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ANIMAL GENETICS

Genome-Wide Association Study Identifies QTLs for EBV of Backfat Thickness and Average Daily Gain in Duroc Pigs[1]

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Abstract—Backfat thickness (BFT) and average daily gain (ADG) are two important economic traits in commercial swine production. Identifying QTLs and uncovering the molecular mechanism for BFT and ADG would greatly help to speed up the breeding progress. In current breeding program, EBV for these two traits are calculated and formulated a comprehensive breeding index, which then be used to improve pig performance. Using Illumina PorcineSNP60 BeadChip, a pilot genomewide association studies (GWAS) for BFT and ADG in 83 Duroc pigs were performed. A total of 31 genome-wise significant SNPs were detected to be associated with BFT on SSC 4, 9, 11, 12 and 14, ten of which were coincident with previously reported QTL regions. There are two genome-wise loci prominently associated with ADG on SSC2 and SSC13, respectively. The two loci on SSC2 are well overlapped with the QTL regions previously reported. All the 31 significant SNPs associated with BFT are verified on 219 outbreed pigs, six SNPs reach an extreme significant level and seven SNP reaches a significant level, *CACNA1E* and *ACBD6* are chosen as positional candidate genes. Our findings not only confirmed previously findings, but also revealed a number of novel SNPs associated with BFT and ADG. Two positional candidate genes *CACNA1E* and *ACBD6* were identified for further study. These results would facilitate the identification of causative genes for BFT and ADG.

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INTRODUCTION

The domestic pig has undergone a long history of extensive natural and artificial selection to meet human dietary needs [1]. In the past decades, conventional genetic improvement was mainly relying on EBV, phenotype, and pedigree information. With the development of modern biotechnology, it is possible to further increase the rate of genetic improvement by understanding the interplay between genetic and environmental factors controlling complex agriculturally important production traits [2]. This information could be integrated with marker-assisted selection (MAS) schemes to increase selection accuracy, shorten generation interval, and accelerate genetic improvement.

Many QTL associated with pig economically important traits have been detected since the 2000s [3]. BFT and ADG are both important economical traits in pig breeding. The heritability of BFT ranges from 0.27 to 0.83 [4–7]. While the heritability of ADG ranges from 0.32 to 0.38 [4, 8]. To date, a total of 210 and 229 QTLs associated with BFT and ADG have been reported respectively (http://www.animalgenome.org/cgi-bin/QTLdb/SS/browse). But there were still no conclusive results showing functional mutations or causal genes affecting BFT and ADG.

With the development of sequencing technology, SNP have been widely used for the detection and localization of QTL for complex traits in many species [9–14]. The objective of this study was to perform a GWAS with the porcine 60K SNP BeadChip and to identify candidate SNPs/genes and chromosomal regions associated with BFT and ADG, which could be used in MAS and genomic selection. Furthermore, this study could contribute to better understand the genetic control of BFT and ADG in pigs.

MATERIALS AND METHODS

Animals and phenotypes for GWAS. A total of 83 duroc belong to 25 families were collected from the breeding stock field of WENS Group, BFT was measured between the 10th and 11th rib of pigs at the weight of 100 ± 5 kg, using the B ultrasound, machine Preg-Alert Pro (Renco Corporation, Minneapolis, MN 55401, United States). ADG is the average daily gain during the period of birth weight to 100 ± 5 kg.

[1] The article is published in the original.

[2] Both authors contributed equally to this study and should be considered as co-first authors.

Table 1. Descriptive statistics for traits measured

Trait	*N*	Mean	SD	Min	Max
Duroc					
BFT	83	−0.43	0.58	−2.01	0.76
ADG	83	9.64	24.53	−22.38	45.82
Combined					
BFT	219	−0.04	0.70	−1.69	3.21

Combined: are consisted of 15 Duroc, 69 Landrace, and 135 Yorkshire.
BFT, backfat thickness; ADG, average daily gain; Min, minimum; Max, maximum.

The EBV rather than raw phenotypes was estimated for the GWAS. EBV has the advantage that they are free of systematic environmental effects on measured phenotypes, as these effects are considered in the statistical model used for estimation of EBV. Additionally, they reflect the genetic makeup more accurately because they do not solely rely on its own records but include information from all measured relatives [15]. The calculation of EBV is listed below:

$$\mathrm{EBV} = b_{AP}(P^* - P),$$

$$b_{AP} = \frac{r_A n h^2}{1 + (n-1)r},$$

n is the number of individuals in the same population, r_A is the relationship coefficient of the individuals which provide information and the evaluated individuals, r is repetitive rate and P is the phenotype.

The mean of BFT (EBV) and ADG (EBV) was −0.43 and 9.64 with a standard deviation of 0.58 and 24.53, respectively (Table 1). The density distribution of values for BFT (EBV) (Fig. 1a) and ADG (EBV) (Fig. 1b) were not significantly deviated from normal distribution.

Animals and phenotypes for verification. Additional 219 pigs (also genotyped on Illumina Beadchip), including 15 Duroc, 69 Landrace and 135 Yorkshire that collected from 72 families in 4 national pig nuclear breeding farms were used for the verification test of significant SNPs for BFT. The measurement standard of BFT is the same with the previous methods used for the 83 Duroc. The mean of BFT (EBV) of 219 pigs are −0.04 with a standard deviation of 0.70 (Table 1).

Genotyping and quality control. DNA was collected from ear tissue using the conventional methods of phenol-chloroform extraction and normalized to 50 ng/μL. The DNA quality was assessed by 260/280 and 260/230 ratios and electrophoresis. Genotyping was performed using the porcine SNP60K Beadchip of Illumina (San Diego, CA, United States) according to Antonio et al. [16]. A total of 83 samples (including sires and dams) were genotyped. Quality control (QC) was performed with MAF > 0.05, call rate per individual > 90%, HWE > 0.01, Missing rate per SNP < 10% using PLINK v. 1.07 [http://pngu.mgh.harvard.edu/purcell/plink/]. Following the quality control, 83 individuals and 37,478 SNPs were selected for the GWAS.

Genome-wide association study. GWAS was performed using Wald test in the software described

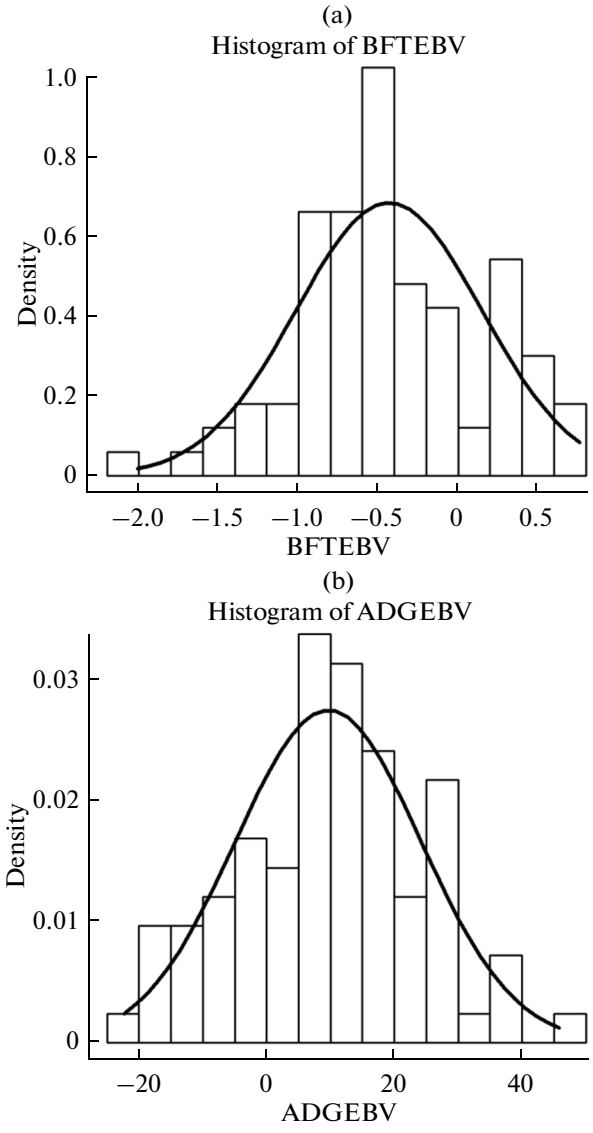

Fig. 1. The distribution of EBV for backfat thickness (BFT) (a) and average daily gain (ADG) (b). The *p*-value is 0.2413 for histogram of BFT and 0.5559 for ADG.

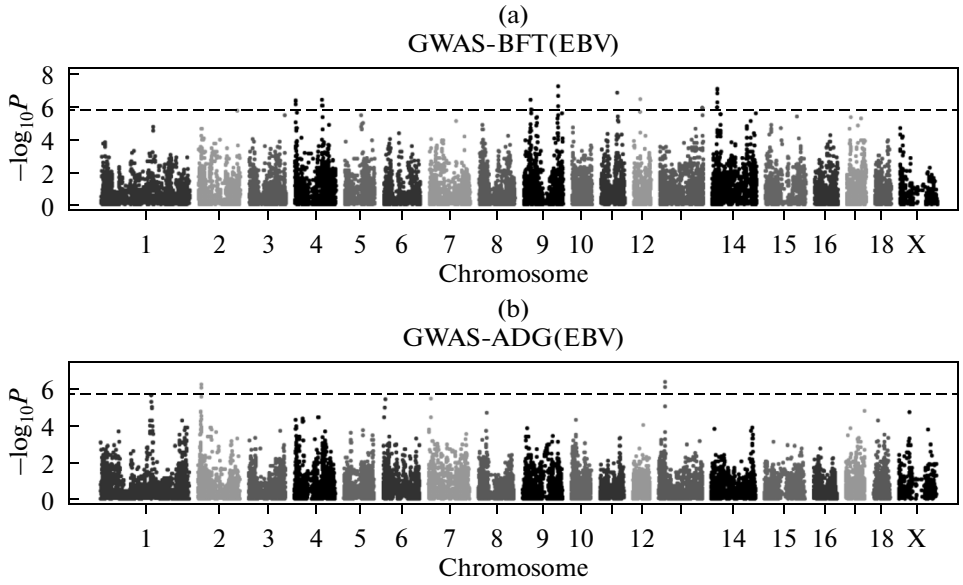

Fig. 2. Genome wide association study for EBV of Backfat thickness (BFT) (a) and Average daily gain (ADG) (b), using the Wald test. Each dot represents one SNP. On the *y*-axis are $-\log_{10}$ (*P*-values), and on the *x*-axis are the physical positions of the SNPs by chromosome. The imaginary line represents the Bonferroni-corrected significance threshold (5.85).

above. The phenotype difference among different genotypes was tested. BFT and ADG were analyzed using the linear regression framework. Linkage disequilibrium (LD) between SNPs was quantified as r^2 on all animals of the GWAS using haploview9 v. 4.2 [17], and the LD block was defined by the criteria of Kent et al. [18]. The Bonferroni corrected *P*-value (P = 0.05/Number of SNPs) was defined as the genome-wise significance threshold.

Candidate genes identification. Significant SNPs detected in GWAS were verified in the extended 219 pigs mentioned above. Candidate genes containing at least one prominent SNP tested in both populations were identified according to their biological function directly or indirectly regulating the development process of the investigated traits.

RESULTS

QC of Phenotypes and Genotypes

The current Porcine 60K Beadchip has 64,232 SNPs [19]. Quality control procedures of the genotype data were carried out using Plink (Version 1.07) (http://pngu.mgh.harvard.edu/purcell/plink/). 19,128 SNPs were excluded as HWE (P < 10E-05), 3531 SNPs were discarded for Call rate <90%, 8745 SNPs removed because of MAF < 0.05. After quality control a subset of 37,478 SNPs excluding SNPs on the Y chromosomes and those ambiguously mapped to the current pig genome assembly (Sscrofa10.02) were used for subsequent GWAS. The average physical distance between any two neighboring SNPs on the same chromosome was approximately 0.07 Mb, ranging from 0.06 Mb (SSC14) to 0.17 Mb (SSCX).

GWAS and Verification

83 Duroc pigs were genotyped using the Illumina Porcine 60K SNP beadchips. The GWAS was performed for the traits of BFT and ADG. After Bonferroni correction (Bonferroni P < 0.05), a total of 31 genome-wise significant SNPs including 11 on SSC4, five on SSC9, one on SSC11, eight on SSC12, and six on SSC14 were identified to be associated with BFT, while the number of genome-wise significant SNPs for ADG is four (Table 2). Linkage disequilibrium (LD) was calculated among all the significant SNPs for BFT in the region between 9.45 and 9.51 Mb on SSC4 and 2.6–2.8 Mb on SSC12. Two large block of strong LD in these regions are observed (Fig. 4). The strong LD region may reflect the action of positive selection for BFT. The most significant SNP ALGA0055091 (P = 6.24E-08) at 13.5 Mb on SSC9 (Fig. 2a). There is a difference of 0.97 between the mean levels of BFT in pigs with genotype AA (BFTEBV, 0.25) and GG (BFTEBV, −0.72) (Fig. 3a). There are two genome-wise significant SNPs showd association with ADG were observed on SSC2 and SSC13, respectively (Fig. 2b). The most significant SNP ASGA0056780 with a *p*-value of 4.08E-07 located at 27.34 Mb on SSC13, the difference between the mean levels of genotype AG and GG is 23.57 (Fig. 3b).

All the 31 significant SNPs correlated to BFT were verified on 219 outbreed pigs. Six SNPs reach an extreme significant level and Seven SNPs reach a significant level (Table 2).

Table 2. Genome-wise significant SNPs associated with EBV of BFT and ADG

Trait	SNP	Chromo-some	Position, Mb	Nearest gene name	Distance	*P*-value	Verification *P*-value
BFT	*MARC0006653*	9	26.28	CHORDC1	With in	4.15E-07**	5.80E-03
BFT	ALGA0118468	9	133.82	*ACBD6*	With out	1.05E-06**	1.53E-02
BFT	MARC0087505	9	133.83	*ACBD6*	With out	1.05E-06**	2.91E-02
BFT	ASGA0044591	9	135.4	*CACNA1E*	With out	2.52E-07**	3.13E-02
BFT	ALGA0055091	9	135.42	*CACNA1E*	With out	6.24E-08**	
BFT	H3GA0011213	4	2.39	EIG2C2	With out	7.92E-07**	
BFT	ASGA0017093	4	2.57	*TRAPPC9*	With out	7.92E-07**	
BFT	ALGA0022167	4	3.29	TRAPPC9	With out	4.60E-07**	
BFT	ALGA0022189	4	3.36	TRAPPC9	With out	5.10E-07**	
BFT	ALGA0026446	4	94.58	NUF2	With out	4.40E-07**	
BFT	ASGA0020645	4	94.78	NUF2	With out	9.63E-07**	
BFT	MARC0064723	4	94.83	NUF2	With out	9.63E-07**	
BFT	ALGA0026469	4	94.85	NUF2	With out	9.63E-07**	
BFT	ASGA0020651	4	94.92	NUF2	With out	9.63E-07**	
BFT	INRA0015436	4	95	*RGS5*	With out	9.63E-07**	
BFT	H3GA0013315	4	95.15	RGS4	With out	9.63E-07**	
BFT	*ALGA0062488*	11	60.09	SLITRK1	With in	1.63E-07**	1.04E-02
BFT	*MARC0072109*	12	24.2	SKAP1	With in	3.90E-07**	4.91E-02
BFT	*ASGA0096968*	12	24.22	*SNX11*	With in	3.90E-07**	
BFT	*DIAS0000287*	12	24.28	*COPZ2*	With in	3.90E-07**	4.69E-02
BFT	*ALGA0065660*	12	24.37	NFE2L1	With in	3.90E-07**	4.14E-02
BFT	*ALGA0065669*	12	24.43	NFE2L1	With in	3.81E-07**	5.49E-03
BFT	*MARC0072078*	12	24.44	NFE2L1	With in	3.81E-07**	
BFT	*ALGA0065654*	12	24.5	STAT5A	With in	3.90E-07**	1.85E-03
BFT	*H3GA0033916*	12	24.67	HOXB1	With in	3.90E-07**	
BFT	ALGA0075614	14	17.49	HAND2	With out	1.03E-07**	1.59E-03
BFT	INRA0042850	14	17.59	HAND2	With out	6.24E-07**	8.47E-05
BFT	ALGA0075813	14	17.92	*GALNT7*	With out	1.79E-07**	1.11E-03
BFT	H3GA0039195	14	17.98	GALNT7	With out	1.21E-06**	
BFT	ALGA0075803	14	18.08	ENSSSCT00000024965	With out	8.93E-08**	
BFT	ASGA0061791	14	18.12	ENSSSCG00000009706	With out	1.03E-07**	
ADG	*ALGA0012049*	2	12.94	YPEL4	With in	5.58E-07**	
ADG	*ASGA0105274*	2	12.39	OR5B17	With in	8.10E-07**	
ADG	ASGA0056780	13	27.35	ZNF621	With out	4.08E-07**	
ADG	M1GA0017429	13	27.24	ZNF621	With out	7.70E-07**	

SNPs in italic are located within the QTL regions reported previously. Genes in italic show that this gene including the SNP. With in means the SNP is overlap with the previous QTL. The verification *P*-value is the *P* value that verified in 219 pigs including 15 Duroc, 69 Landrace and 135 Yorkshire. BFT, backfat thickness; ADG, average daily gain, ** 5% genome-wide significant.

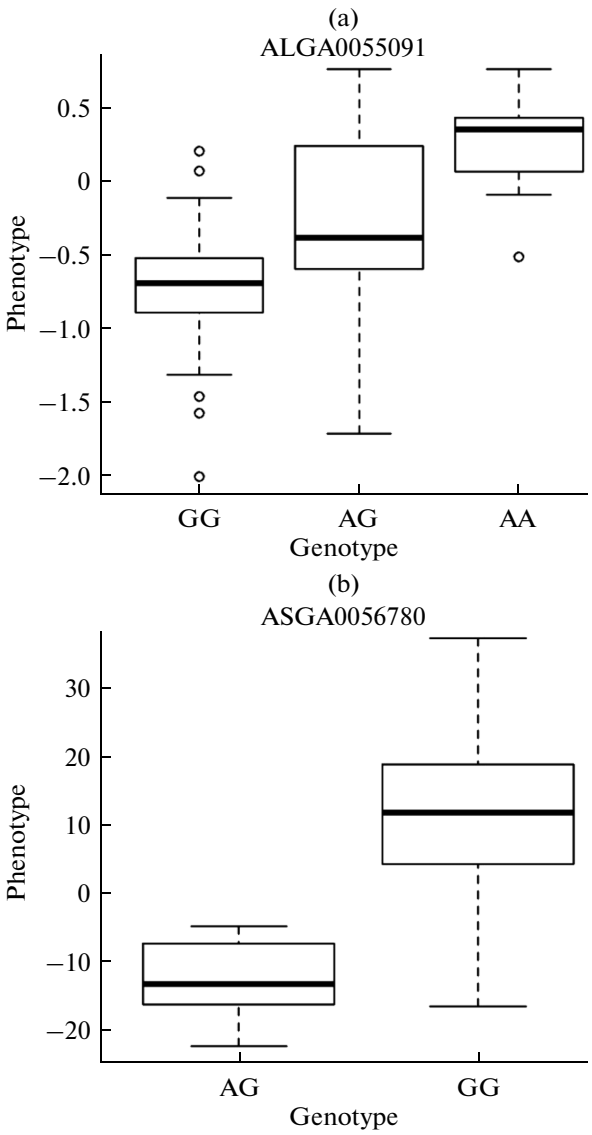

Fig. 3. The distribution of phenotype for the genotype of the most significant SNP for backfat thickness, and average daily gain (a, b).

DISCUSSION

QTLs for EBVs of BFT and ADG

In this study, four chromosomes regions mainly significantly associated with BFT were detected. Whereas QTL effects related to average BFT on SSC9, 11, and 12 have been described previously, respectively. In an experimental population of the three genetically diverse founder groups, Meishan (M), Pietrain (P) and European Wild Boar (W), one QTL for BFT on SSC11 have been identified and were partially overlapped with the area identified herein and one for ADG on SSC2 [20]. Additionally, Liu et al. [21] reported a QTL for ADG in a Duroc × Pietrain cross covered all the 8 significant SNPs on SSC12 associated with EBVs of BFT detected in the current study. The remaining 20 significant SNPs for BFT were reported for the first time.

The SNPs on SSC13 associated with ADG did not confirm the previous studies. But the region is close to a QTL fragment that was detected by Liu and Jennen el al. [22]. The distance between the SNP loci and the QTL region was about 1 Mb.

Several factors might be responsible for the differences between the current study and other studies: firstly, the experimental populations were different in heterogeneity of genetics; the resource population was mainly used in previous experiments, while pure bred Duroc population were used in the current study. Second, QTL mapping approach assumed that the genotype is fixed in two found breeds in F2 cross, and might fail to detect significant association when the causal variant is segregated in the founders, while GWAS could discover more loci which were in the state of linkage disequilibrium. Third, the molecular markers used in the current were different from previous studies. In previous studies microsatellites were mainly used as molecular makers, while procine 60 K beadchips with better genome coverage were used in the current study. Moreover, previous study used linkage analysis, while the current study used association analysis.

Candidate Genes

Gene *ACBD6* including the SNP ALGA0118468, with a *p*-value of 1.05E-06, is an Acyl-CoA-binding domain-containing protein and binds long-chain acyl-coenzyme A molecules with a strong preference for unsaturated C18:1-CoA, lower affinity for unsaturated C20:4-CoA and saturated C16:0-CoA [23]. Coenzyme A(CoA, CoASH, or HSCoA) is an important coenzyme for its role in the synthesis and oxidation of fatty acids and the oxidation of pyruvate in the citric acid cycle. Therefore, *ACBD6* was selected as a candidate gene for backfat thickness.

Gene *CACNA1E* (calcium channel, voltage-dependent, R type, alpha 1E subunit) including the SNP ALGA0055091 which shows a largely additive effects (Fig. 3a), and SNP ASGA0044591 is another candidate gene for BFT for its association with type 2 diabetes and which is tied to obesity and imprison of insulin secretion [24]. Fat deposition trait in pigs is a direct measure of obesity but may only serve to be an indirect measure of diabetes. Subcutaneous fat deposition traits (BFT measurements) are highly correlated with direct chemical measures of subcutaneous, retroperitoneal, and visceral fat in pigs [25, 26]. Pigs with different backfat thickness exhibit clearly distinct plasma concentrations of insulin and glucose, total cholesterol, postprandial triglycerides, low- to high-density lipoprotein cholesterol ratio, growth hormone, and insulin growth factor-1, as well as the appearance of hypertension and hyperplasia of coronary arteries [25, 27].

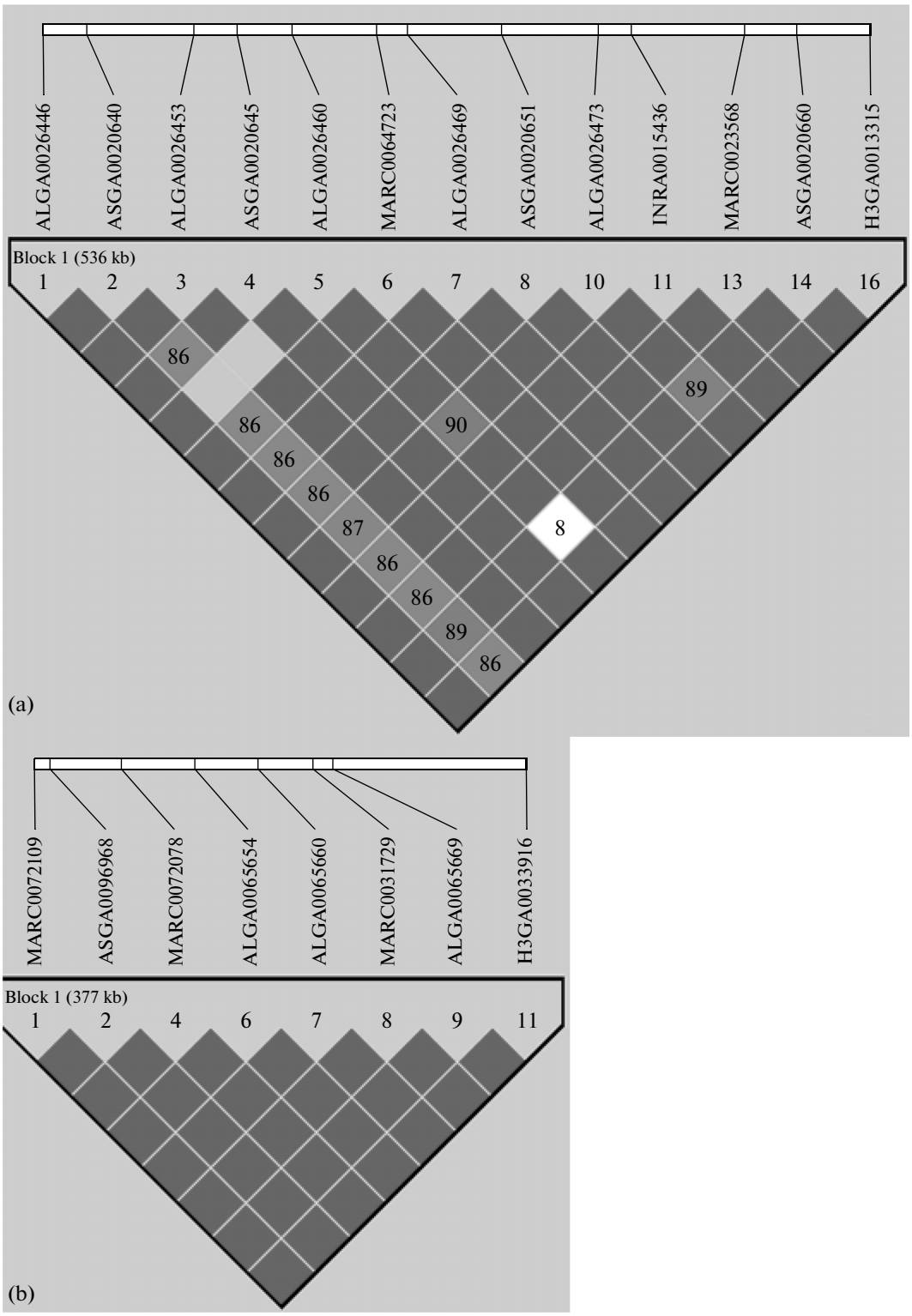

Fig. 4. The linkage disequilibrium plot for the region between 9.45 and 9.51 Mb on SSC4 and 2.42 to 2.46 Mb on SSC12 (a, b). 4 out of 11 significant SNPs on SSC4, all the 8 significant SNPs on SSC12, are located in a large LD block. The values in the boxes are pair wise SNP correlations (r^2) and the box color reflects the degree of correlation.

A problem remaining in the current study is the considerable number of false negatives of genetic associations. The SNPs associated with BFT and ADG were identified by introducing the conservative multiple testing with Bonferroni correction. Certainly, this correction reduced a large number of spurious genetic associations but it may also have produced with many false negatives.

Fine-mapping will be required to confirm the genome-wise significant SNPs founded in the current study and identify the causal variants. The findings of association in this study are expected to be found again in large samples. All of these studies will be stepping stones for the future application in marker-assisted selection for genetic improvement of pig breeding.

The present study revealed 31 genome-wise significant SNPs for BFT in 5 autosomes (SSC4, 9, 11, 12, 14), and 4 for ADG in 2 autosomes (SSC2, 13) in Duroc, using PLINK software. Ten of the 31 SNPs showed significant association with BFT located within the previously reported QTL (affecting average backfat thickness) regions, and 2 SNPs affecting ADG overlapped with the previous study. 13 out of 31 significant SNP associated with BFT are verified in 219 outbred pigs. The general consistence of the significant SNPs detected herein with the reported QTL and candidate genes provide strong support for the outcomes of this study. Our findings lay a preliminary foundation for guiding follow-up replication studies, and eventually revealing the causal mutations underlying BFT and ADG traits in Duroc.

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