Mapping quantitative trait loci for metabolic and cytological fatness traits of connected F_2 crosses in pigs

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Mapping quantitative trait loci for metabolic and cytological fatness traits of connected F_2 crosses in pigs[1]

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ABSTRACT: In the present study 3 connected F_2 crosses were used to map QTL for classical fat traits as well as fat-related metabolic and cytological traits in pigs. The founder breeds were Chinese Meishan, European Wild Boar, and Pietrain with to some extent the same founder animals in the different crosses. The different selection history of the breeds for fatness traits as well as the connectedness of the crosses led to a high statistical power. The total number of F_2 animals varied between 694 and 966, depending on the trait. The animals were genotyped for around 250 genetic markers, mostly microsatellites. The statistical model was a multi-allele, multi-QTL model that accounted for imprinting. The model was previously introduced from plant breeding experiments. The traits investigated were backfat depth and fat area as well as relative number of fat cells with different sizes and 2 metabolic traits (i.e., soluble protein content as an indicator for the level of metabolic turnover and NADP-malate dehydrogenase as an indicator for enzyme activity). The results revealed in total 37 significant QTL on chromosomes 1, 2, 4, 5, 6, 7, 8, 9, 14, 17, and 18, with often an overlap of confidence intervals of several traits. These confidence intervals were in some cases remarkably small, which is due to the high statistical power of the design. In total, 18 QTL showed significant imprinting effects. The small and overlapping confidence intervals for the classical fatness traits as well as for the cytological and metabolic traits enabled positional and functional candidate gene identification for several mapped QTL.

Key words: candidate gene, fatness trait, imprinting, pig, quantitative trait locus

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INTRODUCTION

Fat-related traits are frequently included as a goal of pig breeding programs. Many QTL mapping experiments have been conducted to find loci affecting fat traits, and numerous QTL have been reported (Hu et al., 2005). Most studies used fat traits defined in a rather classical way (e.g., backfat thickness or intramuscular fat). These traits can be seen as end products within a cascade of physiological steps, which are controlled by gene products, such as enzymes. For the interpretation of QTL results and the identification of genes and pathways underlying the QTL, it might be advantageous to have some trait measurements of the direct gene products. Specifically, body fat tissue results from development of adipocytes and deposition of fat into these cells, with the latter mainly influenced by lipogenesis and lipolysis. It was shown that the adipocytes of pigs with a greater propensity to fatten had a greater volume of fat cells (Etherton, 1980; Scott et al., 1981). Lipogenic enzyme activities have also been associated with different amount of fat deposition in pigs (Hood and Allen, 1973). Following this, it would be desirable to have trait measurements of adipocyte characteristics as well as specific enzyme activities regulating lipogenesis to better understand mapped fat trait QTL.

The advantage of using metabolic and cytological traits was demonstrated by Demars et al. (2007), who were able to better characterize the underlying nature of a QTL for body fatness mapped on SSC7 than using solely classical fatness traits. Geldermann et al. (2010) considered numerous classical fat traits as well as measurements of fat-related enzyme activity and number and volume of fat cells. Geldermann et al. (2010) analyzed 3 porcine F_2 crosses that are connected by the same founder breeds and animals. The founder breeds were Chinese Meishan, European Wild Boar, and Pietrain. For analyzing fat traits, these founder breeds are especially well suited because it is known that they differ markedly in fat deposition (e.g., Mourot et al.,

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Table 1. Description of the traits with abbreviations (Abbr.) and summary statistics within and across the 3 crosses, number of observations (n), mean, SD, minimum (Min), and maximum (Max) of the phenotypic observations

Trait	Abbr.	Cross[1]	n	Mean	SD	Min	Max
Average backfat depth, mm	BFD	M×P	316	27.93	6.68	8.70	46.00
		W×P	315	22.82	4.98	10.30	40.00
		W×M	335	31.82	6.68	8.30	48.70
		Joint	966	27.61	7.19	8.30	48.70
Fat area, cm^2	FA	M×P	316	20.85	5.89	5.52	38.80
		W×P	313	16.71	5.52	4.24	37.45
		W×M	335	24.42	6.59	4.18	47.44
		Joint	964	20.75	6.80	4.18	47.44
Soluble protein content, mg/g of tissue	SPC	M×P	315	3.59	1.54	0.63	12.97
		W×P	315	4.86	1.73	2.30	13.30
		W×M	326	3.49	1.12	1.47	8.79
		Joint	956	3.98	1.60	0.63	13.30
NADP-malate dehydrogenase, units/g of tissue	MDH	M×P	315	0.61	0.27	0.07	2.18
		W×P	315	0.45	0.18	0.11	1.22
		W×M	326	0.51	0.19	0.14	1.33
		Joint	956	0.52	0.23	0.07	2.18
Relative number of fat cells with medium cell sizes, 73 to 146 μm, %	FCL	M×P	307	46.37	19.02	5.11	79.48
		W×P	296	56.41	14.80	7.06	82.99
		W×M	91	30.99	17.99	1.90	76.41
		Joint	694	48.63	19.08	1.90	82.99
Relative number of fat cells with large cell sizes, >146 μm, %	FCH	M×P	307	16.86	13.93	0.37	63.21
		W×P	296	5.57	5.78	0.36	39.48
		W×M	91	16.16	12.51	0.41	50.25
		Joint	694	11.85	12.27	0.36	63.21

[1]M×P, Meishan × Pietrain cross; W×P, European Wild Boar × Pietrain cross; W×M, European Wild Boar × Meishan cross.

1996), with Meishan being a fatty and Pietrain a lean breed. The statistical model applied by Geldermann et al. (2010) was simple and treated each cross separately although they are connected. Additionally, Geldermann et al. (2010) ignored putative parent-of-origin effects, which are, however, frequently reported for fat traits in pigs.

The aim of this study was to conduct a joint QTL study of the 3 connected F_2 crosses described by Geldermann et al. (2010) using selected metabolic, enzymatic, and cytological fat traits. For this purpose, the multi-QTL, multi-allele model of Rückert and Bennewitz (2010) was used, which also modeled imprinting effects. This model is tailored to analyze connected F_2 crosses jointly, leading to a greater statistical power to detect QTL. Based on QTL results across traits, positional and functional candidate genes are suggested.

MATERIALS AND METHODS

The research protocol was approved by the German Ethical Commission of Animal Welfare of the Provincial Government of Baden-Wuerttemberg. Care of the animals used in this experiment was in accordance with the guidelines issued by the German Regulation for Care and Treatments of Animals.

Animals and Traits

The experimental design was described in detail by Geldermann et al. (2010). Briefly, the first cross (**MxP**) was obtained by mating 1 Meishan boar with 8 Pietrain sows. The second cross (**W×P**) was generated by mating 1 European Wild Boar boar with 9 Pietrain sows, and the third cross (**W×M**) was obtained by mating the same European Wild Boar boar with 4 Meishan sows. The number of F_2 individuals in the M×P (W×P, W×M) was 316 (315, 335), but varied for some traits (Table 1).

Backfat tissue was collected between the skin and LM at the 13th/14th rib at slaughter. After some preparation, enzyme activity and the soluble protein content were measured in the fat tissues. Additionally, fat cells were extracted from fat tissue, and the diameter of each cell was determined. See Geldermann et al. (2010) for details regarding the protocols used. The traits backfat depth (**BFD**), measured as an average of measurements at the 10th rib, shoulder, and loin and the backfat area (**FA**) at the 13th/14th rib, were considered representative of classical backfat performance traits in this study. The total soluble protein content (**SPC**) and the NADP-malate dehydrogenase (**MDH**) activity were used as indicators for metabolic and enzyme activities, respectively. The 2 cytological traits relative number of fat cells with medium cell size (**FCL**, calculated as the proportion of fat cells with a diameter between 73 and 146 μm) and large cell size (**FCH**, calculated as the proportion of cells with a cell size larger than 146 μm) were used. For summary statistics within and across the 3 crosses, see Table 1. The phenotypes were precorrected for the effect of sex, litter, season, and slaughter age before QTL analysis.

Statistical Analysis

The animals were genotyped genomewide for around 250 markers, mainly microsatellites, but also SNP. A genetic map was calculated across the 3 crosses as described in detail by Rückert and Bennewitz (2010). Because many markers were genotyped in all 3 or in at least 2 crosses, the estimation of a common map was straightforward. The map can be found in Rückert and Bennewitz (2010) and is in agreement with other published maps. The QTL analysis was done using the model of Rückert and Bennewitz (2010), which was adapted from plant breeding experiments and is tailored to analyze connected multiple experimental crosses. The model assumed that 2 founder breeds of a certain cross are divergent homozygous at a certain QTL. For each F_2 individual of a certain cross, 4 genotype probabilities $pr(Q_i^pQ_i^m)$, $pr(Q_j^pQ_i^m)$, $pr(Q_i^pQ_j^m)$, and $pr(Q_j^pQ_j^m)$ were calculated for each chromosomal position. The superscripts denote the parental origin of the alleles [i.e., paternal (p) or maternal (m) derived], and the subscripts denote the breed origin of the alleles (i.e., breed i or j, with i, j being breed M, Pietrain, or W, respectively, depending on which cross is considered). From these genotype probabilities, the probability of an F_2 individual k from a certain cross, for example, W×M receiving a QTL allele from one founder breed, for example, M, from its father, was calculated as $z_{M,k}^p = pr(Q_M^pQ_M^m) + pr(Q_M^pQ_W^m)$. Similarly, the probability of receiving the founder breed allele M from its mother was calculated as $z_{M,k}^m = pr(Q_M^pQ_M^m) + pr(Q_W^pQ_M^m)$. The calculation for the founder breed allele W was done in the same manner. These probabilities were also calculated for the offspring of the other 2 crosses M×P and W×P. The probability of an F_2 individual being heterozygous was calculated as the sum of the 2 heterozygous genotype probabilities [i.e., $z_{ijk} = pr(Q_i^pQ_j^m) + pr(Q_j^pQ_i^m)$]. These probabilities can be used to establish a regression model. However, because the sum of the additive effects within each parental origin is equal to zero, such a model would be overparametrized (see Rückert and Bennewitz, 2010). Therefore, a reparametrization was done as $\tilde{z}_{M,k}^p = z_{M,k}^p - z_{W,k}^p$, $\tilde{z}_{P,k}^p = z_{P,k}^p - z_{W,k}^p$, $\tilde{z}_{M,k}^m = z_{M,k}^m - z_{W,k}^m$, and $\tilde{z}_{P,k}^m = z_{P,k}^m - z_{W,k}^m$. Thus, the final regression model was

$$y_{ijk} = cross_{ij} + a_M^p\tilde{z}_{M,k}^p + a_M^m\tilde{z}_{M,k}^m + a_P^p\tilde{z}_{P,k}^p + a_P^m\tilde{z}_{P,k}^m + d_{MW}z_{MW,k} + d_{MP}z_{MP,k} + d_{WP}z_{WP,k} + e_{ijk}.$$

The term $cross_{ij}$ denotes the fixed effect of the F_2 cross. The residual variance was assumed to be heterogeneous [i.e., $e_{ijk} \sim N(0, \sigma_{ij}^2)$]. The model produced estimates of the additive breed effects of breeds M and Pietrain considering the parental origin of the alleles ($\hat{a}_M^p, \hat{a}_M^m, a_P^p, \hat{a}_P^m$). The additive effects of the W breeds were estimated as $\hat{a}_W^p = -(\hat{a}_M^p + \hat{a}_P^p)$ and $\hat{a}_W^m = -(\hat{a}_M^m + \hat{a}_P^m)$. Combined Mendelian additive effects (i.e., ignoring parental origin of the alleles) were calculated as $\hat{a}_M = \hat{a}_M^p + \hat{a}_M^m$, $\hat{a}_P = \hat{a}_P^p + \hat{a}_P^m$, and $\hat{a}_W = -(\hat{a}_M^p + \hat{a}_M^m + \hat{a}_P^p + \hat{a}_P^m)$. The 3 d terms represent the dominant QTL effects. The model was fitted every centimorgan on the autosomes by adapting the z terms accordingly. The test statistic was an F-test; the F-values were converted into a logarithm (base 10) of odds (**LOD**) score test statistic as $LOD \approx (np \times F) / [2 \times \log(10)]$, with np being the number of estimated QTL effects (i.e., $np = 7$; 4 additive and 3 dominance effects). The global null hypothesis was that at the chromosomal position with the highest test statistic every estimated parameter is equal to zero. The 5% threshold of the test statistic corrected for multiple testing on the chromosome was obtained using the quick method of Piepho (2001). This low significance level was chosen because a large number of QTL with small effects are segregating in this design (Bennewitz and Meuwissen, 2010). Once the global null hypothesis was rejected, the following subhypotheses were tested at significant chromosomal positions by building linear contrasts:

Test for an additive QTL:

$$H_0: a_M^p + a_M^m = 0 \text{ and } a_P^p + a_P^m = 0,$$

H_1: at least 1 of the 2 sums is different from zero.

Test for dominance at the QTL:

$$H_0: d_{MW} = d_{MP} = d_{WP} = 0,$$

H_1: at least 1 is different from zero.

Test for imprinting at the QTL:

$$H_0: a_M^p = a_M^m \text{ and } a_P^p = a_P^m,$$

H_1: at least 1 of the 2 expressions is unequal.

The test of the 3 subhypotheses resulted in the 3 error probabilities p_{add}, p_{dom}, and p_{imp} for additive, dominance, and imprinting QTL, respectively. Additionally, it was assessed how many QTL alleles could be distinguished based on their additive effects. This was done by testing the segregation of the QTL in each of the 3 crosses, considering only additive Mendelian effects (i.e., ignoring imprinting and dominance). For each significant QTL a confidence interval was calculated using the 1 LOD drop method. Multiple QTL were included as cofactors in the model using a forward selection approach. For details, see Rückert and Bennewitz (2010).

RESULTS AND DISCUSSION

Numerous QTL have been mapped with remarkably short confidence intervals. These intervals often showed an overlap across the traits, which can also be seen when comparing the plots of the test statistic against the chromosomal position for those chromosomes with QTL for several traits (Figures 1 and 2). This enabled a joint interpretation of the results.

The summary statistics (Table 1) showed that there is substantial variation within and across the 3 crosses. For BFD and FA, the greatest and least mean was in the W×M and W×P cross, respectively. The greatest and least mean for soluble protein content was observed for W×P and W×M, respectively.

The QTL results are presented in Tables 2, 3, and 4. In general, numerous QTL were reported, most of them on SSC 1, 2, 6, 7, 17, and 18. Several QTL showed significant imprinting effects, especially on SSC 2 and 6. In many cases 3 QTL alleles could be distinguished. The confidence intervals were sometimes remarkably small, given that only linkage information is used.

For BFD and FA (Table 2) QTL were found on SSC 1, 2, 6, 7, 9, 14, 17, and 18. All QTL showed a significant additive effect, and the QTL on SSC 2, 6, and 17 also showed highly significant imprinting effects. The order of breed QTL effects is often (but not always; see QTL for BFD on SSC7, Table 2) M over Pietrain over W. For MDH and SPC, QTL results are shown in Table 3. For SPC, QTL were found on SSC 2, 3, 7, 14, 17, and 18, with an overlap of confidence intervals with the QTL for the fat performance traits reported in Table 2. For the QTL on SSC 2, 17, and 18, imprinting was also significant. For MDH, 3 QTL were found, 2 on SSC7. Interestingly, the QTL on SSC 2 was only significant due to its imprinting effect and on SSC7 due to its dominance effects. The breed QTL effect was typically Pietrain over W over M, if the additive effect was significant. For FCL, 8 QTL were found (Table 4). Only 2 alleles could be distinguished for each QTL; the breed QTL effects of Pietrain and W were often similar. The QTL on SSC 2 reported for the other traits was not significant. For FCH, 5 QTL were found (Table 4). In contrast to FCL, each QTL for FCH showed an overlap of confidence intervals with the performance QTL listed in Table 2.

General Breed and Imprinting Effects

The Meishan breed is known for its high propensity to accumulate backfat. The greater M breed allelic effects for the backfat traits (Table 2) were therefore expected. On the contrary, Pietrain has been selected for growth and meat content and less fat. This is also documented in the differences in the cross mean of these traits. The mean of the M×P cross was in between the mean of the W×M and W×P cross. The trait soluble protein content accumulates the effect of nonspecific enzyme activities and the greater number of mapped QTL than was expected (Table 3). High soluble protein content is attributable to an increased metabolic turnover. Following this, the greater mean of protein content in W×P and lesser mean in W×M (Table 1) is a consequence of selection direction within these breeds. The allelic breed effects (Table 3) also pointed in this direction. This clear pattern of breed allelic effects and cross means was not observable for the remaining traits, which may also be due to limited statistical power to unravel small, but true, differences.

A substantial proportion of QTL showed significant imprinting effects. However, as discussed in Rückert and Bennewitz (2010), some cautions have to be made when interpreting the statistically significant imprinting effects because these might not always reflect true imprinting but may be a result from within-founder breed segregation. Especially if the mode of imprinting is not consistent across the breeds, this can be seen as evidence against real imprinting effects because it is unlikely that real imprinting differs across breeds. However, some imprinted QTL are within well-known porcine imprinting regions (e.g., on SSC2; Nezer et al., 1999; Van Laere et al., 2003).

QTL Results and Candidate Genes on SSC2

The proximal region of SSC2 contains the *IGF2* locus. The gene is imprinted, and only paternally inherited alleles are expressed (e.g., de Koning et al., 2000; Boysen et al., 2011). The QTL found in our study on SSC2 within this chromosomal region (Tables 2 to 4) are in good agreement with this.

The second QTL on SSC2 for BFD and FA matches to the chromosomal position of the gene ***InsR*** (insulin receptor), which is a glycoprotein. It belongs to the receptor tyrosine kinase family. The receptor is located in the membrane (Gu et al., 1992). Binding of insulin to its receptor stimulates lipogenese and inhibits lipolysis. Blüher et al. (2002) investigated the physiological role of insulin in adipose tissue by creating fat-specific *InsR* knockout mice and found that knockout mice had markedly reduced fat mass and exhibited heterogeneity in fat cell size. Hence, *InsR* plays an important role in the pathway from insulin to fatty acid in adiposities and is also a functional candidate gene for this QTL, which should be considered in further functional studies.

QTL Results and Candidate Genes on SSC5

Many studies mapped QTL for fat-related traits on SSC5 (Bidanel et al., 2001; de Koning et al., 2001; Malek et al., 2001a,b; Nii et al., 2006; Ramos et al., 2009; Tomás et al., 2011). In contrast to this, no QTL for BFD or FA was found in our study, but an imprinted QTL for FCL (Table 4). The chromosomal position is close to the *IGF1* gene. The *IGF1* gene has been detected as a candidate gene in pigs (Roehe et

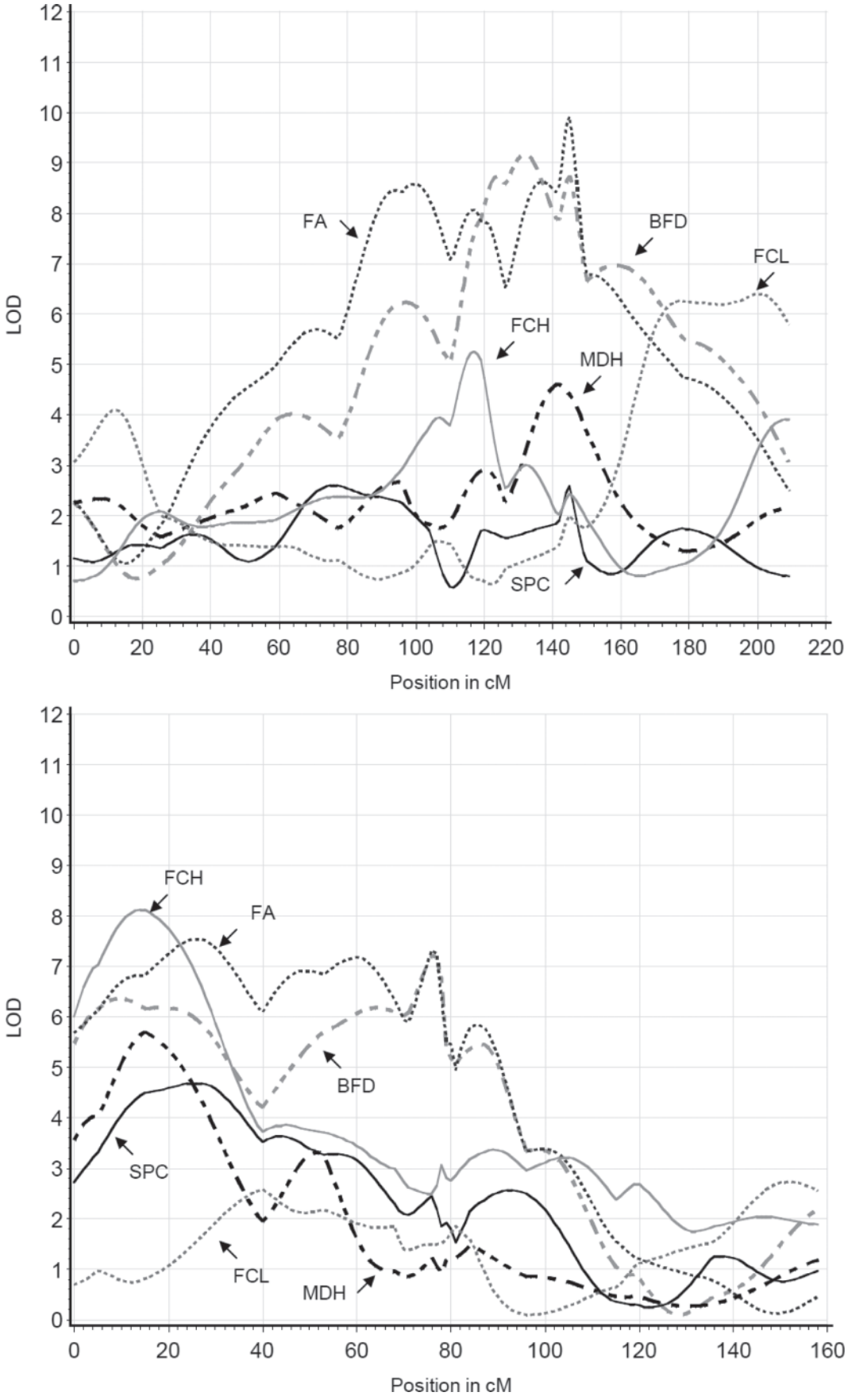

Figure 1. Plot of logarithm (base 10) of odds (LOD) score QTL test statistic for SSC1 (top) and SSC2 (bottom). BFD = backfat depth; FA = fat area; FCL = relative number of fat cells with medium cell sizes; FCH = relative number of fat cells with large cell sizes; MDH = NADP-malate dehydrogenase; SPC = soluble protein content.

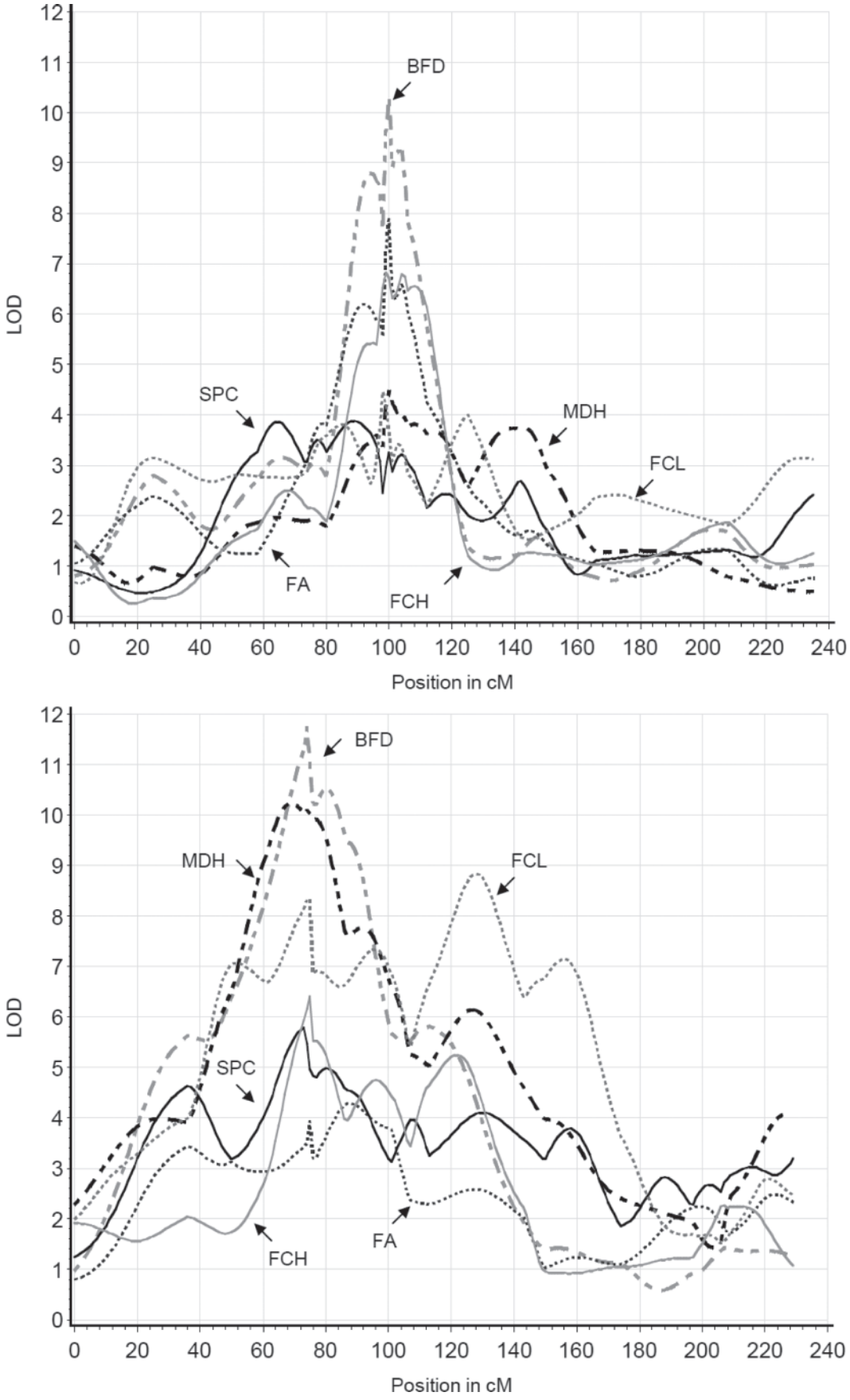

Figure 2. Plot of logarithm (base 10) of odds (LOD) score QTL test statistic for SSC6 (top) and SSC7 (bottom). BFD = backfat depth; FA = fat area; FCL = relative number of fat cells with medium cell sizes; FCH = relative number of fat cells with large cell sizes; MDH = NADP-malate dehydrogenase; SPC = soluble protein content.

Table 2. The QTL results for average backfat depth (BFD) and fat area (FA) with confidence intervals (CI), test statistics, error probabilities, and order of estimated breed QTL effects

Trait	SSC	Position	CI	*F*-value	p_{add}[1]	p_{dom}[2]	p_{imp}[3]	Mode[4]	Order of effects[5]
BFD	1	131	[SW307; SW803] [110.3; 141.7]	5.52	<0.001	0.801	0.905	(–)	$\hat{a}_M = \hat{a}_P > \hat{a}_W$
	2	9	[SW2443; S0141] [0.0; 39.9]	4.21	0.014	1.000	<0.001	(mat)	$\hat{a}_M > \hat{a}_P = \hat{a}_W$
	2	76	[S0141; SW395] [39.9; 81.0]	5.03	<0.001	0.183	0.404	(–)	$\hat{a}_M > \hat{a}_P > \hat{a}_W$
	6	100	[RYR; A1BG] [96.4; 101.2]	7.07	<0.001	0.001	0.002	(pat)	$\hat{a}_M > \hat{a}_P = \hat{a}_W$
	7	75	[ID4SMA; TNFB] [61.3; 76.2]	8.02	<0.001	0.172	0.076	(–)	$\hat{a}_P > \hat{a}_W > \hat{a}_M$
	9	194	[EAE; SW1349] [187.4; 194.6]	3.59	0.019	0.002	0.290	(–)	$\hat{a}_M = \hat{a}_P > \hat{a}_W$
FA	1	145	[SW803; TGFBR1] [141.7; 149.6]	6.52	<0.001	0.097	0.033	(nc)	$\hat{a}_M = \hat{a}_P > \hat{a}_W$
	2	25	[SWC9; S0141] [5.2; 39.9]	4.85	0.014	0.702	<0.001	(nc)	$\hat{a}_M > \hat{a}_P = \hat{a}_W$
	2	77	[MYOD1; SW395] [70.6; 81.0]	3.66	0.001	0.087	0.315	(–)	$\hat{a}_M = \hat{a}_P > \hat{a}_W$
	6	100	[RYR; A1BG] [96.4; 101.2]	5.04	<0.001	0.125	0.014	(pat)	$\hat{a}_M > \hat{a}_P = \hat{a}_W$
	7	87	[CYPA; PLIN] [73.3; 106.8]	3.31	0.002	0.152	0.082	(–)	$\hat{a}_M > \hat{a}_P = \hat{a}_W$
	14	53	[SW2038; SW540] [43.8; 60.7]	3.11	0.002	0.024	0.320	(–)	$\hat{a}_M = \hat{a}_P > \hat{a}_W$
	17	92	[SJ063; SW2427] [69.9; 97.7]	2.86	0.061	0.484	0.004	(nc)	$\hat{a}_M > \hat{a}_P = \hat{a}_W$
	18	29	[EAI; SW787] [10.9; 43.6]	2.79	0.003	0.102	0.457	(–)	$\hat{a}_M > \hat{a}_P = \hat{a}_W$

[1]Error probability for additive effects.
[2]Error probability for dominant effects.
[3]Error probability for imprinting effects.
[4]Mode of imprinting [(–) imprinting not significant, (mat) maternal imprinting, (pat) paternal imprinting, (nc) not consistent].
[5]$\hat{a}_P$: estimated effect of Pietrain breed; $\hat{a}_M$: estimated effect of Meishan breed; $\hat{a}_W$: estimated effect of wild boar breed.

al., 2003) and is involved in the regulation of growth and differentiation of different cell types (e.g., the replication and differentiation of preadiposities), and in the control of BW (Kopecny et al., 2002). Additionally, Estany et al. (2007) investigated a polymorphic $(CA)_n$ sequence repeat, located at the first intron of *IGF1* in a Landrace and a Duroc population. The authors found a significant association between the length of the polymorphism and circulating IGF1 concentrations at 160 d. Furthermore, a negative correlation between intramuscular fat content and IGF1 concentration at an age of 185 d was found. Rajkumar et al. (1999) investigated the role of IGF1 in the accumulation of fat tissue in transgenic mice. They partially inhibited IGF1 action by overexpression of IGFBP1, which binds IGF1 and limits its bioavailability. The authors could demonstrate that transgenic mice, which overexpress IGFBP1, had a reduced epidermal fat mass and adipocyte size compared with wild-type mice. To confirm *IGF1* as a candidate gene underlying this QTL for FCL, the abundance of this gene expression in Pietrain should be compared with Meishan.

QTL Results and Candidate Genes on SSC6

Paternally imprinted QTL were found on SSC6 in the distal region for both fat performance traits and for FCH with a high overlap of confidence intervals (Tables 2 and 4, and Figure 2). The lower boundary of the confidence interval is the halothane gene ***RYR1***, which is a well-known major gene for meat quality. To investigate if this gene is responsible for the QTL in this study, we included the gene as a fixed effect in our QTL model and repeated the analysis. The results revealed that, although *RYR1* was significant for all traits ($P < 0.01$), the QTL were still significant as well (Table 5). This indicates that *RYR1* is not the only causative gene underlying the QTL. These results support the finding of Mohrmann et al. (2006), who also found evidence for additional QTL closely linked to *RYR1* for several fatness traits, including side fat thickness, external shoulder fat weight, belly weight, and loin fat depth.

Another candidate gene is the transforming growth factor-β-1 (***TGF-β-1***), which is located within the confidence intervals. In mice (Samad et al., 1997) and

Table 3. The QTL results for soluble protein content (SPC) and NADP-malate dehydrogenase (MDH) with confidence intervals (CI), test statistics, error probabilities, and order of estimated breed QTL effects

Trait	SSC	Position	CI	*F*-value	p_{add}[1]	p_{dom}[2]	p_{imp}[3]	Mode[4]	Order of effects[5]
SPC	2	22	[SWC9; S0141] [5.2; 39.9]	3.56	0.016	0.825	0.001	(mat)	$\hat{a}_P = \hat{a}_W > \hat{a}_M$
	3	96	[SW828; SW349] [74.0; 138.6]	3.24	0.001	0.842	0.050	(mat)	$\hat{a}_P = \hat{a}_W > \hat{a}_M$
	7	73	[ID4SMA; S0102] [61.3; 86.5]	3.39	<0.001	0.665	0.418	(–)	$\hat{a}_P = \hat{a}_W > \hat{a}_M$
	14	105	[SW210; SW55] [84.3; 122.1]	2.69	0.001	0.295	0.923	(–)	$\hat{a}_W > \hat{a}_M = \hat{a}_P$
	17	87	[SJ063; SW427] [69.9; 97.9]	3.69	0.003	0.035	0.039	(nc)	$\hat{a}_P > \hat{a}_M = \hat{a}_W$
	18	33	[EAI; S0062] [10.9; 58.8]	3.98	0.015	0.003	0.030	(mat)	$\hat{a}_P > \hat{a}_M > \hat{a}_W$
MDH	2	15	[SWC9; S0141] [5.2; 39.9]	3.80	0.658	0.159	<0.001	(mat)	$\hat{a}_M = \hat{a}_P = \hat{a}_W$
	7	69	[ID4SMA; S0102] [61.3; 86.5]	6.71	<0.001	0.831	0.151	(–)	$\hat{a}_P > \hat{a}_W > \hat{a}_M$
	7	225	[PI2; IGH2] [208.8; 229.5]	3.19	0.659	0.001	0.152	(–)	$\hat{a}_M = \hat{a}_P = \hat{a}_W$

[1]Error probability for additive effects.
[2]Error probability for dominant effects.
[3]Error probability for imprinting effects.
[4]Mode of imprinting [(–) imprinting not significant, (mat) maternal imprinting, (nc) not consistent].
[5]$\hat{a}_P$: estimated effect of Pietrain breed; $\hat{a}_M$: estimated effect of Meishan breed; $\hat{a}_W$: estimated effect of wild boar breed.

humans (Fain et al., 2005), increased abundance of cytokine molecule TGF-β-1 was found in individuals suffering from obesity. Both groups were able to detect a significant correlation between body fat content and subsequent release of TGF-β in subcutaneous adipose tissue. To confirm this gene as a functional candidate gene underlying the QTL found in this study, the abundance of TGF-β-1 in Meishan should be compared with Pietrain because the Meishan-breed allelic effect is greater compared with Pietrain for fat area and backfat (Table 2).

QTL Results and Candidate Genes on SSC7

For FCL the mapped QTL in the distal region on SSC7 showed a significant imprinting effect, although the mode was not consistent (Table 4). This region contains probably the ortholog ovine chromosomal region encompassing the callipyge gene (Boysen et al., 2010), which is known to show imprinting effects in sheep. Kim et al. (2004) found several imprinting QTL for growth and meat quality traits in pigs in this chromosomal region. In contrast, Boysen et al. (2010) found an imprinted QTL for ham weight in close proximity, but not within the callipyge ortholog region.

The QTL on SSC7 for BFD and FA was also found in all other traits (SPC, MDH, FCL, and FCH) with a strong overlap of confidence intervals (see also Figure 2) and a congruent mode of inheritance (i.e., purely additive). This QTL was previously reported by other groups (e.g., de Koning et al., 2001; Meidtner et al., 2009). For BFD (Table 2), a phenomenon defined as transgressive variation (de Koning et al., 2001) is observed (i.e., Meishan-allelic effect is larger than the Pietrain-allelic effect for the QTL), which is not in agreement with the breed history and the greater backfat mean of Meishan pigs. This paradox was also reported by Rohrer and Keele (1998), de Koning et al. (1999), and de Koning et al. (2001). Meidtner et al. (2009) investigated PPAR delta gene (***PPARD***) as a candidate gene and found *PPARD* haplotype associations with backfat thickness in a Mangalitsa × Pietrain F_2 cross. The *PPARD* gene has been assigned between SW1856 and S0102 on SSC7 (Barbosa et al., 2004; Tanaka et al., 2006) and is located in the confidence intervals of QTL for FA, SPC, MDH, and FCH. Another candidate gene, tumor necrosis factor α (***TNF-α***), is located near the maximum test statistic for these QTL. An increased concentration of TNF-α contributes to an increased basal lipolysis, which is typical for adipocytes of obese pigs. Several studies were conducted to investigate the effect of TNF-α. Knockout mice were created (Uysal et al., 1997), and exogenous TNF-α was applied in vivo and in vitro to demonstrate that the *TNF-α* abundance is positively correlated with the triglyceride and FFA circulating concentrations (Green et al., 1994; Souza et al., 1998). Chen et al. (2004) investigated the expression of *TNF-α* in dorsal subcutaneous tissue of Tongcheng pigs (obese) and Dabai pigs (lean). They found that *TNF-α* gene expression was significantly increased in obese pigs and overexpressed during the development of obesity.

Table 4. The QTL results for relative number of medium-sized fat cells (FCL) and of large-sized fat cells (FCH) with confidence intervals (CI), test statistics, error probabilities, and order of estimated breed QTL effects

Trait	SSC	Position	CI	*F*-value	p_{add}[1]	p_{dom}[2]	p_{imp}[3]	Mode[4]	Order of effects[5]
FCL	1	176	[TGFBR1; EAA] [149.6; 209.1]	3.82	0.001	0.053	0.254	(–)	$\hat{a}_P = \hat{a}_W > \hat{a}_M$
	4	98	[EAL; AGL] [93.7; 121.5]	3.20	0.109	0.007	0.035	(mat)	$\hat{a}_P = \hat{a}_W > \hat{a}_M$
	5	110	[SW152; DCN] [92.2; 120.1]	3.12	0.009	0.828	0.003	(pat)	$\hat{a}_P = \hat{a}_W > \hat{a}_M$
	6	25	[S0035; SW1057] [0.0; 58.1]	3.27	0.002	0.397	0.056	(–)	$\hat{a}_W > \hat{a}_M = \hat{a}_P$
	7	75	[ID4SMA; S0102] [61.3; 86.5]	2.37	0.001	0.687	0.644	(–)	$\hat{a}_M > \hat{a}_P = \hat{a}_W$
	7	128	[S0066; S0115] [113.0; 143.3]	5.79	0.001	0.001	0.023	(nc)	$\hat{a}_M > \hat{a}_P = \hat{a}_W$
	8	116	[S0144; SW61] [85.0; 127.1]	4.14	0.192	<0.001	0.306	(–)	$\hat{a}_M = \hat{a}_P = \hat{a}_W$
	17	46	[GHRH; SJ063] [43.6; 69.9]	2.57	0.027	0.606	0.021	(pat)	$\hat{a}_W > \hat{a}_M = \hat{a}_P$
	18	37	[SW1808; SW787 [0.0; 43.6]	2.36	0.326	0.005	0.557	(–)	$\hat{a}_M = \hat{a}_P = \hat{a}_W$
FCH	1	118	[SW307; SW780] [110.3; 126.3]	3.65	0.001	0.070	0.021	(nc)	$\hat{a}_P > \hat{a}_M = \hat{a}_W$
	2	14	[SW2443; S0141] [0.0; 39.9]	5.32	0.004	0.386	<0.001	(mat)	$\hat{a}_M = \hat{a}_W > \hat{a}_P$
	6	99	[ETH5001; HFABP] [94.4; 124.9]	5.13	0.016	0.001	0.013	(pat)	$\hat{a}_M > \hat{a}_P > \hat{a}_W$
	7	75	[ID4SMA; S0102] [61.3; 86.5]	4.68	<0.001	0.007	0.433	(–)	$\hat{a}_P = \hat{a}_W > \hat{a}_M$
	14	53	[SW2038; SW540] [43.8; 60.7]	2.91	0.022	0.016	0.260	(–)	$\hat{a}_P > \hat{a}_M = \hat{a}_W$

[1]Error probability for additive effects.
[2]Error probability for dominant effects.
[3]Error probability for imprinting effects.
[4]Mode of imprinting [(–) imprinting not significant, (mat) maternal imprinting, (pat) paternal imprinting, (nc) not consistent].
[5]$\hat{a}_P$: estimated effect of Pietrain breed, $\hat{a}_M$: estimated effect of Meishan breed, $\hat{a}_W$: estimated effect of wild boar breed.

QTL Results and Candidate Genes on SSC18

For the traits FA, SPC, and FCL, QTL on SSC18 next to the *Leptin* locus were found. Leptin contributes to the regulation of appetite, and subsequently of feed intake in pigs and is secreted from adipose tissue (Ramsay and Richards, 2004). Together with insulin and GH, it affects lipid syntheses (Ramsay, 2004). In a study of McNeel et al. (2000), the expression of different proteins expressed in adipocytes, including Leptin, were measured during differentiation of adipocytes. The authors found that the *Leptin* transcript concen-

Table 5. The QTL results for backfat depth (BFD), fat area (FA), and of relative number of large-sized fat cells (FCH) with confidence intervals (CI), test statistics, error probabilities, and order of estimated breed QTL effects, results from a model that adjusted the phenotypes for the effect of the halothane gene *RYR1*

Trait	SSC	Position	CI	*F*-value	p_{add}[1]	p_{dom}[2]	p_{imp}[3]	Mode[4]	Order of effects[5]
BFB	6	100	[LIPE; A1BG] [98.3; 101.2]	3.91	0.117	0.009	0.005	(pat)	$\hat{a}_M > \hat{a}_P = \hat{a}_W$
FA	6	100	[LIPE; A1BG] [98.3; 101.2]	3.99	0.002	0.096	0.006	(pat)	$\hat{a}_M > \hat{a}_P = \hat{a}_W$
FCH	6	97	[S0087; TGFB1] [80.0; 99.5]	4.05	0.022	0.01	0.014	(nc)	$\hat{a}_P > \hat{a}_M > \hat{a}_W$

[1]Error probability for additive effects.
[2]Error probability for dominant effects.
[3]Error probability for imprinting effects.
[4]Mode of imprinting [(pat) paternal imprinting, (nc) not consistent].
[5]$\hat{a}_P$: estimated effect of Pietrain breed, $\hat{a}_M$: estimated effect of Meishan breed, $\hat{a}_W$: estimated effect of wild boar breed.

tration increased over the period of differentiation. The increase was accompanied with an increase of adipocyte size and correlated with BW and adipocyte volume. Studies in humans showed that an increase of plasma leptin concentration is associated with an increase of total body fat (Ellis and Nicolson, 1997; Jensen et al., 1999). Ramsay et al. (1998) found the same results when comparing lean and obese pigs. In our study the breed allelic effect of Meishan is large compared with European Wild Boar and Pietrain for the QTL for FA (Table 2), which is in good agreement with the results of Ellis and Nicolson (1997), Ramsay et al. (1998), and Jensen et al. (1999).

Conclusions

The application of the joint QTL mapping approach applied to the powerful porcine connected F_2 crosses revealed several QTL for classical fat traits as well as for fat-related cytological, metabolic, and enzyme activity traits. The use of this trait combination enabled us to identify some functional and positional candidate genes underlying the QTL. These genes are involved in signaling cascades, which affect fat trait determination. Most promising candidate genes are *TNF-α* on SSC7, *IGF1* on SSC5, and *TGF-β-1* on SSC6, which need further functional investigation.

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