caution is still justified with respect to 17465.1h on SSC3. Although its additive effect was large and significant, the allelic frequency is quite low. Virtually no individuals homozygous for the minor allele were detected or predicted from GenoProb. Therefore, the additive estimate is based on the difference between a low frequency heterozygous class (mostly from related animals) and the major allele homozygous class. Fitting polygenic effects in the model should have reduced the effect of genetic relationships creating spurious associations, but this marker should still be validated in a separate population to see whether this association is confirmed.

Parent-of-origin effects have not, as yet, been extensively studied for production traits in swine though some associations have been detected for candidate genes (e.g., *DLK1* and *IGF2*; Nezer et al., 1999 and Kim et al., 2004), and some QTL scans for backfat have included parent-of-origin effects (e.g., Rattink et al., 2000; Milan et al., 2002; Thomsen et al., 2004). When examining parent-of-origin trends from the single SNP analyses in this study (Table 2), patterns seem to follow a paternal origin trend on SSC10 and a maternal origin tendency on SSC7. Thomsen et al. (2004) suggested paternal and maternal inheritance of carcass measures in this same region on SSC10, and maternally expressed QTL were identified for muscle depth at 56 cM (Rattink et al., 2000) and belly weight at 63 cM (Milan et al., 2002) on SSC7. Both studies imply that parent-of-origin effects may be present in these regions. However, neither of these genomic regions shows parent-of-origin effects in the final model suggesting these effects are either spurious or too small to be detected in conjunction with a Mendelian model of inheritance. Parent-of-origin patterns on SSC3 are less clear with the genotypes expressing more of a polar overdominance pattern (especially in the final model; Table 4). In the murine genome, imprinted genes have been identified in the corresponding regions to where markers 6559.2h, 16963.2h, and 27514.1h are located on SSC3 (Nikaido et al., 2003), suggesting the possibility of imprinting in this region. However, the genes that contain these markers have not been implicated directly. On SSC7, a QTL for backfat with possible parent-of-origin effects has been detected (Rattink et al., 2000) at approximately 57 cM—close to 45796.161h and 45796.385h at 63.0 cM on SSC7 (Table 2). These SNP did not remain in the final, backward-selected model, suggesting that the additive effects from 17281.1h were more indicative of variation in backfat on SSC7.

The final backfat model contained a total of 5 markers when parent-of-origin effects were allowed in the model (Table 4) and 3 markers when parent-of-origin effects were not allowed (Table 5). Selecting markers using backward selection after identifying SNP with individual effects is useful to identify markers with the most potential for use in breeding schemes or for pinpointing areas of the genome to continue sequencing for polymorphisms in linkage disequilibrium with QTL. No multiple comparison procedures were used to account for false discovery rates when testing SNP individually; with the number of tests conducted on 64 robust SNP, 8 to 13 associations were expected at the $P < 0.10$ level. The backward selection process resulted in a more conservative estimate of markers with actual associations. Also, the backward selection process avoids overstating the number of significantly associated SNP resulting from high correlations between regressions of closely linked markers; in this case, the number of associated markers was reduced from 12 to 4 (5 including TBG) when parent-of-origin effects were allowed and from 5 to 2 (3 with TBG) without parent-of-origin effects. When simultaneously fitting multiple linked markers in the same model, it is probable that these markers will explain similar variation in the trait of interest, resulting in multicollinearity. Systematic removal of SNP resulted in a more concise and likely more meaningful model and leads to isolating regions to concentrate resources in finding new SNP. As theory and software evolve, and as increased numbers of SNP are identified, a model fitting haplotypes (Meuwissen and Goddard, 2000) and their interactions may be preferable. At any rate, targeting areas under QTL peaks for SNP markers appears to be an effective strategy for developing usable industry markers. Using the results from this study, the areas where associated SNP were discovered can be further saturated with SNP from sequence data to identify markers with stronger associations with backfat and further detail the gene action in areas exhibiting parent-of-origin effects. This step will bring us closer to fine mapping these regions and to developing more robust genetic markers.

Results in this study were derived using a model with polygenic effects. As such, phenotypic resemblance between related animals due to factors beyond SNP marker genotypes was accounted for through the numerator relationship matrix. Related animals are often the main source of some of the genotypes, especially when alleles have low frequencies (i.e., less than 5%). Therefore, fitting a polygenic model reduces the likelihood of significant but spurious associations for some of the SNP arising due to animal relationships rather than the marker being examined.

In the final model, 3 markers were retained with additive effects: 17465.1h on SSC3, 17281.1h on SSC7, and TBG (also expressed dominance) on SSC X. Adding markers 6559.2h and 27514.1h, which showed effects of parent-of-origin, seems to be of little consequence relative to the effects of these additive markers. However, it is unlikely that any of them except possibly TBG represent a causative mutation for backfat deposition. They should be validated in other populations before extensive use. Since the TBG marker is located on SSC X, an examination of its effect on male backfat deposition is also warranted. The QTL region of SSC1 where no associated SNP were discovered should also be exam-

ined more closely to detect markers in linkage disequilibrium with a causative mutation or to determine whether the QTL detected there actually exists (i.e., the detected QTL was false).

In conclusion, single nucleotide polymorphisms that are associated with backfat deposition and, thereby, carcass leanness have been identified on swine chromosomes 3, 7, and X. Their effects should be validated in other populations and fine mapping in these regions should continue to develop robust genetic markers. These markers were identified by targeting areas of the genome where quantitative trait loci had been detected in previous studies. Targeting areas detected by QTL scans for SNP discovery is the next logical step in developing markers that will be useful for selection and should be continued in advanced generations of other populations where QTL have been identified. Backward selection procedures helped to identify markers with the strongest associations with backfat; potential problems with over-selection of correlated markers were avoided as a result.

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Chromosome	Relative Position	Marker Name	Gene Symbol	Gene Name	Hsa	Start	Probe	Accession No.	SS No.	Beginning position in contig	Ending position in contig	Observed alleles	No. of animals genotyped
1	103.863	16871.1h	HGFAC	HGF activator	4	3,475,103	GGGATGCTCTTGTCTCCTTA	BV677879	23130674	178	178	G/T	279
1	107.835	14124.1h2	AMBP	alpha-1-microglobulin/bikunin precursor	9	112,198,452	CACGTTACAGAATGGAAGGA	BV103453	23130307	360	360	G/T	284
1	107.835	14124.2h2	AMBP	alpha-1-microglobulin/bikunin precursor	9	112,198,452	TTGACTCCCAGAGAGGCCA	BV103453	23130309	428	428	A/G	287
1	119.031	26059.1h	GSN	gelsolin (amyloidosis, Finnish type)	9	119,406,452	TGGGGTGGAGGTGGAGGGAC	BV102948	23132309	117	117	A/G	267
1	132.705	23317.1h2	C9orf78	chromosome 9 open reading frame 78	9	127,946,870	CAAGCCTGGGGTGGGG	BV102732	23131608	150	150	C/T	218
1	137.735	14350.1h	RAPGEF1	Rap guanine nucleotide exchange factor (GEF) 1	9	129,728,202	GCACGAGCGAGTTTGCGGG	BV103468	23130335	553	553	C/T	288
3	0.000	17465.1h	NA	NA	7	3,073,641	GGCTGTAGGAGAGGGT	BV102762	23132038	675	675	A/G	288
3	11.518	6559.2h	DKFZp761L1417	hypothetical protein DKFZp761L1417	7	97,429,762	TGCCTTTGGCTTTTTG	BV106141	16337308	316	316	C/T	265
3	11.518	6559.3h	DKFZp761L1417	hypothetical protein DKFZp761L1417	7	97,429,762	CCTGTCTAGGTGGCCTTAC	G72565	16337309	397	397	C/T	293
3	13.932	13815.1h	PRKRIP1	PRKR interacting protein 1 (IL11 inducible)	7	101,030,294	GGGGAATGACTTGCCCCAG	BV103432	23130266	423	423	A/C	260
3	13.932	24269.1h	TTC11	tetratricopeptide repeat domain 11	7	99,874,453	GCTGCACCCCCGTGCT	BV103145	23132126	526	526	C/G	295
3	14.520	6945.1h	TMPIT	transmembrane protein induced by tumor necrosis factor alpha	7	75,260,959	CAGGAAGCGGCCGTCACTCC	G72507	16337199	150	150	A/G	293
3	18.206	7907.2h	NA	NA	7	6,818,881	TCCTTAACTCACAGCACAA	BV104094	48398182	452	452	C/G	294
3	32.954	16963.2h	SULT1A1	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	16	28,469,356	TGGCCCCTTCTTCAACT	BV103265	23131089	373	373	A/G	291
3	32.954	27514.1h	LOC112869	hypothetical protein BC011981	16	28,501,139	GATTGTAAGGTTCCTTAGTGCTA	BV102888	23132881	610	610	A/G	278
3	32.954	27514.2h	LOC112869	hypothetical protein BC011981	16	28,501,139	TTGCCAACAATGACAGCCA	BV102888	23132883	745	745	G/T	295
3	88.890	8079.2h	FLJ20254	LOC54867	2	27,167,473	TGTGCCCTCTTTTCAGCTC	BV686588		253	253	A/C	291
3	87.737	17340.1h	SLB	selective LIM binding factor, rat homolog	2	27,641,779	ACCTACACACCGAAATTTCACA	BV103308	23131537	221	221	C/T	296
3	89.100	49986.785h	PREB	prolactin regulaory element binding protein	2	27,265,278	TGAATCCGCTCTAGCT	BV680509	52051931	785	785	A/C	274
3	89.200	49990.454h	PREB	prolactin regulaory element binding protein	2	27,265,278	TGTGCTGCTTGTCCAGGA	BV680516	52051943	454	454	A/G	276
3	99.383	16997.1h	WDR35	WD repeat domain 35	2	20,097,513	CCCTCGAGGGCGATCCA	BV103309	23130691	237	237	C/T	297
3	100.551	15655.1h	KCNS3	potassium voltage-gated channel, delayed-rectifier, subfamily S, member 3	2	18,044,458	TGCATCCGCTGGGCATA	BV102681	23129852	500	500	C/T	297
7	44.995	13438.1h	CDKAL1	CDK5 regulatory subunit associated protein 1-like 1	6	20,642,736	GCTCTGTTGGCTCTTCTGT	BV103643	23130590	503	503	G/T	290
7	51.355	5845.4h	PPP1R11	protein phosphatase 1, regulatory subunit 11	6	30,144,361	TCAGGGAGAGAAGGTT	G72658	16337936	259	259	A/G	194
7	54.582	6551.2h	GTF2H4	general transcription factor IIH, polypeptide 4	6	30,982,253	CCCTCTCTCAGGGTCA	BV106137	16337305	251	251	C/G	187
7	53.517	2535.3h	NUTF2	nuclear transport factor 2	16	67,657,055	TCAGCTCCCTCCCTCC	BV104825	48398432	470	470	A/G	223
7	53.517	26623.1h	BAT2	HLA-B associated transcript 2	6	31,692,874	GGGAATTGACATTGCTCCC	BV103054	23132640	695	695	A/G	277
7	53.517	45804.685h	PPARD	peroxisome proliferative activated receptor, delta	6	35,418,313	CCAGGACACCATCCTGCG	BV677929	48397625	685	685	G/T	296
7	59.019	11807.1h	ITPR3	inositol 1,4,5-triphosphate receptor, type 3	6	33,636,199	CTGGACCTGCCGGAA	BV686589		205	205	C/T	279
7	59.019	17281.1h	ZNF76	zinc finger protein 76	6	35,335,488	ACCAGTGTAAGAGGGAACCTA	BV104069	23131456	373	373	A/G	291
7	53.517	45796.161h	PPARD	peroxisome proliferative activated receptor, delta	6	35,418,313	CTCCCAAGTAGGAATTCCGCC	BV678004	48397593	161	161	C/T	297
7	53.517	45796.385h	PPARD	peroxisome proliferative activated receptor, delta	6	35,418,313	AACGTTTCCTTTTTTATCGGAG	BV678004	48397595	385	385	A/G	296
7	64.920	14325.1h	CUL7	cullin 7	6	43,052,210	GGAAGGGCTTTCCCTC	BV103461	23130318	267	267	C/T	290
7	64.920	21351.1h	CUL7	cullin 7	6	43,052,210	CCACGTGCCAGCTGCCACCA	BV102746	23131827	303	303	C/T	288
7	64.920	21351.2h	CUL7	cullin 7	6	43,052,210	GAAGACCAGGGGAAAGGAGGC	BV102746	23131826	263	263	C/T	284
8	1.428	16348.1h	RGS12	regulator of G-protein signalling 12	4	3,347,251	CCTTTCCCGCCGGTTGCCC	BV103401	23130930	651	651	A/G	293
8	5.784	27863.1h	PGR1	T-cell activation protein	4	6,706,987	ACGCAGCATGTTTCACTC	AY596450		246	246	A/G	280
8	5.784	27863.2h	PGR1	T-cell activation protein	4	6,706,987	CTCAGGAAGCTAGAGG	AY596450		329	329	A/G	243
8	5.784	MAN-12P1h	MAN2B2	mannosidase alpha, 2B2	4	6,694,974	CCCATACACGCTGCGGTAC	BV680482	52052035	423	423	A/C	285
8	5.784	MAN-8P2h	MAN2B2	mannosidase alpha, 2B2	4	6,694,974	GCGCTCAGGAACCGG	NM_213849		1344	1344	A/G	242
8	5.784	MAN-Sh	MAN2B2	mannosidase alpha, 2B2	4	6,694,974	GGCCCTGCAGGCAGACTGA	NM_213849		156	156	C/T	255
8	31.864	PPARGC1-8.2	PPARGC1A	peroxisome proliferative activated receptor	4	23,469,914	TCTTCCACAGACTCAGACCAG	BV680483	52052039	84	84	A/T	0
8	31.864	PPARGC1-9.1	PPARGC1A	peroxisome proliferative activated receptor	4	23,469,914	CGCACGCACCGAAATTCT	AY346131S1		1933	1933	A/C	47
10	67.229	26051.1h	AQP3	aquaporin 3	9	33,431,158	TCTGTCTGCCCCCAGGAC	BV102944	23132269	71	71	A/G	288
10	67.229	16023.1h	GALT	galactose-1-phosphate uridylyltransferase	9	34,636,635	GGGCGTGCACCTCCTT	BV103319	23129420	687	687	A/G	289
10	67.959	20663.1h	UBAP1	ubiquitin associated protein 1	9	34,169,011	GAAGCACAGTCAGGATGG	BV102774	23131686	318	318	C/T	291
10	67.959	20663.2h	UBAP1	ubiquitin associated protein 1	9	34,169,011	AAGCACAGTCAGGATGG	BV102774	23131686	318	318	C/T	294
10	80.626	31672_466.1	EPC1	enhancer of polycomb homolog 1 (Drosophila)	10	32,561,865	CACGCTGTTTCCATATAA	BV102644		193	193	C/T	297
10	80.626	31672_509.2	EPC1	enhancer of polycomb homolog 1 (Drosophila)	10	32,561,865	GGTGGGGCCGAGAGAGCA	BV102644		236	236	A/G	297
10	76.343	37843_638.2	BAMBI	BMP and activin membrane-bound inhibitor homolog (Xenopus laevis)	10	28,970,459	GGCTTCAAAATGCTGAAAAAAAC	BV677799	48397841	638	638	C/T	295
10	76.343	38189.341h	BAMBI	BMP and activin membrane-bound inhibitor homolog (Xenopus laevis)	10	28,970,459	AGACAGCAGTTCTCGTGGCC	BV677966	48397688	341	341	C/T	295
10	80.626	16865.01h	MRC1	mannose receptor, C type 1	10	18,102,358	TGTCTAGCTGATATCAGAGG	BV103215	23130683	48	48	A/C	293
10	80.626	16865.05h	MRC1	mannose receptor, C type 1	10	18,102,358	ATGTCAGGAAGCTGAGGGTG	BV103215	23130684	68	68	A/C	296
10	80.626	16865.1h	MRC1	mannose receptor, C type 1	10	18,102,358	GGTTACATCTTTGGGTACC	BV103215	23130672	97	97	A/G	292
10	80.626	16865.2h2	MRC1	mannose receptor, C type 1	10	18,102,358	CCACTGAAAGGTTATTCAATA	BV103215	23130673	139	139	A/T	295
10	80.626	27741.1h	MRC1	mannose receptor, C type 1	10	18,102,358	CAGGCAGGAAGATTGT	AY368183		1280	1280	A/G	298
10	85.871	16857.1	NMT2	N-myristoyltransferase 2	10	15,153,909	GGTGCCTGGTGGGGC	BV103211	23131306	94	94	C/T	129
10	85.871	16857.2h	NMT2	N-myristoyltransferase 2	10	15,153,909	ACCTGTTTTCCTCACC	BV103211	23131309	173	173	C/T	210
10	90.595	13739.1h	GAD2	glutamate decarboxylase 2	10	26,545,600	TATCACCTCTCCAAAGTTAGT	BV103636	23130238	420	420	A/G	269
10	90.595	15438.1h	BMI1	B lymphoma Mo-MLV insertion region (mouse)	10	22,614,146	GGTAAATTCTCTAGGAAATGTATT	BV102649	23130099	126	126	A/T	268
10	98.701	41180.232h	CREM	cAMP responsive element modulator	10	35,456,394	TGTGAGTATTAAATCAGATCATGT	BV677861	48397581	232	232	A/G	297
10	98.701	41395.182h	CREM	cAMP responsive element modulator	10	35,456,394	AACAAATGTGGCAGGAA	BV677783	48397788	182	182	A/G	266
10	106.989	16863.1h	CAMK1D	calcium/calmodulin-dependent protein kinase ID	10	12,395,592	AACCTTCTGTTCTAACCA	BV103214	23131348	465	465	A/G	293
10	126.022	36969_772.1	AKR1C4	aldo-keto reductase family 1, member C4 (chlordecone reductase; 3-alpha hydroxysteroid dehydrogenase, type I; dihydrodiol dehydrogenase 4)	10	5,192,798	CTATTGCCAAGAAACACAA	BV680543	52052006	278	278	C/T	177
10	127.794	49422.1h	AKR1C2	aldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid binding protein; 3-alpha hydroxysteroid dehydrogenase, type III)	10	4,985,965	CCAAATCCCAGTACAGGAA	BV680531	52052004	42	42	A/T	298
10	126.022	49431_198.2	AKR1C4	aldo-keto reductase family 1, member C4 (chlordecone reductase; 3-alpha hydroxysteroid dehydrogenase, type I; dihydrodiol dehydrogenase 4)	10	5,192,798	GTGATTCTTCCCTTCCT	BV680533	52052007	198	198	C/T	291
10	127.794	33391_261	AKR1CL2	aldo-keto reductase family 1, member C-like 2	10	4,858,402	CCTCCGGTGCCCGCGCT	BV102614	23133169	261	261	C/T	295
10	127.794	36962.1h	AKR1C2	aldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid binding protein; 3-alpha hydroxysteroid dehydrogenase, type III)	10	4,985,965	TGGGATCCCAGGGCAGCAT	DQ474066		779	779	C/T	283
10	127.196	14984_99	AKR1C4	aldo-keto reductase family 1, member C4 (chlordecone reductase; 3-alpha hydroxysteroid dehydrogenase, type I; dihydrodiol dehydrogenase 4)	10	5,192,798	TCTGTGAAAATGTGGGT	BV680523	52051950	99	99	G/T	296
10	127.196	20502_681.2	AKR1C4	aldo-keto reductase family 1, member C4 (chlordecone reductase; 3-alpha hydroxysteroid dehydrogenase, type I; dihydrodiol dehydrogenase 4)	10	5,192,798	CTTCTCAATCTTTTAGCTA	DQ494489		6582	6582	C/T	275
10	127.196	20502_722.1	AKR1C4	aldo-keto reductase family 1, member C4 (chlordecone reductase; 3-alpha hydroxysteroid dehydrogenase, type I; dihydrodiol dehydrogenase 4)	10	5,192,798	GGACCAGAAGAAATTAAAAATG	DQ494489		6623	6623	C/T	297
10	127.196	27648_208.1	AKR1C4	aldo-keto reductase family 1, member C4 (chlordecone reductase; 3-alpha hydroxysteroid dehydrogenase, type I; dihydrodiol dehydrogenase 4)	10	5,192,798	TGCGTGAAGCTGAATGA	BV680527	52051993	280	280	C/T	278
10	127.196	31503.1h	AKR1C4	aldo-keto reductase family 1, member C4 (chlordecone reductase; 3-alpha hydroxysteroid dehydrogenase, type I; dihydrodiol dehydrogenase 4)	10	5,192,798	GAGATAAGGGTGAGTAGTGG	BV680525	52051987	40	40	A/G	292
10	127.196	31063.1h	AKR1C4	aldo-keto reductase family 1, member C4 (chlordecone reductase; 3-alpha hydroxysteroid dehydrogenase, type I; dihydrodiol dehydrogenase 4)	10	5,192,798	GAAATAACAGAATCAGAGTACT	BV680526	52051991	354	354	G/T	295