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Genome-wide detection of runs of homozygosity in Ding'an pigs revealed candidate genes relating to meat quality traits

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Abstract

Background Ding'an (DA) pig, a native Chinese breed, is renowned for its excellent meat quality, disease resistance, high reproductive performance, and adaptability. Its meat quality traits hold significant economic value. However, its conservation population has been declining due to the impact of commercialized breeds and African swine fever, which is not conducive to its development and utilization.

Results This study utilized whole-genome resequencing data from 15 DA pigs to reveal their genetic characteristics and current resource status. We analyzed the length, number, and distribution patterns of Runs of Homozygosity (ROH) in DA pigs, as well as high-frequency ROH regions. The results identified 23,208,098 single nucleotide polymorphisms (SNPs), 4,497,242 insertion and deletion (InDels), 13,622 copy number variation (CNVs), and 399,934 structure variation (SVs). Further analysis revealed relatively high genetic diversity and low inbreeding levels in DA pigs. Through functional gene enrichment analysis of high-frequency ROH regions, we identified multiple candidate genes associated with specific traits in DA pigs, including meat quality (*ANKRD1*, *CPNE5*, *MYOM1*), fat deposition (*OBSCN*, *MAPK4*, *PNPLA1*, *PACSIN1*, *GRM4*), and skeletal muscle development (*LRPPRC*, *WNT9A*).

Conclusions This study conducted whole-genome sequencing and ROH analysis on DA pigs, revealing high genetic diversity and low inbreeding levels within the population. Through functional gene enrichment analysis of high-frequency ROH regions, we identified multiple candidate genes associated with meat quality, fat deposition, and skeletal muscle development. These findings not only enhance our understanding of the genetic mechanisms underlying the unique traits of DA pigs but also provide valuable insights for practical applications. Specifically, the identified candidate genes and genomic regions can guide conservation efforts to maintain genetic diversity and mitigate inbreeding risks. Meanwhile, these genetic insights can be integrated into breeding programs to improve meat quality and other economically important traits, thereby supporting the sustainable development and utilization of DA pigs.

Keywords Ding'an pigs, Runs of homozygosity, Selection signature, Meat quality traits, Inbreeding coefficients

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Background

Ding'an (DA) pig, named after its origin in Ding'an County, Hainan Province, is one of the four major groups of Hainan pigs. It is renowned for its excellent meat quality, strong disease resistance, high reproductive performance, and robust adaptability [1]. Among these characteristics, its meat quality traits are of particularly high economic value. DA pigs is rich in various nutritional components, including proteins, amino acids, and fatty acids. These proteins not only form body tissues but also repair damaged tissues and provide energy. Moreover, these nutrients can effectively reduce the occurrence of muscle symptoms, which is especially crucial for the elderly. Therefore, gaining a deeper understanding of meat quality-related genes will provide valuable information for DA pig breeding programs.

However, in recent years, due to the impact of commercialized breeds and African swine fever, the conservation population of DA pigs has been continuously decreasing, potentially leading to inbreeding issues. Insufficient control of inbreeding within the DA pig population may result in reduced genetic diversity and variability, which is detrimental to the protection of this valuable genetic resource and the sustainable development of the livestock industry. Consequently, the conservation of DA pigs has become an urgent and important task.

Runs of Homozygosity (ROH) are continuous homozygous segments in the genome, formed by identical haplotypes inherited from common ancestors. These segments can be inherited within a population and provide insights into population history [2]. Typically, long ROH segments indicate recent common ancestry, while short ROH segments suggest ancient common ancestors in the lineage. ROH detection effectively estimates inbreeding levels across the entire genome, making it a valuable method for assessing animal inbreeding [3]. By studying ROH patterns and distribution in pig populations, precise genetic breeding and conservation strategies can be developed to maintain genetic diversity and mitigate the adverse effects of inbreeding.

Numerous studies have utilized ROH to reveal genetic mechanisms in various animals, including cattle [4], pigs [5] and chickens [6]. For example, Xu et al. [7] conducted ROH analysis on Xidu black pig genomes, identifying candidate genes associated with fat deposition, muscle development, and reproduction. Wu et al. [8] analyzed ROH in Wannan Black pigs using genome resequencing data, revealing high inbreeding levels due to ancient inbreeding. Tian et al. [9] analyzed ROH in Wenchang chickens using genome resequencing data, identifying candidate genes related to growth and meat quality traits, providing valuable reference for Wenchang chicken conservation and utilization. In summary, these studies have demonstrated that ROH analysis can effectively identify genomic regions under selection and highlight candidate genes associated with economically important traits. While these studies have demonstrated the effectiveness of ROH analysis in identifying genomic regions under selection and revealing candidate genes associated with economically important traits, research on the genetic characteristics of DA pigs is still relatively limited.

This study aims to fill this gap by investigating the genomic characteristics of DA pigs and assessing their inbreeding levels using ROH and single nucleotide polymorphism (SNP) information. We employ ROH and nucleotide diversity (pi) to detect selection signatures and identify candidate genes potentially associated with meat quality traits in DA pigs. By revealing the genetic characteristics of DA pigs, this study contributes to understanding their current genetic resources and provides valuable references for future genetic improvement and efficient utilization.

Materials and methods

Sample data sources and variant detection

The whole-genome resequencing data of DA pigs used in this study were obtained from previous studies in our laboratory [1]. The samples of DA pigs came from a local farm in Hainan Province, China. In simple terms, the DNA was extracted by a commercial kit (Tiangen Biotech Co., Ltd., Beijing, China) and then quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The DNA was randomly fragmented using an ultrasonic high-efficiency sample processing system. After fragment selection, fragments of approximately 500 bp were obtained. Subsequently, end repair was performed on the fragments, an "A" base was added to the 3' end, and library adapters were ligated to both ends. After single-strand separation, circularization, and rolling circle amplification, the library was converted into DNA nanoballs (DNBs). Following quality control, libraries that met the requirements were subjected to sequencing. The average sequencing depth was more than 15×. Additionally, we collected whole-genome resequencing data from 77 pigs, comprising 52 individuals from local breeds (Lingao and Tunchang pigs) and 25 from commercial breeds (Large White and Landrace pigs) (see Supplementary Table S1 for details). The data processing pipeline was as follows: First, quality control of the resequencing data was performed using fastp (v0.20.1) [2]. Subsequently, the processed data were aligned to the pig reference genome (Sscrofa11.1, https://www.ncbi.nlm.nih.gov/assembly/GCF_000003025.6/, accessed on December 5, 2023) using BWA (v0.7.17) [3]. The paired data were then sorted using SAMtools (v1.9) [4], duplicates were removed using Picard (v1.9) [5], and variant calling for DA pig samples was conducted using GATK (v4.1.6.0) [6].

The variant analysis process included: identifying single nucleotide polymorphisms (SNPs) and insertions and deletions (InDels) from all variants using the SelectVariants tool; detecting copy number variations (CNVs) using CNVnator (v0.4.1) [7], and identifying structural variations (SVs) using Delly (v0.7.8) [8]. We filtered for very chromosomal regions (e.g. sex chromosomes) when identifying and analyzing insertions and deletions, copy parameters, and structural variations. CNVnator captured the read-depth signal in genomic regions with different CNVs and genotyped both deletions and duplications with correction for GC bias. For each pig, the aligned files were processed to identify genome-wide CNVs using standard parameters and 200-bp bins. The minimum segment size was set to -1 kb, and all other parameters were set to default [9]. Delly's SV with a "QUAL" score below 20 was discarded to ensure the credibility of structural variation detection. To ensure high-quality variant sites, stringent filtering criteria were applied to SNPs, with a maximum missing rate set at 0.9 and a minor allele frequency (MAF) of 0.01. Considering the relative rarity of InDel, CNV, and SV variants, all relevant information was retained to maintain data integrity and accuracy.

Analysis of genetic diversity and linkage disequilibrium

The genetic diversity of DA pigs was estimated using Expected Heterozygosity (H_E), Observed Heterozygosity (H_O), MAF, nucleotide diversity (*pi*) and effective population size (Ne). H_E, H_O and MAF were calculated using PLINK (v1.9) [10], and *pi* was calculated using VCFTOOLS (v0.1.16) [11]. Ne was calculated using SNeP (v1.1) software [12]. The squared correlation between pairs of SNPs (r^2) was used to measure Linkage disequilibrium (LD) decay. r^2 was calculated individually for each breed using PopLDdecay [13] with default parameters. To visualise the LD decay, the distribution of LD was plotted using the R package ggPlot 2. In addition, we also conducted calculations of H_E, H_O, MAF, *pi*, Ne, and LD for local breeds such as Lingao pig (LG) and Tunchang pig (TC), as well as commercial breeds including Large White pig (LW) and Landrace pig (LR), for comparison with the genetic diversity of DA pigs.

ROH detection and analysis

The ROH on all autosomes of each individual was identified using PLINK (v1.90) [10] software with the following parameters: (1) the shortest length that defines the ROH is 50 Kb; (2) only ROHs longer than 25 SNPs are detected; (3) the sliding window size is 50 SNPs, moving one SNP at a time; (4) the maximum spacing of consecutive SNPs in the ROH is 100 Kb; and (5) a maximum of one heterozygous SNP and five missing SNPs are allowed to appear in the sliding window. We referred to some other literatures [14] and ROH fragments extracted from sequence data were further classified into 0.05–0.5 Mb, 0.5-1 Mb and >1 Mb according to their lengths. total number of ROHs and lengths of all individuals were counted.

Genomic inbreeding coefficient

Two methods (F_{ROH} and F_{HOM}) were used to estimate inbreeding coefficients for the DA pig population. First, F_{ROH} was calculated according to the method proposed by McQuillan et al. [15], which is defined as the ratio of the total length of ROH to the total length of the genome covered by the analysed SNP or sequence. The formula is:

$$F_{ROH} = \frac{\sum L_{ROH}}{L_{auto}},$$

Where ΣLROH represents the sum of the lengths of ROH fragments on the autosomes of a single individual, and Lauto represents the total physical length of the autosomes of the analysed species, which in this study was 2265.77 Mb. In addition, We statistically analysed ROH fragments of different lengths and calculated the correlation coefficients between these segments.

Secondly, the F_{HOM} was calculated using PLINK (v1.9) software [10] with the specific formula:

$$F_{HOM} = \frac{N_{HOM}}{N}$$

Where N is the total number of SNPs detected and NHOM is the number of homozygous SNPs detected.

Identification of high-frequency ROH candidate genes

To investigate candidate genes associated with meat quality traits in DA pigs, we employed two population-based selection signature methods: ROH and *pi*. We further analyzed the top 1% candidate loci using two cross-signal methods. Based on the pig reference genome (Sscrofa 11.1), we annotated the selected loci for gene identity using ANNOVAR (version: June 7, 2020) [16].

Subsequently, we conducted functional enrichment analysis using DAVID (version 6.7, https://david.ncifcrf.gov/) to further elucidate the functions of candidate genes [17]. Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways with P-values less than 0.05 were considered significantly enriched. Finally, we conducted a literature review on the functions of the enriched genes to gain insights into the unique genetic characteristics of DA pigs.

Results

Variant detection analysis

Following rigorous quality control, we identified a total of 24,499,654 SNPs, 4,497,242 InDels, 13,622 CNVs, and 399,934 SVs from the whole genomes of 15 DA pigs. Genomic variations exhibited distinct chromosomal distribution patterns (Fig. 1). SNPs were unevenly distributed, with higher density on chromosomes 1, 7, and 9. InDels clustered prominently on chromosomes 1, 6, 7, 8, 9, 10, and 17. CNVs showed elevated mutation rates on chromosomes 2, 5, 6, and 12, while SVs were concentrated on chromosomes 5, 6, 7, and 9. These prominent peak regions may serve as focal points for future research. To ensure data accuracy and reliability, we implemented stringent quality control measures for each type of genomic variation. The samples and variation

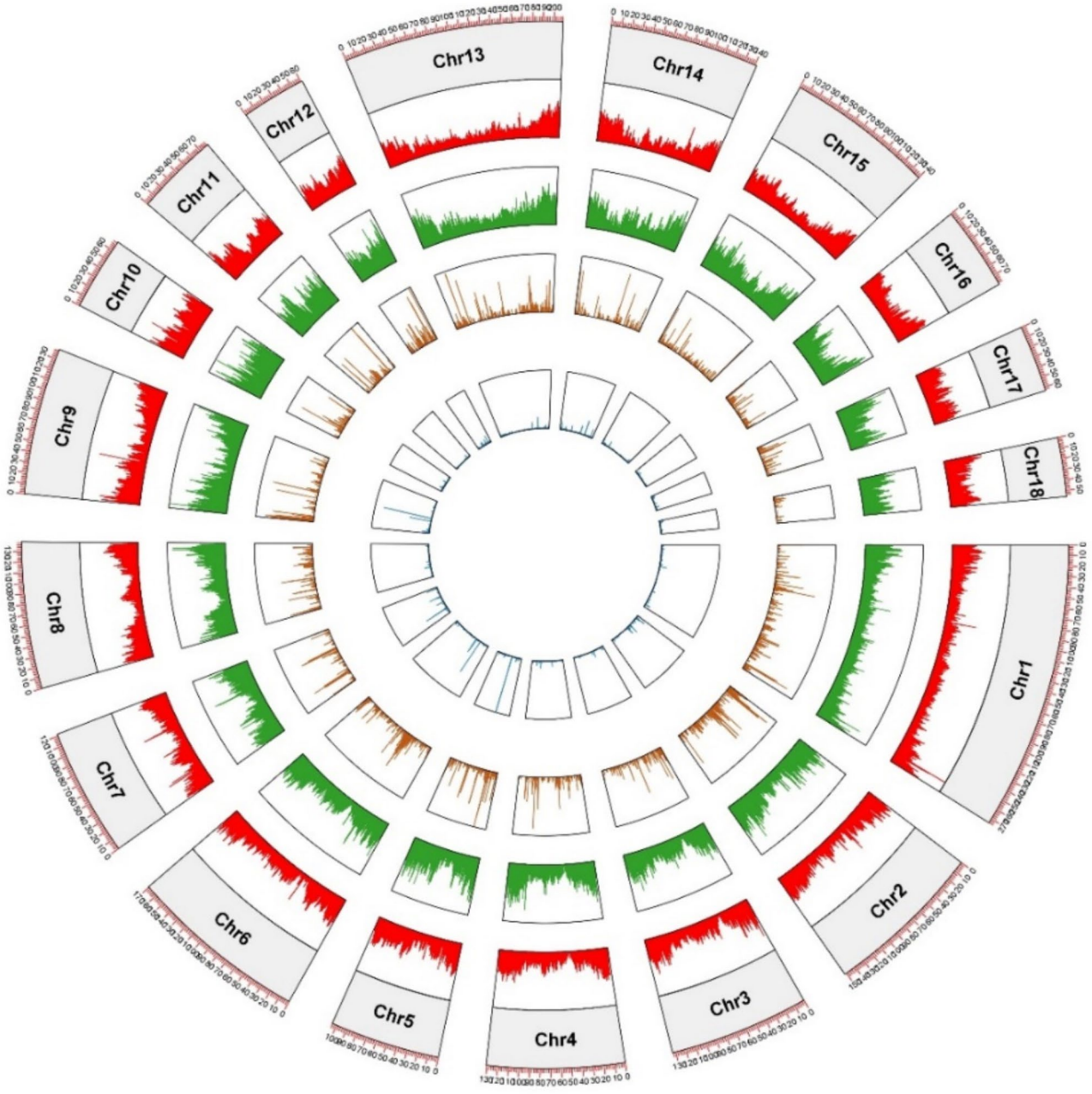

Fig. 1 Density Plots of SNP, InDel, CNV and SV Variations in Ding'an pigs, where the outermost circle represents the length of each chromosome, with a window size of 100,000. The first inner circle represents the density distribution of SNPs, the second inner circle represents the density distribution of InDels, the third inner circle represents the density distribution of CNVs, and the fourth inner circle represents the density distribution of SVs. Abbreviations: SNP = Single Nucleotide Polymorphism; InDel = Insertion and Deletion; CNV = Copy Number Variation; SV = Structure Variation

Table 1 Genetic diversity index of different populations of pigs

Breed	Number of Individuals	H_E	H_O	MAF	pi	Ne
DA	15	0.364 ± 0.120	0.407 ± 0.173	0.273 ± 0.130	0.0034 ± 0.0018	35
TC	32	0.313 ± 0.148	0.309 ± 0.165	0.229 ± 0.141	0.0033 ± 0.0022	73
LG	20	0.299 ± 0.155	0.339 ± 0.199	0.217 ± 0.144	0.0017 ± 0.0008	43
LR	13	0.125 ± 0.177	0.108 ± 0.166	0.091 ± 0.141	0.0014 ± 0.0011	46
LW	12	0.123 ± 0.178	0.135 ± 0.207	0.089 ± 0.143	0.0014 ± 0.0010	38

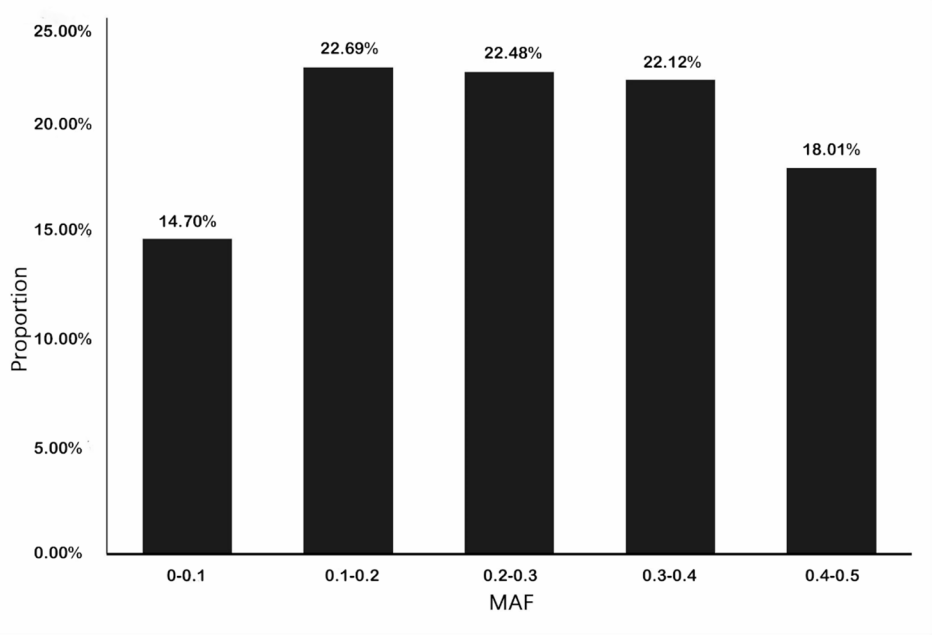

Fig. 2 Distribution of minor allele frequency in Ding'an pigs. The horizontal coordinate indicates the value of MAF, ranging from 0 to 0.5, and the vertical coordinate indicates the proportion. Abbreviations: MAF = Minor Allele Frequency

data that passed quality control were subsequently used for statistical analysis of each phenotype.

Analysis of genetic diversity

To understand the genetic diversity of DA pigs, we calculated the genetic diversity index for each of the five pig breeds: *Ho*, H_E, MAF, *pi*, and Ne (Table 1). Genetic diversity indices varied significantly across breeds. Expected heterozygosity (H_E) ranged from 0.123 in LW pigs to 0.364 in DA pigs, whereas observed heterozygosity (H_O) spanned from 0.108 in LR pigs to 0.407 in DA pigs, highlighting their higher genetic variability. Domestic breeds generally exhibit higher H_E and H_O values compared to foreign breeds. The MAF ranges from 0.089 (LW) to 0.273 (DA), and *pi* ranges from 0.0014 (LR and LW) to 0.0034 (DA). Notably, DA pigs consistently show higher genetic diversity indices across all measures compared to other breeds. Ne can be used to assess the impact of genetic drift and inbreeding on populations. DA pigs have a relatively small Ne value of 35, indicating a small population size that may be susceptible to issues such as genetic drift. Therefore, strengthening conservation efforts for DA pigs is crucial to prevent a decline in their genetic diversity. The MAF distribution for each marker in DA pigs, revealing a relatively uniform distribution (Fig. 2).

Table 2 Descriptive statistics of ROH number and length (Mb)

ROH length(Mb)	ROH number	Percent(%)	Mean length(Mb)
0.05–0.5	36,454	96.87%	0.129 ± 0.088
0.5-1	1046	2.78%	0.651 ± 0.125
> 1	130	0.35%	1.439 ± 0.573
Total	37,630	100.00%	0.148 ± 0.149

LD decay analysis revealed a decline in r^2 values as SNP distances increased (Figure. S1). At an r^2 threshold of 0.3, the physical distances between paired SNPs varied from 0.44 Kb in TC pigs to 178 Kb in LW pigs. For DA pigs, this distance was 6.4 Kb, reflecting their rapid LD decay and high recombination rates compared to commercial breeds. In conclusion, these results all indicate that the DA pig's genetic diversity is at a high level.

Length and number distribution statistics for ROH

In this study, we performed a statistical analysis of ROH length and quantity in 15 DA pigs (Table 2). A total of 37,630 ROH were detected, with an average of 2,508 ROH

per pig, and an average total length of about 2.19 Mb. The average length of each ROH segment is 0.148 Mb, ranging from 0.05 Mb to 4.90 Mb. ROH segments of 0.05-0.5 Mb account for 96.87%, 0.5-1 Mb for 2.78%, and those exceeding 1 Mb for 0.35%. The longest ROH segment was observed on chromosome 1, containing 10,748 SNPs.

In addition, we also analyzed the relationship between the total number of ROH in a single DA pig and the total length of the genome covered by ROH, as well as the chromosomal distribution of the number and coverage of ROH in all individuals (Fig. 3) (Fig. 4). Each individual contains an average of 2,509 ROH, with a maximum of 3,670 and a minimum of 1,610. The ROH lengths range from 200 to 600 Mb. Considerable variation in the total number and length of ROH among individuals is observed, consistent with findings from other studies. Chromosome 1 harbors the highest number of ROH (5,361), while chromosome 18 has the least (768). The highest ROH coverage rate was observed on chromosome 8 (4.18%), while chromosome 10 showed the lowest (1.61%).

Inbreeding coefficients of DA pigs

The inbreeding coefficients were analysed using different calculation methods (Table 3). The F-values of this DA pig were not the same. The mean value of F_{ROH} (ALL) (F_{ROH} (ALL) means inbreeding coefficient of all lengths of ROH.)calculation was the largest with 0.164 and the mean value of F_{ROH} (> 1 Mb) was the smallest with 0.006. The maximum value of inbreeding coefficient of F_{ROH} (ALL) was 0.269 and the minimum value was 0.088, its range is 0.087–0.26. The mean value of F_{HOM} was – 0.119, it ranges from – 0.225 to 0.038. In addition, we performed correlation analyses on ROH fragments of different lengths (Fig. 5). The strongest correlation was between F_{ROH} (0.05-0.5 Mb) and F_{ROH} (ALL) (at 0.98), while the correlation between F_{ROH} (> 1 Mb) and F_{ROH} (ALL) was weak (0.68). The results suggest that the 0.05-0.5 Mb and 0.5-1 Mb ROH fragments may be the main source for calculating F_{ROH}.

Candidate genes and pathway analysis

To identify candidate genes associated with meat quality traits in DA pigs, we employed two population-based selection signals: ROH and *pi*. We identified potentially selected SNPs and marked the top 1% regions for each method. The intersection of these top 1% regions yielded 510 candidate genes (Fig. 6). Additionally, we calculated SNP frequencies within ROH, selecting the top 1% (present in at least 53.8% of samples). For *pi* values, we performed a logarithmic transformation with a threshold of 3.4, represented by a red line in the figure.

We conducted GO functional enrichment and KEGG pathway enrichment analyses on these 510 annotated genes using the DAVID database. The results, shown in Fig. 7, reveal significant enrichment in 17 GO terms and

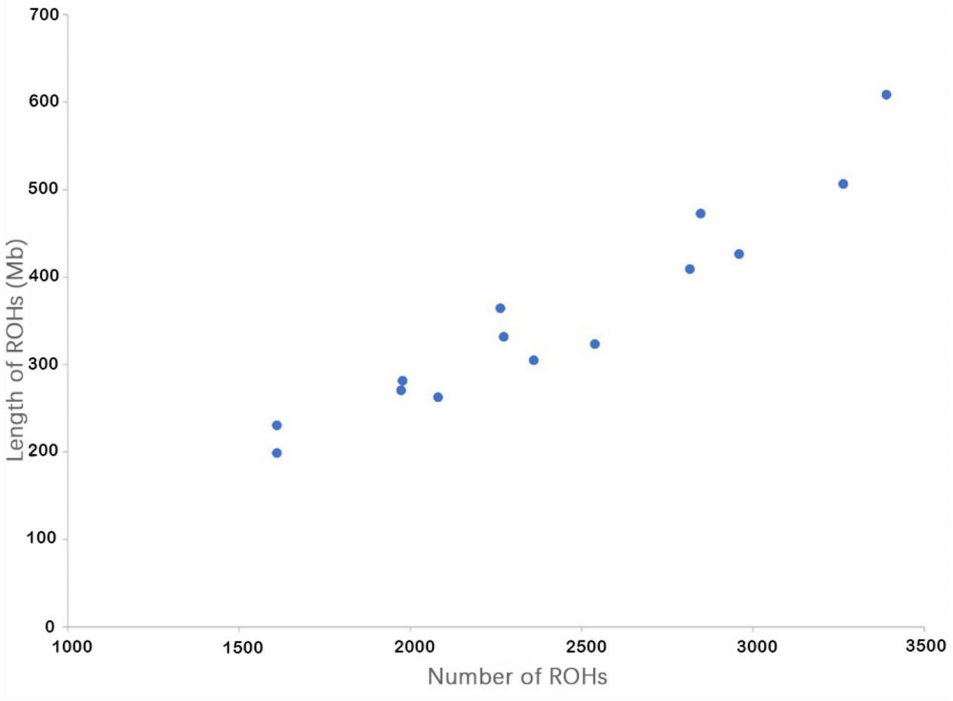

Fig. 3 Relationship between total number of ROH fragments (x-axis) and total ROH genome length (Mb) (y-axis) for all Ding'an pig individuals. Each point represents an individual. Abbreviations: ROH = Runs of Homozygosity

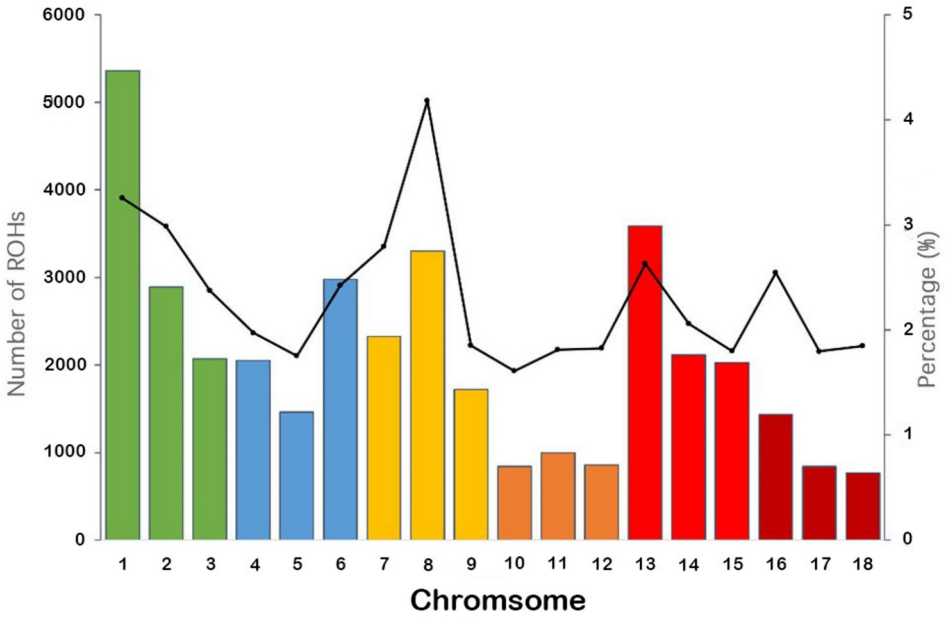

Fig. 4 The number of ROHs and percentage coverage per chromosome in the Ding'an pig population. The vertical bars represent the total number of ROH per chromosome, while the line indicates the percentage of the chromosome covered by ROH

Table 3 Average genomic inbreeding coefficients of F_{HOM} and F_{ROH} for ROH of different length types in DA pigs

F_{ROH}(0.05-0.5 Mb)	F_{ROH}(0.5-1 Mb)	F_{ROH}(>1 Mb)	F_{ROH}(ALL)	F_{HOM}
0.139 ± 0.003	0.02 ± 0.007	0.006 ± 0.005	0.164 ± 0.054	-0.119 ± 0.081

4 KEGG pathways (Supplementary Table S2). GO clustering analysis was mainly enriched in the regulatory processes (GO:0050714 ~ positive regulation of protein secretion; GO:0007346 ~ regulation of mitotic cell cycle; GO:2000049 ~ positive regulation of cell-cell adhesion mediated by cadherin; GO:0010524 ~ positive regulation of calcium ion transport into cytosol; GO:2000279 ~ negative regulation of DNA biosynthetic process), development processes (GO:0001890 ~ placenta development) and binding processes (GO:0005109 ~ frizzled binding; GO:0005524 ~ ATP binding; GO:0046872 ~ metal ion binding). The KEGG pathway is significantly enriched in signalling pathways that regulating pluripotency of stem cells; proteoglycans in cancer; hippo signaling pathway and IL-17 signalling pathway.

In addition, our analyses identified several candidate genes (*ANKRD1*, *CPNE5*, *MYOM1*, *OBSCN*, *DECR1*, *MAPK4*, *PNPLA1*, *PACSIN1*, *GRM4*, *LRPPRC, and WNT9A*) that may be closely associated with meat quality traits in DA pigs. These findings provide valuable insights into the potential mechanisms underlying their unique characteristics.

Discussion

SNP, InDel, CNV, and SV variations of DA pigs

This study conducted whole-genome resequencing of DA pigs, yielding comprehensive genomic information and enabling an in-depth analysis of SNPs, InDels, CNVs, and SVs within their genome. The observed polymorphisms in the study population reflect existing genetic variations that may be closely associated with the unique genetic characteristics of DA pigs. These diverse types of variations have exerted significant influences on the genetic diversity and evolutionary trajectory of DA pigs. Notably, SNPs, being the most common autosomal variations, received particular attention in our analysis. The findings of this study not only lay a solid foundation for comparative and functional genomic analyses of DA pigs but also provide valuable genetic information for future selective breeding of pigs with specific traits.

Analysis of genetic diversity and linkage disequilibrium in DA pigs

This study conducted a comprehensive evaluation of the genetic diversity of DA pigs by assessing genetic diversity parameters (including heterozygosity, allele frequency, and effective population size) and LD. Gene heterozygosity (H) is an important indicator for measuring the richness of population genetic diversity. In this study, H_O and H_E of DA pigs (average H_O = 0.41 ± 0.17, average H_E = 0.36 ± 0.12) were higher than those of commercial pigs (H_O = 0.27) [18] and some local breeds, such as Laiwu pigs (H_O = 0.23) [18] and Jinhua pig (H_O = 0.31 ± 0.16, H_E = 0.32 ± 0.14) [19]. Furthermore, the H_E and H_O of DA

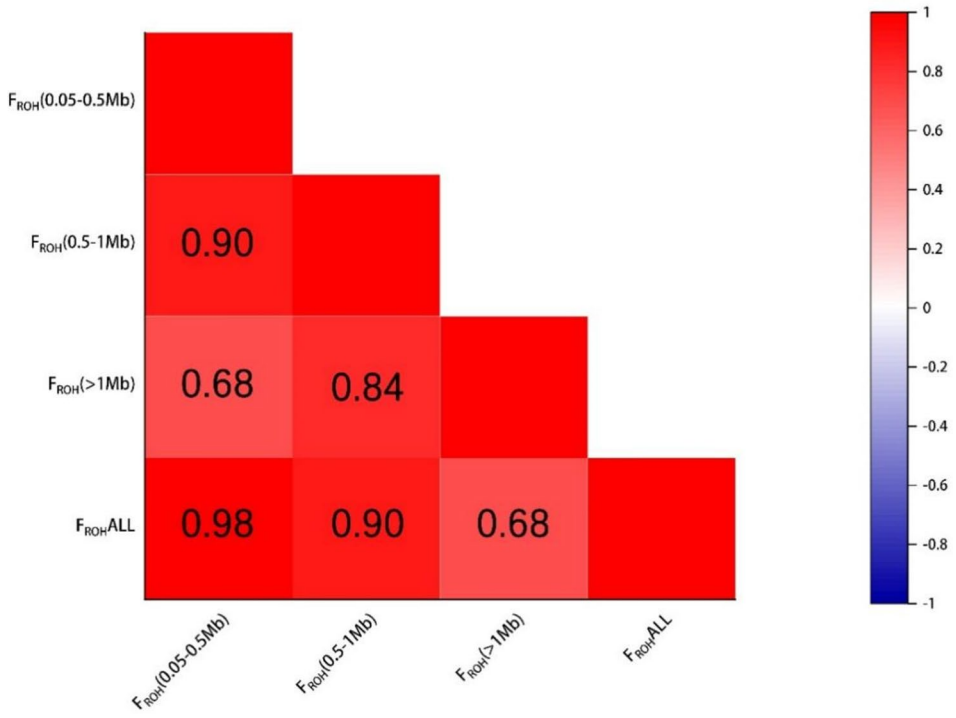

Fig. 5 Plot of ROH correlation coefficients for different lengths of Ding'an pigs, decreasing from red to blue, with stronger correlations the closer the colour is to red

pigs were higher than domestic breeds (LG and TC) and significantly higher than foreign commercial pigs (LR and LW). MAF of DA pigs was 0.27 ± 0.13, higher than the other four breeds studied. The proportion of SNPs with MAF below 0.1 was relatively high (approximately 14.7%, see Fig. 1), further confirming the high level of genetic diversity in DA pigs [20]. A high degree of genetic diversity may mean a higher degree of genetic variation, which may affect the expression of genes or functions, thus affecting traits such as fleshy traits. Meanwhile, *pi* is another widely used genetic diversity evaluation index. The *pi* value of DA pigs (0.0034 ± 0.0018) was higher than some local breeds, such as LG (*pi* = 0.0017 ± 0.0008) and TC (*pi* = 0.0033 ± 0.0022), and also exceeded some commercial breeds, such as LR (*pi* = 0.091 ± 0.141) and LW (*pi* = 0.089 ± 0.143). The higher *pi* value indicates a rapid decay of LD in DA pigs.

Ne is used to measure the actual number of effective breeding individuals in a population, helping researchers determine whether a population needs protection. In this study, the Ne of the DA pig population was relatively small, possibly due to limited population size and a certain degree of genetic drift within the population, which may threaten the genetic diversity within the group. Therefore, when protecting DA pigs, scientific breeding plans should be formulated to prevent the loss of independent family lineages.

LD analysis can reveal the history and evolution of a population. Comparing LD levels between different populations can reflect differences in overall genetic diversity [21]. In this study, when the r^2 value was 0.3, the distance between paired SNPs in DA pigs was 6.4 kb, close to local breeds LG (0.7 kb) and TC (1.7 kb), and smaller than some commercial breeds such as LR (16.6 kb) and LW (178 kb). The decay rate of the chain imbalance of domestic varieties is much lower than that of foreign varieties, which clearly distinguishes between domestic and foreign varieties. This result is consistent with the aforementioned *pi* analysis, indicating that the genetic diversity of DA pigs is relatively stable [22]. However, given that genetic diversity is influenced by multiple factors, continuous monitoring of genetic variation and structure is necessary to maintain the stability of its diversity.

Statistical analysis of genomic ROH in DA pigs

Analysis of the distribution, length, and number of ROH in the genome provides valuable insights into the inbreeding history of a population [23]. Typically, longer ROH segments are associated with recent inbreeding events, while shorter ROH segments indicate more distant inbreeding occurrences [24]. It is noteworthy that whole-genome sequencing, compared to SNP chips, may result in the detection of higher SNP density due to its higher resolution, potentially leading to the identification of shorter ROH lengths [25, 26].

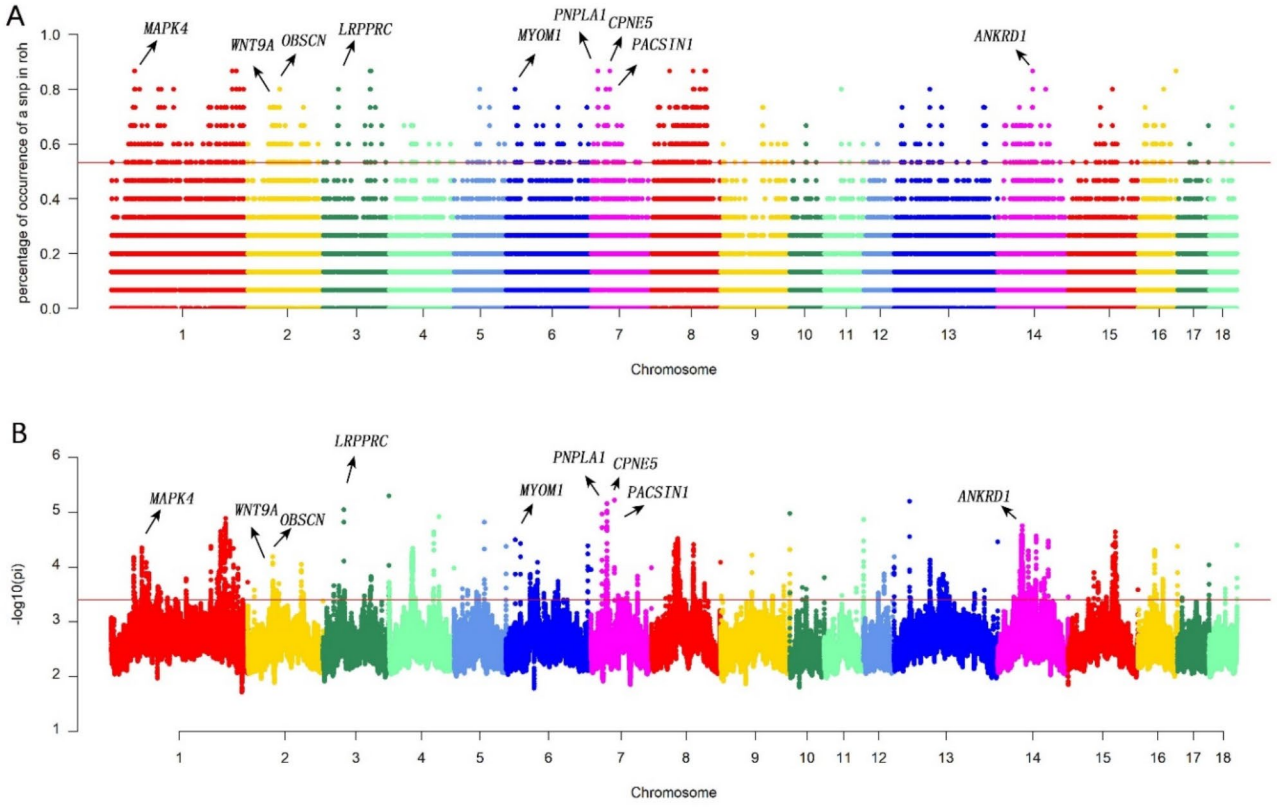

Fig. 6 Manhattan plot of selection signatures in Ding'an pigs. (**A**) Manhattan plot of the incidence of each SNP in ROH in different individuals. The red line represents the highest 1% threshold. (**B**) Genome-wide distribution of selection signatures detected by *pi*. The red line represents the top 1% threshold level. Abbreviations: *pi* = Nucleotide diversity Analysis

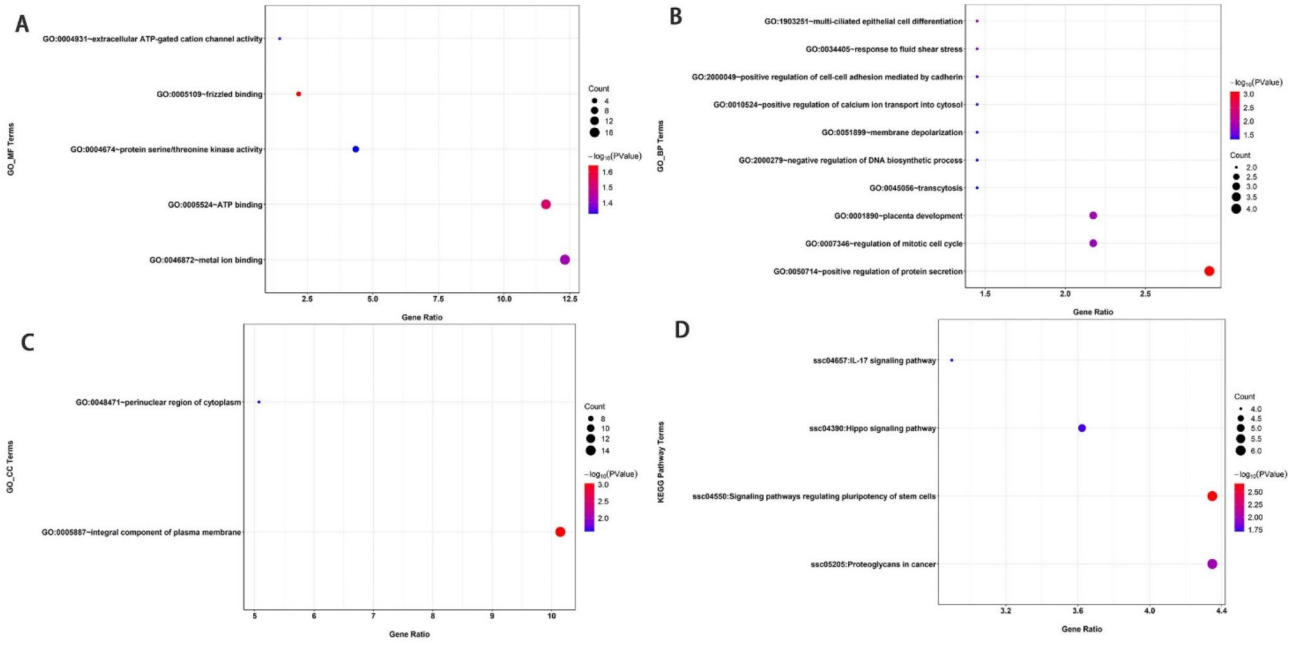

Fig. 7 Enrichment analysis of the variants located in gene exons. (**A**) Bubble plot of enriched GO Molecular Function (MF) for candidate genes. (**B**) Bubble plot of enriched GO Biological Process (BP) for candidate genes. (**C**) Bubble plot of enriched GO Cellular Component (CC) for candidate genes. (**D**) Bubble plot of enriched KEGG pathways for candidate genes. Abbreviations: GO = Gene Ontology; KEGG = Kyoto Encyclopedia of Genes and Genomes

In this study, the ROH segments in the DA pig population were predominantly short (length < 1 Mb), which is consistent with previous findings from whole-genome sequencing studies on other pig breeds [14, 27, 28]. Although shorter ROH fragments reflect long-distance inbreeding, long-term accumulation may increase the homozygous probability of recessive deleterious alleles. These deleterious alleles may affect fecundity, growth performance, or disease resistance, especially in closed populations [3]. Short ROH fragments have a small single effect, but a large number (e.g. 0.05-0 Mb of ROH in DA pigs accounted for 96.87%, Table 2), which may reduce the overall heterozygosity of the genome in the long term and limit the potential of polygenic regulation of traits such as fat deposition [2]. The presence of short ROH fragments indicates that the population has historically experienced bottlenecks or genetic drift. If Ne is persistently low (e.g. Ne = 35 in DA pigs), genetic drift may accelerate the loss of favourable alleles or the fixation of harmful alleles, affecting the stability of breeding targets [28]. This observation suggests that DA pigs may have experienced inbreeding in more distant generations, aligning with our understanding of their historical background. However, it is crucial to emphasize that ROH length is not solely determined by inbreeding events but is also influenced by the randomness of gamete formation and genomic recombination rates [29]. Therefore, when interpreting ROH data, these factors should be considered comprehensively.

Genomic inbreeding coefficients in DA pigs

Traditionally, inbreeding coefficients have been estimated based on pedigree data (F_{PED}). However, F_{PED} cannot be considered an accurate estimate of true inbreeding levels due to various limitations, such as errors in pedigree records and the failure to account for random recombination events during meiosis [30]. In contrast, genomic information provides a more comprehensive and precise method. By analyzing SNPs and other genetic variations, it is possible to compare and analyze the relationships between individuals within a population, thereby calculating more accurate inbreeding coefficients. Numerous studies have demonstrated that inbreeding coefficients can be estimated based on ROH segments in the absence of pedigree information [31].

In this study, the average inbreeding coefficient calculated based on ROH (F_{ROH}) was 0.164, lower than some foreign breeds such as Złotnicka Spotted pigs (0.287) and Polish LR pigs (0.171) [32]. This may indicate that DA pigs have undergone relatively low selection intensity in recent years. Compared to commercial pigs, the DA pig population exhibits lower levels of inbreeding and higher genetic diversity, consistent with the previous findings from genetic diversity and LD analyses.

Additionally, we calculated the F_{HOM} value for DA pigs and found it to be negative. This may be due to F_{HOM} inability to distinguish between identical by descent (IBD) and identical by state (IBS) alleles [33]. In comparison, F_{ROH} is less influenced by external factors and is therefore considered more accurate in estimating inbreeding levels [34, 35].

Identification of high frequency ROH regions and candidate genes

We investigated high-frequency regions in the DA pig population, and a total of 510 candidate functional genes were identified in these regions. The identified genes were analysed for GO terms and KEGG signalling pathways, which resulted in the identification of several pathways related to tissue development and immunity. For example, among the KEGG pathways, we identified the Hippo signalling pathway, a highly conserved pathway that controls organ size by participating in tissue development and regeneration and regulating cell proliferation and apoptosis [36]. The IL-17 signalling pathway plays an important role in biological processes such as the immune response, and it has been shown that IL-17 is essential for processes such as host defence, tissue repair and the pathogenesis of inflammatory diseases [37], this may well explain the excellent disease resistance of the DA pig. Therefore, these signalling pathways may help researchers to better understand the genetic characteristics of DA pigs.

In addition, we conducted a literature search for the identified candidate genes to explore the possible biological functions of the candidate genes identified through ROH and *pi* analysis. Some of these genes may be related to meat quality traits in DA pigs. Several studies have shown that the *ANKRD1* gene is a candidate gene for meat quality traits, and understanding the biological processes involved in this gene may provide insights into the prediction of meat quality-related traits [38]. In addition, the *CPNE5* gene affects meat colour, and it has been suggested that this gene may be involved in the synthesis of melanin, which is then transported to the muscles through the circulatory system. This ultimately affects meat colour traits such as brightness [39]. Typically, meat traits vary between different colours of pork. Other studies have found that the *MYOM1* gene is associated with pork meat texture and loin texture, and mutations in this gene have been found to have an effect on meat and loin texture parameters by genome-wide association analysis [40]. Several studies have explored the correlation between the *DECR1* gene and pork texture characteristics, finding that the gene encodes an enzyme involved in the β-oxidation of polyunsaturated fatty enoyl-CoA ester [41]. In summary, these results may explain the good meat quality of DA pigs.

Fat deposition directly affects the taste and tenderness of pork, and pork that is high in fat is usually more tender and flavourful. In our study, we identified several genes that may be related to fat deposition among the enriched genes in DA pigs. The *OBSCN* gene was reported to be associated with intramuscular fat content in pigs, and the expression of this gene showed a correlation with muscle fat [42]. *MAPK4* and *PNPLA1* genes are associated with pig body size. It has been shown that the *MAPK4* gene is involved in the regulation of skeletal growth and development as well as nutrient uptake, and is associated with fat deposition [43].The *PNPLA1* gene has also been associated with body size, and this gene influences changes in body size in pigs by being involved in lipid deposition [44]. The *PACSIN1* gene is associated with body height, body length and hip circumference [45]. A closely related region of this gene associated with body size in pigs has been identified [46]. Other studies have found that this gene plays a role in adipose and muscle tissue development, glutathione metabolism, and lipid metabolism [47]. In addition, the *GRM4* gene is a pleiotropic gene associated with carcass length and backfat thickness in pigs [48]. It has been suggested that this gene may play an important role in adipogenesis by regulating *MAPK* activity and thus in adipogenesis [49]. Therefore, these genes may explain the good meat quality of DA pigs in terms of fat deposition.

It has been shown that skeletal muscle has an effect on meat tenderness [50, 51]. This study identified several genes associated with skeletal muscle growth and development. For example, the *LRPPRC* gene may control conformational traits by regulating skeletal muscle development, and the gene is involved in skeletal development at different levels [52]. In addition, the *WNT9A* gene acts as a key gene for satellite cell differentiation, and it attenuates the differentiation of senescent satellite cells during skeletal muscle development through synergistic effects [53]. These results may provide a side explanation for the meat quality traits of DA pigs.

Stress resistance is another feature of interest in DA pigs, and four genes in the KEGG pathway are involved in the disease resistance pathway. We also identified candidate genes related to adaptability. For example, the *CADPS2* gene is associated with adaptation to the rearing environment [54]. The *PIK3IP1* gene plays an inhibitory role in T cell activation, which in turn is involved in the immune response [55]. Moreover, the *SLC26A7* gene can regulate intracellular pH via chloride channels, which may be related to environmental adaptation [56]. In addition, we identified candidate genes related to growth and development of livestock, such as *ELAVL2* [57] and *JMJD4* [58]. As well as candidate genes related to reproductive traits, such as *IGF1R* [59, 60] and *MAPK14* [61]. These reference genes provide an understanding of stress resistance, growth traits and reproductive traits in DA pigs.

In summary, the candidate functional genes identified in this study provide assistance in the study of good meat quality traits in DA pigs. These findings may provide new ideas for further understanding of the genetic characteristics of DA pigs and provide valuable references for later conservation programmes and efficient use of DA pigs. These results can explain the superior meat quality traits of DA pigs. Overall, the regions identified in this study provide a molecular basis for the excellent germplasm characteristics of DA pigs.

Conclusions

This study conducted whole-genome sequencing and ROH analysis on DA pigs, revealing high genetic diversity and low inbreeding levels within the population. Through functional gene enrichment analysis of high-frequency ROH regions, we identified multiple candidate genes associated with meat quality, fat deposition, and skeletal muscle development. These findings not only enhance our understanding of the genetic mechanisms underlying the unique traits of DA pigs but also provide valuable insights for practical applications. Specifically, the identified candidate genes and genomic regions can guide conservation efforts to maintain genetic diversity and mitigate inbreeding risks. Meanwhile, these genetic insights can be integrated into breeding programs to improve meat quality and other economically important traits, thereby supporting the sustainable development and utilization of DA pigs.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

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Author contributions

Q.X. and F.W. conceived and designed this study. Z.Y.W. performed the statistical analysis and wrote the manuscript. D.Y.P. carried out data collection and a portion of the statistical analysis and offered some of the paper writing viewpoints. Z.Q.Z. gives assistance to the statistical analysis. X.X.F. collected a part of the data. All the authors were involved in developing, writing, and commenting on the manuscript, and all read and approved the final version of the manuscript.

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Data availability
The data analyzed in this study was derived from previous study (Zhong et al., 2023). Additional data details are provided in the supplementary information (Table S1).

Declarations

Ethics approval
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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