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Identification of novel variants and candidate genes associated with porcine bone mineral density using genome-wide association study

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Abstract

Pig leg weakness not only causes huge economic losses for producers but also affects animal welfare. However, genes with large effects on pig leg weakness have not been identified and suitable methods to study porcine leg weakness are urgently needed. Bone mineral density (**BMD**) is an important indicator for determining leg soundness in pigs. Increasing pig BMD is likely to improve pig leg soundness. In this study, porcine BMD was measured using an ultrasound bone densitometer in a population with 212 Danish Landrace pigs and 537 Danish Yorkshires. After genotyping all the individuals using GeneSeek Porcine 50K SNP chip, genetic parameter estimation was performed to evaluate the heritability of BMD. Genome-wide association study and haplotype analysis were also performed to identify the variants and candidate genes associated with porcine BMD. The results showed that the heritability of BMD was 0.21 in Landrace and 0.31 in Yorkshire. Five single-nucleotide polymorphisms on chromosome 6 identified were associated with porcine BMD at suggestive significance level. Two candidate quantitative trait loci (74.47 to 75.33 Mb; 80.20 to 83.83 Mb) and three potential candidate genes (*ZBTB40*, *CNR2*, and *Lin28a*) of porcine BMD were detected in this study.

Key words: bone mineral density, candidate gene, heritability, pig, single-nucleotide polymorphism

Introduction

The incidence of leg weakness is high in pig production, which causes severe economic losses and seriously affects animal welfare. Leg weakness is the second leading cause of pig elimination, next to the reproductive diseases (Le et al., 2017). According to the data from a large-scale pig farm (personal data), about 10% of farrowed sows between parities one and four were eliminated due to leg weakness. A previous study reported that the prevalence of lameness ranged from 8.8% to 16.9% (Heinonen et al., 2013). Low bone mineral density (**BMD**) is one of the main causes of pig leg weakness (Storskrubb et al., 2010). Leg soundness has usually been used to evaluate the leg health, and it was reported that the heritability of leg soundness was between 0.1 and 0.5 (Guo et al., 2013). Pig leg soundness has been determined using many methods, including leg score (Fukawa et al., 2008), gait score (Guo et al., 2013), bone mineral content (Mitchell et al., 2001), BMD (Rothammer et al., 2014), osteochondrosis

Abbreviations

BMD	bone mineral density
DXA	dual-energy X-ray absorptiometry
GWAS	genome-wide association study
Q–Q	quantile–quantile
QTL	quantitative trait loci
SSC	sus scrofa chromosome
SNP	single-nucleotide polymorphisms
SOS	speed of sound

score (Lundeheim, 1987; Fukawa et al., 2008), and biceps brachii muscle length (Guo et al., 2013).

The BMD is defined as the mineral content per unit volume of bone, which can indicate bone health in humans (Marshall et al., 1996). Studies have reported that BMD could accurately predict the risk of fracture (Chevalley et al., 1991; Schott et al., 1998). Additionally, leg score and gait score were also intensively used in the studies of leg weakness in pigs. However, these scores are highly subjective and making them difficult to repeat the measurements. Compared with these score measurements, an objective measurement, BMD, has great potential in the study of pig leg weakness. However, few BMD studies have been reported in pigs, possibly due to the difficulty of applying BMD measurement in practice and the high cost associated with it.

At present, a method suitable for measuring BMD is urgently needed for porcine BMD study. In previous studies, dual-energy X-ray absorptiometry (**DXA**) has been used to measure porcine BMD, which may be harmful to animal health (Kaufman et al., 2007). Besides, DXA measurement is difficult to perform on a live animal and causes great stress for pigs, which limited the use of DXA in the pig BMD study. A portable ultrasonic bone densitometer could overcome those shortcomings because whose result is highly correlated with those of DXA, quantitative computed tomography, single-photon absorptiometry, and dual-photon absorptiometry, which are easier to use (Yamazaki et al., 1994). BMD has been estimated by measuring the speed of sound (**SOS**) in bone tissue using an ultrasonic bone densitometer (Lees and Stevenson, 1993). Ultrasound bone densitometry may be a practical method for the pig BMD study.

Genome-wide association study (**GWAS**) is a method aimed at detecting variants associated with complex traits (Visscher et al., 2012). Recently, GWAS as one of the most popular methods has been used for studying the genetic mechanisms of complex traits. Many significant variants associated with human BMD have been identified in various GWAS studies (Richards et al., 2008; Wu et al., 2013; Mo et al., 2018; Trajanoska et al., 2018 However, there are very few reports of GWAS related to pig BMD.

In this study, we used a Sunlight MiniOmni Ultrasound Bone Densitometer (Sunlight Medical Ltd. Israel, Tel-Aviv, Israel) to measure the pig BMD. Genetic parameter estimation was performed to evaluate the heritability of pig BMD. GWAS and haplotype analysis were used to detect variants and candidate genes significantly associated with pig BMD.

Materials and Methods

Use of animals and the procedures performed in this study were approved by the Scientific Ethics Committee of Huazhong Agricultural University (Approval Number HZAUSW-2019-006).

Phenotypes and single-nucleotide polymorphism genotyping

Bone mineral density data were determined using a Sunlight MiniOmni Ultrasound Bone Densitometer, which measured the SOS in the bone, using Ultrasound Omnipath Axial Transmission technology with a proprietary multi-transducer probe. In humans, an ultrasound bone densitometer is usually used for BMD measurement, where values of SOS are used to study BMD (Wu et al., 2000; Jones and Boon, 2008). This study also followed this approach to evaluate BMD in pigs. In this study, SOS was measured in multiparous sows (one to seven parities) between 1 and 3 d after parturition. System quality verification was performed before the first measurement of the day to ensure the reliability of results. The SOS measurements were repeated three to five times on the sow metatarsus, and the average of the measurements was used as the final result. In the current study, all the pigs were reared in the fully slatted floor, with the same feeding and management condition. The diet of the same type was provided for the Landrace and Yorkshire pigs, with the same calcium and phosphorus levels and no growth hormones. The porcine BMD variation in different breeds was studied using a one-way ANOVA analysis after adjusting the parity effect. Also, the variation in different parities was studied after adjusting the effect of the breed.

In this study, genotyping was performed using the GeneSeek Porcine 50K SNP chip (Neogen Corporation, Lansing, MI) on the DNA samples obtained from 293 Landrace and 603 Yorkshires pig populations. In total, 48,909 single-nucleotide polymorphisms (**SNP**) were genotyped. The genotyping data were processed with a quality control process, where the SNP call rates less than 0.90 and minor allele frequencies less than 0.01(Huang et al., 2017) were removed. For sample quality control, the samples with call rates less than 0.90 and a significant deviation from the population were filtered out. After the quality control, 212 Danish Landrace and 537 Danish Yorkshires pigs were remained in the subsequent GWA meta-analyses, each with 39499 SNP and 42391 SNP, respectively.

Variance component analysis and estimation of heritability

Heritability estimation was performed using the GREML algorithm of GCTA v1.93.0 beta software (Yang et al., 2010; Lee et al., 2011; Yang et al., 2011b). The statistical model for estimating variance components was as follows:

$$y = \mathbf{X}b + \mathbf{Z}g + e,$$

in which y is a vector of SOS measurements; b is the fixed effect for the parity of the sow; g is the additive genetic effect, with the assumption that $g \sim N(0, \mathbf{G}\sigma^2_g)$, in which σ^2_g is the genetic variance and **G** is the genomic relationship matrix as described by VanRaden (2008); **X** and **Z** are the incidence matrices for the fixed effect b and the additive genetic effect g, respectively; e is the residual error, assumed to follow a normal distribution $e \sim N(0, \mathrm{I}\sigma^2_e)$, in which I is an identity matrix and σ^2_e is the residual error variance.

Genome-wide association study

Considering that genetic differences existed between Landrace and Yorkshire, single-population GWAS and GWA meta-analysis were performed in this study. GWAS was performed using the MLMA-LOCO (leaving-one-chromosome-out) algorithm of GCTA (Yang et al., 2011a, 2014). MLMA-LOCO algorithm is also called MLM LOCO analysis. The model can be described as follows:

$$y = \mathrm{a} + \mathbf{X}b + g^{-} + \mathbf{C}d + e$$

in which y is a vector of SOS measurements, a is the mean of y, b is the additive genetic effect, g^{-} is the accumulated effect of all

SNP except those on the chromosome where the candidate SNP is located, *d* is the fixed effect for parity of sows, *e* is the residual error, and **X** and **C** are the respective incidence matrices of *b* and d. Meta GWA analyses were conducted by metal (Willer et al., 2010) software. To further control the population stratification, we divided the chi-square value by inflation factor (λ) (Yang et al., 2014), then corrected *P*-values were derived from a chi-square distribution with degree freedom (df) of 1 (Devlin and Roeder, 1999). In this study, Manhattan and quantile–quantile (**Q–Q**) plots were made using CMplot (https://github.com/YinLiLin/R-CMplot; accessed December 3, 2019).

Haplotype analysis

To identify the candidate quantitative trait loci (**QTL**) region, haplotype analysis was performed for the flanking SNP within 1 Mb of the suggestive significant SNP, using Haploview 4.0 (Barrett, 2009). Haplotype blocks were defined according to default confidence intervals of haploview (Gabriel et al., 2002). In this study, single-population haplotype analysis was performed, and the regions with significant SNP in linkage disequilibrium both in Landrace and Yorkshire were considered as the candidate QTL.

SNP and candidate QTL functional analysis

To study the function of SNP and candidate QTL, SNP and candidate QTL were mapped to pig chromosomes using Sscrofa11.1 (http://asia.ensembl.org/index.html; accessed March 20, 2018). Also, the genes within candidate QTL regions were searched and annotated using Ensembl BioMart tools (http://asia.ensembl.org/index.html; accessed September 20, 2018) and references (https://www.ncbi.nlm.nih.gov/pubmed; accessed September 20, 2018), respectively. The genes within the candidate QTL were considered as potential candidate genes. Furthermore, the function of all potential candidate genes was studied from the previous publications related to the bone metabolism study. The genes reported to be associated with bone metabolism in animals were considered as the candidate genes for BMD.

Results

Descriptive statistical analysis of bone mineral density in Landrace and Yorkshire pigs

The descriptive statistical analysis of SOS measurements is shown in Table 1. The results showed that the mean of SOS measurements in Landrace pigs was similar to Yorkshires (Table 1). In Landrace pigs, the mean SOS of the second parity was the lowest (4,175.30 m/s) and the mean SOS of the fourth parity with only one pig was the highest (4,343.82 m/s). In Yorkshires, the mean SOS of the first parity was the lowest (4,238.82 m/s) and the fifth parity was the highest (4,352.82 m/s). One-way ANOVA analysis identified a significant difference ($P < 0.01$) in Yorkshire but no significant differences were detected in Landrace. No significant difference between Landrace and Yorkshire.

Variance component estimation and calculation of heritability

The variance components and the SE of BMD were estimated in Landrace and Yorkshire pigs. The results showed that the heritability of BMD was 0.21 and 0.31 in Landrace pigs and Yorkshires and each associated with SE of 0.13 and 0.08, respectively (Table 2).

Genome-wide association study

Firstly, single-population GWAS was performed on Landrace and Yorkshire population separately, and the results are shown in Supplementary Figure S1. Two SNP on chromosome 6 were significantly associated with porcine BMD in Yorkshire. But no SNP was identified in Landrace. Secondly, the GWA meta-analysis was performed, and the results are shown in Supplementary Figure S3. Five SNP were found significantly associated with porcine BMD. However, population stratification was detected (Supplementary Figure S2). Thus, to control the population stratification, an adjustive analysis was performed in GWAS. The Q–Q plot and Manhattan plot of adjusted *P* values are shown in Figure 1 and Figure 2, respectively. The expansion coefficients are 1.263 and 1 before and after adjusting of the Q–Q plot, respectively. Unfortunately, GWAS showed that no SNP was strongly associated with BMD after adjusting, with five SNP on chromosome 6 suggested significantly associated with BMD, as shown in Figure 2. ASGA0028695 is an intron mutation of *Man1c1*, explaining 4.47% phenotypic and 21.58% genetic variance in landrace, 3.94% phenotypic and 12.73% genetic variance in Yorkshire. WU_10.2_6_75058017 is an intergenic mutation with explaining 4.47% phenotypic and 21.58% genetic variance in Landrace, 4.01% phenotypic and 12.73% genetic variance in Yorkshire. MARC0002557, an upstream gene variant, explained 4.47% phenotypic and 21.58% genetic variance in Landrace, 3.97% phenotypic and 12.82% genetic variance in Yorkshire. MARC0021944 is an intron variant of *Nipal3*, with explaining 4.47% phenotypic and 21.58% genetic variance in Landrace, 3.84% phenotypic and 12.42% genetic variance in Yorkshire. ASGA0099279 is an intron variant of *Fam131c* explaining 3.78% phenotypic and 18.23% genetic variance in Landrace, 3.54% phenotypic and 11.45% genetic variance in Yorkshire.

Table 1. Porcine BMD descriptive statistics in Landrace and Yorkshire pigs (SOS [m/s])

	Mean (age)		BMD, mean (SD) (*n*)		
Parity	Landrace	Yorkshire	Landrace		Yorkshire
1	365.24	362.84	4,294.60 (188.378) (70)		4,238.82 (172.623) (163)
2	557.60	529.58	4,175.30 (249.07) (20)		4,253.92 (177.73) (78)
3	687.92	692.27	4,327.80 (205.45) (39)		4,295.45 (202.08) (94)
4	813.77	808.66	4,343.82 (181.18) (39)		4,298.68 (176.934) (126)
5	989.02	981.54	4,309.02 (258.110) (44)		4,352.82 (189.56) (76)
P-value (SOS ~parity in Landrace)*			0.191	*P*-value (SOS ~parity in Yorkshire)*	2.48×10^{-6}
P-value (SOS ~breed) adj**			0.235		

*P-value (SOS ~parity) indicates that a significant difference exists in different parities SOS in Yorkshire, with no significant difference in Landrace.
**P-value (SOS~breed) adj indicates that no significant difference is obtained in Landrace and Yorkshire SOS after adjusting for the effects of parities.

Haplotype analysis

Considering that some genetic difference existed between Landrace and Yorkshire, haplotype analysis was performed within each single population. The haplotype analysis, conducted for a 1-Mb region flanking the significant SNP, is shown in Figure 3. Four haplotype blocks were detected around ASGA0099279 (in block 2) in the Landrace population (Figure 3a). The detailed haplotype analysis were shown in Supplementary File 1. Three haplotype blocks were identified around ASGA0099279 (in block 2), with ASGA0099279 in linkage disequilibrium with block 1 (r^2∈[0.02, 0.41]) and 3(r^2∈[0.05, 0.35]) in Yorkshire population (Figure 3b). Seven haplotype blocks were found around four significant SNP (WU_10.2_6_75058017 in block 2, MARC0002557 in block3,and MARC0021944 and ASGA0028695 in block 5) in Landrace population (Figure 3c), with that WU_10.2_6_75058017 is in linkage disequilibrium with blocks 1(r^2∈[0.06, 0.59]). Nine haplotype blocks were identified around four significant SNP (WU_10.2_6_75058017 in block 2, MARC0002557 not in any blocks, MARC0021944 in block 4, and ASGA0028695 in block 6) in Yorkshire population (Figure 3d). Interestingly, WU_10.2_6_75058017 is in linkage disequilibrium with all blocks (r^2∈[0.04, 1]); MARC0002557 is in linkage disequilibrium with blocks 3 and 4; MARC0021944 is in linkage disequilibrium with block 1 (r^2∈[0.03, 0.63]) and 9 (r^2∈[0.03, 0.86]); and ASGA0028695 is in linkage disequilibrium with block 1 (r^2∈[0.03, 0.66]), 7 (r^2∈[0.20, 0.96]), 8 (r^2∈[0.36, 0.58]), and 9 (r^2∈[0.08, 0.61]). The regions with significant SNP in linkage disequilibrium were considered as the candidate regions of BMD both in Landrace and Yorkshire population. In the result, 74.47 to 75.33 Mb and 80.20 to 83.83 Mb on chromosome 6 were considered as the candidate QTL of porcine BMD.

Table 2. Variance components and the heritability of BMD at the metatarsus

Breed	σ^2_g [1] (SE)	σ^2_e [2] (SE)	h^2 (SE)
Landrace	9,409.04 (6,313.66)	36,017.74 (6,092.26)	0.21 (0.13)
Yorkshire	10,654.46 (3,092.05)	23,757.57 (2,433.80)	0.31 (0.08)

[1]σ^2_g = genetic variance.
[2]σ^2_e = error variance.

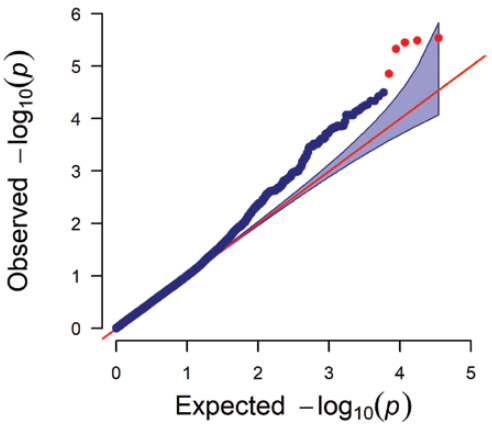

Figure 1. Quantile–quantile plot of adjusted *P* values.

Positional candidate genes at GWAS loci

The function of genes in the candidate QTL were queried, in which the genes *CNR2* (Karsak et al., 2009; Sophocleous et al., 2014, 2017; Woo et al., 2015; Zhang et al., 2015), *ZBTB40* (Richards et al., 2008; Rivadeneira et al., 2009), and *Lin28a* (Shyh-Chang et al., 2013) were previously reported to be associated with BMD in humans, as shown in Table 4.

Discussion

The genetic study of BMD is essential for breeding for sow leg soundness. In this study, BMD was measured using a Sunlight MiniOmni Ultrasound Bone Densitometer and SOS measurements. In the last century, the correlation between SOS measured using ultrasound bone densitometry and BMD measured using DXA was analyzed, confirming that SOS and BMD were correlated moderately well (Lees and Stevenson, 1993). Studies have shown that SOS measured using quantitative ultrasound and BMD measured using DXA were correlated moderately well at the hip, lumbar spine, total body, and heel (Jones and Boon, 2008).

The SOS was not significantly different between Landrace and Yorkshire pigs. A significant difference was found at different parities Yorkshire SOS, but no significant difference was detected at different parities Landrace SOS, which may be due to some individuals in the Landrace population were eliminated because of leg weakness. Thus, studies in a large population are necessary to study the relationship between parity and BMD between the two breeds. In one study (Lees and Stevenson, 1993), the SOS of normal humans and osteoporosis

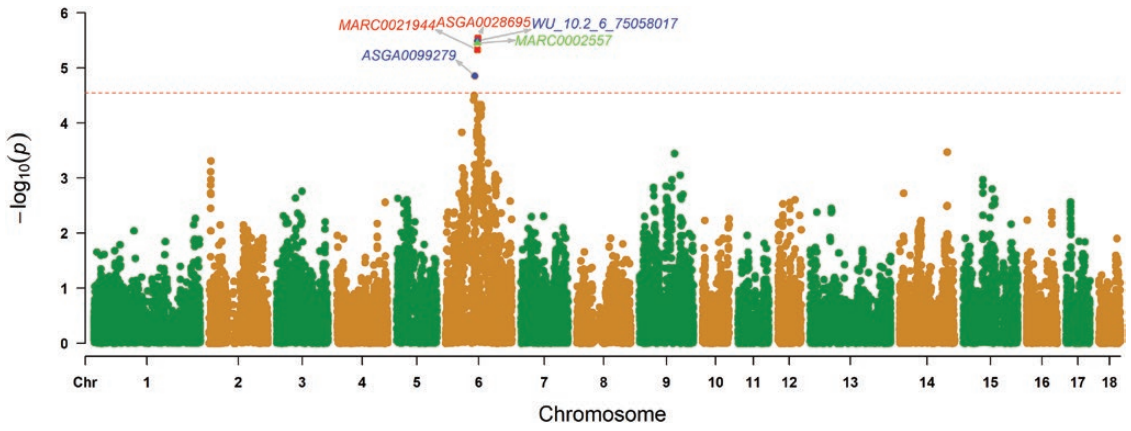

Figure 2. Manhattan plots for the single-marker analysis of BMD at the metatarsus after adjusting. The x-axis indicates the chromosomes in the genome. The dotted lines suggest the Bonferroni-corrected suggestive significant threshold (2.86×10^{-5}). The suggestive significant SNP are marked with their names.

Table 3. The summary statistics of genetic and phenotypic variance explained by significant SNP

SNP ID	Chr[1]	Bp[2]	*P*-value	MAF		Effect		The genetic variance explained by SNP[3]		The phenotypic variance explained by SNP[4]	
				Landrace	Yorkshire	Landrace	Yorkshire	Landrace	Yorkshire	Landrace	Yorkshire
ASGA0028695	6	83,184,006	1.46×10^{-7}	0.014	0.106	−271.09	−85.34	0.2158	0.1273	0.0447	0.0394
WU_10.2_6_75058017	6	81,122,702	1.68×10^{-7}	0.014	0.158	−271.09	−71.09	0.2158	0.1294	0.0447	0.0401
MARC0002557	6	81,562,859	1.90×10^{-7}	0.014	0.144	−271.09	−74.96	0.2158	0.1282	0.0447	0.0397
MARC0021944	6	82,148,927	2.69×10^{-7}	0.014	0.11	−271.09	−83.12	0.2158	0.1242	0.0447	0.0384
ASGA0099279	6	75,202,125	1.05×10^{-6}	0.017	0.24	−231.24	−59.96	0.1823	0.1145	0.0378	0.0354

[1] and [2] indicate the chromosome and position of significant SNP, respectively.
[3] and [4] indicate the genetic and phenotypic variance components explained by each SNP in Landrace and Yorkshire, respectively, suggesting strong linkage existed in ASGA0028695, WU_10.2_6_75058017, MARC0002557, and MARC0021944 in Landrace population.

was measured, and the mean of those results was 1,551 vs. 1,513 m/s, respectively. According to our study, porcine SOS was higher than that of humans.

The heritability of SOS measurements at the metatarsus in Landrace pigs and Yorkshires was 0.21 and 0.31, respectively, indicating that the heritability of porcine BMD was medium. However, the SE was large, due to the small sample size used in this analysis. Therefore, studies with a large sample size are encouraged to obtain more accurate measures of the heritability of porcine BMD. Many reports indicated that the heritability of BMD was different in different parts of the human body. For instance, a study reported that the heritability of BMD was 0.46 to 0.78 for different parts using DXA based on 250 pairs of female twins of ages 50 to 70 yr (Arden et al., 1996). Lenchik et al. (2004) reported that, based on a study of 124 women and 120 men from 101 families, the heritability of BMD was 0.42 to 0.56 for different parts using the QCT technique (Lenchik et al., 2004). Hernandez-de Sosa (2014) reported that the heritability of BMD was 0.252 to 0.537 for different parts using DXA. However, there is no research reporting the heritability of BMD in sows.

Considering some genetic differences existed between the two breeds, single-population GWAS and GWA meta-analysis were performed in this study. In single-population GWAS results, five SNP on chromosome 6 were associated with porcine BMD in Yorkshire but none were identified in Landrace. We speculate that the differences in the results from the two populations were caused by different population sizes and different genetic backgrounds but caution that additional in-depth studies are desperately needed. Otherwise, adjusting analysis was performed after the GWA meta-analysis to control the population stratification. The expansion coefficient was declined after adjusting, which indicated that population stratification was controlled effectively. Unfortunately, no SNP obtained were strongly associated with BMD in this study. Five SNP all on chromosome 6 were detected were suggestively associated with BMD. In a previous study, a QTL associated with pig BMD was mapped between 36,937,640 and 37,714,128 bp on Sus scrofa chromosome (**SSC**) 6 (Rothammer et al., 2014); this QTL was not identified in this study.Significant SNP of this study were not within previously reported QTL regions for porcine BMD, this may be due to the small sample size used in this study or may be due to the genetic differences between our studied population and their population.

In this study, haplotype analysis was performed in a single population, indicating that the difference was found in the haplotypes between Landrace and Yorkshire. The regions with significant SNP in linkage disequilibrium were considered as the candidate regions of BMD both in Landrace and Yorkshire populations. In the result, 74.47 to 75.33 and 80.20 to 83.83 Mb on chromosome 6 were considered as the candidate QTL of porcine BMD. Otherwise, the functions of genes within candidate QTL were queried. Among those genes, three candidate genes (*ZBTB40*, *Lin28a*, and *CNR2*) were reported to be associated with BMD in humans. *ZBTB40* gene is located at 637324 bp upstream of WU_10.2_6_75058017. *ZBTB40* was reported to be associated with human BMD in several studies (Richards et al., 2008; Rivadeneira et al., 2009; Chao et al., 2012). *Lin28a* gene is located 622116 bp upstream of ASGA0028695. The *Lin28a* was reported that it could enhance tissue repair in some adult tissues by reprogramming cellular bioenergetics and accelerate the regrowth of cartilage and bone after ear and digit injuries (Shyh-Chang et al., 2013).

The gene *CNR2* is located at 569,848 bp downstream of WU_10.2_6_75058017. *CNR2* encodes the cannabinoid receptor 2, which has a significant role in regulating bone metabolism (Sophocleous et al., 2014). *CNR2* encodes CB2, one of cannabinoid

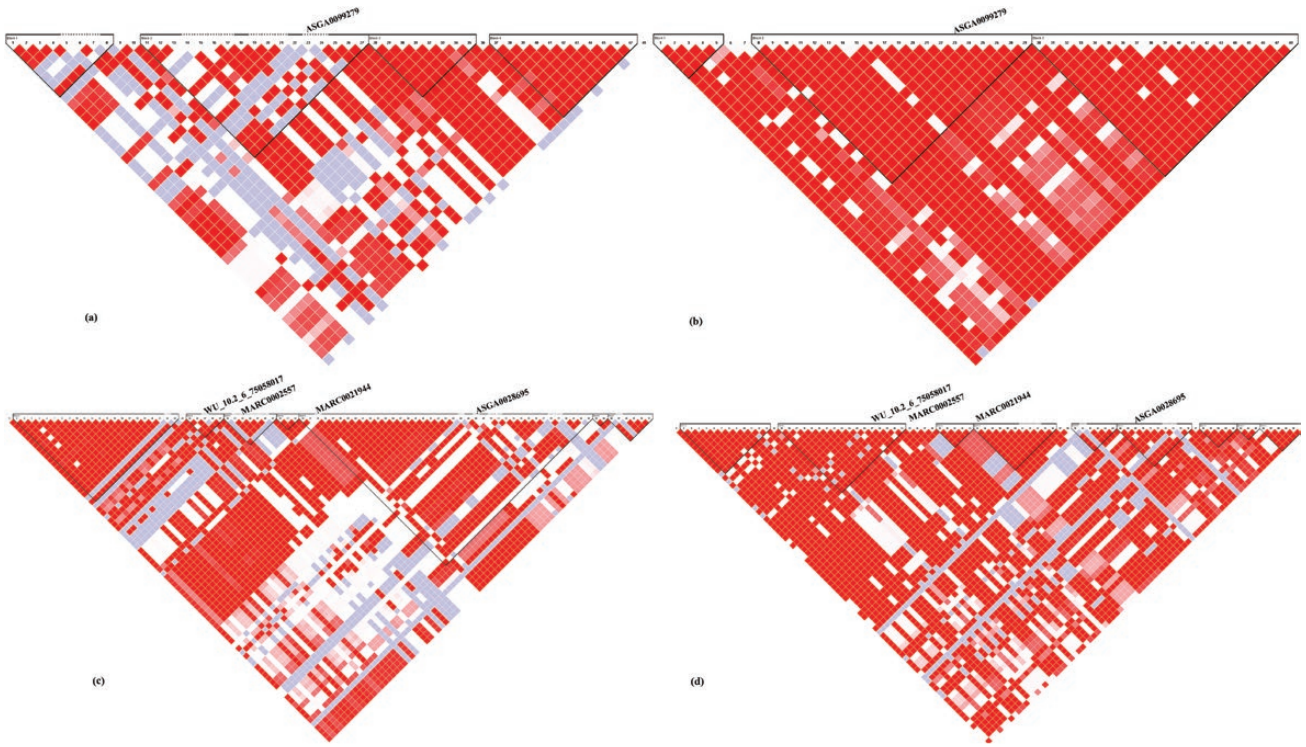

Figure 3. The haplotype of the flanking region of suggestive significant SNP. (a) and (b) Haplotype of the flanking region of ASGA0099279 in Landrace and Yorkshire population, respectively. (c) and (d) indicate the haplotype of the flanking region of ASGA0028695, WU_10.2_6_75058017, MARC0002557, and MARC0021944 in Landrace and Yorkshire population, respectively.

Table 4. The candidate genes reported being associated with BMD in humans

Gene symbol	Gene name	The adjacent SNP	Distance,1 bp	Reference
CNR2	Cannabinoid receptor 2	WU_10.2_6_75058017	–569,848	(Karsak et al., 2009; Sophocleous et al., 2014, 2017; Woo et al., 2015; Zhang et al., 2015)
ZBTB40	Zinc finger and BTB domain containing 40	WU_10.2_6_75058017	–637,324	(Richards et al., 2008; Rivadeneira et al., 2009; Chao et al., 2012)
Lin28a	Lin-28 homolog A	ASGA0028695	–622,116	(Shyh-Chang et al., 2013)

[1]Distance indicates the distance between the significant SNP and the genes; a positive number suggests the gene is located upstream of the SNP.

system numbers. The cannabinoid system is well known to tune important steps of cell communication in bone (Karsak et al., 2009). In recent studies, *CNR2* was identified as being related to BMD in Han Chinese (Zhang et al., 2015), Russian (Karsak et al., 2009), and Korean (Woo et al., 2015) populations. Moreover, some experiments confirmed that *CNR2*-deficient mice had higher trabecular bone mass by the age of 3 mo and reduced age-related bone loss (Sophocleous et al., 2017).

Our study indicates that differences were existed in different parities pigs, suggesting that more reliable results can be obtained doing GWAS in the population of the same parity. Otherwise, this study suggests that *Lin28a*, *CNR2*, and *ZBTB40* may be potential candidate genes of porcine BMD. But in-depth study with a large sample size is necessary to identify the function of *Lin28a*, *CNR2*, and *ZBTB40* in porcine BMD.

Conclusion

In this study, the heritability of BMD was estimated and BMD was confirmed to be a moderately heritable trait. Five SNP on SSC 6 detected were suggestive significantly associated with BMD. Two candidate QTL on chromosome 6 were identified. Three genes in candidate QTL were considered as the potential candidate genes for porcine BMD.

Supplementary Data

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

Supplementary Figure S1. Manhattan plots of single-population GWAS. The dotted line and solid line suggest the Bonferroni-corrected suggestive significant threshold (2.86 ×1 0^{-5}) and significant threshold (1.43 × 10^{-6}), respectively. The significant SNP are in red.

Supplementary Figure S2.Quantile–quantile plot before adjusting.

Supplementary Figure S3. Manhattan plots before adjusting. The dotted line and solid line suggest the Bonferroni-corrected suggestive significant threshold (2.86 × 10^{-5}) and significant threshold (1.43 × 10^{-6}), respectively. The Landrace and Yorkshire were marked in yellow and blue, respectively. The significant SNP are marked with their names.

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Conflict of interest statement

The authors declare that they have no competing interests.

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