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Mining of candidate genes related to body size in Chinese native pig breeds based on public data

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To study the key genes that influence the body size of local pig breeds in China. Genome-wide SNP chip data from a total of 129 pigs from eight breeds, consisting of four large body size breeds (MZ, HT, ST, RC) and four small body size breeds (XI, BX, WZ, DN) were analyzed. Principal Component Analysis (PCA) was employed to assess the genetic clustering of the eight breeds. Fst and XP-CLR were used to detect selective signals between the large ans small body size breeds groups. The PCA results indicated a clear clustering of small breeds and a dispersion distribution among large breeds. Fst and XP-CLR identified 142 overlapping regions within a 500 kb up & down stream of significant loci. These regions encompassed 520 annotated genes, which were enriched in 34 biological pathways. Gene network analysis highlighted nine key genes, of which five (*NPR3*, *TNFSF11*, *TBC1D7*, *FGF2*, *IGF1R*) are known to be associated with bone growth and body size traits in animals. Additionally, four novel candidate genes (*IKBKB*, *SFRP1*, *LRP6*, *SPRY1*) were identified that might be related to pig body size. Our findings provide a theoretical basis for further revealing the genetic mechanism of pig body size traits.

Keywords Principal component analysis, Population structure, SNP Chip, Body size, Pig breeds, Candidate genes

Due to its extensive history, wide-scale distribution and large population, pigs have become a significant commercial species in China. In 2020, China topped the world rankings with 41.13 million tons of pork produced and 527.04 million pigs sold, accounting for 53.84% of the country's annual meat production[1]. As a result, scientists have placed significant emphasis on studying key characteristics of pigs, such as growth, reproduction, and meat quality[2].

Pig is also a valuable animal model due to its similarities with humans in terms of anatomy, tissue, and physiology. Miniature pigs are particularly small in size and are commonly used as experimental animal models in biomedical and human disease research[3]. China possesses immensely rich local pig germplasm resources, and the diverse geomorphic environment that contributes to the variation in body size among local pig breeds in the country has become an important research focus. Body size traits are highly important and complex quantitative attributes, encompassing body length, body width, and body height. Currently, there are few reported candidate genes associated with pig body size traits in pigs. Previous studies have indicated that body size is more closely related to genetic factors than to environmental conditions[4].

In mammals, variations in the *NCAPG* and *PLAG1* genes are closely linked to body size regulation, particularly demonstrating significant effects in cattle[5]. *RBFOX1* is related to small body size in pigs[6]. The continuous expression of the *IGF2BP1* gene after birth can increase body size by 15% and feed efficiency by 6% in duck[7]. *IGFBP7* is associated with body size increase in giant whales[8]. Larger bird species exhibit higher evolutionary rates of the *IGF2BP1* gene. *IGFBP7* and *PLXDC2* genes demonstrated accelerated evolution in large- and medium-sized birds, respectively[9]. In a study by Li et al., it was found that the *IGFBP6* gene influences the growth and development of pigs through haplotype analysis of Bamaxiang pigs[10].

Another study identified seven candidate genes related to pig body size, including *PLIN1*, *LIPE*, and *PNPLA1*, through gene network interaction analysis[11]. In a genome-wide association analysis of Licha black pigs, *PLAG1* and *BMP2* genes were identified as potential candidates for pig size[12]. Additionally, a whole-genome sequencing analysis of pig breeds in various regions of China, as well as wild boars in northern and southern China, found that genes such as *LEPR*, *FANCC*, *COL1A1*, and *PCCA* may be related to pig body size[13]. Furthermore, genome-

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wide association study on 43 growth and carcass traits in 315 Bamaxiang pigs revealed candidate genes associated with corresponding body size traits, such as *HMGA1*, *NUDT3*, *EIF2AK1*, *TMEM132C*, and *AFF2*[14].

The body size traits of pigs not only play a crucial role in breeding but also have close relationships with other traits. For instance, studies have found that large-sized pigs tend to exhibit a relatively low carcass lean meat rate due to thicker skin, larger bodies, and thicker bones[15]. Seideman discovered a significant correlation between body size traits and carcass traits in their research on the carcass quality of Landrace pigs[16]. Research results by Zhang indicated that body size traits (including body length, body height and tube circumference) in pigs exhibited a weak negative genetic correlation with reproductive traits but a strong positive genetic correlation with growth traits[17]. Moreover, numerous studies have demonstrated that the body size traits of pigs not only reflect their body size and structure but are also closely linked to their health status and fattening effects[18,19]. Simultaneously, these body size traits also exhibit certain correlations with the production and reproductive performance of pigs. Some researchers have proposed that identifying genes related to pig size is crucial for improving the economic benefits of farms and is a strategic direction for pig breeding genes in China's future[20].

There is a certain correlation between the body size traits and the reproductive and carcass traits of pigs, which is very important for the economic benefit of breeding. Compared with carcass traits, the measurement of body size traits does not require slaughter, the method is simple, the results are accurate, and the cost is lower. In the production practice, the selection of body size traits can indirectly carry out the selection of carcass traits, and effectively reduce the breeding cost. Therefore, elucidating the formation mechanisms of pig body size and studying its growth and developmental characteristics have become research hotspots. In this study, eight Chinese local pig breeds with significant differences in body size and genetic stability were selected. By comparing the genomic genetic differentiation of large and small Chinese local pig breeds with SNP chip data, significant signal loci were screened out, and important candidate genes related to pig body size traits were excavated. This study lays a foundation for revealing the genetic mechanism of pig size, and has important guiding significance for selecting pig carcass traits and improving economic benefits in production practice.

Methods

Ethics statement

This research use of Chinese local pig data from public Illumina 60 K chip (https://doi.org/10.5061/dryad.30tk6). Animal samples were collected in accordance with the current laws of the country. All samples and data are exchanged in accordance with national and international regulations.

Pig population classification

This study focused on selecting pig breeds with significant differences in body size and genetic stability, as the body size of pig breeds can vary under different feeding levels. The body size parameters were classified according to the guidelines provided by the 'Journal of Animal and Poultry Genetic Resources in China: Swine'. The large and small pig populations were classified as follows: The large pig population ($n = 71$) consisted of MZ, HT, ST, and RC, with adult length ≥ 130 cm, adult weight ≥ 140 kg, and adult height ≥ 75 cm. The small pig population ($n = 58$) consisted of XI, BX, WZ, and DN, with adult length ≤ 100 cm, adult weight ≤ 50 kg, and adult height ≤ 55 cm. For detailed information regarding the body size of each pig breed, refer to Table 1.

Data download and collation

This study utilized data from Chinese local pig breeds obtained through the publicly available Illumina 60k chip, with the dataset sourced from the academic team of Huang (https://doi.org/10.5061/dryad.30tk6)[21]. The dataset included 71 large-sized pigs (MZ, $n = 20$; HT, $n = 16$; ST, $n = 15$; RC, $n = 20$) and 58 small-sized pigs (XI, $n = 13$; BX, $n = 16$; WZ, $n = 16$; DN, $n = 13$).

Control of chip data quality

We used PLINK 1.90 software to update the SNP location of *Sus scrofa version 10.2* in the database to *Sus scrofa version 11.1*. Subsequently, the updated data was filtered using Plink v1.90 software, with the following quality control standards: (1) Removal of marker sites at unknown locations; (2) Exclusion of marker sites on sex chromosomes; (3) Exclusion of marker sites with a minimum allele frequency (MAF) lower than 0.01.

Group	Breed	Code	weight (kg)	Length (cm)	Chest circumference (cm)	Height (cm)
Large-size	Min	MZ	227.10	152.20	147.30	89.10
	Hetaodaer	HT	147.05	143.85	120.30	92.10
	Sutai	ST	198.56	172.45	149.54	87.28
	Rongchang	RC	170.60	148.40	130.30	76.00
Small-size	Xiang	XI	43.94	92.78	80.76	47.44
	Bamaxiang	BX	34.80	75.28	76.56	40.87
	Wuzhishan	WZ	28.17	69.55	70.38	48.13
	Diannanxiaoer	DN	48.81	91.18	84.99	51.46

Table 1. Classification and body size of Chinese local pig populations used in this study.

Principal component analysis and population genetic structure analysis

We performed Principal Component Analysis (PCA) on the SNP data obtained after quality control using Plink v1.90 software, and visualized it using the ggplot2 package in R v4.2.0. Additionally, we utilized ADMIXTURE v1.3.0 software to analyze the population structure of these eight local Chinese varieties, and the population structure was plotted using R v4.2.0.

Selective signal analysis and enrichment analysis

We employed two methods to identify the selection signals in large and small pigs. Firstly, we utilized VCFtools v4.2.0 (parameter "-fst") software to analyze the Fst (Fixation Index) between the large and small pigs, and selected these sites with the top 1% of Fst values as potential candidates. Additionally, we employed the XP-CLR (cross-population composite likelihood ratio) software developed by Chen[22] to detect the selection signals (parameter "-w1 0.005 200 2000 -p0 0.95"). This method detects the selected signals based on the difference in allele frequency between populations, and these sites with the top 1% XP-CLR values were considered as potential selected sites.

Subsequently, we utilized the 500 kb downstream overlapping region of the likely selected SNP sites identified by Fst and XP-CLP as the selected region. We then conducted a search for candidate genes in the selected area using the Ensemble Biomart biological information website (http://www.ensembl.org/biomart).

To further analyze the functions of these candidate genes, we conducted a GO enrichment analysis using the DAVID database (http://david.ncifcrf.gov/home.jsp) and a KEGG pathway enrichment analysis using KOBAS 3.0 (http://kobas.cbi.pku.edu.cn/kobas3).

Gene interaction network analysis

We selected terms and pathways with p-values less than 0.05 to identify key genes within significant signaling pathways. The OmicsNet 2.0 online website (https://www.omicsnet.ca/OmicsNet/UploadView.xhtml) was utilized for the analysis and visualization of the protein-protein interaction (PPI) network, filtering out the key genes. Finally, relevant literature was consulted to identify the candidate genes most strongly associated with pig body size traits.

Results

Significance test of pig population scale data

Significant differences were observed in the four traits of body weight, body length, chest circumference, and body height between the large and small pig populations ($P<0.01$, $P<0.001$). The results, depicted in Fig. 1, validate the accuracy and reliability of our criteria for categorizing pig population sizes.

SNP quality control

The dataset used in this study consisted of 129 Chinese local pigs, encompassing 61,773 SNP loci. Subsequent analysis was carried out using 48,288 high-quality SNP loci, after excluding loci on abnormal and sex chromosomes, as well as loci with a minor allele frequency (MAF) below 0.01 and a typing success rate below 0.9.

Principal component analysis and population genetic structure analysis

Based on the two principal components, PCA1 (explaining 8.75% of the variance) and PCA2 (explaining 8.98% of the variance), it is evident that the four small pig populations form a distinct cluster. In contrast, the other four large-size pig populations demonstrate greater dispersion compared to the small-size pig populations, as depicted in Fig. 2. Additionally, the population genetic structure analysis conducted using ADMIXTURE software determined the optimal number of populations to be eight, as illustrated in Fig. 3. When K = 2, a clear separation between the small and large pig populations is observed. When K = 3, the BX subgroup in the small pig population separates first. At K = 4, the MZ subgroup in the large pig population is separated. At K = 5, the DN subgroup separates. At K = 6, the HT subgroup separates. At K = 7, the ST and XI subgroups separate, and at K = 8, the RC subgroup separates. As the value of K increases (from K = 2 to K = 8), the sample population exhibits different characteristics in terms of population structure.

Selective signal analysis

In order to identify genes associated with body size in Chinese local pigs, this study utilized two methods, namely Fst and XP-CLR, to detect genomic selection signals between the large and small pig populations (as illustrated in Fig. 4A and B). The 500 kb regions upstream and downstream of the top 1% SNPs identified by Fst and XP-CLR were designated as potential selected regions. A total of 142 overlapping regions were identified, mainly located on chromosomes 1, 3, 6, 7, and 13. Annotation of these overlapping regions using the Ensemble database (https://www.ensembl.org/biomart/martview/05d3f1407fb70a6cf56988a72f63b164) identified a total of 520 potential candidate genes.

GO enrichment and KEGG pathway enrichment analysis

We identified a total of 520 potential candidate genes and further characterized them by enriching them into 18 significant Gene Ontology (GO) categories and 16 significant KEGG signaling pathways (as depicted in Fig. 5A and B; Tables 2 and 3). Notable enrichments were observed in various cellular compartments such as the nucleus (GO:0005634) and membrane (GO:0016020), as well as functional annotations such as RNA binding (GO:0003723) and mRNA binding (GO:0003729). Additionally, significant enrichments were observed

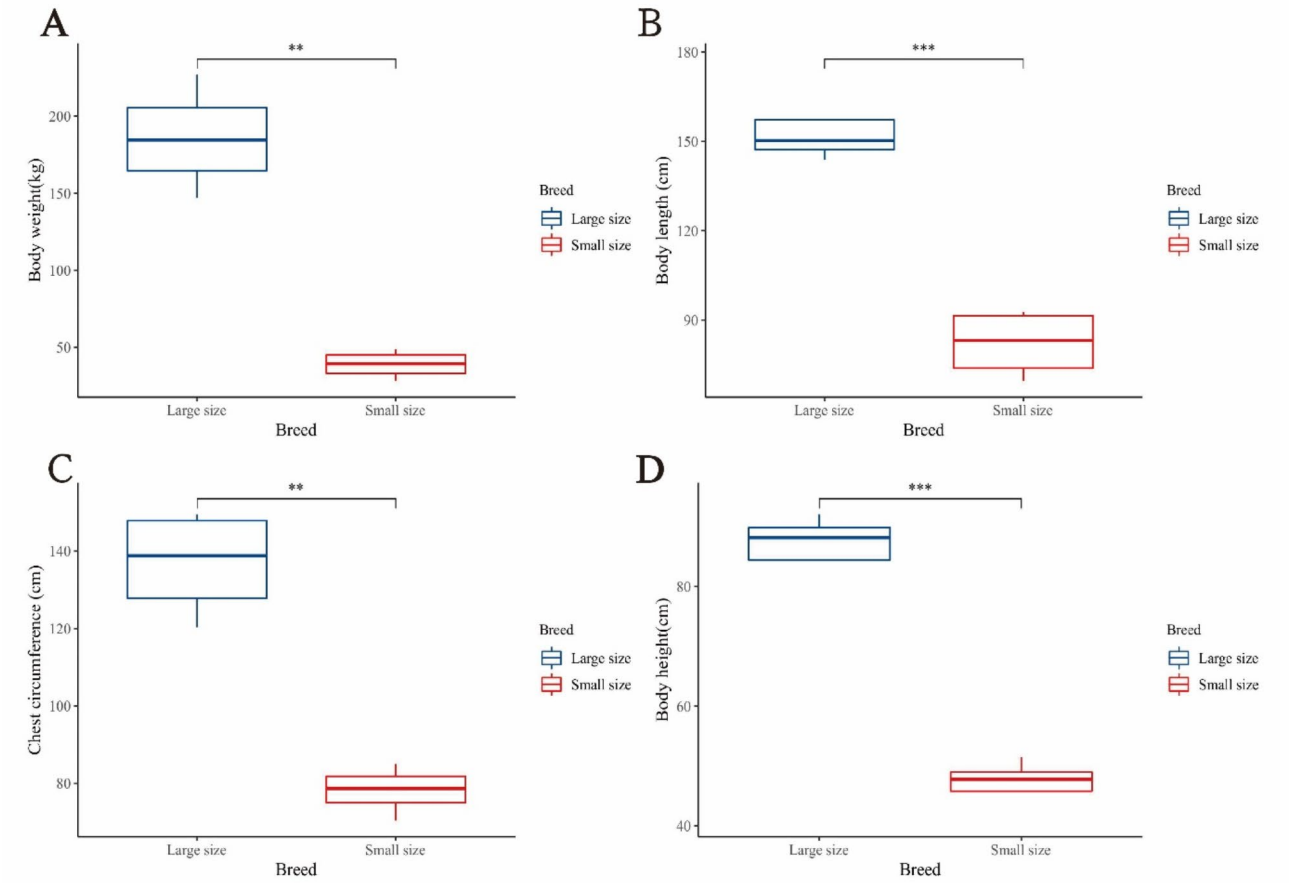

Fig. 1. Significance test statistics of body size data of large and small pigs. Among them, (**A**, **B**, **C** and **D**) were statistically significant test statistics of body weight, body length, chest circumference and body height of large and small pigs, respectively, and ** and *** represented extremely significant differences ($P < 0.01$, $P < 0.001$).

in processes such as nuclear speck (GO:0016607), transmembrane transport (GO:0055085), and negative regulation of ERK1 and ERK2 cascade (GO:0070373) as well as osteoclast proliferation (GO:0002158).

Furthermore, KEGG enrichment analysis revealed that the identified genes were primarily associated with signaling pathways such as PI3K-Akt signaling pathway (ssc04151), Cytokine-cytokine receptor interaction (ssc04060), mTOR signaling pathway (ssc04150), Parathyroid hormone synthesis, secretion and action (ssc04928), Wnt signaling pathway (ssc04310), Ras signaling pathway (ssc04014), Notch signaling pathway (ssc04330), and Calcium signaling pathway (ssc04020).

In reviewing relevant literature, we discovered that the processes related to negative regulation of ERK1 and ERK2 cascade (GO:0070373) and osteoclast proliferation (GO:0002158), as well as the Wnt[23,24], mTOR[25–27], and PI3K-Akt[28–30] signaling pathways described in the KEGG analysis, have significant implications in bone development. These findings suggest that these processes and pathways may play a direct or indirect role in the regulation of bone development, providing essential insights for future investigations into the mechanisms underlying bone development.

Gene interaction network analysis

Furthermore, we integrated the previously obtained GO and KEGG enrichment analysis results to gain deeper insights into the gene terms and pathways that may be associated with bone development. Key genes from these categories and pathways were identified and screened, followed by gene interaction network analysis using the online tool OmicsNet 2.0. Subsequently, candidate genes most strongly associated with body size traits were identified by referencing relevant literature (as shown in Fig. 6). A total of 9 candidate genes were identified as potentially related to pig body size traits (*NPR3*, *TNFSF11*, *FGF2*, *IKBKB*, *SFRP1*, *SPRY1*, *IGF1R*, *LRP6*, *TBC1D7*). Among these, five genes (*NPR3*, *TNFSF11*, *FGF2*, *TBC1D7*, *IGF1R*) have been previously validated as directly related to body size traits in various organisms. The remaining four genes (*IKBKB*, *SFRP1*, *SPRY1*, *LRP6*) are newly discovered candidate genes that may be associated with body size traits in this study, and these genes are directly or indirectly linked to bone growth and development in different animals.

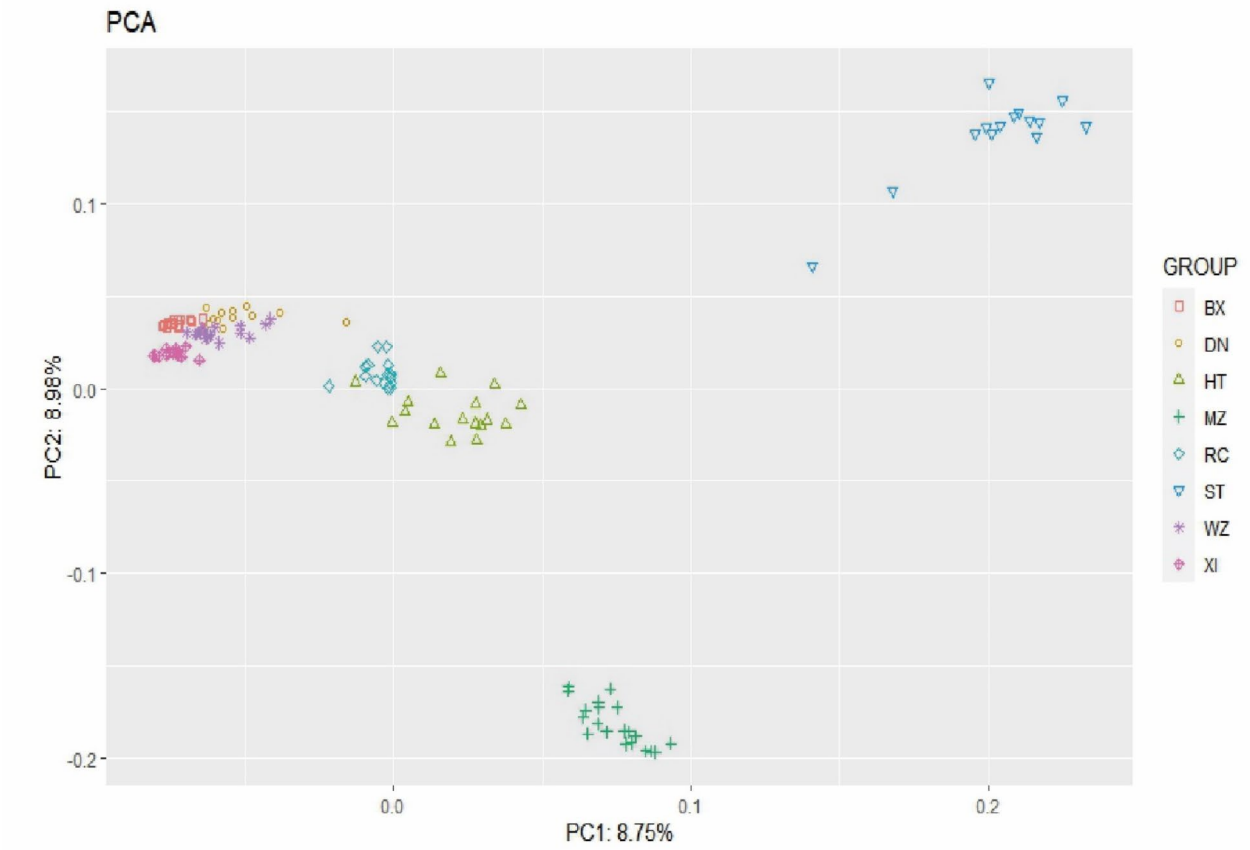

Fig. 2. PCA structure of 8 Chinese local pig breeds.

Discussion

The four small-sized pig breeds selected in this study (WZ, BX, DN, and XI) are all representative of small-sized pig strains within local pig breeds in China. These breeds are characterized by adult body length ≤ 100 cm, adult body weight ≤ 50 kg, and adult body height ≤ 55 cm, and are primarily distributed in southwest China and Hainan Island[21]. On the other hand, the four selected large-sized pig breeds (MZ, HT, ST, and RC) are typical of large pigs found in various regions of China, exhibiting adult body length ≥ 130 cm, adult body weight ≥ 140 kg, and adult body height ≥ 75 cm. These large pig breeds are renowned for high reproduction rates, resilience to coarse feeding, stress resistance, and excellent meat quality, making them exceptionally high-quality local pig breeds[21].

The results indicate an extremely significant difference in body size between the large and small pig populations in this study ($P < 0.01$, $P < 0.001$). Consequently, the 129 individuals from the eight pig breeds were divided into two populations of large and small pigs for analysis. The principal component analysis (PCA) results revealed that the small pig population distinctly clustered together, while the large pig population exhibited greater dispersion, primarily attributed to regional differences resulting in diverse breeding and selection practices, which hindered cohesive clustering. The analysis of population genetic structure demonstrated a clear separation between small-sized pigs and large-sized pigs when K = 2. Furthermore, with an increase in the K value, each group displayed distinct population structure characteristics, thereby supporting the rationale behind the grouping in this study.

In this study, the Fst and XP-CLR methods were employed to identify the selection signals within the large and small pig populations. The Fixation selection index (Fst) is a parameter utilized to gauge population differentiation based on population genetic structure. Fst values range from 0 to 1, where 0 denotes identical genetic structures between the two populations and 1 indicates complete separation. The range $0.05 < Fst < 0.15$ signifies a moderate degree of genetic differentiation, while $0.15 < Fst < 0.25$ indicates a marked degree of genetic differentiation. $Fst > 0.25$ signals a high degree of genetic differentiation between the two populations. In this study, Fst values were computed between the large and small pig populations to assess the level of genetic differentiation. The approach based on the population differentiation index (Fst) is adept at accurately identifying genes that are fixed or have undergone fixation in distinct populations. For example, identified candidate genes potentially influencing litter size traits in sheep by evaluating Fst values between local sheep populations with high and low litter production[31]. Similarly, pinpointed candidate genes associated with the growth regulation of Pacific oysters using both Fst and XP-CLR methods[32]. In this study, the top 1% Fst value exceeded 0.25, indicating a high degree of genetic differentiation between the large and small pig populations. Meanwhile, higher XP-CLR values in both large and small populations denote greater selection intensity at a site between

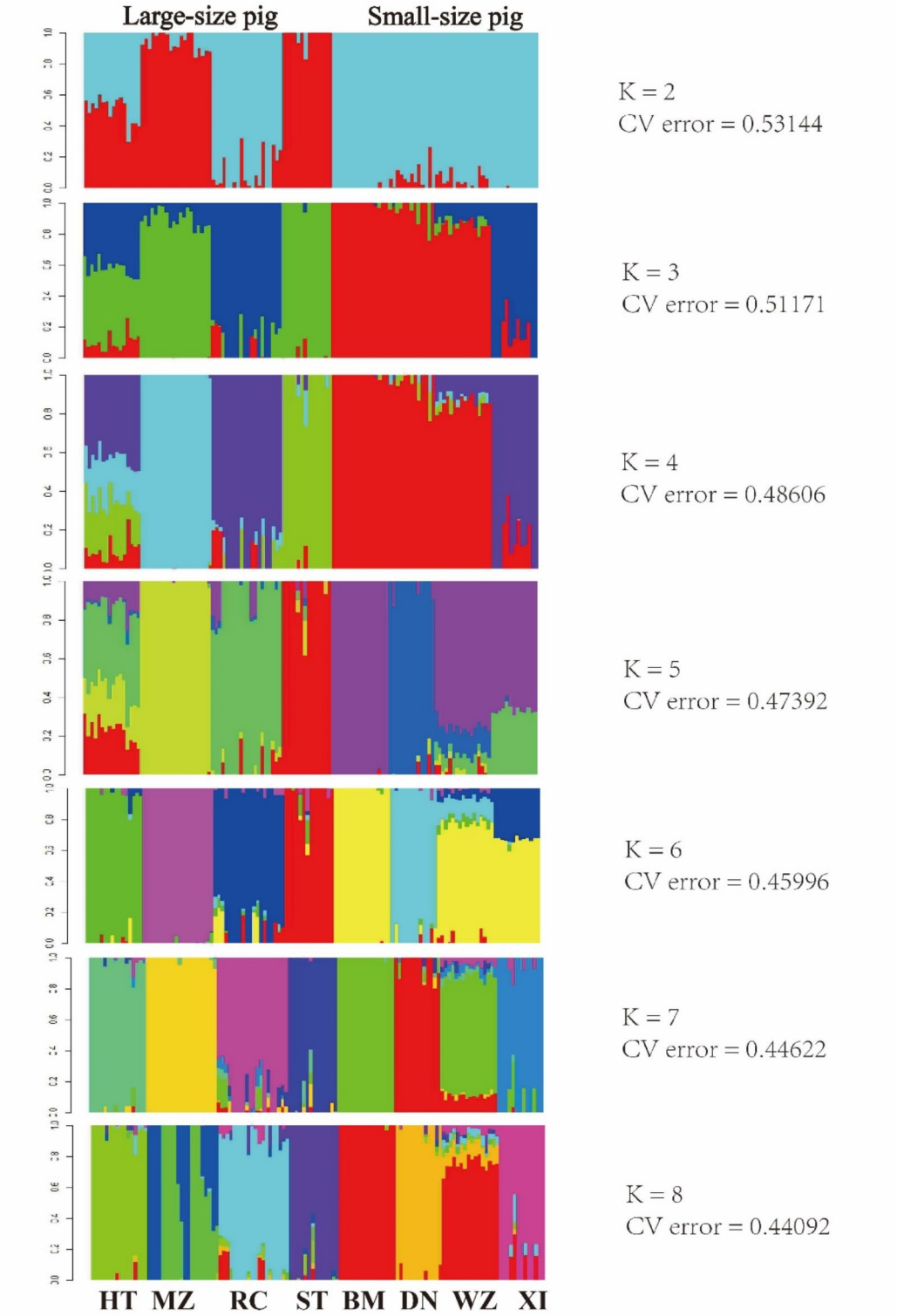

Fig. 3. Population genetic structure characteristics detected by ADMIXTURE when K = 2–8. Each color represents a progenitor group, each vertical bar represents a pig breed, and the length of the color portion of the vertical bar represents the genetic contribution of the progenitor group.

the two populations. Therefore, the combination of these two methods facilitated the identification of selected regions between the large-sized and small-sized pig populations.

To further elucidate the genetic mechanisms underlying pig body size traits, 520 selected genes were identified through gene annotation in the specified region. By employing GO and KEGG enrichment analyses, along with gene interaction network analysis, a total of nine candidate genes associated with pig body size traits

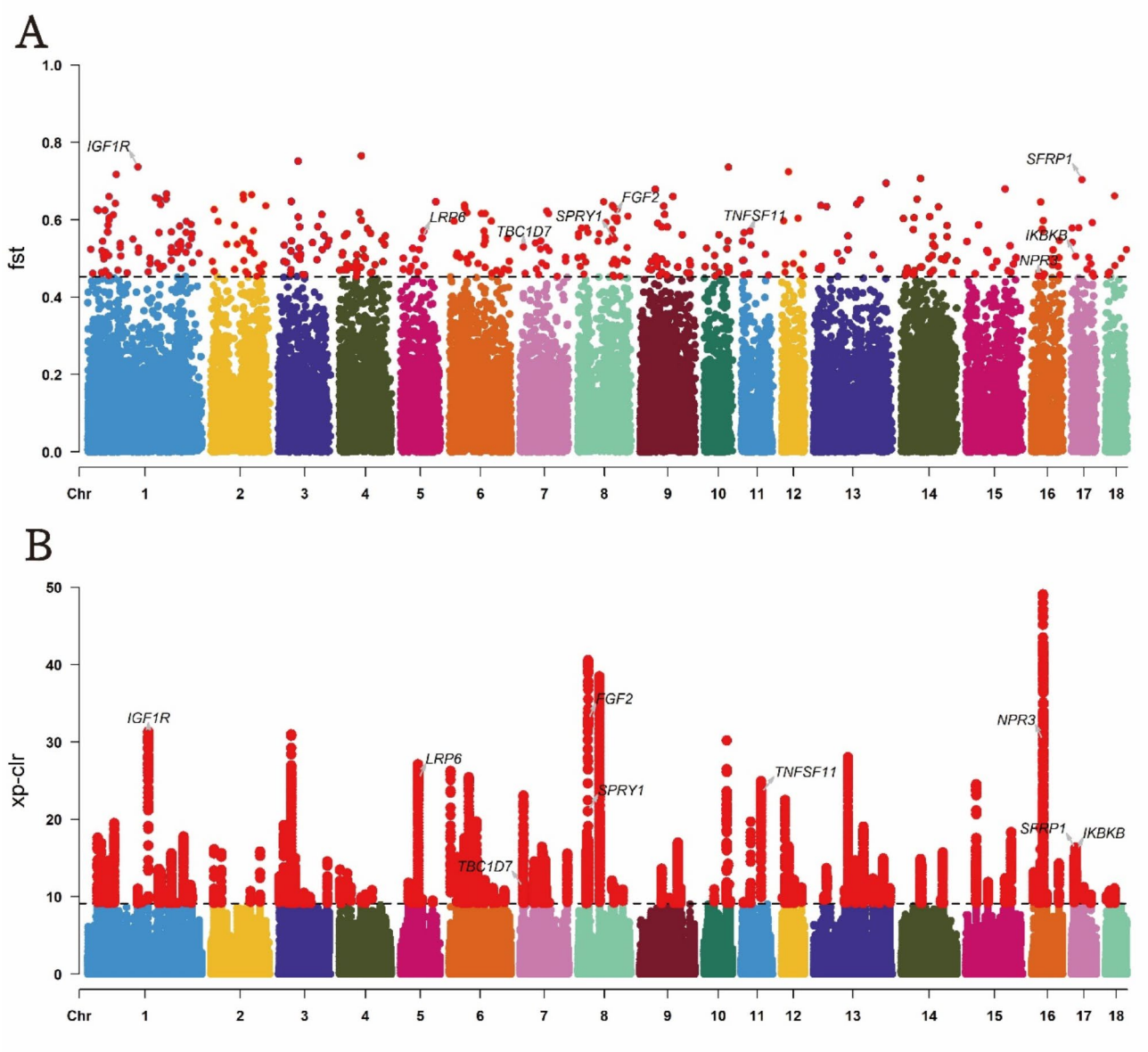

Fig. 4. **(A)** Manhattan map of Fst for large pigs and small pigs. The X-axis represents the position of the snp and the Y-axis represents the Fst value. The black line represents the threshold for statistical significance. **(B)** Manhattan chart of Fst for large and small pigs. The X-axis represents the position of the snp and the Y-axis represents the XP-CLR value. The black line represents the threshold for statistical significance.

were uncovered: *NPR3*, *TNFSF11*, *FGF2*, *IKBKB*, *SFRP1*, *SPRY1*, *IGF1R*, *LRP6*, and *TBC1D7*. Previous studies have directly linked the functions of these genes (*NPR3*, *TNFSF11*, *TBC1D7*, *FGF2*, *IGF1R*) to body size and the growth and development of bones in various organisms. The *NPR3* gene, known as the diuretic natriuretic peptide receptor 3, encodes one of the three natriuretic peptide receptors. Overproduction of natriuretic peptides can result in bone overgrowth and skeletal abnormalities in children, particularly affecting height, vertebrae, and finger length[33]. In 2023, the team led by Huang conducted haplotype analysis using a total of 1,096 high-depth resequencing sample data from 43 pig breeds worldwide, and found that the *NPR3* gene is a key candidate gene that affects bone development and growth in pigs and other animals[34]. Additionally, during the osteogenic differentiation process of human bone marrow mesenchymal stem cells, *NPR3* is up-regulated along with *RUNX2*, *DLX5*, and other osteogenic genes, further indicating that *NPR3* plays an important regulatory role in bone growth[35]. The *TNFSF11* gene, also known as tumor necrosis factor (ligand) superfamily member 11, functions as an essential cytokine for osteoclast differentiation. It induces osteoclast generation, triggers osteoclast precursor cell survival, nucleation, and osteoclast activity. It is currently the only known cytokine that directly stimulates the development and activation of osteoclasts. *TNFSF11* gene strongly promotes the formation, differentiation, and maturation of osteoclasts, inhibiting apoptosis and prolonging their survival[36]. By employing PCR-RFLP combined with DNA sequencing technology, Xianghui detected that the *TNFSF11*

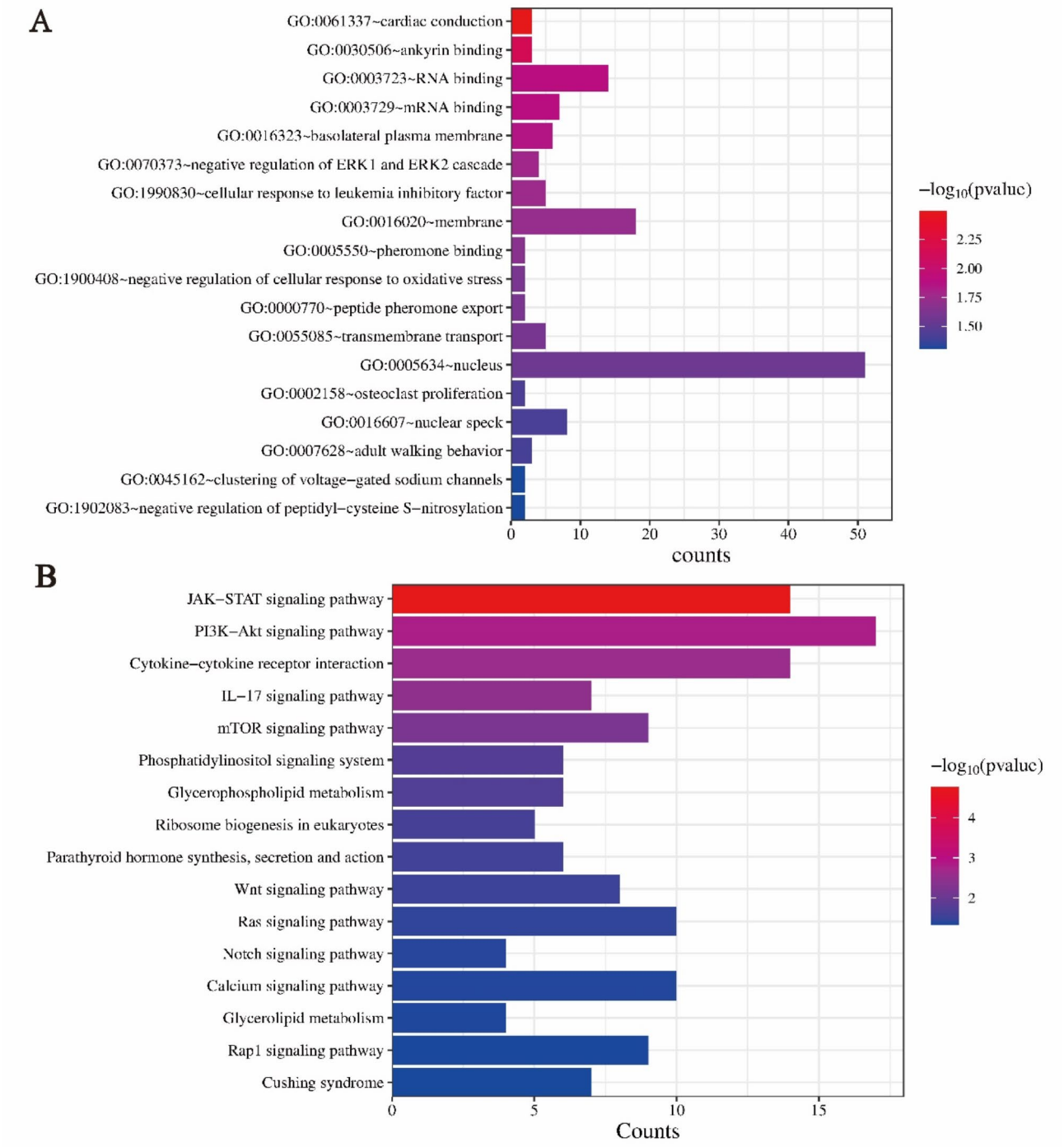

Fig. 5. GO (**A**) and KEGG (**B**) analysis of candidate genes annotated from selected regions in large and small pigs.

gene had a significant impact on body size traits in Qinchuan cattle[36]. This gene was utilized as a candidate gene for early selection of new Qinchuan cattle strains. Furthermore, a study by Burgess utilized scanning electron microscopy to observe that the *TNFSF11* gene could significantly increase the absorption lacunae of osteoclasts on the bone surface[37]. It specifically binds to mature osteoclasts and induces the formation of actin rings in these cells. It is concluded that *TNFSF11* can directly activate mature bone cells and regulate their function. Therefore, the *TNFSF11* gene is also likely to be a key candidate gene affecting the body size of pigs. The *TBC1D7* gene, a member of the TBC1 domain family, plays an important role in regulating cell growth and differentiation. Liu conducted a growth trait correlation analysis on 116 Guanling cattle and found that *TBC1D7* had significant effects on the body oblique length, chest circumference, and rear hip circumference of Guanling cattle[38]. Other studies have demonstrated that the *TBC1D7* gene can form a *TSC* complex with tuberous sclerosis proteins

Categary	Enriched Term	*P*-Value	Gene Symbols
Go term	RNA binding	0.012	*RBFOX1*, *YTHDF3*, *RNMT*, *SAMD4B*, *TARBP1*, *NXF1*, *SYNCRIP*, *RPS16*, *PUF60*, *U2AF2*, *NOVA1*, *TUT1*, *RBM34*, *SNRPA*
Go term	mRNA binding	0.013	*NXF1*, *SYNCRIP*, *RBFOX1*, *YTHDF3*, *NOVA1*, *SAMD4B*, *SUPT5H*
Go term	negative regulation of ERK1 and ERK2 cascade	0.016	*RAPGEF1*, *RANBP9*, *SPRY1*, *PTPN2*
Go term	membrane	0.019	*RALBP1*, *SLC45A2*, *RAB4B*, *SLC44A1*, *SSC5D*, *SCRIB*, *NAPG*, *RAP1GAP*, *TMPRSS15*, *PLPP7*, *RAB31*, *PIEZO2*, *PRRT1B*, *PAF1*, *PLCH1*, *SPRY1*, *PFKP*, *PLEC*
Go term	transmembrane transport	0.025	*SLC22A6*, *SLC25A39*, *SLC44A1*, *SLC4A1*, *SLC22A8*
Go term	nucleus	0.028	*NAA40*, *ZC3H3*, *PDCD5*, *POLB*, *SYNCRIP*, *PRX*, *MEIOC*, *UBTF*, *PHACTR1*, *ZNF444*, *SOX5*, *MEF2A*, *NKX6-3*, *RBFOX1*, *TIGD5*, *WDR37*, *NSUN2*, *PRKCD*, *REV1*, *UBE2QL1*, *SIRT5*, *MTAP*, *SUB1*, *PLAA*, *ZFPM2*, *OTUB1*, *TXNDC9*, *RNMT*, *NOL7*, *PRDM12*, *RLF*, *NXF1*, *SERTAD3*, *SERTAD1*, *MED30*, *PUF60*, *U2AF2*, *ZNF628*, *NLRP5*, *ZNF546*, *ZNF623*, *ZNF787*, *ZNF784*, *SRPK2*, *SUPT5H*, *OXR1*, *NOVA1*, *ZFP64*, *PPT1*, *NCAPD3*, *RCOR2*
Go term	osteoclast proliferation	0.037	*NPR3*, *TNFSF11*
Go term	nuclear speck	0.038	*SRPK2*, *NXF1*, *TMEM179B*, *U2AF2*, *TUT1*, *SCAPER*, *PIAS1*, *TIMM50*

Table 2. GO enrichment term of candidate genes in large-size and small-size pigs.

Categary	Enrichment Pathway	*P*-Value	Gene Symbols
KEGG	PI3K-Akt signaling pathway	0.0016	*TEK*, *LAMC3*, *ITGA2B*, *ITGA11*, *IL4G6PC3*, *FGF2*, *CREB3L2*, *CHUK*
KEGG	Cytokine-cytokine receptor interaction	0.0026	*TNFSF11*, *IL4*, *IL28B*, *IL17RB*, *IL13*, *IFNA1*, *GDF9*
KEGG	mTOR signaling pathway	0.0065	*WNT16*, *TBC1D7*, *SLC3A2*, *SEH1L*, *LRP6*, *IKBKB*, *IGF1R*, *FZD2*, *CHUK*
KEGG	Parathyroid hormone synthesis, secretion and action	0.028	*TNFSF11*, *MEF2A*, *LRP6*, *GNAQ*, *GATA3*, *CREB3L2*
KEGG	Wnt signaling pathway	0.031	*WNT16*, *VANGL2*, *SFRP1*, *MAP3K7*, *LRP6*, *FZD2*, *DKK4*, *APCDD1*
KEGG	Ras signaling pathway	0.036	*TEK*, *RALBP1*, *PLAAT3*, *PAK4*, *IKBKB*, *IGF1R*, *FGF2*, *CHUK*, *CALML4*, *ABL1*
KEGG	Notch signaling pathway	0.040	*NUMBL*, *NCSTN*, *HEY2*, *DLL3*
KEGG	Calcium signaling pathway	0.043	*RYR3*, *PLCD3*, *ITPKC*, *GNAQ*, *GNA14*, *FGF2*, *CHRM5*, *CASQ1*, *CALML4*, *CACNA1D*

Table 3. KEGG enrichment pathways of candidate genes in large-size and small-size pigs.

1 (*TSC1*) and 2 (*TSC2*), participating in cell and body growth and development[39]. In this study, the *TBC1D7* gene was found to be enriched in the mTOR signaling pathway (ssc04150). After forming the TSC complex, *TBC1D7* regulates the core complex 1 (mTORC1) in the mTOR signaling pathway, thereby controlling cell cycle and growth. Additionally, *TBC1D7* also regulates the core complex 2 (mTORC2) in the mTOR signaling pathway, affecting cell skeleton[40,41]. The *FGF2* gene (Fibroblast Growth Factors, FGFs) plays a crucial role in the proliferation, differentiation, growth, and survival of osteoblasts. It has been confirmed that *FGF2* is expressed in osteoblasts and stromal cells, facilitating the recruitment of bone marrow stromal cells[42,43]. Other studies have demonstrated that *FGF2* promotes bone formation and stimulates bone regeneration[44]. *FGF2* also acts as a significant growth factor, regulating the BMP signaling pathway of subchondral bone during chondrocyte differentiation and proliferation. This process promotes the regeneration and repair of articular cartilage[45]. Previous studies have identified the *IGF1R* gene as a key gene determining dog size[46]. *IGF1* primarily binds to insulin-like growth factor receptors *IGF1R* and *IGF2R* to mediate the growth-promoting effects of growth hormone. This regulation influences osteocyte proliferation, differentiation, and apoptosis[47]. Additionally, *IGF1R*-deficient mice exhibit a certain degree of dwarfism and affect chondrocyte proliferation, leading to excessive hypertrophy and apoptosis of the growth plate in the bone elongation region. Knocking out *IGF1R* in mice results in *IGF1* affecting bone cell-mediated bone mineralization, which is crucial for bone formation[47]. Consequently, *IGF1R* may be a key candidate gene influencing pig body size.

The remaining four genes (*IKBKB*, *SFRP1*, *LRP6*, *SPRY1*) are newly discovered candidate genes that may be associated with body size traits in this study. These genes have been found to have direct or indirect relationships with bone growth and development in various animal species. For instance, *IKBKB* gene plays important roles in cell growth, proliferation, differentiation and apoptosis[48]. The absence of the *IKBKB* gene in mice leads to an increase in bone formation rate. The inhibition of *IKBKB* expression promotes the proliferation and differentiation of osteoblasts[49], enhance the synthesis of cartilage extracellular matrix[50].

SFRP1 gene has been shown to inhibit the Wnt signaling pathway, making it a key player in cell apoptosis and early embryonic development. As a negative regulator of the Wnt signaling pathway, *SFRP1* also plays a significant regulatory role in osteoblast differentiation, cell proliferation, and the expression of various bone morphogenetic proteins[51]. The inhibition of *SFRP1* promotes bone formation[52,53]. *LRP6* gene is closely associated with the regulation of bone mineral density and the glucolipid metabolism pathway, and it plays a crucial role in development[54,55]. Studies by Mani demonstrated that conditional knockout of the *LRP6* gene in osteoblasts in rats leads to slow osteoblast development and a significant reduction in bone mass[56] and osteoblasts in embryos[57].

SPRY1 protein in mammals could inhibit cell growth and differentiation by negatively regulating growth factor signaling pathways[58]. Within the pool of skeletal muscle stem cells, the *SPRY1* gene is crucial for maintaining the undifferentiated state and cellular homeostasis, thereby contributing to the normal development and functional maintenance of skeletal muscle[59]. In this study, the *SPRY1* gene was found to be involved in the

Fig. 6. PPI gene interaction network of candidate genes related to body size traits in large and small pigs.

negative regulation (GO:0070373) of the ERK1 and ERK2 cascade. *SPRY1* binds to and inhibits the activity of *ERK1* and *ERK2*, and this negative regulatory activity can impact processes such as cell growth, differentiation, migration, and survival. Moreover, *SPRY1* is implicated in the regulation of FGF-ERK-mediated organogenesis and limb formation[60]. In conclusion, *SPRY1* negatively regulates the osteogenic differentiation of bone marrow mesenchymal stem cells and plays a crucial role in bone formation. It is also likely to have an impact on bone development in pigs.

All the above genes are core genes in the GO and KEGG enrichment pathways and interaction networks, and they are enriched in the Wnt, mTOR, PI3K-Akt and other important signaling pathways. These signaling pathways play a crucial role in growth, development, and basic metabolism. For example, the Wnt signaling pathway is highly conserved in the process of biological evolution, and its activation or inhibition controls the expression of numerous genes related to growth and metabolism[23]. The PI3K-Akt signaling pathway is widely present in cells and plays a pivotal role in the regulation of cell growth, proliferation, and differentiation[61]. Recent studies have indicated its regulatory role in the proliferation, differentiation, and apoptosis of osteoblasts and osteoclasts, as well as its correlation with MAPK, MEK/ERK, mTOR, Src, and other signal transduction pathways[28]. Research on PI3K signaling has shown that Akt and its downstream target proteins play a key regulatory role in bone formation and remodeling. Knockout mice for Akt1/Akt2 exhibited delayed ossification, while Akt1 knockout mice showed shorter bone length and delayed formation of secondary ossification centers[29]. Furthermore, the PI3K-Akt signaling pathway can also enhance the proliferative activity of osteoblasts and regulate their differentiation ability[62]. The mTOR signaling pathway regulates cell size and protein synthesis and controls the growth of limb bones[63,64]. The insulin signaling pathway, also known as the growth signaling pathway, regulates ontogenesis starting from the binding of insulin to receptors, triggering a series of intracellular signal transductions that eventually reach various effector organs to exert their effects[65].

In conclusion, it is reasonable to speculate that these 9 genes are potential candidate genes associated with pig body size traits. In follow-up studies, I propose to implement marker-assisted selection (MAS) to focus on some candidate genes that may be closely related to growth, development and body size characteristics of pigs, such as *IKBKB*, *SFRP1*, *LRP6* and *SPRY1*. By developing molecular markers associated with these genes, breeders can screen out individuals with good growth potential at an early stage. This method not only improves the selection efficiency, but also reduces the reproduction of inferior individuals, thus improving the production performance of the whole population. In addition, we believe that genomic selection (GS) can also further improve the accuracy and efficiency of breeding programs. It is recommended to combine genotype and phenotype data to construct a comprehensive selection model that enables breeders to more accurately predict the growth potential and production performance of individuals. At the same time, genome selection also helps to shorten the selection cycle and achieve a fast-breeding cycle. We are also considering the use of gene editing technologies such as CRISPR-Cas9 for functional validation of key candidate genes. The application of these techniques will help us verify the specific role of these genes in body size and productivity, and then modify the target traits by targeted editing of these genes. This will allow breeders to breed pigs with good production characteristics more quickly.

Conclusion

In this study, we have identified seven potential genomic regions that may be under selection between small and large pigs. Through a comprehensive analysis involving gene annotation, GO enrichment analysis, KEGG pathway analysis, and interaction network analysis, we have identified a total of nine significant candidate genes that could potentially regulate pig body size traits. Among these genes, *NPR3*, *TNFSF11*, *TBC1D7*, *FGF2*, and *IGF1R* have been previously reported to be associated with bone development and body size traits in various animal species. On the other hand, the remaining four genes, *IKBKB*, *SFRP1*, *LRP6*, and *SPRY1*, represent newly discovered candidate genes that may be linked to body size traits based on our investigation.

Data availability

The SNP chip genotype data used in this article was public data (http://dx.doi.org/10.5061/dryad.30tk6).

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Author contributions
Ben Zhang was responsible for the writing and drawing of the manuscript. Ruimin Qiao and Yuan Hong checked the manuscript. All authors reviewed the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

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