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Whole-genome Sequencing Reveals the Genetic Relationships and Selection Signatures of the Min Pig

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Key words
Min pig, Northeast wild boar, Whole genome sequencing, Genetic relationship, Selection signatures

ABSTRACT

The Min pig is indigenous to China. The genetic background of this breed was previously unclear, limiting the utility of the Min pig. In this study, the whole genomes of ten Min pigs and four Northeast wild boars were sequenced and the analysis yielded 8,988,338 non-redundant SNPs plus 1,231,680 InDels. A phylogenetic tree was constructed and a principal component analysis (PCA) was performed based on previously published SNP data from 66 individual pigs. Both analyses indicated the Min pig fell between the European and Asian pigs, while the Northeast wild boar was closely related to the Asian domestic and wild pig breeds. Selective sweep analysis indicated that 181 genes in the Min pig genome had been subjected to selection, including several genes encoding zinc finger proteins. Additional genes associated with myokinesis and lipid metabolism were also identified as under selection. Only SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) interactions in the vesicular transport pathway were identified as under selection (P=0.0029). This study describes the genomic framework of the Min pig and identifies signatures of selection. These results provide a useful genomic background for further studies of the genetic mechanisms associated with important economic traits in the Min pig.

INTRODUCTION

Pigs are one of the most important human-domesticated animals worldwide. Globally, pork accounts for a high proportion of human meat consumption, especially in China. In 2018, 62% of all meat consumed in China was pork. Pigs are also useful animal models for biomedical research (Yang *et al*., 2015). Pigs have been domesticated for thousands of years, and were originally domesticated in multiple regions (Giuffra *et al*., 2000; Bosse *et al*., 2014). Thus, domestic pigs represent a rich genetic resource.

China is one of the original regions of pig domestication. Wild boars began to be domesticated in China about 10,000 years ago (Xiang *et al*., 2017). At present, there are 76 indigenous pig breeds in China. Comparing commercial pigs, the indigenous Chinese breeds are highly fertile, have high disease resistance, tolerate coarse feed, have a slow growth rate, and have a low percentage of lean meat (China National Commission of Animal Genetics Resources, 2011). However, Chinese pig breeds have been subjected to almost no high-intensity artificial breeding programs. As the phenotypic and genetic diversities of Chinese pigs are richer than those of commercial pig breeds, the Chinese pigs represent ideal models for the improvement of economic traits and genetic research (Guo *et al*., 2015; Kirthika *et al*., 2017).

The Min pig is one of the oldest and most famous indigenous pig breeds in China. Although this breed is mainly distributed in the northeastern China, it was introduced into the United States in the last century (Young, 1992). The genetic diversity of the Min pig is very rich. According to historical records, the Min pig migrated from Shandong province to northeastern China with humans, about 400 years ago. However, pigs in northeastern China were independently domesticated 8,000–3,500 BP (before present) (Xiang *et al*., 2017). Therefore, the Min pig may have been crossed with native pig breeds. A recent study identified a long fragment (~48Mb) in the X chromosome of the Min pig that was derived from ancient interspecies introgression. At the same time, the Min pig has two haplotypes: The European and the Northern Chinese (Ai *et al*., 2015). These results illustrate the complexity of the genetic background of the Min pig. Understanding the genetics of this breed is a prerequisite for making full use of its germplasm. The development of next-generation sequencing technology has made sequencing the complete

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genome of a species easy and cheap. Next-generation sequencing is a fast and accurate way to discover the hereditary basis of important breed characteristics.

In this study, the whole genomes of ten Min pigs and four Northeast wild boars were sequenced, and the genomic characteristics of these pigs were analyzed. The SNPs data of Northeast wild boar, Min pig and Yorkshire were compared, because their genomic differences represented the various degrees of artificial selection. The evolutionary identity of the Min pig was investigated by comparing Min pig genomes with the previously published genomes of other pig breeds, including various Chinese indigenous pigs, European pig breeds, and some wild boars. Finally, artificially or naturally selected genes were identified.

MATERIALS AND METHODS

Ethical aspects

The experimental protocol used in this study was approved by the Heilongjiang Academy of Agricultural Sciences, Harbin, China (2015-5).

Animals and sampling

Ear tissue samples were collected from ten Min pigs at the scientific research base of the Institute of Animal Husbandry, Heilongjiang Academy of Agricultural Sciences (Harbin, China). No lineage relationships existed among any of the sampled pigs within three generations. Ear tissue samples were also collected from four Northeast wild boars in the Xiao Xing'an Ling forest zone of Heilongjiang province, China.

Genomic sequencing strategy

DNA was extracted from each sample of ear tissue. Total DNA was isolated using the QIAamp DNA Mini Kit (Qiagen 51304) following the manufacturer's instructions. The genomes were sequenced using an Illumina (San Diego, CA, USA) Hiseq2000 with PE500 libraries, in paired-end 2×100 mode. The raw reads obtained after library construction and sequencing were first cleaned by removing the 3'-ends using cut adapt 1.2.1, allowing a 10% base error rate and requiring ≥10 bp overlap (AGATCGGAAG). Next, sequences were removed if they had an average quality score ≤ Q20 within a 5-bp sliding window or ambiguous ("N") bases, or if they were shorter than 50 bp.

High-quality paired-end reads were mapped to the pig reference genome sequence (Sus scrofa 10.2) using Burrows-Wheeler Aligner (BWA 0.7.12). The reference sequence was indexed and a suffix array (SA) coordinate alignment was generated for each read using the "aln–k2–l32–i5-o1–q0" command. The SA coordinates were converted to chromosomal coordinates and output in sequence alignment map (SAM) format with command "sampe–a2000." The alignments were improved using Picard (Picard tools 1.119) (http://sourceforge.net/projects/picard/): The "Fix Mate Information" command was used to ensure that all mate-pair information was synchronized between each read and its mate pair, and the "Mark Duplicates" command was used to mark potential polymerase chain reaction (PCR) duplicates. Where multiple read pairs had identical external coordinates, only the pair with the best mapping quality was retained, and the others were marked as PCR duplicates. A local realignment of the mapped reads around InDels was performed using GATK (GATK3.0): First, the "Realigner Target Creator" command was used to identify suspicious intervals that were likely in need of realignment, and the "IndelRealigner" command was then used to realign the identified intervals.

SNP and InDel calling from sequencing data

After alignment, SNP calling for the two populations (Min pigs and Northeast wild boars) was performed using the Bayesian approach as implemented in the GATK. GATK uses a Bayesian genotype likelihood model to estimate the most likely genotypes and allele frequencies in a given population simultaneously, and generates an accurate posterior probability of a segregating variant allele at each locus, as well as an accurate posterior probability of each genotype in each sample. The "UnifiedGenotyper" command with parameters "-stand_call_conf 50-stand_emit_conf 30.0" was used to identify SNPs. SNPs were filtered, and only high-quality SNPs were retained. High-quality SNPs were defined as those which met several criteria: coverage depth ≥8 and ≤1000; root mean square (RMS) mapping quality ≥40; variant confidence/quality by depth ≥2; Phred-scaled P-value, using Fisher's exact test to detect strand bias ≤60; consistency of the site with at most two segregating haplotypes ≤13.0; Z-score from the Wilcoxon rank sum test of Alt vs. Ref read mapping qualities ≥-12.5; Z-score from the Wilcoxon rank sum test of Alt vs. Ref read position bias ≥-8.0; and the missing percentage of samples within each group ≤50%.

InDel calling was performed using the "UnifiedGenotyper" command with the parameters "-stand_call_conf50-stand_emit_conf30.0" in GATK. Only high-quality InDels were retained, where high-quality InDels were defined as those which met four criteria: coverage depth ≥8 and ≤1000; Phred-scaled P-value, using Fisher's exact test to detect strand bias ≤200; variant confidence/quality by depth ≥2; and Z-score from Wilcoxon rank sum test of Alt vs. Ref read position bias ≥-20.0.

Genomic population sequences

To explore the genetic relationships among worldwide pig populations, genome sequence data for 66 individual pigs (including indigenous pigs and wild boars) were downloaded from the NCBI Genbank and these sequences were analyzed as described above. A phylogenetic tree was constructed using MEGA5 with the novel sequencing data and previously published data and the application of the neighbor-joining method. Tree reliability was assessed using 1000 bootstrap replicates. The numbers presented for each clade represent bootstrap support values given as percentages.

A principal component analysis (PCA) of the autosomal SNPs was performed using EIGENSOFT 5.0.2. Eigen vectors were obtained from the covariance matrix using reigen in R, and the significance of differences among vectors was determined using the Tracy-Widom test. SNPs were compared among the Min pig, the Northeast wild boar, and the pig dbSNP138 data (NCBI: txid9823). SNPs from the Min pig, Yorkshire, and Northeast wild boar populations were called and filtered as described above, except that coverage depth was set to ≥8 and ≤1000 for Min pigs, ≥8 and ≤500 for Yorkshire boars, and ≥8 and ≤400 for Northeast wild boars.

Selective sweep analysis

A sliding-window approach (500-kb windows sliding in 50-kb steps) was used to calculate polymorphism levels (using θπ pairwise nucleotide variation as a measure of variability), genetic differentiation (Fst), and selection statistics (Tajima's D, a measure of selection in the genome) between the Min pig and the Northeast wild boar. The distributions of the θπ ratios (θπ, Min/θπ, northeast) and the Fst values were used to identify regions with significant signatures of the selective sweeps. An empirical procedure was used to select windows in the empirical distribution with significant low and high θπ ratios (5% left and right tails, where the θπ ratios were 0.728 and 1.367, respectively) in conjunction with significantly high Fst values (5% right tail, where Fst was 0.361). These regions of the genome were considered to have strong signals of selective sweep, and might harbor genes that have undergone selection.

Functional enrichment of selected genes

Functional enrichments in Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, and InterPro domains were explored using the Database for Annotation, Visualization and Integrated Discovery (DAVID). Genes showing evidence of selection were mapped to their respective human orthologs, and the lists were submitted to DAVID to test the enrichment of various biological categories, including GO biological processes (GO-BP), GO molecular functions (GO-MF), KEGG pathways, and InterPro domains. For each test, all known genes were used as the background, and the Benjamini-corrected modified Fisher Exact P values (EASE score) were calculated to determine the significance of the enrichment. Only categories with a P-value <0.05 were considered to be significantly enriched.

RESULTS

Genomic sequencing information

Based on the size of the *Sus scrofa* genome (2.8 Gb), the genomic sequencing coverage was about 7.8× for the Min pigs and 9× for the Northeast wild boars. The analysis identified 8,988,338 non-redundant SNPs plus 1,231,680 InDels from the Min pigs, and 11,345,128 non-redundant SNPs plus 1,121,653 InDels from the Northeast wild boars (Fig. 1A).

In both the domesticated Min pigs and the Northeast wild boars, many SNPs were intergenic and intronic; only a few SNPs were identified in the exonic regions or the untranslated regions (UTRs) (Table I). In the Min pigs, 6,199,989 heterozygous SNPs (68.98% of 8,988,338) and 2,788,349 homozygous SNPs (31.02% of 8,988,338) were identified. In the Northeast wild boars, 6,166,077 heterozygous SNPs (54.35% of 11,345,128) and 5,179,051 homozygous SNPs (45.65% of 11,345,128) were identified. The number of SNPs distributed on each chromosome was positively correlated with chromosome length (Fig. 1C).

Table I. Distribution of SNPs in Min pigs and Northeast wild boars.

Category	Min pig	Northeast wild boar
Exonic	43,108	51,017
nc_transcript	42,276	46,904
Intronic	1,991,046	2,499,832
UTR	31,872	38,293
Splicing	261	308
Upstream/Downstream	574,446	702,931
Intergenic	5,572,866	6,935,773

Exonic, A transcript variant occurring within an exon; nc_transcript, A transcript variant of a non coding RNA; Intronic, A transcript variant occurring within an intron; UTR, A UTR variant of the 5' UTR or 3' UTR; Splicing, A splice variant that changes the 2 base region at the 5' end of an intron; Upstream/downstream, A sequence variant overlaps with the 1 kb region downstream or upstream of a gene; Intergenic, A sequence variant located in the intergenic region, between genes.

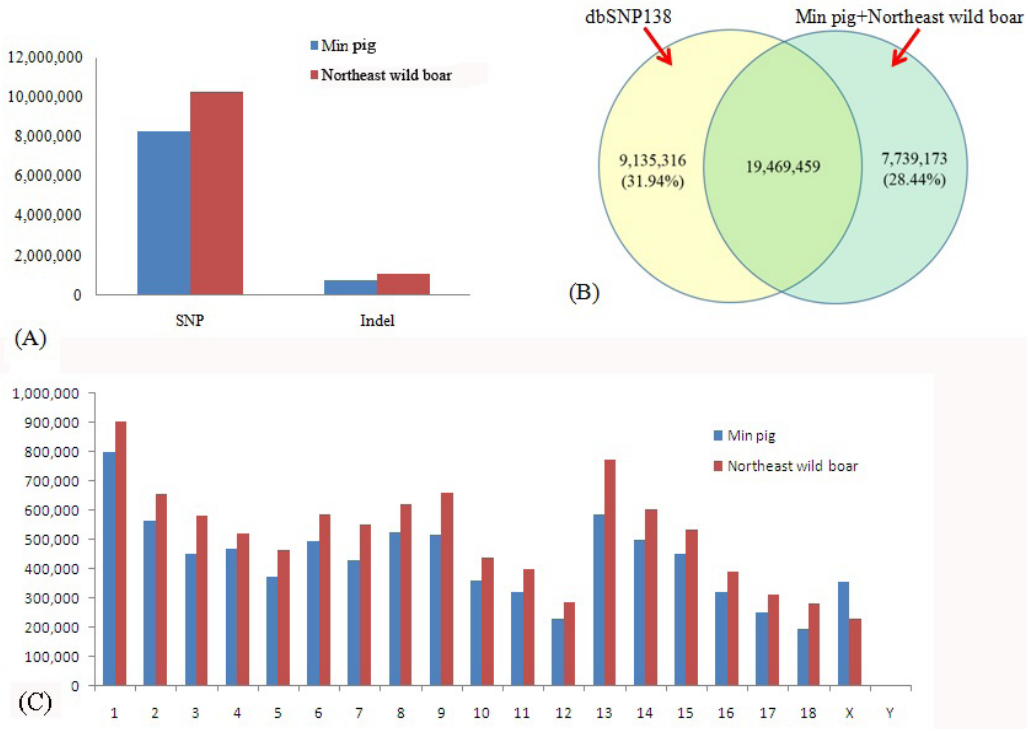

Fig. 1. SNPs and InDels in the Min pigs and the Northeast Wild boars. (A) Numbers of SNPs and InDels in the Min pig and the Northeast wild boar. Blue represents Min pig and red represents Northeast wild boar. (B) Shared and unique SNPs between the Min pig with the Northeast wild boar and the pig dbSNP database (build138): 9,135,316 SNPs are unique in dbSNP138, 7,739,173 SNPs are unique in Min pig and Northeast wild boar, and 19,469,459 are shared between them. (C) The distribution of SNPs on the pig chromosomes. Blue represents Min pig and red represents Northeast wild boar.

SNPs from Min pigs and Northeast wild boars were called and filtered as described above, by setting coverage depth ≥8 and ≤1400. This yielded 27,208,632 SNPs across all fourteen individual genomes, of which 18,190,197 (66.85%) were intergenic, 6,722,554 (24.71%) were intronic, and 143,272 (0.53%) were exonic (Fig. 1B). The identified SNPs were compared to those in the pig dbSNP 138 database, which showed that 68.06% of the SNP variants in pig dbSNP (19,469,459 SNPs) were in the SNP dataset generated in this study, but 7,739,173 of the SNP variants identified in this study were absent from the dbSNP 138 database (Fig. 1B). These novel SNPs increased the number of known porcine genetic variants.

Yorkshire pigs are a mature cultivated pig breed. SNPs from Min pigs, Yorkshire pigs (ERS177318, ERS177319, ERS177320, ERS177322, ERS177325), and Northeast wild boars were called and filtered as described above, with coverage depths set to ≥8 and ≤1000 for Min pigs; to ≥8 and ≤500 for Yorkshire pigs; and to ≥8 and ≤400 for Northeast wild boars. This analysis identified 22,368,881 SNPs across the ten Min pigs; 17,585,639 SNPs across the four Northeast wild boars; and 9,854,629 SNPs across the five Yorkshire pigs. Of the identified SNPs, 5,398,231 were shared among all three breeds, while 6,858,528 SNPs were unique to the Min pigs; 4,642,136 SNPs were unique to the Northeast wild boars; and 1,092,046 SNPs were unique to the Yorkshire pigs.

Phylogenetic analysis and demographic distribution

This study identified 28,411,844 SNPs in the 66 previously published pig genomes. A phylogenetic tree was constructed based on the novel sequencing data generated here and the previously published data (Fig. 2), which shows that the Min pig clustered together with the Northeast wild boars and several European pig breeds (e.g., Duroc, Yorkshire, and Landraces). The constructed phylogeny was then analyzed with the PCA (Fig. 3). In the PCA, the first eigenvector geographically distinguished 36 Asian individuals from 26 European individuals and one African individual; while the second eigenvector captured the biological differences between the pigs. Notably, a previous study also showed that Min pigs have a European haplotype in their X-linked selective sweep region, and this region has an exceptionally low recombination rate (Ai *et al*., 2015).

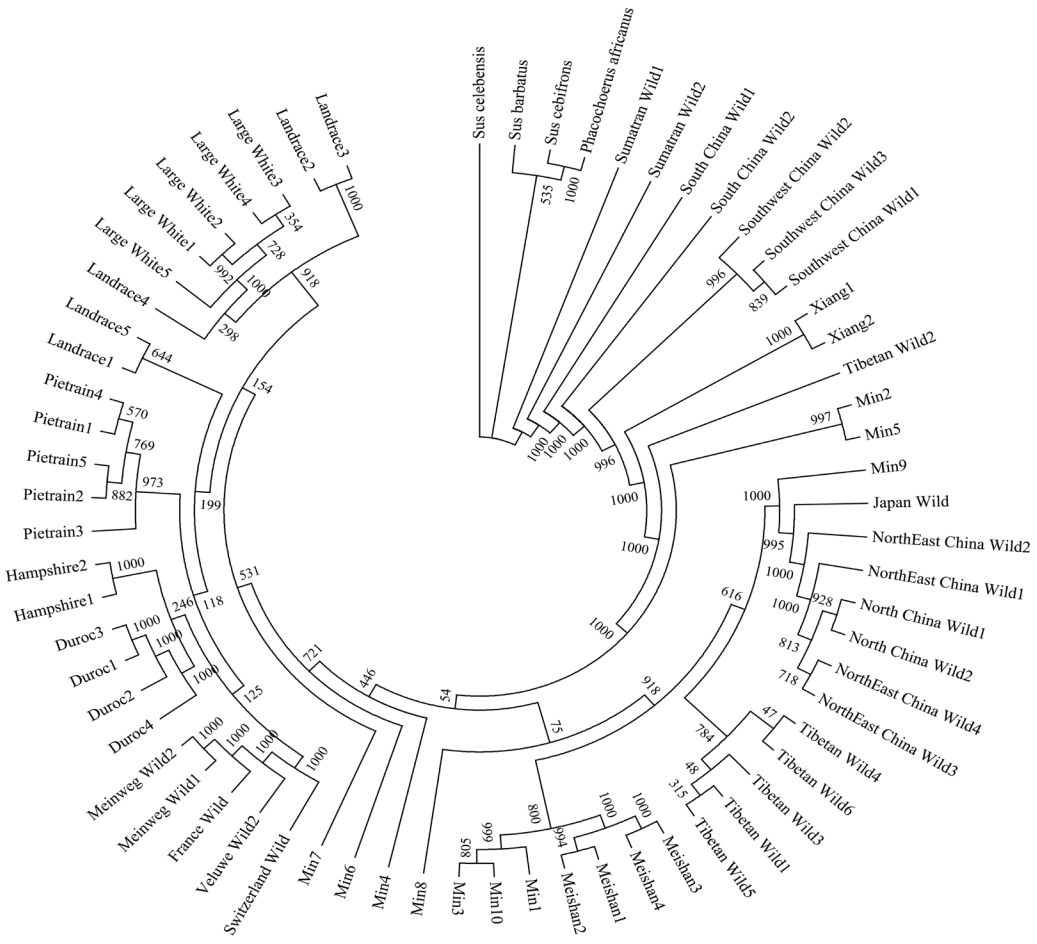

Fig. 2. Phylogenetic tree built using re-sequenced and previously published data. The numbers behind the name of each pig breed represent numbers of individuals. The numbers on each clade represent bootstrap support values. *Sus celebensis*, Warty pig in Sulawesi; *Sus barbatus*, Bearded pig; *Sus cebifrons*, Visayan Warty pig; *Phacochoerus africanus*, Common Warthog; Sumatran Wild, wild boar in Sumatra; South China Wild, wild boar in South China; Southwest China Wild, wild boar in Southwest China; Xiang, Xiang pig, another native pig breed in China; Tibetan Wild, wild boar in Tibetan; Min, Min pig; Japan Wild, wild boar in Japan; North East China Wild, wild boar in North East China; North China Wild, wild boar in North China; Meishan, Meishan pig, another native pig breed in China; Switzerland Wild, wild boar in Switzerland; Veluwe Wild, wild boar in Veluwe; France Wild, wild boar in France; Meinweg Wild, wild boar in Meinweg.

Screening of selected genes

The results of these analyses suggested that the Min pig had slightly lower levels of polymorphism as compared to the Northeast wild boar (median θπ; Min/θπ; Northeast=0.9855) (Fig. 4A). Genomic regions with strong signals of selective sweep in the Min pig spanned 15.71 Mb (0.559% of the genome, containing 181 genes). In Northeast wild boar, the regions with strong signals of selective sweep spanned 29.81 Mb (1.061% of the genome, containing 411 genes) (Fig. 4B). Of the 181 genes showing evidence of selection in the Min pig, 118 have been annotated (Table II). The proteins encoded by these 118 candidate genes included several zinc finger proteins: *ZNF259* (zinc finger protein 259), *ZNF365* (zinc finger protein 365), *ZNF384* (zinc finger protein 384), *ZNF410* (zinc finger protein 410), *ZNF646* (zinc finger protein 646), *ZNF668* (zinc finger protein 668), *ZNF713* (zinc finger protein 713), *ZCCHC9* (zinc finger, CCHC domain containing 9), and *ZBTB11* (zinc finger and BTB domain containing 11). Signatures of selection were also identified in some genes associated with myokinesis and lipid metabolism, including *CKMT2* (creatine kinase mitochondrial 2), *MYO1C* (myosin 1C), *LCN9* (lipocalin 9), *LCN15* (lipocalin 15), APOA5 (apolipoproteinA-V), and CRHR2 (corticotropin- releasing hormone receptor 2). In addition to these, other genes identified as having been under selection were VRTN (vertebrae development

homolog), which is related to the number of vertebrae (Ren *et al*., 2012), and OPA1 (opticatrophy 1), which is associated with autosomal dominant optic atrophy (Jin *et al*., 2015). In addition, *OR2B6* (olfactory receptor, family 2, subfamily B, member 6) and *ARHGAP 32* (Rho GTPase activating protein 32) overlapped with the top signals of selective sweep.

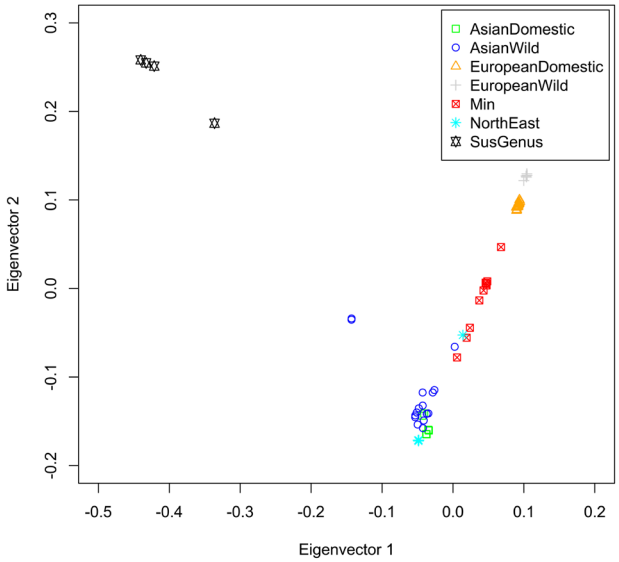

Fig. 3. Principal component analysis (PCA). Each point represents an individual, and 80 individuals were divided into seven pig populations. Different populations are represented by different colors and shapes. Green represents Asian domestic pigs, blue represents Asian wild boars, yellow represents European domestic pigs, gray represents European wild boars, red represents Min pig, wathet blue represents Northeast wild boars, and black represents the domestic pig's close relatives.

Of the 411 genes showing evidence of selection in the Northeast wild boar, 279 were annotated. These genes were mainly related to natural selection. Some of them are associated with the nervous system, including olfactory receptors (e.g., *OR2B6*, *OR8b8*, and *OR8b4*) (Monahan *et al*., 2015), *PDCL3* (Srinivasan *et al*., 2015), and *TAC3* (Shankar *et al*., 2015); some are related to immunity, including NLR family genes, pyrin domain containing genes (e.g.,*NLRP4* and *NLRP11*) (Eibl *et al*., 2012; Tadaki *et al*., 2011), *IFN-OMEGA-2* (Zhao *et al*., 2009), *Foxn1* (Ruan *et al*., 2014), and *OSCAR* (Barrow *et al*., 2015); some are related to male reproduction, including *HSD17B6* (Ishizaki *et al*., 2013), *ACRBP* (Vilagran *et al*., 2013), *ELSPBP1*, and *PRM1* (D'Amours *et al*., 2012; Dogan *et al*., 2015); and some are related to mitochondrial energy metabolism, including *COX6A1* and *NDUFA9*. Of all the genes showing evidence of selection, only *Arhgap32* was identified in both the Min pig and the Northeast wild boar.

Functional enrichment of selected genes

The enrichment of genes showing evidence of selection in Min pigs and Northeast wild boars was explored with DAVID. Some pathways, GO terms, and Interpro domains were significantly enriched (P<0.05). In the Min pig, only soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) interactions

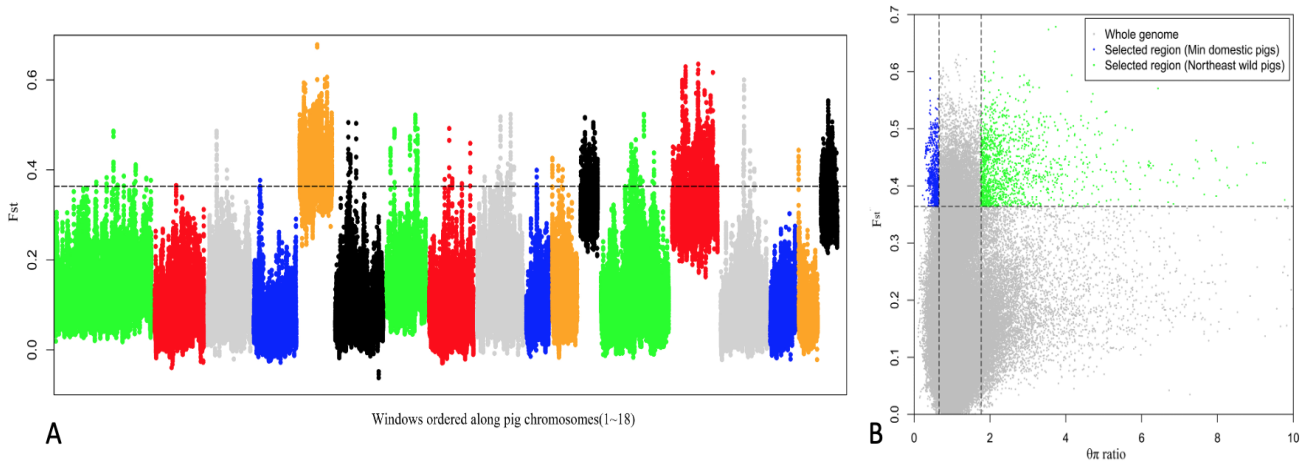

Fig. 4. Selective sweeps in the Min pig and the Northeast wild boar. (A) θπ distribution across the pig chromosomes. (B) The ratio of Fst to θπ. The significant low and high θπ ratios of 0.728 and 1.367, and significantly high Fst values of 0.224 were used to screen the selected region of Min pig and Northeast wild boar. Blue points represent the selected region of Min pig, and green points represent the selected region of Northeast wild boar.

Table II. Key selected genes in Min pigs.

Gene	Fst	Theta_Pi	Description	Pathways from biosystems
SPTSSB	0.41	0.37	Serine palmitoyltransferase, small subunit B	Metabolism of lipids and lipoproteins
LDHB	0.31	0.61	L-Lactate dehydrogenase	Glucagon signaling pathway
HIBCH	0.31	0.43	3-Hydroxyisobutyryl-CoA hydrolase	Amino Acid metabolism, Carbon metabolism
PSPH	0.29	0.62	Phosphoserine phosphatase	Amino acid synthesis and interconversion (transamination)
CAPZA2	0.29	0.35	Capping protein (actin filament) muscle Z-line, alpha 2	Advanced glycosylation endproduct receptor signaling
ZCCHC9	0.28	0.58	Zinc finger, CCHC domain containing 9	Unknown
PLCH1	0.28	0.58	Phospholipase C, eta 1	D-myo-inositol (1,4,5)-trisphosphate biosynthesis
CKMT2	0.28	0.61	Creatine kinase, mitochondrial 2 (sarcomeric)	Arginine and proline metabolism; Creatine metabolism
PISD	0.28	0.62	Phosphatidylserine decarboxylase	FOXA1 transcription factor network; Glycerophospholipid biosynthesis
ZNF384	0.26	0.56	Zinc finger protein 384	Unknown
ZNF713	0.26	0.51	Zinc finger protein 713	Gene Expression
MYO1C	0.26	0.55	Myosin IC	Insulin Signaling
CERS6	0.25	0.58	Ceramide synthase 6	Ceramide biosynthesis
SEMA3C	0.24	0.34	Semaphorin 3C	Axon guidance
ZNF410	0.23	0.33	Zinc finger protein 410	Unknown
COQ6	0.23	0.29	Coenzyme Q6 monooxygenase	Metabolism of lipids and lipoproteins; Ubiquinol biosynthesis
LCN15	0.23	0.48	Lipocalin 15	Transmembrane transport of small molecules
VRTN	0.21	0.32	Vertebrae development homolog	Unknown
LCN9	0.21	0.62	Lipocalin 9	Transmembrane transport of small molecules
STRBP	0.21	0.36	Spermatid perinuclear RNA binding protein	Unknown
OPA1	0.20	0.63	OPA1 mitochondrial dynamin like GTPase	Regulation of Apoptosis
APOA5	0.20	0.60	Apolipoprotein A5	Chylomicron-mediated lipid transport
CRHR2	0.20	0.53	Corticotropin releasing hormone receptor 2	Corticotropin-releasing hormone signaling pathway

in the vesicular transport pathway were identified (P=0.0029); and the *VAMP1*, *STX4*, *STX6*, and *STX1B* genes were involved. Several GO terms and Interpro domains were enriched, including SNAP receptor activity (enriched with *STX4*, *STX6*, and *STX1B*), phospho lipid binding (enriched with *PIK3C2G*, *ZFYVE28*, *APOA5*, *TRIM72*, *ARHGAP32*, and *SNX19*), thiolester hydrolase activity (enriched with *PSMD14*, *USP13*, *ACOT12*, and *HIBCH*), and progesterone metabolic processes (enriched with *AFP* and *DHRS9*). In the Northeast wild boar, only the ABC transporter pathway was identified (P=0.0036). However, many genes were identified in the GO and Interpro analyses (Table III).

DISCUSSION

Many diverse pig breeds are indigenous to China. The appearances, body sizes, and productive capacities of these breeds differ significantly due to contrasting geographical environments, customs, and ethnicities. Pigs have been an important human food resource for many thousands of years. However, due to slow growth and a low percentage of lean meat, many indigenous pig breeds have been replaced by introduced pig breeds, including the Landrace, Duroc, and Yorkshire. Some Chinese pig breeds have become useful resources for academic research, including the Wuzhishan pig (Xu *et al*., 2019), Laiwu pig (Liu *et al*., 2019), Tibetan pig (Zhang *et al*., 2019), Taihu pig (Wang *et al*., 2019), and the Min pig (Liu *et al*., 2017).

In this study, the complete genomes of ten Min pigs and four Northeast wild boars were sequenced. Northeast wild boars have inhabited the same localities as Min pigs for hundreds of years. Here, 6,858,528 SNPs unique to the Min pig were identified, as were 4,642,136 SNPs unique to

Table III. GO, pathway and interpro analysis results.

Category	Term	P-Value	Genes cluster in this term
Min pig			
GOTERM_MF_FAT	SNAP receptor activity	0.0114	STX4, STX6, STX1B
GOTERM_MF_FAT	Phospholipid binding	0.0123	PIK3C2G, ZFYVE28, APOA5, TRIM72, ARHGAP32, SNX19
GOTERM_MF_FAT	Thiolester hydrolase activity	0.0451	PSMD14, USP13, ACOT12, HIBCH
GOTERM_BP_FAT	Progesterone metabolic process	0.0465	AFP, DHRS9
KEGG_PATHWAY	SNARE interactions in vesicular transport	0.0029	VAMP1, STX4, STX6, STX1B
INTERPRO	Target SNARE coiled-coil region	0.0158	STX4, STX6, STX1B
INTERPRO	Potassium channel	0.0213	KCNAB1, KCNAB3
INTERPRO	HAD-superfamily hydrolase	0.0213	PSPH, PHOSPHO2
Northeast wild boar			
GOTERM_MF_FAT	ATPase activity, coupled to transmembrane movement of substances	0.0073	ABCA3, ABCB1, ABCC5, ATP5F1B, ABCB4, ABCC1, ATP6V0C
GOTERM_BP_FAT	Negative regulation of protein kinase activity	0.0129	PDCD4, PDPK1, CBLC, PAK2, TSC2, NF2
GOTERM_BP_FAT	Negative regulation of protein kinase cascade	0.0197	SOCS1, TSC2, NF2, IL1RL1
GOTERM_BP_FAT	Cell-substrate adhesion	0.0207	TNFRSF12A, BCAM, PDPK1, ANTXR1, PKD1, ECM2
GOTERM_BP_FAT	Response to endogenous stimulus	0.0304	TXN2, SLC18A2, CAV2, SI, ADCY9, PDPK1, SOCS1, ABCC5, MGP, MSI1, EIF2B5, PLA2G1B, ABCB4
GOTERM_BP_FAT	Regulation of cell-substrate adhesion	0.0373	TSC2, COL8A1, NF2, ECM2
GOTERM_BP_FAT	Ion transport	0.0401	SLC40A1, CAV2, GABRD, KCNV1, SCN3B, KCTD5, CNNM3, PKD1, TRPM4, STEAP1, KCNK1, CNNM4, HTR3C, SLC12A6, NMUR2, SLC8A2, ATP5F1B, SLC12A7, SLC17A7, ATP6V0C
GOTERM_BP_FAT	DNA integration	0.0403	HMGA1, PPFIBP1, XRCC4
GOTERM_BP_FAT	Neurotransmitter transport	0.0444	SLC18A2, PCLO, PPFIA3, SLC6A20, SLC17A7
GOTERM_BP_FAT	Regulation of phosphorus metabolic process	0.0490	PDCD4, PKMYT1, SOCS1, CBLC, BMPR1A, NF2, AXIN1, PLA2G1B, ADCY9, SMG6, PDPK1, MAP3K21, TSC2, PAK2
KEGG_PATHWAY	ABC transporters	0.0036	ABCA3, ABCB1, ABCC5, ABCB4, ABCC1
INTERPRO	ABC transporter	0.0021	ABCA3, ABCB1, SMC3, ABCC5, ABCB4, ABCC1

the Northeast wild boar. More SNPs were identified in the Northeast wild boar than in the Min pig, and more SNPs were identified in the Min pig than in the Yorkshire pig. This pattern was consistent with the degrees of artificial selection imposed upon each breed.

The phylogeny developed here suggested that European pig breeds (including wild boars and domestic pigs) are distinct from Asian pig breeds (including wild boars and domestic pigs), consistent with previous studies (Choi *et al*., 2014). We were surprised that Min pig fell between the European and Asian pig breeds, and did not form a cluster with the Asian pig breeds like the Northeast wild boar. We therefore speculate that Min pig might have hybridized with European pig breeds at some point since the earliest domestication of this breed. Currently, the Min pig is primarily bred in Heilongjiang province, in the northernmost part of China. Only the Heilongjiang River separates Heilongjiang province from Russia, and cross-border trade has been common for hundreds of years. In addition, the Heilongjiang River is frozen for almost four months of the year, providing convenient conditions for gene flow among different pig breeds. Previous studies of the 60K SNP genotype data indicated that pig breeds indigenous to Russia, Belorussia, Kazakhstan, and Ukraine

clustered with European pig breeds and that the Min pig clustered with Chinese breeds, including the Luchuan, Erhualian, Laiwu, Hetao large ear, and Tibetan (Traspov *et al*., 2016). However, in the neighbor-joining tree produced here, the Min pig is the Chinese pig breed that is closest to the European pig breeds.

Next-generation sequencing technology allows a clearer understanding of the origin and evolution of a species (Groenen *et al*., 2012; Li *et al*., 2013). The current study found little genetic overlap among the genomes of different pig breeds. It is possible that many genomic differences exist among breeds due to different breeding goals and living environments (Choi *et al*., 2015; Ma *et al*., 2015). These results also highlight the molecular mechanisms that potentially underly the various pig phenotypes. For example, the Yorkshire and Landrace breeds have both been subjected to intensive selection for fast growth, a high percentage of lean meat, and large litters. Therefore, the identification of the genes *GHR*, *IGF1R*, and *IGF2R* as showing evidence of genetic selection was not surprising (Wang *et al*., 2018). In addition, the genes of Tibetan wild boars, which live in high altitudes, show evidence of selection on genes associated with hypoxia, olfaction, energy metabolism, and the drug response (Li *et al*., 2013).

Min pig has a very special trait, which is cold tolerance. It lives in high-latitudes (47–53° N, 121–141° E), in regions that are very cold, with long winters. Min pigs are well adapted to temperatures that range from 39°C in summer to -25°C in the winter. Thus, that the identification of genes associated with myokinesis and lipid metabolism as being under selection was not surprising. Indeed, muscle and adipose tissues are the primary producers of heat in cold environments (Virtanen, 2014). Genes such as CKMT2, MYO1C, LCN9 and LCN15, are all related to energy metabolism. *CKMT2* is responsible for the transfer of high energy phosphate from mitochondria to the cytosolic carrier (Qin *et al*., 1999). *MYO1C* is a widely expressed motor protein that links the actin cytoskeleton to cell membranes, acts as a slow transporter, and is associated with numerous cellular processes (Greenberg *et al*., 2012). The functions of *LCN9* and *LCN15* remain unknown, but another gene in the same Lipocalin superfamily (*LCN2*) is known for regulating thermogenic activation in adipose tissue (Flower, 2000; Guo *et al*., 2016). *APOA5* is related to lipid transfer (Lim *et al*., 2014). *CRHR2* plays an important role in white fat tissue loss and lipid metabolism under hypoxia (Xiong *et al*., 2014).

Another new discovery in this study was that many zinc finger protein genes were selected, such as *ZNF259*, *ZNF365*, *ZNF384*, *ZNF410*, *ZNF646*, *ZNF668*, and *ZNF713*. ZNF proteins play important roles in many biological processes (Cassandri *et al*., 2017).

In pathway analysis, only one SNARE interaction pathway in the vesicular transport pathway was identified; and this pathway included the *VAMP1*, *STX4*, *STX6*, and *STX1B* genes. *VAMP1*, a synaptobrevin family protein, is one of the main components of the SNARE complex. In mammalian cells, the SNARE complex consists of more than 60 member proteins (Salpietro *et al*., 2017). The SNARE complex drives membrane fusion (Zhang, 2017). However, it is unclear why this pathway was identified as under selection in the Min pig. This section pressure may be related to the high adaptability of this breed.

CONCLUSION

In this study, a total of 8,988,338 non-redundant SNPs plus 1,231,680 InDels were identified in Min pig. Both the PCA and phylogenetic tree results supported the notion that the genetic relationship of Min pig is between Asian pigs and European pigs. A total of 181 genes were subjected to selection pressure, especially some genes related to energy metabolism, such as *CKMT2, MYO1C, LCN9, LCN15, APOA5* and *CRHR2*. In addition, many *ZNF* protein genes were also under selection pressure.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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