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Identify known and novel candidate genes associated with backfat thickness in Duroc pigs by large-scale genome-wide association analysis

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Abstract

Backfat thickness (BFT) is complex and economically important traits in the pig industry, since it reflects fat deposition and can be used to measure the carcass lean meat percentage in pigs. In this study, all 6,550 pigs were genotyped using the Geneseek Porcine 50K SNP Chip to identify SNPs related to BFT and to search for candidate genes through genome-wide association analysis in two Duroc populations. In total, 80 SNPs, including 39 significant and 41 suggestive SNPs, and 6 QTLs were identified significantly associated with the BFT. In addition, 9 candidate genes, including a proven major gene *MC4R*, 3 important candidate genes (*RYR1*, *HMGA1*, and *NUDT3*) which were previously described as related to BFT, and 5 novel candidate genes (*SIRT2*, *NKAIN2*, *AMH*, *SORCS1*, and *SORCS3*) were found based on their potential functional roles in BFT. The functions of candidate genes and gene set enrichment analysis indicate that most important pathways are related to energy homeostasis and adipogenesis. Finally, our data suggest that most of the candidate genes can be directly used for genetic improvement through molecular markers, except that the *MC4R* gene has an antagonistic effect on growth rate and carcass lean meat percentage in breeding. Our results will advance our understanding of the complex genetic architecture of BFT traits and laid the foundation for additional genetic studies to increase carcass lean meat percentage of pig through marker-assisted selection and/or genomic selection.

Lay Summary

Backfat thickness (BFT) is a complex and economically important trait in the pig industry because it reflects fat deposition and can be used to measure the carcass lean meat percentage in pigs. In this study, two Duroc populations were genotyped using SNP chips, and genome-wide association analysis was used to identify SNPs and candidate genes related to BFT. A number of genetic markers and candidate genes including *MC4R*, *RYR1*, *HMGA1*, *NUDT3*, *SIRT2*, *NKAIN2*, *AMH*, *SORCS1*, and *SORCS3* were identified to be significantly related to BFT. Our data suggest that many of the candidate genes can be directly used for genetic improvement through molecular markers.

Key words: backfat thickness, candidate gene, Duroc, genome-wide association analysis, pigs

Abbreviations: BFT, backfat thickness; QTL, quantitative trait locus; GWAS, genome-wide association analysis; SNP, single nucleotide polymorphisms; MAS, molecular marker-assisted selection; GS, genomic selection; GO, Gene Ontology; SSC, sus scrofa chromosome; LD, linkage disequilibrium; D100, days adjusted to 100 kg; Q-Q, quantile-quantile

Introduction

Growth and fatness traits are important economic traits that are highlighted in the global pig industry, and they have been intensively selected for several decades especially for Western commercial breeds (Guo et al., 2017). Backfat thickness (BFT) refers to the thickness of subcutaneous fat on the back, which directly reflects pig fat deposition (Newcom et al., 2005; Yang et al., 2019). In pig breeding programs, lean meat percentage is difficult to directly measure and showed a significant negative correlation with BFT, so it is usually evaluated by measuring BFT (Chen et al., 2019; Lopez et al., 2018). BFT can be measured in vivo by ultrasound and has a high heritability. Therefore, dissecting the genetic structure of BFT not only helps us to improve the carcass lean content of pigs, especially Chinese domestic pigs, but also helps us to understand the obesity of human, because pigs are more physiologically similar to human than rodents and other model animals (Miller and Ullrey, 1987).

To understand the molecular bases of phenotypic traits in pigs, many quantitative trait locus (QTL) mapping studies have been conducted in the past few decades. To date, there were 33,143 QTLs associated with 699 different pig traits in previous reports. Among them, 3,289 QTLs associations respectively for fatness traits have been deposited in the pig QTL

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database (http://www.animalgenome.org/cgi-bin/QTLdb/index, Release 44, April 26, 2021). For BFT, previous research has identified many QTLs and important candidate genes through linkage analysis to help clarify the genetic basis and accelerate the breeding process of BFT in pigs, such as *IGF2* (Nezer et al., 1999; Van Laere et al., 2003), *MC4R* (Kim et al., 2000; Kim et al., 2006), and *LEPR* (Ovilo et al., 2002; Ovilo et al., 2005). With the advent of high-throughput genotyping technology, genome-wide association analysis (GWAS) based on high-density single nucleotide polymorphism (SNP) chips has gradually become a powerful strategy for detecting genetic variants associated with complex traits. SNPs that are significantly associated with phenotypic traits detected by GWAS can be directly applied to modern breeding systems through molecular marker-assisted selection (MAS) or genomic selection (GS) (Zhang et al., 2014). Based on GWAS, additional QTLs and potential candidate genes with large effects were also identified for BFT traits, including *HMGA1*, *NUDT3* (Liu et al., 2014; Qiao et al., 2015), *DHCR7*, *FGF23*, *MEDAG*, *DGKI*, and *PTN* (Gozalo-Marcilla et al., 2021).

In the present study, we performed a GWAS based on the Geneseek Porcine 50K SNP Chip (Neogen, Lincoln, NE) to identify known and new SNPs, QTLs, and candidate genes in two large-scale Duroc populations, and evaluated their molecular value. The main goal of this research is to analyze the complex genetic architecture of BFT traits and identify important QTLs, genes, and pathways behind the genetic variation of BFT traits.

Materials and Methods

Animals and phenotype

The experimental procedures used in this study met the guidelines of the Animal Care and Use Committee of the South China Agricultural University (SCAU, Guangzhou, China). The Animal Care and Use Committee of the SCAU (Approval number SCAU#0017) approved all the animal experiments described in this study.

During the period of 2013 to 2018, BFT phenotypic data were collected for Duroc pigs (U.S. Duroc, n = 3,769; Canadian Duroc, n = 2,781) from the Guangdong Wen's Foodstuffs Group, Co., Ltd. (Guangdong, China) as described previously (Qiu et al., 2021; Zhou et al., 2021). Briefly, the two Duroc populations come from two different core breeding farms, and all pigs eat and drink ad libitum. When the live weight of the pigs reached about 100 kg (100 ± 5 kg), the backfat thickness of the pigs is measured between the 10th and 11th ribs using an Aloka 500V SSD B ultrasound (Corometrics Medical Systems, USA) and then corrected to 100 kg. The corrected 100 kg BFT was calculated by the following formula (http://www.ccsi.ca/Reports/Reports_2007/Update_of_weight_adjustment_factors_for_fat_and_lean_depth.pdf):

$$BFT(mm) = Measured\ BFT * \frac{A}{A + B * (Measured\ body\ weight - 100kg)}$$

where A = 13.468 and B = 0.111528 in sires, and A = 15.654 and B = 0.156646 in dams.

Genotyping and Quality Control

All 6,550 Duroc pigs were genotyped using the Geneseek Porcine 50K SNP Chip (Neogen, Lincoln, NE). Genotype data were further quality controlled using PLINK v1.90 software (Chang et al., 2015), and its threshold is set as follows: individual and SNP call rates ≥ 95%; minor allele frequency ≥ 0.01; Hardy–Weinberg equilibrium test $P \geq 10^{-6}$. Moreover, only autosomal SNPs are retained. Subsequently, the missing genotypes were imputed using Beagle software with default parameters based on the remaining SNP genotypes (Pook et al., 2020). A final set of 38,959 and 35,972 informative SNPs and 3,769 and 2,781 individuals were used for further GWAS analysis in the U.S. and Canadian original Duroc populations, respectively.

Genome-Wide Association Analysis

The single-population analysis of these two different populations used the same univariate linear mixed model that was performed by using GEMMA for genome-wide association analysis (Zhou and Stephens, 2012). The statistical model used was as follows:

$$y = W\alpha + X\beta + u + \varepsilon$$

where y refers to a vector of phenotypic value of BFT for each population; W is the incidence matrices of covariates, including fixed effects of sex, BFT determination batch, pigsty, and year-season; α represents the vector of corresponding coefficients including the intercept; X is the vector of marker genotypes; β specifies the corresponding effect size of the marker; u stands for the vector of random effects, with $u \sim N(0, G\sigma_\mu^2)$, where G is the genomic relationship matrix, which has been calculated by GEMMA in advance.; ε is the vector of random residuals, with $\varepsilon \sim N(0, I\sigma_e^2)$, where I is an identity matrix.

The significance cutoff was defined as the Bonferroni test threshold; a stringent genome-wide threshold (significant) as well as a more lenient chromosome-wide threshold (suggestive) were $P < 0.05/N$ and $P < 1/N$, respectively, where N is the number of SNPs tested in the analyses.

The GCTA tool was used to compute the genomic heritability and phenotypic variances contributed by significant SNPs by dividing the estimated genetic variance by the total variance measured (Yang et al., 2011).

Haplotype Block Analysis

The software PLINK (Chang et al., 2015) and Haploview (Barrett et al., 2005) were used for haplotype blocks analysis to detect linkage disequilibrium (LD) between significant SNPs. Linkage disequilibrium blocks were defined using the solid spin algorithm by the criteria of Gabriel et al. (2002) and require at least two significant SNPs.

Candidate Gene Search and Functional Enrichment Analysis

The physical locations of all SNPs and functional genes refer to the latest version of the Sus scrofa 11.1 genome [http://ensembl.org/Sus_scrofa/Info/Index]. The range of searching for the functional gene closest to the position of the significant SNP is based on the LD decay distance ($r2$ = 0.2) of the two populations. Our previous studies on these two populations showed that the average LD decay distance across the whole genome of U.S. Duroc and Canadian Duroc populations was 540 and

800 kb, respectively (Zhuang et al., 2020). In addition, potential candidate genes are identified based on the physiological and biochemical functions of functional genes. Gene Ontology (GO) analyses were performed with all functional genes using the KOBAS online tool version 3.0 (Xie et al., 2011).

Results

Descriptive statistics and heritability estimates

Summary statistics for BFT trait and estimated genomic heritability are presented in Table 1. The coefficients of variation of the BFT traits of U.S. and Canadian Duroc populations are 12.72% and 21.20%, respectively. The estimated genomic heritability (±SD) of BFT traits of American and Canadian Duroc pigs is moderate, 0.25 ± 0.02 and 0.34 ± 0.03, respectively.

Genome-Wide Association Analysis

The significant threshold and suggestive threshold of the U.S. Duroc population were 1.28E-06 (0.05/38,959) and 2.57E-05 (1/38,959), respectively, whereas the significant threshold and suggestive threshold of the Canadian Duroc population were 1.39E-06 (0.05/35,972) and 2.78E-05 (1/35,972), respectively. GWAS results and quantile-quantile (Q-Q) plots are shown in Figure 1, Supplementary Table S1, and Supplementary Figure S1. The estimated genomic inflation factors (λ) and Q-Q plots show that there is no obvious evidence of population stratification. In total, 80 SNPs, including 39 significant and 41 suggestive SNPs, were identified on sus scrofa chromosome (SSC) 1, 2, 5, 7, 9, 13, 14. Among them, in U.S. Duroc population, a total of 16 significant SNPs and 20 suggestive SNPs were identified; in the Canadian Duroc population, 23 significant SNPs and 21 suggestive SNPs were identified.

A total of 6 significant QTLs were found to be associated with BFT traits in two Duroc populations (Table 2). Among them, two QTLs (1:158,309,021-161,824,864; 1:158,309,021-162,192,627) with an interval of 3.88Mb on SSC 1 were detected in both Duroc populations. Two QTLs (6:47,357,966-48,289,460; 7:30,213,771-30,497,305) on SSC 6 and 7, were found to partially overlap with QTLs were previously detected by GWAS or linkage mapping for BFT traits. The remaining two QTLs (1:38,161,769-38,185,044; 2:76,416,246-76,440,900) are located on SSC 1 and 2, and contain 5 significant SNPs that were situated in two haplotype blocks (Figure 2).

Candidate Genes Search and Functional Analysis

Among the identified 80 SNPs, 43 SNPs are located within 21 functional genes and 37 SNPs are located in the intergenic region, surrounded by 23 closest functional genes and the longest distance is 426 kb. In addition, an additional 57 genes were included in the 6 QTLs identified above. Finally, a total of 91 functional genes were initially considered candidate genes related to BFT traits. Of these, 9 highlighted candidate genes, which seem to have biochemical and physiological effects related to the characteristics of BFT traits or fat deposition, including *MC4R*, *RYR1*, *SIRT2*, *HMGA1*, *NUDT3*, *NKAIN2*, *AMH*, *SORCS1*, and *SORCS3*.

In order to better understand the potentially functions and mechanisms of candidate genes identified by GWAS that affect BFT traits, we also performed gene set enrichment analysis on the above functional genes. Interestingly, several KEGG pathways and GO terms are significantly enriched for the functional genes, including NF-kappa B signaling pathway, negative regulation of insulin secretion, cardiac muscle cell development, negative regulation of lipid catabolic process, positive regulation of glucose metabolic process, growth factor activity, among others (Supplementary Table S2).

Table 1. Phenotype and heritability statistics for BFT traits in two Duroc populations

Population	Unit	*N*	Mean(SD)	C.V.	h^2(SD)
U.S. Duroc	mm	3916	10.30 ± 1.31	12.72	0.25 ± 0.02
Canadian Duroc	mm	2119	11.04 + 2.34	21.20	0.34 ± 0.03

Unit, Number (*N*); Mean(SD), Mean(standard deviation); C.V., coefficient of variation; h^2(SD), heritability (standard deviation), phenotypic correlations and genetic correlations (standard deviation) of BFT traits.

Estimate the Genotypic Effect of Peak SNPs

We comprehensively evaluated the backfat thickness phenotypic effects of the Top SNP genotypes of the above 9 highlighted candidate genes in two Duroc populations (Table 3) and also considered the trait days adjusted to 100 kg (D100), which is a very important growth trait in breeding. For *MC4R*, top SNPs with GG genotypes had lower BFT phenotypic values than those with genotypes AG and AA, but D100 is significantly higher in two Duroc populations. It implies that the *MC4R* gene has an antagonistic effect on improving lean meat rate and growth rate. The D100 phenotype values of the other 5 top SNPs with different genotypes have no obvious difference, indicating that the use of these SNPs in subsequent molecular breeding will not affect the growth rate.

Discussion

GWAS has proven to be an efficient tool for genetic analysis and identification of causal genes of important traits in plants and livestock animals. GWAS has been successfully applied to the identification of causal genes for pig coat color (Ren et al., 2011), meat quality (Cho et al., 2019; Ma et al., 2014), and genetic defects (Noskova et al., 2021). Recently, some authors have reported the results of GWAS for BFT traits in pig (Chen et al., 2019; Fabbri et al., 2020; Guo et al., 2017; Kim et al., 2000; Kim et al., 2015; Liu et al., 2014; Long et al., 2014; Okumura et al., 2013; Qiao et al., 2015; Xu et al., 2020; Yang et al., 2019), and three well-studied genes (*MC4R*, *IGF2*, and *LEPR*) (Kim et al., 2000; Nezer et al., 1999; Ovilo et al., 2005; Ovilo et al., 2002; Van Laere et al., 2003) and several popular candidate genes (*HMGA1* and *NUDT3*) (Liu et al., 2014; Qiao et al., 2015) that have been identified many times are related to BFT traits. In particular, Gozalo-Marcilla et al. (2021) used large-scale populations with different genetic backgrounds including 275,590 pigs to systematically identify the major genes of BFT traits and discovered 5 new candidate genes (*DHCR7*, *FGF23*, *MEDAG*, *DGKI*, and *PTN*).

In this study, two Duroc populations, a total of 6,550 Duroc pigs were used for GWAS analysis of BFT traits. Previous studies have shown that the two Duroc populations have different genetic backgrounds (Zhou et al., 2021; Zhuang et al., 2020), so the two Duroc populations performed GWAS

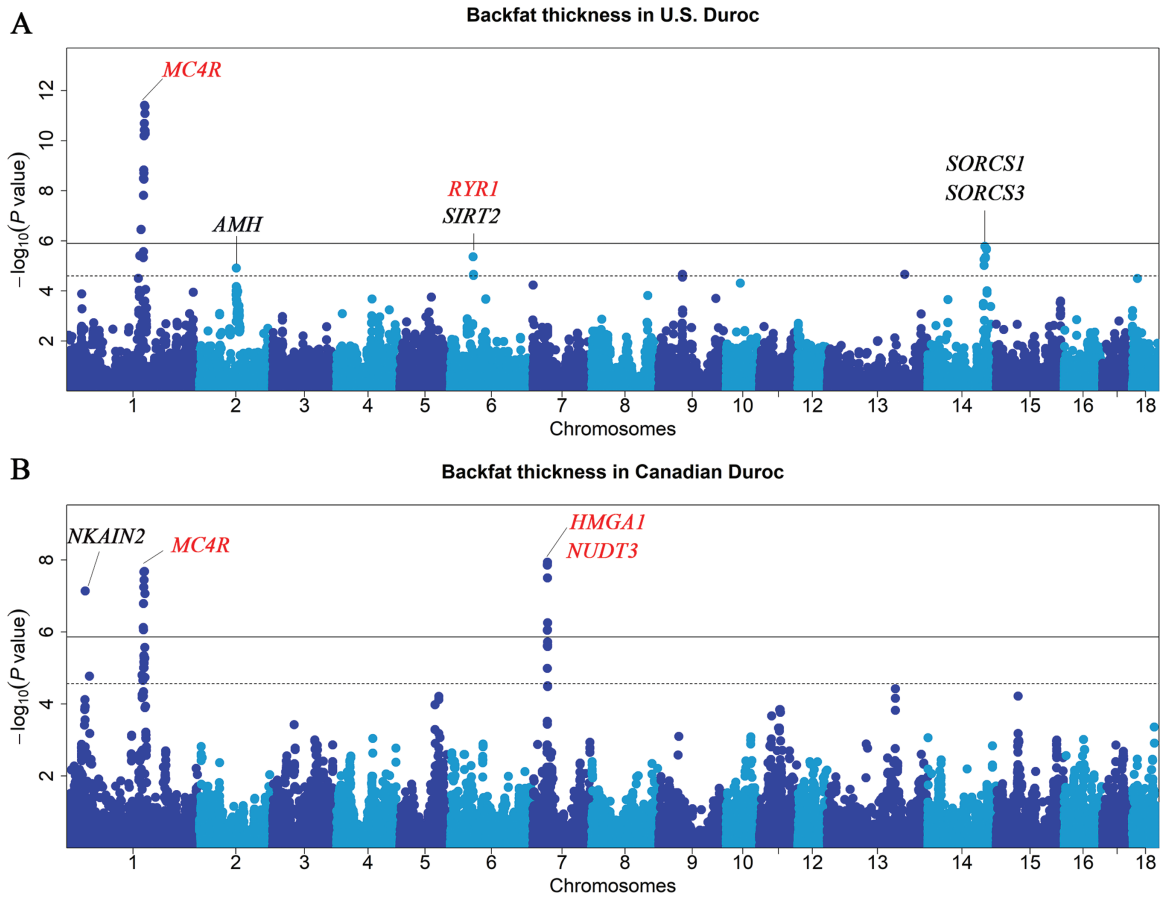

Figure 1. Manhattan plots of GWAS for BFT traits in the two Duroc pig populations. In the Manhattan plots, negative log10 *P*-values of the quantified SNPs were plotted against their genomic positions. Different colors indicate various chromosomes. The solid and dashed lines represent the 5% genome-wide and chromosome-wide (suggestive) Bonferroni-corrected thresholds, respectively. Candidate genes are denoted with different colors, red for candidate gene previously identified, and black for candidate gene newly found in the current study. Manhattan plot for (A) U.S. Duroc, and (B) Canadian Duroc.

Table 2. Significant QTLs and candidate genes associated with BFT traits in two Duroc populations

Population	QTL	N[1]	Top SNP	Position	*P*-value	CPV%[2]	Candidate genes
U.S. Duroc	1:158309021-162192627	16	mc4r	160773437	3.83E-12	2.41	*MC4R*
	2: 76416246-76440900	2	10006986	76416246	1.22E-05	1.70	*AMH*
	6:47357966-48289460	3	Hal	47357966	4.32E-06	0.68	*RYR1*, *SIRT2*
Canadian Duroc	1:38161769-38185044	3	ASGA0002401	38161769	7.18E-08	2.33	*NKAIN2*
	1:158309021-161824864	22	mc4r	160773437	2.07E-08	2.75	*MC4R*
	7:30213771-30497305	6	ASGA0032536	30476054	1.14E-08	1.42	*HMGA1*, *NUDT3*

[1] The number of significant SNPs.
[2]CPV%, contribution to phenotypic variance of Top SNP.

analysis separately. A total of 6 significant QTLs were found to be associated with BFT traits in two Duroc populations. Among them, 4 QTLs (1:158,309,021-162,192,627, 6:47,357,966-48,289,460 and 7:30,213,771-30,497,305) located on SSC 1, 6, and 7 overlap with the QTLs discovered by previous researchers (Kim et al., 2000; Kim et al., 2006; Liu et al., 2014; Qiao et al., 2015; Yue et al., 2003), including a major gene *MC4R* and 3 important candidate genes (*RYR1*, *HMGA1*, and *NUDT3*). The *MC4R* gene is considered to be one of the major genes affecting pig BFT traits and is also related to growth and feed intake (Kim et al., 2000). Interestingly, the causal mutation site in the *MC4R* gene is a missense mutation at amino acid position 298 of the *MC4R* protein (Kim et al., 2000), which is also the top SNP in the two Duroc populations in this study. In related studies in humans and mice, the *MC4R* gene is also a key gene that affects weight, fat deposition, and obesity (Cowley et al., 2001; Fan et al., 1997). The QTLs associated with BFT traits located on chromosome 6 (QTL6) have been identified many times (Cherel et al., 2011; Gozalo-Marcilla et al., 2021; Sanchez et al., 2011), and the results are consistent in many Pietrain hybrid populations (Edwards et al., 2008; Harmegnies et al., 2006; Yue et al., 2003). The most attractive gene in this QTL on SSC 6 is *RYR1*. In pigs, it was first discovered that *RYR1*

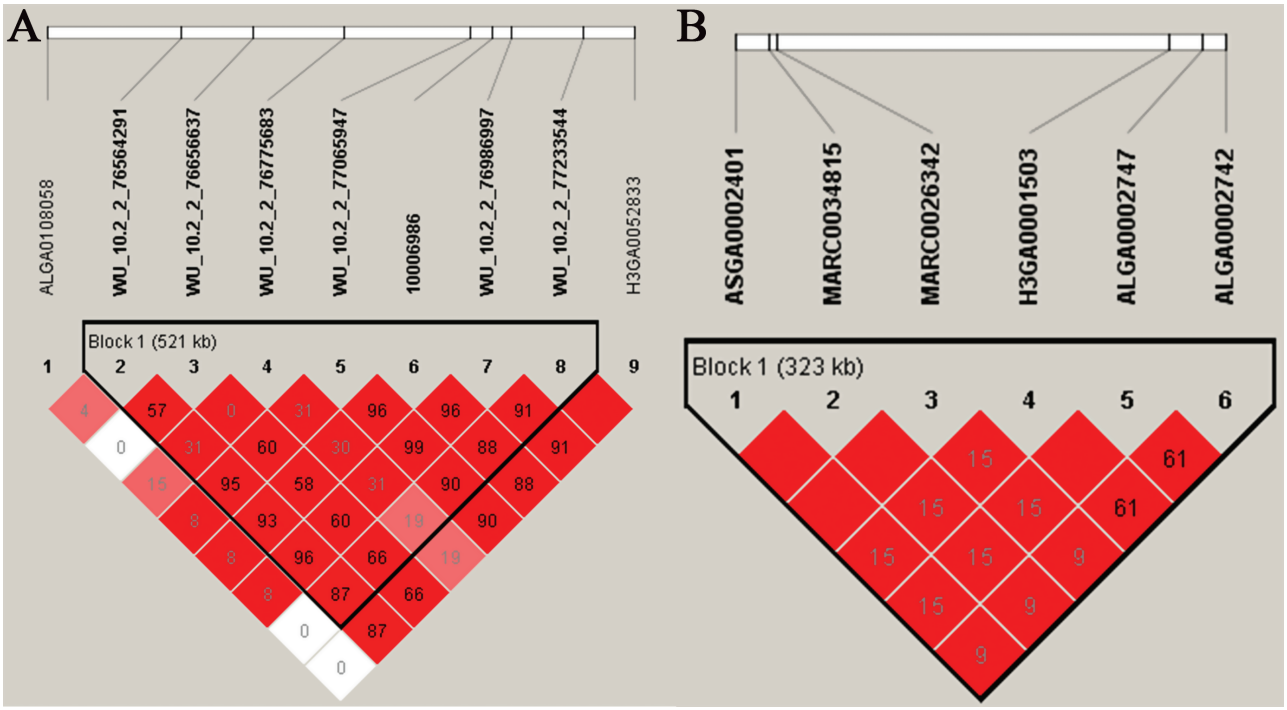

Figure 2. The novel LD block in the significantly associated region on SSC1 and 2. The complete red boxes with no numbers indicated that SNP pairs have complete linkage disequilibrium. The LD block on (A) SSC 1:38,161,769-38,185,044, (B) SSC 2:76,416,246-76,440,900.

Table 3. Estimation of the molecular value of top SNP related to potential candidate genes of BFT traits

Canadate Genes	Top SNP	Top SNP Allele A/B	BFT (mean + sd)[2]			D100 (mean + sd)[2]		
			AA	AB	BB	AA	AB	BB
MC4R	mc4r	A/G	$10.94^{A} \pm 1.45$ (181)	$10.46^{B} \pm 1.37$ (1330)	$10.15^{C} \pm 1.24$ (2258)	$157.4^{a} \pm 9.0$ (181)	$158.4^{a} \pm 8.7$ (1330)	$159.6^{b} \pm 8.2$ (2258)
AMH	10006986	A/G	$10.19^{A} \pm 1.24$ (2678)	$10.54^{B} \pm 1.44$ (1034)	$10.85^{B} \pm 1.49$ (57)	$159.3^{a} \pm 8.3$ (2678)	$158.7^{a} \pm 8.8$ (1034)	$158.6^{a} \pm 8.7$ (57)
RYR1/ SIRT2	Hal	C/T	$10.34^{A} \pm 1.32$ (3092)	$10.12^{B} \pm 1.27$ (654)	$9.34^{C} \pm 0.66$ (23)	$159.0^{a} \pm 8.4$ (3092)	$159.4^{a} \pm 8.4$ (654)	$160.6^{a} \pm 8.7$ (23)
SORCS1/ SORCS3	WU_10.2_14_ 128415606	A/G	$10.28^{A} \pm 1.28$ (3693)	$11.31^{B} \pm 1.27$ (75)	11.20 (1)	$159.1^{a} \pm 8.41$ (3693)	$159.0^{a} \pm 10.14$ (75)	155.3 (1)
MC4R	mc4r	A/G	$11.27^{A} \pm 2.37$ (1965)	$10.52^{B} \pm 2.23$ (745)	$9.81^{C} \pm 1.82$ (71)	$160.9^{a} \pm 11.6$ (1965)	$162.4^{b} \pm 10.8$ (745)	$163.6^{ab} \pm 10.3$ (71)
NKAIN2	ASGA0002401	A/G	$10.05^{AB} \pm 1.99$ (16)	$10.32^{A} \pm 2.26$ (522)	$11.21^{B} \pm 2.34$ (2243)	$163.6^{a} \pm 11.6$ (16)	$162.1^{a} \pm 10.8$ (522)	$161.2^{a} \pm 11.6$ (2243)
HMGA1, NUDT3	ASGA0032536	A/C	$11.16^{A} \pm 2.39$ (1688)	$10.91^{B} \pm 2.27$ (964)	$10.52^{B} \pm 2.31$ (129)	$161.7^{a} \pm 11.3$ (1688)	$160.8^{a} \pm 11.6$ (964)	$161.9^{a} \pm 11.6$ (129)

[1]Canadian Duroc population are in bold.
[2]Different letters indicate a significant difference at $P < 0.05$.

is a major gene affecting malignant hyperthermia, which can cause nerve, liver, and kidney damage and death (Fujii et al., 1991). Yue et al. (2003) found the *RYR1* gene related to many meat quality traits such as BFT, pH value, meat color, lean cuts, and fat cuts by QTL mapping. However, Cherel et al. (2011) found that the *RYR1* gene in the QTL6 is not a key contributor to muscle development and backfat traits, and its effect may be the result of adjacent independent QTL linkage with these traits. Although Cherel et al. (2011) did not provide further potential candidate genes, we further searched the QTL6 and found one other potential candidate gene *SIRT2* that is related to fat deposition. *SIRT2* and *SIRT1* have been shown to be involved in the differentiation of rodent adipocyte precursors (Lantier et al., 2018; Xu et al., 2016). In humans, the decreased expression of *SIRT1* and *SIRT2* may enhance the differentiation ability of visceral adipose stem cells in human obesity and promote the expansion of visceral adipose tissue (Perrini et al., 2020). Potential candidate genes for the QTL (7:30,213,771-30,497,305) located on SSC 7 are *HMGA1* and *NUDT3*, because of which these two genes are functionally related to growth and fat metabolism (Iiritano et al., 2012; Speliotes et al., 2010).

In addition, another QTL (1:38,161,769-38,185,044) significantly affects BFT traits on SSC 1. All three significant SNPs

in this QTL are located in the intron of the gene *NKAIN2*. *NKAIN2* has been reported to be related to body mass index in Japanese population (Yasukochi et al., 2018), and it has also been reported to be related to human dietary total fat intake (Rudkowska et al., 2015). Therefore, *NKAIN2* may be a novel potential candidate gene affecting BFT traits that is involved in growth and fat development. The size of the QTL (2:76,416,246-76,440,900) is 24.7 kb on chromosome 2, and this region contains three functional genes (*AMH*, *JSRP1*, and *AP3D1*). Among them, the gene *AMH* plays an important role in the development of adult female follicles (Merhi et al., 2020). Aly et al. (2019) found that obesity can lead to an increase in adipokines, such as leptin, produced in adipose tissue, and the increase in leptin is related to the decrease in serum *AMH*. The expression of *AMH* in the blood may be related to obesity, and it is a potential candidate gene related to fat deposition.

A total of 9 suggestive SNPs were detected on SSC 14 associated with BFT traits, but they did not constitute a haplotype block. Interestingly, the 14 most recent gene has two genes (*SORCS1* and *SORCS3*) belonging to the same family of genes. *SORCS1* and *SORCS3* are two related sorting receptors expressed in neurons of the arcuate nucleus of the hypothalamus and are related to fat deposition in mice (Subkhangulova et al., 2018). In addition, Purfield et al. (2019) found that the *SORCS1* gene was associated with fat deposition in beef cattle of different breeds. Júnior et al. (2016) found that the *SORCS2* gene was significantly related to the backfat traits of Nellore cattle through GWAS. Therefore, *SORCS1* and *SORCS3* are promising candidate genes whose functions are related to fat deposition.

Gozalo-Marcilla et al. (2021) found that the candidate genes related to BFT traits mainly involve biological processes such as energy homeostasis, adipogenesis, fatty acid metabolism and insulin signaling, phosphate, calcium, and vitamin D homeostasis. The significantly enriched GO pathways found in this study are also related to energy homeostasis, adipogenesis, and metabolism, including positive regulation of glucose metabolic process, negative regulation of mitochondrial membrane potential, energy reserve metabolic process, negative regulation of insulin secretion, response to lipid, and negative regulation of lipid catabolic process. Energy homeostasis and adipogenesis play an important role in the growth of backfat (Jiang et al., 2018). It is conceivable that the candidate genes involved in the above pathways may have potential effects on BFT traits.

Although the identification of QTLs associated with traits can help us better understand the underlying genetic mechanism, the final use in molecular breeding is still peak SNPs. Therefore, further evaluation of the molecular value of peak SNPs can lay the foundation for subsequent genetic improvement of the Duroc population through MAS or GS. At present, pig breeding companies are mainly concerned about the characteristics of growth rate and lean meat percentage, so D100 is also used to evaluate the molecular value of peak SNPs. Although the *MC4R* gene is one of the major genes affecting backfat thickness, *MC4R* gene has an antagonistic effect on improving lean meat rate and growth rate, which is consistent with the results of previous studies (Kim et al., 2000). Van den Maagdenberg et al (2007) also found that the *MC4R* mutation has a significant positive effect on average daily gain ($P < 0.001$), accompanied by higher fat thickness ($P < 0.05$) and lower carcass lean content ($P < 0.01$) in 1,155 four-way hybrid pigs. The other seven important candidate genes can be used for genetic improvement of BFT traits by GS or MAS.

Conclusion

In summary, our data show that *MC4R*, *RYR1*, *SIRT2*, *HMGA1*, *NUDT3*, *NKAIN2*, *AMH*, *SORCS1*, and *SORCS3* are important genes for understanding Duroc pig BFT traits. Among them, five candidate genes, *SIRT2*, *NKAIN2*, *AMH*, *SORCS1*, and *SORCS3*, are newly discovered. The identified SNPs, especially peak SNPs, can be used as potential molecular markers to improve the lean percent of pigs through MAS and GS. In addition, these results can increase our understanding of the potential molecular biology of fat deposition.

Availability of Data and Material

The SNP genotyping data containing variant information in two Duroc populations are not publicly available because the genotyped animals belong to commercial breeding companies, but they can be obtained from the corresponding author under reasonable requirements.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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Authors' Contributions

JieY and Z.W. conceived and designed the experiment. R.D., Z.Z., L.C., S.Z., E.Z., and JianY collected the samples and recorded the phenotypes. R.D., Z.Z., S.Z., J.W., JianY, and D.R. extracted the DNA for genotyping. R.D., W.H., and Y.Q. analyzed the data. R.D., W.H., and JieY wrote the manuscript. Z.W. contributed to the materials. All authors reviewed and approved the manuscript.

Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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