

# Refining genomewide association for growth and fat deposition traits in an F<sub>2</sub> pig population<sup>1</sup>

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**ABSTRACT:** The identification of genomic regions that affect additive genetic variation and contain genes involved in controlling growth and fat deposition has enormous impact in the farm animal industry (e.g., carcass merit and meat quality). Therefore, a genome-wide association study was implemented in an F<sub>2</sub> pig population using a 60,000 SNP marker panel for traits related to growth and fat deposition. Estimated genomic EBV were linearly transformed to calculate SNP effects and to identify genomic positions possibly associated with the genetic variability of each trait. Genomic segments were then defined considering the markers included in a region 1 Mb up- and downstream from the SNP with the smallest *P*-value and a false discovery rate < 0.05 for each trait. The significance for each 2-Mb segment was tested using the Bonferroni correction. Significant SNPs were detected on SSC2, SSC3, SSC5, and SSC6, but 2-Mb segment

significant effects were observed on SSC3 for weight at birth (wt\_birth) and on SSC6 for 10th-rib backfat and last-rib backfat measured by ultrasound at different ages. Furthermore, a 6-Mb segment on SSC6 was also considered because the 2-Mb segments for 10 different fat deposition traits were overlapped. Although the segment effects for each trait remain significant, the proportion of additive variance explained by this larger segment was slightly smaller in some traits. In general, the results confirm the presence of genetic variability for wt\_birth on SSC3 (18.0–20.2 Mb) and for fat deposition traits on SSC6 (133.8–136.0 Mb). Within these regions, fibrosin (*FBRS*) and myosin light chain, phosphorylatable, fast skeletal muscle (*MYLPF*) genes could be considered as candidates for the wt\_birth signal on SSC3, and the SERPINE1 mRNA binding protein 1 gene (*SERPINE1*) may be a candidate for the fat deposition trait signals on SSC6.

**Key words:** fat, genome association, growth, pig, single nucleotide polymorphism

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J. Anim. Sci. 2016.94  
doi:10.2527/jas2015-0182

<sup>1</sup>This project was supported by Agriculture and Food Research Initiative Competitive Grant number 2010-65205-20342 from the USDA National Institute of Food and Agriculture and by funding from the National Pork Board Grant number 11-042. Partial funding was also provided by the US Pig Genome Coordination Program. Computer resources were provided by the Michigan State University High Performance Computing Center (HPCC). R. J. C. Cantet was funded by UBACyT 20020100100861 from Universidad de Buenos Aires and PIP 11220120100621CO from CONICET (Argentina). J. L. Gualdrón Duarte and Y. L. Bernal-Rubio were funded by COLCIENCIAS (Colombia).

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Received December 8, 2015.

Accepted January 31, 2016.

## INTRODUCTION

Genomewide association studies (GWAS) using high-density SNP genotypes on phenotypic data from complex traits (e.g., growth and fat deposition: Choi et al., 2010; Lee et al., 2011; Fontanesi et al., 2012, 2014; Okumura et al., 2013; Gualdrón Duarte et al., 2014; feed intake: Jiao et al., 2014; Howard et al., 2015) allows identifying genes that determine the expression of economically relevant traits. A practical method to perform GWAS is to linearly transform the predictions of genomic EBV into the SNP effects (Garrick, 2007; Strandén and Garrick, 2009; Sun et

al., 2011; Gualdrón Duarte et al., 2014). After plotting those SNP effects, genomic positions that suggest association with the genetic variability of the trait may be identified. Moreover, testing the significance of the segments formed from the most relevant genome position under linkage disequilibrium (LD) improves the identification of specific regions or segments (Hayes et al., 2010; Gualdrón Duarte et al., 2014) responsible for a fraction of the variability in the trait. If the experimental population has measurements for different phenotype characteristics, it is feasible to find a relevant genomic region associated with the expressions of multiple traits. For that reason, the main objective of this research was to identify genomic regions that are associated with the additive variance in traits related to growth and fat deposition at different ages from an  $F_2$  generation of a pig population using linear transformation of genomic EBV and posterior selection of the candidate segments.

## MATERIALS AND METHODS

Animal protocols were approved by the Michigan State University All University Committee on Animal Use and Care (Animal use form number 09/03-114-00).

### Data Set

Data for the analysis were from an experimental population from the Michigan State University Swine Teaching and Research Farm, East Lansing, MI (Edwards et al., 2008). The initial generation ( $F_0$ ) was raised with 4 unrelated Duroc boars mated to 15 Pietrain sows by AI. The  $F_0$  animals were confirmed to be homozygous normal for the *RYR1* gene (Edwards et al., 2008) by DNA test (Fujii et al., 1991). From all resulting progeny, 50 females and 6 males were selected and mated as  $F_1$  parents by avoiding full- or half-sib matings. The total number of piglets born alive in the  $F_2$  was 1,259 out of 142 litters from 11 farrowing groups. Growth and fat deposition phenotypes were obtained only for  $F_2$  animals, and the traits were 10th-rib backfat (**bf10**), last-rib backfat (**lrf**), and LM area; all traits were estimated using B-mode ultrasound and recorded at 10, 13, 16, 19, and 22 wk of age. Weight was recorded at birth and wk 3, 6, 10, 13, 16, 19, and 22. Measures of fat-free total lean, total body fat tissue, empty body protein, and empty body lipid were recorded at wk 22. Average daily gain between 10 and 22 wk of age and the number of days to reach 105 kg were calculated from these BW measures (for more details, refer to Edwards et al. [2008] and Choi et al. [2010]). A descriptive summary of all phenotypes is presented in Supplemental Table S1 (see the online version of the article at <http://journalofanimalscience.org>), and

correlations of traits are presented in Supplemental File S1 (see the online version of the article at <http://journalofanimalscience.org>).

### Genotyping and Data Editing

Deoxyribonucleic acid was isolated from white blood cells using standard procedures as previously described for this population (Edwards et al., 2008). Quantity and quality of DNA samples were determined using a Qubit fluorometer (Invitrogen by Life Technologies, Carlsbad, CA). The population was genotyped using 2 SNP marker panels. The first set consisted of 411 animals (4  $F_0$  Duroc boars, 15  $F_0$  Pietrain sows, 6  $F_1$  males, 50  $F_1$  females, and 336  $F_2$  pigs), which were genotyped with the Illumina PorcineSNP60 beadchip (Ramos et al., 2009), using the pig genome *Sus scrofa* Build 10.2 assembly ([http://www.ensembl.org/Sus\\_scrofa/Info/Annotation](http://www.ensembl.org/Sus_scrofa/Info/Annotation); accessed March 1 2015). The other set comprised 612  $F_2$  animals that were genotyped using a 9,000 tag SNP panel, the GeneSeek Genomic Profiler for Porcine LD (GeneSeek, a Neogen Company, Lincoln, NE; Badke et al., 2013). A set of 5,350 SNP out of  $M = 62,163$  were eliminated from all analyses, as their physical positions were unknown. Mendelian inconsistencies ( $\leq 0.01\%$ ) were taken as missing genotypes, and 21 animals (1  $F_1$  and 20  $F_2$ ) with more than 10% of SNP missing were not used for any analysis. By similar considerations, 2,978 SNP were removed from the analyses because they had more than 10% missing data. Additionally, 9,877 SNP were excluded because their minor allele frequency was below 0.01. The editing procedure was performed following Badke et al. (2012) and Gualdrón Duarte et al. (2013, 2014), using the program PLINK version 1.07 (Purcell et al., 2007). The  $F_2$  animals genotyped with the 9,000 SNP panel were imputed to the Illumina PorcineSNP60 beadchip following procedures discussed by Gualdrón Duarte et al. (2013, 2014), using the software AlphaImpute (Hickey et al., 2012). The accuracy of imputation was, on average, 0.99 (Gualdrón Duarte et al., 2013). Genotypes imputed in the  $F_2$  received a second editing procedure by a minor allele frequency  $< 0.05$ , which excluded 4,244 virtually monomorphic SNP. The editing policies and genotype imputation resulted in a data set with records from 1,002 pigs ( $F_0$ ,  $F_1$ , and  $F_2$ ) having 40,569 SNP per animal.

### Estimation of Genomic Relationship Matrix

The genomic relationship matrix was estimated from observed and imputed (approximately 40,000) SNP genotypes. Genotypes were expressed using the

allelic dosage (0, 1, and 2; Badke et al., 2013; Gualdrón Duarte et al., 2013, 2014), and genotypes were entered into a marker matrix  $\mathbf{M}$  ( $n \times m$ ), such that  $n$  is the number of animals and  $m$  the number of SNP from the reference allele. Therefore, following procedures used by Gualdrón Duarte et al. (2014),  $\mathbf{M}$  was standardized to matrix  $\mathbf{Z}$  with generic elements equal to  $Z_{ij} = (\mathbf{M}_{ij} - 2p_j) / (\{m[2p_j(1 - p_j)]\}^{1/2})$ . Therefore, calculation proceeded by subtracting twice the frequency of the reference allele at the  $j$ th marker ( $p_j$ ) to the  $\mathbf{M}_{ij}$  (VanRaden, 2008) and then dividing the resulting difference by the square root of the expected variance  $2p_j(1 - p_j)$  of each element in the column multiplied by the number of columns ( $m$ ) in  $\mathbf{M}$ . The allele frequency  $p_j$  was obtained from the 19  $F_0$  animals. The genomic relationship matrix was then calculated as

$$\mathbf{G} = \mathbf{Z}\mathbf{Z}' \quad [1]$$

### Prediction Model

Using the genomic relationship matrix from Eq. [1], the centered animal model for genomic evaluation can be written as

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{a} + \mathbf{e}, \quad [2]$$

in which  $\mathbf{y}$  is the phenotypic vector containing the data from each growth trait,  $\mathbf{X}$  is the incidence matrix that relates records to the fixed effects of sex in  $\boldsymbol{\beta}$ , vector  $\mathbf{a}$  contains the random breeding values such that  $\mathbf{a} \sim N(\mathbf{0}, \mathbf{G}\sigma_A^2)$ ,  $\mathbf{e}$  is the random error vector such that  $\mathbf{e} \sim N(\mathbf{0}, \mathbf{I}\sigma_e^2)$ , and  $\mathbf{I}$  is the identity matrix. Variance components were estimated with REML using the regress version 1.3-10 R package (Clifford and McCullagh, 2006).

### Genome Screening

The  $P$ -values were assessed as 1 minus the cumulative probability density of the absolute value of the standardization of SNP effects ( $\hat{g}_j$ ); then,  $\text{SNP}_{ej} = \hat{g}_j / \{\text{var}(\hat{g}_j)\}^{1/2}$  (Gualdrón Duarte et al., 2014), a number that was then multiplied by 2 so as to obtain  $P\text{-value}_j = 2[1 - \Phi(|\text{SNP}_{ej}|)]$ , in which  $\Phi(x)$  is the cumulative density function of the normal distribution for the random variable  $x$ . When analyzing the growth and fat deposition traits, the  $P$ -values for each SNP were plotted across the genome as  $-\log_{10}(P\text{-value})$  using the absolute SNP position in megabase pairs. We have shown (Gualdrón Duarte et al., 2014; Bernal Rubio et al., 2015) that this test is a computationally ultrafast implementation of the EMMAX procedure (Kang et al., 2008).

### Proportion of Variance Explained by Segments with Large Effect

After the genome screen using model [2], the SNP with the smallest  $P$ -values (peak SNP) and a false discovery rate ( $\mathbf{FDR}$ )  $< 0.05$  (Storey and Tibshirani, 2003) were selected to form SNP segments. The segments were defined by 2 methods: 1) taking all SNP within 1 Mb upstream and 1 Mb downstream of the peak SNP (Gualdrón Duarte et al., 2014), which essentially would imply a minimal contribution to the additive variance from markers located beyond such distance, and 2) if 2 or more peak SNP were located within a close range, then a segment including all markers between the flanking peak SNP and the markers located 2 Mb up- and downstream was formed.

The proportion of variance associated with each segment was estimated by building a genomic relationship matrix  $\mathbf{G}_1$  (as described in Eq. [1]) using all SNP that belonged to the segment, whereas the genomic relationship matrix  $\mathbf{G}_2$  was built using all remaining SNP. The model fitted can be represented as

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{a}_1 + \mathbf{a}_2 + \mathbf{e}, \quad [3]$$

in which  $\mathbf{a}_1$  is the vector of additive random effects associated with those SNP located in the segment, such that  $\mathbf{a}_1 \sim N(\mathbf{0}, \mathbf{G}_1\sigma_{A_1}^2)$ , and  $\mathbf{a}_2$  is the vector of additive random effects associated with all SNP except those involved with  $\mathbf{a}_1$ , such that  $\mathbf{a}_2 \sim N(\mathbf{0}, \mathbf{G}_2\sigma_{A_2}^2)$ . Model [3] assesses the proportion of variance explained by the segment of interest (local variance) from the genome variance explained by all SNP (global variance). The variances estimated in model [3] were compared with those estimates from model [2] as in Hayes et al. (2010) or Gualdrón Duarte et al. (2014).

To adjust the level of significance for multiple comparisons, a Bonferroni correction ( $\mathbf{BC}$ ) was performed. In this context, if the pig genome is approximately 2,800 Mb long and the average size of the segment is  $\lambda$  Mb in length, there are  $2,800/\lambda$  segments along the genome with corresponding multiple tests. Therefore, for  $\alpha = 0.05$ , the BC was equal to  $0.05/(2,800 \text{ Mb}/\lambda) = \alpha^*$  (adjusted  $\alpha$  or critical value). Hence, to evaluate the significance of the segments, a second  $P$ -value for the likelihood ratio test ( $P\text{-value}_{\text{LRT}}$ ) was calculated to compare with BC. This  $P\text{-value}_{\text{LRT}}$  was assessed as 1 minus the distribution function of a  $\chi^2$  random variable with 0.5 df (Self and Liang, 1987; Liang and Self, 1996) as follows:

$$P\text{-value}_{\text{LRT}} = 1 - \chi^2(\text{LRT}),$$

in which  $\chi^2(x)$  is the distribution function of a random variable having the  $\chi^2$  as density and  $\text{LRT}$  is

the likelihood ratio test obtained by contrasting appropriate models.

### Candidate Gene Screening

For the significant segments that were obtained, a candidate gene screening was performed as follows. A genomic region was delimited using the maximum and minimum position of the flanking SNP markers of each segment. Next, within this genomic region, the names of genes that could be involved in growth and fat depositions traits were extracted. The genes names were obtained from Ensembl ([http://ensembl.org/Sus\\_scrofa/Info/Index](http://ensembl.org/Sus_scrofa/Info/Index); accessed June 1, 2015).

## RESULTS

### Genome Screening

The  $P$ -values for the 40,569 SNP of each growth and fat deposition trait were obtained as described in the Materials and Methods section. The  $P$ -values for each trait were then plotted along the genome to identify genomic positions that are associated with variation in each trait (Supplemental Fig. S1 through S4; see the online version of the article at <http://journalofanimalscience.org>). Large peaks ( $-\log_{10}(P\text{-value})$  above 5) can be seen for the traits weight at birth (**wt\_birth**); weight at wt\_13wk; ADG; empty body lipid; total body fat tissue; bf10 at 10, 13, 19, and 22 wk; and lrf at 10, 13, 16, 19, and 22 wk, suggesting additive genetic variation by trait. Within these, only SNP for wt\_birth and bf10 and lrf measured at 10, 13, 16, 19, and 22 wk were significant using a FDR  $< 0.05$ . However, Manhattan plot peaks for traits weight at wt\_13wk, ADG, empty body lipid, and total body fat tissue (Supplemental Table S2; see the online version of the article at <http://journalofanimalscience.org>) are suggestive as possible QTL for those traits. In addition, the SNP ALGA0045948 (SSC7) was the peak SNP for empty body protein and had the highest  $-\log_{10}(P\text{-value})$  for days the number of days to reach 105 kg and fat-free total lean, whereas the SNP ALGA0045724 (SSC7) was the peak SNP for weight at wt\_13wk and had the highest  $-\log_{10}(P\text{-value})$  for weight at wt\_16wk and wt\_19wk (Supplemental Table S2; see the online version of the article at <http://journalofanimalscience.org>). Therefore, the influence of these SSC7 regions on days the number of days to reach 105 kg, fat-free total lean, and weight at wt\_16wk and wt\_19wk should not be dismissed.

### Tests of Segment Effects

There were 117 SNP with a FDR  $< 0.05$ , including wt\_birth (SSC3); bf10 at 10, 13, 19, and 22 wk (SSC2,

SSC3, and SSC6); and lrf at 10, 13, 16, 19, and 22 wk (SSC2, SSC5, and SSC6). For each trait and chromosome, we selected the SNP with the smallest  $P$ -values (peak SNP) from the 117 SNP (Table 1). Eight peak SNP were then chosen to form segments of 2 Mb (1 Mb on each side of the SNP with the smallest  $P$ -value).

Chromosome 6 displayed 4 peak SNP (10 traits in total) located in a common region of 2.2 Mb (133.8–136.0 Mb). Here, the peak SNP M1GA0008917 and ASGA0029651 are consecutively located and have a LD ( $r^2$ ) of 1. This was also observed for the other 2 peak SNP, ALGA0122657 and ALGA0104402 (Supplemental Fig. S5, S6, and S7; see the online version of the article at <http://journalofanimalscience.org>). Also, despite not being adjacent, SNP pairs M1GA0008917/ASGA0029651 and ALGA0122657/ALGA0104402 had substantial LD ( $r^2 > 0.6$ ; Supplemental Fig. S5, S6, and S7; see the online version of the article at <http://journalofanimalscience.org>). Then, using the region of 2.2 Mb (that include the 4 peak SNP), a longer segment was considered that included markers positioned 2 Mb up- and downstream from the extreme SNP, to cover the LD of the flanking markers from the region. As a result, a longer segment of 6 Mb (physical position on SSC6: 131.9–137.9 Mb) was additionally tested for the significant traits.

The LRT was performed to test the segment effects. The estimates of the variance components and the log-likelihood obtained from model [3] were compared with those from model [2]. Results for LRT of 2-Mb segments indicated that the segment on SSC3 was significant for wt\_birth, explaining 30% of the total additive variance. Similarly, the segments on chromosome 6 were significant for bf10 at 10, 13, 16, 19, and 22 wk and for lrf at 10, 13, 16, 19, and 22 wk, explaining between 4 and 10% of the total additive variance (Table 2). The  $P\text{-value}_{\text{LRT}}$  values of these traits were smaller than the critical Bonferroni threshold or  $BC\ 3.571429e \times 10^{-5}$  (for 2,800 Mb/2 Mb = 1,400 segments, then  $P_{\text{critical}} = \alpha^* = 0.05/1,400 = 3.571429e \times 10^{-5}$ ). Regarding the wt\_birth, the proportion of additive variance explained by the 2-Mb segment was remarkably high (30%); hence, a probable overestimation should be considered. Notably, the value for the peak SNP “ALGA0075667” exceeded the others within the genomic region ( $-\log_{10}(P\text{-values}) = 7.27$ ; see Supplemental Fig. S2 [see the online version of the article at <http://journalofanimalscience.org>]) and also was not in LD with the adjacent SNP (Supplemental Fig. S8; see the online version of the article at <http://journalofanimalscience.org>).

When peak SNP on SSC2 (bf10 at 16 and 19 wk), SSC3 (bf10 at 13 wk), and SSC5 (lrf at 16 wk) were evaluated through segment analysis of 2 Mb, they did not display significant effects, and hence, the further LD analysis and candidate gene screening was not

**Table 1.** Significant SNP markers by trait

SNP-ID <sup>1</sup>	Chromosome	Position, Mb <sup>2</sup>	Trait <sup>3</sup>	<i>P</i> -value <sup>4</sup>
MARC0087200	2	146.7230	bf10_16wk lrf_19wk	4.73 × 10 <sup>-6</sup> 7.27 × 10 <sup>-6</sup>
ALGA0075667	3	19.1643	wt_birth	5.33 × 10 <sup>-8</sup>
H3GA0010564	3	119.3397	bf10_13wk	1.07 × 10 <sup>-6</sup>
ALGA0031990	5	58.3026	lrf_16wk	9.03 × 10 <sup>-7</sup>
MIGA0008917	6	133.8855	bf10_22wk lrf_16wk lrf_22wk	6.42 × 10 <sup>-7</sup> 4.84 × 10 <sup>-8</sup> 5.22 × 10 <sup>-7</sup>
ASGA0029651	6	133.9292	bf10_10wk lrf_10wk	9.00 × 10 <sup>-7</sup> 4.28 × 10 <sup>-0</sup>
ALGA0122657	6	136.078566	lrf_13wk	3.04 × 10 <sup>-9</sup>
ALGA0104402	6	136.0844	bf10_13wk bf10_16wk bf10_19wk lrf_19wk	1.01 × 10 <sup>-8</sup> 1.42 × 10 <sup>-7</sup> 9.16 × 10 <sup>-7</sup> 7.20 × 10 <sup>-8</sup>

<sup>1</sup>SNP-ID = name of the marker SNP selected by highest  $-\log_{10}(P\text{-value})$  and a false discovery rate < 0.05.

<sup>2</sup>Marker SNP physical position along the chromosome in megabase pairs.

<sup>3</sup>bf10\_10wk, bf10\_13wk, bf10\_16wk, bf10\_19wk, and bf10\_22wk = 10th-rib backfat (mm) at wk 10, 13, 16, 19, and 22, respectively; lrf\_10wk, lrf\_13wk, lrf\_16wk, lrf\_19wk, and lrf\_22wk = last-rib backfat (mm) at wk 10, 13, 16, 19, and 22, respectively; wt\_birth = weight at birth.

<sup>4</sup>*P*-value = *P*-value of the marker SNP selected by trait.

performed. Nonetheless, the possible influence of these regions on SSC2, SSC3, and SSC5 should not be dismissed and could be further evaluated in future studies.

Equally, the LRT for the 6-Mb segment (SSC6) was smaller than the BC = 0.0001073 (for 2,800 Mb/6 Mb = 466 segments, then  $P_{\text{critical}} = \alpha^* = 0.05/466 = 0.0001073$ ) for each trait where it was significant (Table 3). However, when the size of the tested segment increased from 2 to 6 Mb, the proportion of the total variance explained decreased slightly for bf10 at 10 wk (from 9.59 to 7.6%), lrf at 13 wk (from 8.33 to 5.7%), bf10 at 16 wk (from 8.1 to 7.2%), and lrf at 19 wk (from 8.3 to 7.5%; Table 3).

### Candidate Segment Gene Screening

When analyzing the SSC3 signal, the candidate gene screening reveals a couple of genes that, considering their physiological function, could be related to wt\_birth: fibrosin (*FBR5*; located at 18,187,484–18,193,499 bp) and myosin light chain gene phosphorylatable, fast skeletal muscle (*MYLPF*; located at 18,376,798–18,379,771 bp) that are related to cartilaginous tissue and muscle development. Considering the signal detected on SSC6 for fat deposition traits, the search for candidate genes was performed in the 6-Mb segment (131.9–137.9 Mb). As a result in our gene screen, and in addition to the already reported *PDE4B* (phosphodiesterase 4B, CAMP-

specific), *Clorf141* (chromosome 1 open reading frame 141; Lee et al., 2011), and *LEPROT* genes (Okumura et al., 2013), the *SERBP1* gene was identified that could be responsible for the significant signal. *SERBP1* is located at 134,068,990 to 134,081,998 bp, and the protein is associated with the regulation of mRNA and lipid metabolism (Li et al., 2010).

## DISCUSSION

The main goal of this research was to improve the identification of genomic regions (segments) associated with the additive variation in pig growth and fat deposition traits and to identify which of these regions are determining the expression of multiple traits.

### Genomewide Association for Growth and Fat Deposition Traits

A GWAS approach was used on each growth and fat deposition trait to find possible genomic regions affecting these traits. From these candidate regions, SNP with the lowest *P*-values and a FDR < 0.05 within each chromosome were then chosen by trait. Finally, 8 SNP located on SSC2, SSC3, SSC5, and SSC6 were selected for 11 traits (wt\_birth; bf10 at 10, 13, 16, 19, and 22 wk; and lrf at 10, 13, 16, 19, and 22 wk). In particular, a 2.2-Mb region on SSC6 constructed with 4 peak SNP located between 133.8 and 136.2 Mb was significant for 10 traits.

When analyzing wt\_birth, the results revealed a significant position at 19.1 Mb on SSC3. Previous studies in pigs had reported QTL for birth weight in the same region on SSC3. Liu et al. (2007) found a QTL peak located at position between position 0 and 30 cM in a population of similar genetic background (Duroc × Pietrain), and Malek et al. (2001) reported a QTL at position 19 Mb in a Berkshire × Yorkshire population. Therefore, both regions could be considered as candidates to be influencing the expression of the trait.

In relation to the ADG, the trait showed a peak SNP, ASGA0021485 ( $-\log_{10}(P\text{-value}) = 5.56$ ), located on SSC4 position 112.48 Mb (Supplemental Table S2; see the online version of the article at <http://journalofanimalscience.org>). Fontanesi et al. (2014) described in a Duroc population that ADG is significantly associated to several SNP located in this same genomic region between MIGA0006238 and MIGA0006250 (111.5 and 112.7 Mb, respectively, of SSC4). Moreover, Howard et al. (2015) showed a significant association between ADG and markers in SSC3 but in different genomic regions (44.24–46.14 Mb and 82.08–82.89 Mb). Additionally, Jiao et al. (2014), using a Duroc experimental population, described a segment of 1 Mb

**Table 2.** Variance components and log-likelihood for models with or without the segment of 2 Mb

Statistic	Seg-chromosome <sup>1</sup>													
	Trait <sup>2</sup>						SNP-ID <sup>3</sup>							
	2	2	3	3	5	6	6	6	6	6	6	6		
bfi0_16wk	lrf_19wk	wt_birth	bfi0_13wk	lrf_16wk	bfi0_22wk	lrf_16wk	lrf_22wk	bfi0_10wk	lrf_10wk	lrf_13wk	bfi0_13wk	bfi0_16wk	bfi0_19wk	lrf_19wk
MARC-0087200	MARC-0087200	ALGA-0075667	H3GA-0010564	ALGA-0031990	MIGA-0008917	MIGA-0008917	MIGA-0008917	ASGA-0029651	ASGA-0029651	ASGA-0122657	ALGA-0104402	ALGA-0104402	ALGA-0104402	ALGA-0104402
5.33	5.14	7.27	5.97	6.04	6.19	7.32	6.28	6.05	9.37	8.52	7.96	6.85	6.04	7.14
$-\log_{10}(P\text{-value})^4$														
Lk_m1 <sup>5</sup>	-1,434.97	-1,376.41	629.42	-1,228.38	-1,100.18	-1,969.69	-1,100.18	-1,581.85	-888.23	-454.70	-651.44	-1,228.38	-1,434.97	-1,764.37
Lk_m2 <sup>6</sup>	-1,431.30	-1,370.92	618.11	-1,222.47	-1,094.00	-1,960.82	-1,088.80	-1,573.34	-875.83	-440.10	-636.82	-1,214.75	-1,422.70	-1,755.35
LRT <sup>7</sup>	7.33	10.98	22.62	11.82	12.36	17.74	22.75	17.03	24.82	29.20	27.27	24.54	18.04	26.47
$P\text{-value}_{\text{LRT}}^8$	0.00227237	0.000283139	$5.15 \times 10^{-6}$	0.000177285	0.00013151	$7.00 \times 10^{-6}$	$4.81 \times 10^{-7}$	$1.03 \times 10^{-5}$	$1.61 \times 10^{-7}$	$1.56 \times 10^{-8}$	$4.42 \times 10^{-8}$	$1.87 \times 10^{-7}$	$5.97 \times 10^{-6}$	$6.75 \times 10^{-8}$
VarE_m1 <sup>9</sup>	5.57	4.49	0.082	3.73	2.79	17.80	2.79	7.65	1.82	0.75	1.07	3.73	5.57	4.49
VarA_m1 <sup>10</sup>	4.70	5.25	0.022	2.61	2.07	14.64	2.07	6.45	1.18	0.38	0.76	2.61	4.70	5.25
VarE_m2 <sup>11</sup>	5.53	4.51	0.088	3.69	2.79	18.36	2.82	7.84	1.85	0.76	1.07	3.78	5.64	4.56
VarA_m2 <sup>12</sup>	4.46	4.77	0.014	2.33	1.88	11.56	1.68	5.27	0.88	0.27	0.60	1.98	3.73	4.25
segmVA <sup>13</sup>	0.38	0.53	0.039	0.87	0.35	1.47	0.26	0.60	0.29	0.08	0.15	0.53	0.83	0.80
%segmVA <sup>14</sup>	0.04	0.05	0.30	0.13	0.07	0.05	0.06	0.04	0.10	0.08	0.08	0.08	0.05	0.08

<sup>1</sup>Seg-chromosome = number of chromosome where the segment is located.

<sup>2</sup>bfi0\_10wk, bfi0\_13wk, bfi0\_16wk, bfi0\_19wk, and bfi0\_22wk = 10th-rib backfat (mm) at wk 10, 13, 16, 19, and 22, respectively; lrf\_10wk, lrf\_13wk, lrf\_16wk, lrf\_19wk, and lrf\_22wk = last-rib backfat (mm) at wk 10, 13, 16, 19, and 22, respectively; wt\_birth = weight at birth.

<sup>3</sup>SNP-ID = name of the marker SNP selected by highest  $-\log_{10}(P\text{-value})$  and a false discovery rate  $< 0.05$ .

<sup>4</sup> $-\log_{10}(P\text{-value}) = -\log_{10}(P\text{-value})$  selected to create a segment.

<sup>5</sup>Lk\_m1 =  $-\log$ -likelihood for m1; m1 = model [2] without the segment:  $\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{a} + \mathbf{e}$ , in which  $\mathbf{y}$  is the phenotypic vector containing the data from each growth trait,  $\mathbf{X}$  is the incidence matrix that relates records to the fixed effects of sex in  $\boldsymbol{\beta}$ , vector  $\mathbf{a}$  contains the random breeding values such that  $\mathbf{a} \sim N(\mathbf{0}, \mathbf{G}\sigma_A^2)$ ,  $\mathbf{e}$  is the random error vector such that  $\mathbf{e} \sim N(\mathbf{0}, \mathbf{I}\sigma_e^2)$ , and  $\mathbf{I}$  is the identity matrix.

<sup>6</sup>Lk\_m2 =  $-\log$ -likelihood for m2; m2 = model [3] with the segment:  $\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{a}_1 + \mathbf{a}_2 + \mathbf{e}$ , in which  $\mathbf{a}_1$  is the vector of additive random effects associated with those SNP located in the segment, such that  $\mathbf{a}_1 \sim N(\mathbf{0}, \mathbf{G}_1\sigma_{A_1}^2)$ , and  $\mathbf{a}_2$  is the vector of additive random effects associated with all SNP except those involved with  $\mathbf{a}_1$ , such that  $\mathbf{a}_2 \sim N(\mathbf{0}, \mathbf{G}_2\sigma_{A_2}^2)$ .

<sup>7</sup>LRT = likelihood ratio test (for m1 and m2).

<sup>8</sup> $P\text{-value}_{\text{LRT}} = P\text{-value}$  for the LRT.

<sup>9</sup>VarE\_m1 = error variance ( $\sigma_e^2$ ) of m1.

<sup>10</sup>VarA\_m1 = additive variance ( $\sigma_A^2$ ) of m1.

<sup>11</sup>VarE\_m2 = error variance ( $\sigma_e^2$ ) of m2.

<sup>12</sup>VarA\_m2 = additive variance ( $\sigma_A^2$ ) of m2.

<sup>13</sup>segmVA = Additive variance segment ( $\sigma_{A_1}^2$ ) of m2.

<sup>14</sup>%segmVA = proportion in percent of the total variance explained by the segment.

**Table 3.** Variance components and log-likelihood for models with or without the segment of 6 Mb on chromosome 6

Statistic	Peak-SNP: MITGA0008917, ASGA0029651, ALGA0122657, and ALGA0104402										
	bf10_10wk	bf10_13wk	bf10_16wk	bf10_19wk	bf10_22wk	lrf_10wk	lrf_13wk	lrf_16wk	lrf_19wk	lrf_22wk	Trait <sup>2</sup>
Lk_m1 <sup>3</sup>	-888.23	-1,228.38	-1,434.97	-1,764.37	-1,969.69	-454.70	-651.44	-1,100.18	-1,376.41	-1,581.85	
Lk_m2 <sup>4</sup>	-876.13	-1,211.15	-1,423.30	-1,755.65	-1,961.18	-438.80	-638.44	-1,087.88	-1,364.51	-1,570.73	
LRT <sup>5</sup>	24.20	34.47	23.33	17.45	17.02	31.79	26.01	24.59	23.80	22.24	
$P$ -value <sub>LRT</sub> <sup>6</sup>	$2.23 \times 10^{-7}$	$1.03 \times 10^{-9}$	$3.54 \times 10^{-7}$	$8.20 \times 10^{-6}$	$1.03 \times 10^{-5}$	$4.15 \times 10^{-9}$	$8.61 \times 10^{-8}$	$1.82 \times 10^{-7}$	$2.76 \times 10^{-7}$	$6.30 \times 10^{-7}$	
VarE_m1 <sup>7</sup>	1.82	3.73	5.57	11.16	17.80	0.75	1.07	2.79	4.49	7.65	
VarA_m1 <sup>8</sup>	1.18	2.61	4.70	10.06	14.64	0.38	0.76	2.07	5.25	6.45	
VarE_m2 <sup>9</sup>	1.85	3.79	5.67	11.30	18.33	0.75	1.08	2.80	4.60	7.84	
VarA_m2 <sup>10</sup>	0.87	1.79	3.65	8.33	11.40	0.27	0.59	1.64	4.15	5.00	
segmVA <sup>11</sup>	0.22	0.68	0.72	1.25	1.68	0.08	0.10	0.40	0.71	0.73	
%segmVA <sup>12</sup>	0.08	0.11	0.07	0.06	0.05	0.08	0.06	0.08	0.08	0.05	

<sup>1</sup>Peak-SNP = names of the peak SNP located in the segment.

<sup>2</sup>bf10\_10wk, bf10\_13wk, bf10\_16wk, bf10\_19wk, and bf10\_22wk = 10th-rib backfat (mm) at wk 10, 13, 16, 19, and 22, respectively; lrf\_10wk, lrf\_13wk, lrf\_16wk, lrf\_19wk, and lrf\_22wk = last-rib backfat (mm) at wk 10, 13, 16, 19, and 22, respectively.

<sup>3</sup>Lk\_m1 = -log-likelihood for m1; m1 = model [2] without the segment:  $\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{a} + \mathbf{e}$ , in which  $\mathbf{y}$  is the phenotypic vector containing the data from each growth trait,  $\mathbf{X}$  is the incidence matrix that relates records to the fixed effects of sex in  $\boldsymbol{\beta}$ , vector  $\mathbf{a}$  contains the random breeding values such that  $\mathbf{a} \sim N(\mathbf{0}, \mathbf{G}\sigma_A^2)$ ,  $\mathbf{e}$  is the random error vector such that  $\mathbf{e} \sim N(\mathbf{0}, \mathbf{I}\sigma_e^2)$ , and  $\mathbf{I}$  is the identity matrix.

<sup>4</sup>Lk\_m2 = -log-likelihood for m2; m2 = model [3] with the segment:  $\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{a}_1 + \mathbf{a}_2 + \mathbf{e}$ , in which  $\mathbf{a}_1$  is the vector of additive random effects associated with those SNP located in the segment, such that  $\mathbf{a}_1 \sim N(\mathbf{0}, \mathbf{G}_1\delta_A^2)$ , and  $\mathbf{a}_2$  is the vector of additive random effects associated with all SNP except those involved with  $\mathbf{a}_1$ , such that  $\mathbf{a}_2 \sim N(\mathbf{0}, \mathbf{G}_2\delta_A^2)$ .

<sup>5</sup>LRT = likelihood ratio test (for m1 and m2).

<sup>6</sup> $P$ -value<sub>LRT</sub> =  $P$ -value for the LRT.

<sup>7</sup>VarE\_m1 = error variance ( $\sigma_e^2$ ) of m1.

<sup>8</sup>VarA\_m1 = additive variance ( $\sigma_A^2$ ) of m1.

<sup>9</sup>VarE\_m2 = error variance ( $\sigma_e^2$ ) of m2.

<sup>10</sup>VarA\_m2 = additive variance ( $\sigma_A^2$ ) of m2.

<sup>11</sup>segmVA = Additive variance segment ( $\delta_A^2$ ) of m2.

<sup>12</sup>%segmVA = proportion in percent of the total variance explained by the segment.

located on SSC4 position 6 Mb that explained 5.04% of the total variance but did not report association in the same region that we found significant for ADG.

Regarding the fat deposition traits, Edwards et al. (2008) and Choi et al. (2010), using microsatellites, showed a peak position on SSC6 ranging from 134 to 143 cM and from 111 to 143 cM associated with *bf10* and *lrf*, respectively, when measured at 10, 13, 16, 19, and 22 wk. In addition, a recent study in Duroc (Okumura et al., 2013) detected a region on SSC6 located between 135.1 and 136.2 Mb to be significant for backfat thickness and consistent with previously reported studies of QTL mapping and association (Lee et al., 2011; Fontanesi et al., 2012). Moreover, Lee et al. (2011) reported a significant association of 2 SNP (MARC0083918 and ASGA0029677) with backfat thickness, which are located within the 2.2-Mb SSC6 segment identified in the present study, and they propose the gene *PDE4B* involved in the metabolism of fat as candidate gene. In our study, the SNP MARC0083918 and ASGA0029677 did not coincide with the peak *P*-value SNP, but they were in moderate LD ( $r^2 = 0.3$ ) with the 4 peak SNP markers for fat deposition (M1GA0008917, ASGA0029651, ALGA0122657, and ALGA0104402). Furthermore, Sanchez et al. (2014) described a genomic region on SSC 6 (between 134.691 and 135.078 Mb) significantly associated to backfat, ham weight, and lean meat content in a Landrace population. This genomic region partially overlaps genomic region on SSC6 (133.8–136 Mb) described in this study. Finally, Óvilo et al. (2005) had evaluated the *LEPR* gene for the highly significant QTL for fat-related traits reported in a narrow region (130–132 cM) included on the SSC6 segment described in this research, and then the effect of the gene was further evaluated by Muñoz et al. (2009), who also found another signal for fat deposition traits between 60 and 100 cM.

Concerning the peak SNP obtained on SSC2, SSC3, and SSC5 for fat deposition traits, previous results reported QTL on these same chromosomes but in different regions. Using microsatellites, significant regions were obtained on SSC2 (de Koning et al., 1999; Lee et al., 2003; Kim et al., 2006) and SSC5 (Kim et al., 2006) for backfat thickness and on SSC3 for side fat thickness (Liu et al., 2007). Despite the lack of significance obtained for the 2-Mb segment approach applied to the significant SSC2, SSC3, and SSC5 peak SNP (described below), the significance reached for these peak SNP should be taken into account for future research.

### Significant Segment Approach

Following the Genome Wide Association analysis, 2-Mb segments (1 Mb up- and downstream) were created for each of the 7 selected SNP. Variance com-

ponents and the log-likelihood were then estimated from the centered animal models [2] and [3] (Hayes et al., 2010; Gualdrón Duarte et al., 2014), and the performance of both models was compared. Each of the chromosome segment effects was tested using the LRT, and the size of the test was adjusted by the BC. The same methodology was applied for 10 traits for a specific 6-Mb region of chromosome 6.

Segments of 2 Mb located on SSC3 and SSC6 showed significant ( $P\text{-value}_{\text{LRT}} < \text{BC}$ ) effects for *wt\_birth* and *bf10* and *lrf* at different ages. These segment effects explained 4 to 10% of the total variance for each trait. An unusual result was obtained for *wt\_birth*, as the SSC3 segment explained 30% of the total variance, which leads to suspicion of an overestimation; a probable explanation could be the lack of correlation (LD) between the peak SNP and the adjacent SNP (Supplemental Fig. S85; see the online version of the article at <http://journalofanimalscience.org>).

Segments located on SSC2, SSC3, and SSC5 were not significant ( $P\text{-value}_{\text{LRT}} > \text{BC}$ ), despite the fact that some of them explained a high proportion of the additive variance. For example, the segment effects on SSC3 for *bf10* at 13 wk explained 13% of the additive variance although it did not show significance (Table 2). It is worth mentioning that the number of multilocus genotypes included in the 2-Mb segment tested on SSC3 was 171, a value much lower than the 356 genotype effects included in the 2.2-Mb segment on SSC6. Moreover, the average number of “replicates” for the multilocus genotypes observed on SSC6 was more evenly spread than those on SSC3, where a few genotypes accounted for most of the replicates. This resulted in a very small number of multilocus genotypes in the SSC3 QTL region. Similar results were obtained for the trait *lrf* at 16 wk on SSC5 (no significant segment) and SSC6 (significant segment) with 69 and 223 number of genotypes, respectively. Therefore, the small number of genotypes per segment, or degrees of freedom, reduced the power (Christensen, 2011) to test for significant segment effects.

The 6-Mb segment tested on SSC6 was significant for 10 traits. However, the proportion of the additive variance explained by the segment was different for the different traits. For *bf10* at 10 and 16 wk and *lrf* at 13 wk, the explained additive variance slightly decreased when compared with the same trait using a segment of 2 Mb (selected by SNP and trait). In this situation, the inclusion of more SNP (an increase of the length of the window or segment size) may have resulted in adding regions with no effect on the expression of the trait and, therefore, in an increase in the error. On the other hand, for *bf10* at 13, 19, and 22 wk and *lrf* at 10, 16, 19, and 22 wk, the explained additive variance



became greater. Here, the regions included may have had genes that are linked to the quantitative trait.

Furthermore, in this region, 4 markers were responsible for the highest signal for 10 of the traits and they are positionally paired, even though the LD analysis (Supplemental Fig. S5, S6, and S7; see the online version of the article at <http://journalofanimalscience.org>) revealed a high degree of linkage between the 4 of them, which suggests that they are detecting a common signal, located between 133.8 and 136.0 Mb. The results of the present study confirm the presence of genetic variability for traits related to fat deposition in this specific region of SSC6.

### Candidate Segment Gene Screening

Previous studies have reported QTL for birth weight in the same SSC3 region as identified by the present analysis (Malek et al., 2001; Liu et al., 2007). When evaluating the orthologous region in other species, some interesting QTL were reported. In cattle, a QTL that affects calving traits was detected (Sahana et al., 2011) on BTA3, and in sheep, on ovine chromosome 1 [OARI], a QTL was reported that affects bone density (Campbell et al., 2003). This could be related with birth weight, because a negative relationship between bone density and birth weight was observed in humans (Steer et al., 2014). In the SSC3 region containing the wt\_birth QTL, there are some candidate genes with physiological functions related to embryonic or fetal growth, such as the *FBR3* gene that has an influence in the development of myoblasts and involves in embryonic development (Prakash et al., 2007) and the *MYL6F* gene that has been suggested to be related to muscle fiber development, as it was differentially expressed in pig skeletal muscle from gestational Day 33 to 65, a period that encompasses the transition from primary to secondary fiber formation in pigs (Mei et al., 2008); therefore, variation in this gene could conceivably affect prenatal muscle growth and ultimately body size at birth.

Screening for candidate genes for the fat deposition traits on the SSC6 chromosomal region revealed the gene *SERBP1*. This gene produces the PAI1 mRNA binding protein, which may play a role in the PAI1 mRNA regulation and stability and, consequently, favors PAI1 protein translation (Heaton et al., 2001; Heberlein et al., 2012). In humans, overexpression of the PAI1 protein may participate in the onset of the metabolic syndrome that is linked to obesity (Alessi and Juhan-Vague, 2006). In cattle, PAI1 was found to be highly expressed in animals with greater backfat thickness (Jin et al., 2012). Levels of SERBP1 protein were found to be enriched in mice with obesity (Heberlein et al., 2012), and in chickens, the SERBP1 mRNA was found highly

expressed in the abdominal fat of lines selected for fat content (Resnyk et al., 2013). These results suggest that the *SERBP1* gene could be involved in the regulation of fat deposition, as the SERBP1 protein stabilizes PAI1 mRNA and PAI1 is related with lipid metabolism.

Code and data to obtain and reproduce presented results are publicly available at [https://github.com/steibelj/GWA\\_growth](https://github.com/steibelj/GWA_growth); accessed February 1, 2016).

### Applications

The present study reports on a genome scan and posterior selection of candidate genome segments to identify genome regions related to growth and fat deposition in pigs. As a result, specific regions were detected on SSC3 and SSC6 that affect the additive genetic variation of single and multiple traits of growth and fat deposition. Furthermore, the inclusion of the results obtained here with those from similar experimental populations in a meta-analysis would refine the search for QTL for economically relevant traits in the pig industry.

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