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ABSTRACT: Effects of genetic variation in porcine adipocyte and heart fatty acid-binding protein genes, *A-FABP* and *H-FABP*, respectively, on intramuscular fat (IMF) content and backfat thickness (BFT) were examined in F_2 crossbreds of Meishan and Western pigs. The involvement of each FABP gene in IMF accretion was studied to confirm previous results for Duroc pigs. The F_2 crossbred pigs were genotyped for various markers including microsatellite sequences situated within both FABP genes. Linkage analysis assigned the *A-FABP* and *H-FABP* genes to marker intervals *S0001-S0217* (20 cM) on SSC4 and *Sw316-S0003* (16.6 cM) on SSC6, respectively, refining previous chromosomal assignments. Next, the role of both chromosome regions/genes on genetic variation in IMF content and BFT was studied by 1) screening SSC4 and SSC6 for QTL affecting both traits by performing a line-cross analysis and 2) estimation of the effect of individual A-FABP and H-FABP alleles on both traits. In the first analysis, suggestive and chromosome-wise significant evidence for a QTL affecting IMF was detected on SSC6. The *H-FABP* gene is a candidate gene for this effect because it resides within the large region containing this putative QTL. The second analysis showed a considerable but nonsignificant effect of H-FABP microsatellite alleles on IMF content. Suggestive evidence for a QTL affecting BFT was found on SSC6, but *H-FABP* was excluded as a candidate gene. In conclusion, present and previous results support involvement of *H-FABP* gene polymorphisms in IMF accretion independently from BFT in pigs. Therefore, implementation of these polymorphisms in marker-assisted selection to control IMF content independently from BFT may be considered. In contrast to previous findings for Duroc pigs, no evidence was found for an effect of the A-FABP gene on IMF or BFT in this population.

Key Words: Fat Metabolism, Genetic Markers, Loci, Meat Quality, Pigs

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Introduction

Intramuscular fat (**IMF**) content is a major determinant of meat quality. In particular, eating quality traits are influenced by the amount of intramuscular fat (reviewed by Hovenier et al., 1993). Intramuscular fat content is highly heritable (h^1 = .5; Hovenier et al., 1993), but genetic correlations with other production traits are unfavorable but moderate (Hovenier et al., 1992). However, improving IMF content by selective breeding is difficult because this trait is measured on the carcass. Marker or gene-assisted selection is a promising strategy for genetic improvement of such carcass traits (Meuwissen and Goddard, 1996).

Previously, genetic variants of both heart (**H**) and adipocyte (**A**) fatty acid-binding protein (**FABP**) genes (*FABP3* and *FABP4*) were shown to be associated with IMF content, backfat thickness (**BFT**), and growth (Gerbens et al., 1998b and 1999). These findings are consistent with the function and tissue-specific expression of these FABP (Veerkamp and Van Moerkerk, 1993; Veerkamp and Maatman, 1995). Functionally, FABP are intracellular proteins that transport fatty acids from the cell membrane to sites of fatty acid oxidation or phospholipid or triacylglycerol synthesis. The *H-FABP* gene is expressed predominantly in muscle cells, whereas *A-FABP* is expressed almost exclusively in adipocytes. However, whether the observed effects of the genetic variants are due to the FABP genes themselves or closely linked genes is still inconclusive. Analysis of data from other pig breeds, lines, or populations

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might provide additional insight into FABP roles in these traits. Therefore, our objective was to examine the effect of genetic variants of both FABP genes in F_2 pigs from a cross between Meishan boars and Western breed sows that has been shown to segregate for QTL and a major gene affecting IMF content (Janss et al., 1997; De Koning et al., 1999). Furthermore, these data allowed us to estimate the chromosomal position of *FABP3* and *FABP4* more accurately by linkage analysis.

Materials and Methods

Animal Data

Pigs from the F_2 generation of crosses of the Chinese Meishan breed and Western pig lines were available from an experiment involving five Dutch pig breeding companies. Nineteen purebred Meishan boars were crossed with 126 purebred sows either from the Large White or the Dutch Landrace breed to produce F_1 crossbreds. Randomly selected F_1 crossbreds, 39 boars and 264 sows were intercrossed, except that no full sibs were mated. Of the F_2 crossbreds, 1,200 pigs were performance tested and 844 randomly selected pigs were slaughtered at 90 kg live weight to assess meat quality traits. Detailed information about the breeding strategy and the setup of the experiment is given by Janss et al. (1997).

Animals were housed at five test stations. To avoid confounding the background effects of the various Western pig lines and test-station effects, semen of F_1 boars was distributed across test stations. Furthermore, pigs were slaughtered at a single slaughterhouse on 26 slaughter days, and pigs from the different test stations were slaughtered on the same day to avoid confounding the effects of test station with slaughter day.

Pigs from 19 paternal half-sib families had informative segregation of alleles for a major gene affecting IMF content according to Janss et al. (1997) and were used for subsequent statistical analyses. In total, 619 F_2 crossbred pigs were included, of which meat quality traits were recorded from 418 randomly selected pigs.

Performance and Meat Quality Trait Data

Pigs included in this analysis were all performance tested. At approximately 90 kg, age, live weight, and backfat thickness (BFT) were recorded. For pigs selected for meat quality evaluation, carcass weight and BFT were also recorded after slaughter. Backfat thickness was measured with the Hennesy grading probe between the third and fourth ribs, 6 cm from the spine of the carcass.

Twenty-four hours after slaughter, samples of the longissimus muscle were obtained for IMF content evaluation according to Hovenier et al. (1992). Intramuscular fat content was determined using the Soxhlet petroleum-ether extraction method and expressed as the weight percentage of wet muscle tissue.

Genotype Data

In total, 619 F_2 crossbred pigs, their F_1 parents, and Meishan grandparents were genotyped for the described microsatellite sequences present in the porcine *H-FABP* (Gerbens et al., 1998a) and *A-FABP* gene (Gerbens et al., 1998b). For the H-FABP microsatellite, only two alleles were segregating. For the A-FABP microsatellite, eight alleles were segregating in this pig population. Genotypic data from nine and seven additional microsatellites, covering SSC4 and SSC6, respectively, had been evaluated previously (De Koning et al., 1999) and were included in the present analysis. The linkage groups of SSC4 and SSC6 span 111 and 152 cM with an average marker interval of 10 and 20 cM, respectively.

All microsatellite sequences were amplified using PCR as described by Groenen et al. (1996). Fragment lengths were determined upon electrophoresis on an 8% denaturing polyacrylamide gel in an ABI377 automatic sequencer (ABI, Perkin Elmer, Foster City, CA). Genotyping results were independently evaluated by two examiners and by segregation analysis using the pedigree file.

Marker Linkage Analysis

Previously, the *A-FABP* and *H-FABP* genes were assigned to SSC 4 and 6, respectively, using cell hybrid analysis (Gerbens et al., 1997, 1998b). In the present analysis, A-FABP and H-FABP microsatellites were assigned to chromosomes on the basis of two-point and multipoint linkage analysis (CRIMAP 2.4; Green et al., 1990) using genotyping data from all individuals of the 19 half-sib families. Two-point linkage assignments were considered significant when LOD score exceeded 3.0. The most likely multipoint linkage map was based on the highest LOD score value.

Quantitative Trait Loci Analysis

Data were statistically analyzed in two ways. First, the effect of the positions of both FABP genes was evaluated by including microsatellite data of both FABP genes in a chromosome scan for QTL as developed by Haley et al. (1994) for semi-inbred line crosses. This approach assumes that both founder populations are fixed for different alleles of QTL affecting the trait of interest, but this does not exclude breeds having marker alleles in common.

At a given location along the genome for each F_2 animal, the probabilities were estimated whether it inherited two Meishan alleles, two Western alleles, or one of each founder line based on its marker genotypes. These probabilities can be used in a least squares model to investigate the role of a genomic region on the trait of interest. This type of analysis has been applied to several crossbred pig populations (Andersson et al., 1994; Knott et al., 1998; De Koning et al., 1999).

The assumption of QTL allele fixation in both founder populations allows direct estimation of dominance and

additive effects of a putative QTL at any position. Additive effects are defined as half the phenotypic difference between pigs homozygous for the QTL alleles originating from Meishan and the Western breeds. These additive effects are estimated for the Meishan QTL alleles; that is, a positive value indicates an increase of the trait of interest due to the respective Meishan QTL allele. Dominance effects are estimated as the deviation of pigs heterozygous for the QTL alleles from the mean of the two types of homozygous pigs. If the heterozygous animals are closer to the homozygotes with the Meishan alleles, the dominance is defined as positive.

Interval mapping is done with multimarker analysis (Knott et al., 1998) using 1-cM interval lengths. Test statistics were evaluated along both chromosomes, with the highest value appearing at the most likely position of the QTL. Phenotypic data were preadjusted for nongenetic effects of day of slaughter, breeding company, sex, and carcass weight and analyzed in a model assuming polygenic inheritance as described previously by De Koning et al. (1999).

For the QTL analysis, the following model was fitted:

$$y_i = \mu + c_{ai}a + c_{di}d + e_i$$

where y_i is the adjusted trait observation of individual i, μ is the population mean, a and d are the estimated additive and dominant effect of a putative QTL at the given location, c_{ai} and c_{di} are the coefficients for the additive and dominance component for individual i denoting the probability of individual i carrying two Meishan alleles or being heterozygous at the given location, respectively, and e_i is the residual error.

Significance of the chromosome-scan approach was evaluated according to Lander and Kruglyak (1995). Suggestive evidence was defined as the *F*-ratio threshold value that resulted in one expected false-positive in this experiment. Although Lander and Kruglyak (1995) suggest that a genome-wide significance threshold should always be applied, in this case the effect of a specific part of the genome (i.e., two chromosomes) were evaluated to validate earlier findings. Therefore, chromosome-wise significance thresholds were applied. These significance levels do not account for testing multiple traits. Suggestive and chromosomewise significance thresholds were obtained empirically by permutation tests of the data (Churchill and Doerge, 1994). Ten thousand permutations resulted in suggestive and chromosomewise significance thresholds of 5.0 and 5.2 for SSC4 and 4.5 and 5.1 for SSC6, respectively, for both IMF content and BFT data.

Candidate Gene Analysis

The second approach to analyze the data was a straightforward candidate gene analysis for comparison with previous studies in Duroc pigs (Gerbens et al., 1998b, 1999). Here, genetic variation at both FABP genes was assumed to affect the trait of interest directly. Analysis was performed within litters using SAS (1990). Because of complete confounding between litter and company, company could be excluded from all models. For the evaluation of IMF content, prior analysis with SAS indicated that sex and carcass weight should be included in the model. In a separate evaluation of IMF content, BFT was included as a covariate in the model to account for the correlation between these traits (Hovenier et al., 1992) (i.e., to estimate the effect of IMF for the same level of BFT).

For the evaluation of BFT, sex and carcass weight were included in the model. Moreover, an additional analysis included also growth from start of test until day of slaughter to account for differences in growth and fat accretion characteristics between both founder populations.

Because eight A-FABP alleles are segregating in the Meishan population, analysis of the individual genotype classes would be inefficient. Hence, substitution effects of individual alleles were estimated according to Ostergard et al. (1989). Using this procedure, it is possible to estimate all allelic effects simultaneously, provided that the sum of all estimated allelic regression coefficients is constrained. For A-FABP, this constraint was applied by conditioning all other allele classes on the A1 allele class. The A1 allele class was chosen because this was the most abundant allele, and this class was demonstrated to be the less favorable allele in previous analyses for Duroc pigs (Gerbens et al., 1998b). For H-FABP, a similar analysis was applied. Here, the constraint was applied by regressing on allele H1 corrected for H2. In the latter case, H1H1, H1H2, and H2H2 genotypic animals were assigned the score 2, 0, and –2, respectively.

Significant effects of allele classes were determined according to the *F*-statistic of the analysis.

Results

Marker Linkage Analysis

Two-point linkage analysis revealed that the *A-FABP* gene was significantly associated with markers on SSC4. According to multipoint linkage analysis, the most likely order was (sex averaged; Kosambi centimorgans within brackets): *S0227* – [19.9] – *S0301* – [18.1] – *S0001* – [9.9] – ***A-FABP*** – [10.1] – *S0217* – [6.4] – *S0073* – [3.7] – *Sw589* – [2.7] – *S0214* – [20.6] – *Sw445* – [19.8] – *S0097*.

The *H-FABP* gene was significantly associated with markers on SSC6. Multipoint linkage analysis revealed the most likely order to be (sex averaged; Kosambi centimorgans within brackets): *S0035* – [11.7] – *Sw2406* – [29.9] – *Sw1057* – [29.6] – *S0220* – [12.7] – *Sw316* – [5.1] – ***H-FABP*** [11.5] – *S0003* – [51.3] – *Sw2419*.

Quantitative Trait Loci Analysis

Information Content. A QTL analysis was performed with and without microsatellite information from both

FABP genes to detect QTL on SSC4 and SSC6 contributing to genetic variation in IMF content and BFT (Figure 1). According to the Haldane map used in this analysis, the *A-FABP* gene was positioned at 54 cM on SSC4 and the *H-FABP* gene at 106 cM on SSC6 (Figure 1).

Local information content (Figure 1A,B) increased by inclusion of FABP microsatellite information, especially in the case of SSC4/A-FABP. This increase in information content is due to the high number of *A-FABP* microsatellite alleles segregating in this population and the increased coverage of the chromosome in this region. Inclusion of additional *A-FABP* microsatellite information not only increased the information content in the specific interval but also affected flanking intervals due to increased information content in previously uninformative families.

For SSC6/H-FABP, hardly any increase was seen due to its position in a relative densely covered area of SSC6, and the existence of only two alleles for the H-FABP microsatellite in this Meishan crossbred population.

SSC4/A-FABP. For IMF, the most probable position of a QTL on SSC4 was found around 65 cM (Figure 1C). However this test statistic did not exceed suggestive and chromosome-wise significance levels. Strikingly, the *F*-statistic profile was lowered when including A-FABP microsatellite information. Although this QTL did not significantly contribute to the variation in IMF content in this population, the effect of this locus was still considerable (Table 1). Individuals homozygous for the Meishan alleles have on average .44% more IMF than those homozygous for the western breed alleles, and this effect seemed to be additive in nature.

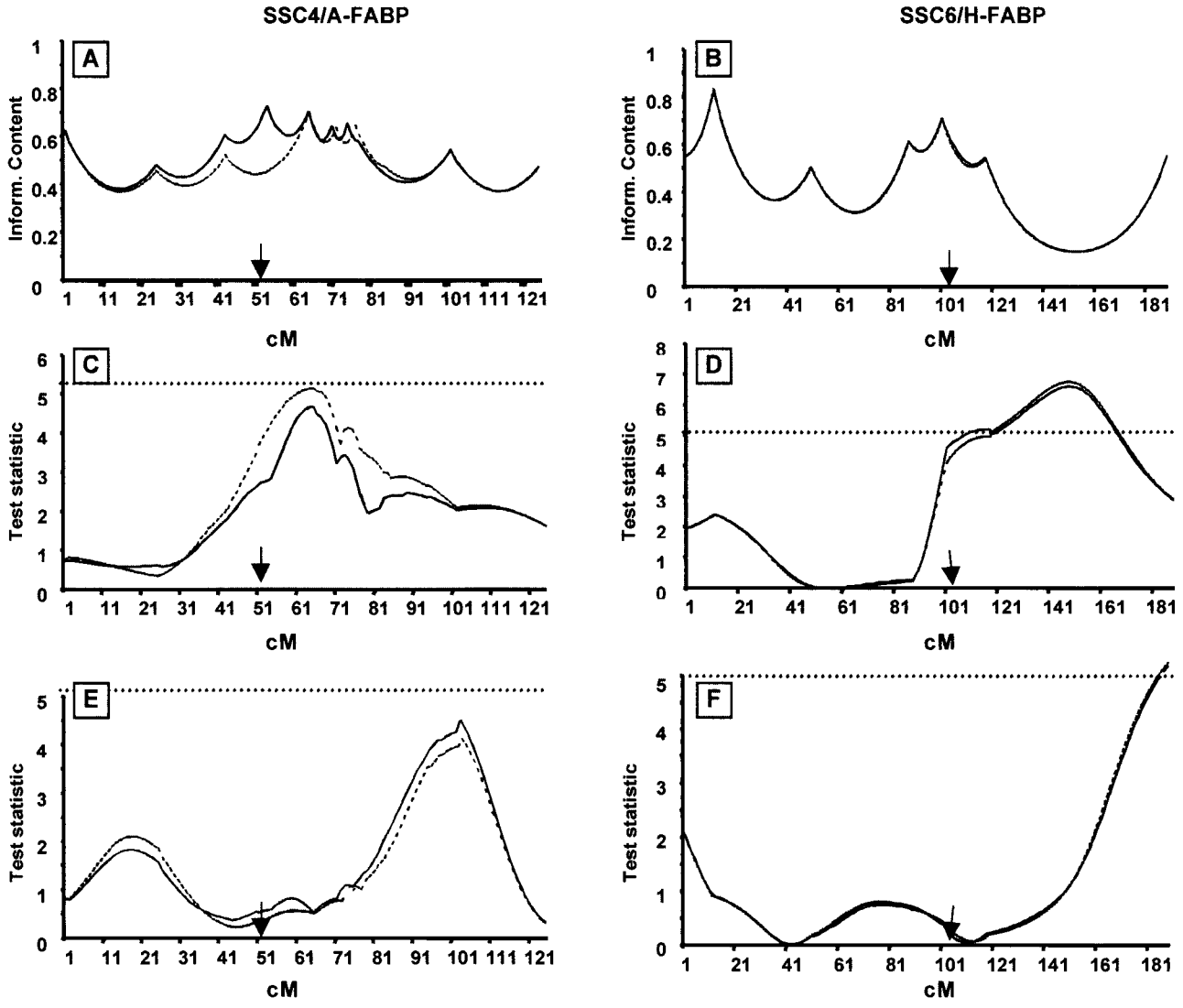

Figure 1. Information content and test statistics for SSC4 (left) and SSC6 (right). In each panel, results are shown without (dashed line) and with (solid line) information of both FABP microsatellites included in the analysis. Panel A and B show the information content along the chromosome. Panel C and D represent the *F*-ratio plot along the chromosome for intramuscular fat (IMF). Similarly, Panels E and F are for backfat thickness (BFT). Haldane map positions of the *A-FABP* and *H-FABP* genes on SSC4 (54 cM) and 6 (106 cM), respectively, are indicated with arrows. Horizontal dotted lines represent the chromosomewise ($P = .05$) significance threshold.

Table 1. Additive and dominance effects of most significant QTL loci on SSC4 and SSC6 for intramuscular fat content (IMF) and backfat thickness (BFT)

Trait	SSC	Position, cM	*F*-ratio	Additive	SD	Dominance	SD
IMF	4	65	4.67	.22	.07	–.05	.10
	6	149	6.61[sc]	–.44	.12	.10	.33
BFT	4	103	4.50	–.22	.41	–2.01	.68
	6	189	5.18[s]	–.61	.40	–1.75	.63

[s]indicates significance at the suggestive level; [c]at the chromosomewise level. For reference, *A-FABP* and *H-FABP* genes are positioned at 54 cM on SSC4 and 106 cM on SSC6, respectively.

There was no evidence of any region on SSC4 significantly affecting BFT (Figure 1E). The highest *F*-statistic became more pronounced after inclusion of *A-FABP* microsatellite information but remained nonsignificant. In particular, around the position of the A-FABP gene, the test statistic was very low.

SSC6/H-FABP. Suggestive evidence for a QTL affecting IMF content was detected on SSC6 around 149 cM, which was significant at the chromosomewise level. The maximum test statistic for this QTL is found in the marker interval adjacent to the *H-FABP* gene. This marker interval covers a very wide range of approximately 70 cM of SSC6 (Figure 1D). This QTL appears to be additive in action, and Meishan alleles are causing a decrease in IMF content (Table 1). Individuals carrying both Meishan alleles have on average .88% less IMF than those homozygous for the Western pig alleles.

Furthermore, there was also suggestive evidence for a QTL on the end of the linkage group of SSC6 affecting BFT that nearly approached the chromosomewise (P = .05) significance threshold (Figure 1F). At the position of the *H-FABP* gene, the test statistic showed no evidence of any contribution to variation in BFT.

Candidate Gene Analysis

SSC4/A-FABP. The effect of each individual allele of *A-FABP* was estimated simultaneously, both for IMF content and BFT. For both traits, inclusion of *A-FABP* microsatellite information did not provide a significantly better explanation of the data. No significant effects of separate *A-FABP* alleles on IMF content were detected in this analysis (Table 2).

In contrast, for BFT, the effects of the *A-FABP* A5 and A9 alleles differed significantly from zero (Table 2). These effects were even more pronounced when fitting growth data in this model (BFT2; Table 2). Both alleles had unfavorable regression coefficients (i.e., BFT increased with increasing number of either the A5 or A9 allele in the individuals). The A5 allele originated almost exclusively from the Meishan population, whereas the A9 allele was present in several subpopulations of the western pigs.

SSC6/H-FABP. Inclusion of *H-FABP* genetic variation data in the model did not provide a significantly better explanation of the data for either IMF content or BFT. For the individual alleles of H-FABP, the effects on IMF content approached significance (Table 2). Because in this analysis only two *H-FABP* alleles were present, the regression coefficient expresses the difference between alleles. Therefore, the contrast between both homozygous *H-FABP* genotype classes H1H1 and H2H2 is .36% IMF where the H2 allele increased the IMF content. Because H1 and H2 alleles both segregate in the Meishan and western pig populations, no conclusions as to the origin of this effect can be drawn.

No evidence for differences in BFT between the H-FABP alleles was found (Table 2).

Discussion

Marker Linkage Analysis

According to genetic linkage analysis, the *A-FABP* and *H-FABP* genes are located on SSC4 and 6, respectively. These findings confirm previous results from cell hybrid analysis (Gerbens et al., 1997, 1998b) and are supported by comparative mapping data. Recently, the human *A-FABP* gene (*FABP4*) was assigned to human chromosome 8q21 (Prinsen et al., 1997), a region syntenic with porcine chromosome 4pter-4q14 as observed by ZOO-FISH analysis (Goureau et al., 1996). The porcine *A-FABP* gene was located between the markers *S0001* and *S0073* that have been assigned to SSC 4p12-p13 (Marklund et al., 1993) and SSC 4q15-q16 (Robic et al., 1996), respectively. Thus, the *A-FABP* gene resides around the centromere of SSC4. So far, the flanking *S0217* marker has not been physically assigned. The *A-FABP* microsatellite sequence resides in the middle of the region between *S0001* and *S0217*, the marker is highly polymorphic, and because it is located within a gene it is also a good reference in genome analysis comparative with other species.

The human *H-FABP* gene (*FABP3*) is located on chromosome 1p32-35 (Troxler et al., 1993; Huynh et al., 1995) a region syntenic with porcine SSC 6q21-q26 and 6q32-qter, as established by comparative mapping analysis and ZOO-FISH analysis (Goureau et al., 1996). To our knowledge, none of the flanking markers has been physically localized on SSC6. Therefore, no distinction can be made whether *H-FABP* is located in the centromeric or telomeric region of chromosome 6 syntenic with human chromosome 1. Moreover, the rearrangements of the gene order between humans and

pigs in this region, presumably by intrachromosomal inversion (Johansson et al., 1995) indicates that physical localization of the *H-FABP* gene should be considered.

QTL and Candidate Gene Analysis

SSC4/A-FABP. In the present study, no significant evidence was detected for QTL affecting either IMF content or BFT on SSC4. In contrast, De Koning et al. (1999) found suggestive linkage between SSC4 and IMF content in the same population using two statistical models. This study adds the *A-FABP* microsatellite information to the line-cross analysis. The overall reduction of the test statistic due to this addition is probably an effect of the increased information content and consequently an increased power to estimate the chromosomal contribution to IMF content. The fact that, in contrast to earlier findings (Gerbens et al., 1998b), no evidence for an effect of *A-FABP* on IMF content was found, may be due to absence of variation in this population, epistatic interaction of genes or the magnitude of the background gene effects. In the QTL analysis, fixation of alleles in the two founder populations was assumed. However, when the QTL alleles are not fixed, the expected contrast decreases and makes the QTL harder to detect.

Previously, a significant contrast between *A-FABP* A1A1 and A1A3 genotype classes was found of approximately 1% IMF in a commercial Duroc population (Gerbens et al., 1998b). In the Meishan crossbred population, the candidate gene analysis showed no significant effect of the *A-FABP* alleles, including the A3 allele, on IMF content. This failure to detect an association between *A-FABP* alleles and IMF content in the present study might be due to the presence of eight distinct *A-FABP* microsatellite alleles in the Meishan crossbred pig population. Most likely, presuming a single mutation event, only two alleles are affecting a trait, in this case, IMF content. Therefore, the high number of *A-FABP* alleles is unrealistic and reduces the statistical power of the candidate gene approach. On the other hand, the use of more marker alleles or multiple marker haplotypes might avoid the possibility of linkage equilibrium between the marker site and the causative mutation (Templeton et al., 1987).

For BFT, no overall significant effect of *A-FABP* allele classes could be detected in the candidate gene analysis. However, the *A-FABP* A5 and A9 alleles were significantly associated with higher BFT. In contrast, no evidence for a QTL affecting BFT was found on SSC4 in the vicinity of the *A-FABP* gene in the QTL analysis. This difference between methods might be due to a false assumption that QTL alleles were fixed in each population in the QTL analysis. Namely, both *A-FABP* alleles increase BFT with similar magnitude, but the A5 allele originates from the Meishan founder boars whereas the A9 allele from the Western founder pigs. However, within-family QTL analysis (De Koning et al., 1999) instead of line-cross QTL analysis showed also no evidence for the presence of a QTL affecting BFT around the *A-FABP* gene on SSC4 in the Meishan crossbred population.

In contrast, others reported and substantiated QTL affecting BFT on SSC4 in Wild pig and Meishan crossbred populations (Andersson et al., 1994; Knott et al., 1998; Walling et al., 1998). According to the reported position of these QTL, *A-FABP* might be a candidate gene involved in genetic variation of BFT in these breeds or populations. The lack of evidence for a clear effect of *A-FABP* on BFT in the candidate gene analysis and QTL analysis in the Meishan crossbred population and also in the Duroc population (Gerbens et al., 1998b) might be due to differences in control of BFT between breeds and(or) that the QTL was not segregating in the selected breeds.

SSC6/H-FABP. In general, screening for QTL on SSC6 should be given special attention because a specific mutation of the ryanodine receptor gene (*Ryr-1*) that resides on SSC6 might be segregating in the population under investigation. This mutation renders pigs halothane-susceptible, a condition that also affects per-

Table 2. Regression coefficients (se) of each individual *A-FABP* or *H-FABP* allele for backfat thickness (BFT) and intramuscular fat (IMF)

Gene	Allele	BFT[a]	BFT2[a]	IMF[a]	IMF2[a]
A-FABP	A1	1.02 (.79)	1.08 (.78)	.19 (.15)	.12 (.15)
	A3	.16 (1.13)	.13 (1.12)	–.10 (.21)	–.11 (.21)
	A4	.74 (.91)	.65 (.90)	–.07 (.17)	–.11 (.17)
	A5	1.49 (.67)*	1.64 (.66)*	.08 (.13)	–.01 (.12)
	A6	.82 (1.88)	.73 (1.86)	–.03 (.37)	–.04 (.35)
	A9	1.91 (.92)*	2.00 (.92)*	–.03 (.17)	–.15 (.17)
	A10	–4.19 (3.10)	–3.89 (3.07)	.04 (.59)	.28 (.57)
	A11	–1.96 (1.65)	–2.35 (1.64)	–.09 (.32)	.02 (.30)
H-FABP	H1	–.06 (.27)	–.05 (.27)	–.09 (.05)†	–.09 (.05)†

[a]BFT: model includes sex and carcass weight; BFT2: model includes sex, carcass weight, and growth from start of test till slaughter; IMF: model includes sex and carcass weight; IMF2: model includes sex, carcass weight and BFT.
†$.05 < P < .10$.
*$P < .05$.

formance and meat quality of pigs (Zhang et al., 1992). However, this mutation was not segregating in the Meishan crossbred population (Janss et al., 1997).

Results from the QTL analysis indicated a suggestive and chromosomewise significant QTL affecting IMF content on SSC6. Although the highest peak is about 50 cM telomeric of the *H-FABP* gene, the test statistic at the *H-FABP* gene was still seen to exceed the suggestive threshold level. The wide region suggested to contain the QTL is very poorly covered with markers, indicating that the position of the QTL peak can still change considerably with additional data. In this respect, the *H-FABP* gene can still be responsible for the additive QTL effect of about one phenotypic standard deviation (.88% IMF) between the homozygotes for the Meishan and Western pig alleles. However, the width of the QTL region also leaves the possibility of two or more QTL on SSC6.

Previously, De Koning et al. (1999) found evidence suggestive of QTL on SSC2, SSC4, SSC6, and SSC7, whereas only the QTL on SSC 6 reached chromosomewise ($P = .05$) and even approached genomewide ($P = .13$) significance. This result was confirmed by our study that contributed an additional marker and candidate gene to this interesting chromosomal region.

In the candidate gene analysis, also effects of the *H-FABP* alleles on IMF content were detected that approached significance. This effect of .36% IMF between either homozygous genotype class is high considering the overall mean of 1.84% IMF (SD .87%) in the Meishan crossbred population and is similar to a previously reported effect in a Duroc population (Gerbens et al., 1999). However, two essential differences are present between both studies. First, the candidate gene approach relies on the presence of localized linkage disequilibrium within a population at the level of the gene (Haley, 1999). In contrast to the Duroc outbred population, this linkage disequilibrium is expected to be much higher in the Meishan F_2 crossbred population; thus, associations may be due to loci some distance away from the *H-FABP* gene on SSC6 and(or) differences between founder breeds. Second, the significant association between genetic variation in the *H-FABP* gene and IMF content in a Duroc population was detected using *H-FABP Msp*I, *Hae*III, and *Hin*fI RFLP alleles as a source of genetic variation (Gerbens et al., 1999), whereas in the present study a dimorphic microsatellite within the *H-FABP* gene was used for practical reasons. These polymorphic sites are all located within a 10-kb region of the *H-FABP* gene and thus genetically closely linked. However, the observed effects with the microsatellite alleles in this study cannot be ascribed to specific *H-FABP* RFLP alleles because linkage phases between these polymorphic alleles are not similar in both founder populations of the Meishan crossbreds (data not shown).

For BFT, results from both the QTL and candidate gene analyses in the Meishan crossbred population did not confirm the involvement of *H-FABP* in backfat accretion as found for Duroc pigs (Gerbens et al., 1999). The suggestive QTL affecting BFT at the end of SSC6, however, could be influencing the significant QTL affecting IMF found in the same region. The difference in both QTL peak positions suggests that the QTL affecting IMF is in fact two QTL of which the most telomeric is affecting both BFT and IMF and the other specifically IMF. Future analyses, by including BFT information in the QTL analysis of IMF content and additional genotyping information of the rest of the Meishan crossbred pig population may confirm this hypothesis.

In conclusion, results of the *H-FABP* gene in the Meishan crossbred population confirm previous results in Duroc pigs for IMF content but not for BFT (Gerbens et al., 1999). The fact that the effects on IMF are detected in two very distinct pig breeds/populations supports the hypothesis that the *H-FABP* gene rather than a closely linked gene will be responsible for these effects. Therefore, it will be of interest to evaluate whether these allelic variants are associated with differences in *H-FABP* gene expression and(or) *H-FABP* protein functionality. Two of the three RFLP together with the microsatellite sequence are located in the second intron of the porcine *H-FABP* gene, whereas the other RFLP site is located in the 5′ untranslated region (Gerbens et al., 1997, 1998a). Both intronic sequences and 5′ untranslated regions have been reported to affect gene expression. Therefore, one of the mutations that cause these *H-FABP* allelic variations might be responsible for the observed effects on IMF itself, although linkage with other sites of genetic variation in or near the *H-FABP* gene cannot be excluded and needs further investigation.

Implications

Findings from this study in Meishan crossbred pigs support the association of heart fatty acid-binding protein (*H-FABP*) genetic variation with intramuscular fat content, but not with backfat thickness, as found in a previous study with Duroc pigs. The evidence that the *H-FABP* gene is responsible for part of the genetic variation in intramuscular fat content in pigs can initiate the search for the causative mutation in this gene. Furthermore, presently known polymorphisms in the *H-FABP* gene can be implemented in marker-assisted selection to improve meat quality of pigs. However, before this implementation 1) the presence of the association, 2) the linkage phase between quantitative trait locus allele and *H-FABP* gene polymorphism, and 3) the linkage with the halothane allele, when present, should be established for the pig population under investigation. In contrast, no evidence was found to support the role of the adipocyte fatty acid-binding protein (*A-FABP*) gene in intramuscular fat content.

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