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Multiple association studies identify 3 novel candidate genes for teat number trait in Danish Landrace and Large White pigs: *BRINP3*, *LIN52*, and *UBE3B*

Zijian Qiu,[†,1] Yanzhen Yin,[†,1] Ruihua Huang,[†,‡,‖] Jin Zhou,[†] Qian Liu,[†] Kaiyue Liu,[†] Chenxi Liu,[†] Pinghua Li,[†,‡,‖,2] and Qingbo Zhao[†,‡,2]

[†]Key Laboratory (Nanjing) for Evaluation and Utilization of Pigs Resources, Ministry of Agriculture and Rural Affairs, Nanjing, China
[‡]College of Animal Science & Technology, Institute of Swine Science, Nanjing Agricultural University, Nanjing, China
[‖]Huaian Academy, Nanjing Agricultural University, Huaian, China

[1]These authors are contributed equally.
[2]Corresponding author: qingbo_zhao@aliyun.com (Q.Z.); lipinghua718@njau.edu.cn (P.L.)

Abstract

Milk is an essential source of nutrition for preweaning piglets. Therefore, in the breeding process, sows were expected to have sufficient teats to suckle their piglets. However, in Danish Landrace and Large White pigs, the number of piglets born currently exceeds the number of teats, making it urgent to select and breed for an increased teat number. In this study, the samples of 491 Danish Landrace pigs and 1,047 Danish Large White pigs with teat number phenotype were used to perform genome-wide association studies to identify SNPs associated with total teat number (**TTN**) based on SNP-chip data and data imputed to the level of whole-genome sequencing (**iWGS**), respectively. In Landrace pigs, the most significant SNP on SSC10 explains 5.14% of the phenotypic variance, while in Large White pigs, the most significant SNP on SSC7 explains 4.46% of the phenotypic variance. Additionally, linkage disequilibrium and linkage analysis (**LDLA**) were used to refine the regions of QTLs on SSC10 to 2.89 to 5.43 Mb in Danish Landrace pigs and to 96.00 to 97.95 Mb on SSC7 in Danish Large White pigs, respectively. To maximize the utility of information from 2 populations, meta-analysis was conducted across multiple populations. A total of 12 protein-coding genes were identified within the candidate QTL regions determined by LDLA and meta-analysis. To supplement the candidate gene set, transcriptome-wide association studies (**TWAS**) based on embryo and placenta tissues identified 7 protein-coding genes associated with TTN in Landrace and Large White pigs. Phenome-wide association studies (**PheWAS**) query was conducted for all the above genes, revealing that nearly all of them are associated with teat number traits. Additionally, some genes showed strong associations with carcass traits, suggesting a potential association between teat number and carcass traits. Through functional annotation and integrated analysis, *BRINP3*, *LIN52*, *ABCD4*, and *UBE3B* were determined as the functional candidate genes regulating TTN. These findings lay the foundation for identifying the genetic loci regulating teat number in Danish pigs, as well as for their molecular breeding.

Lay Summary

Piglets depend on milk of sows for survival, making it crucial for sows to have enough teats to feed them. However, in Danish Landrace and Large White pigs, the number of piglets often exceeds the number of teats. In this study, the samples of 491 Danish Landrace pigs and 1,047 Danish Large White pigs were performed genome-wide associate studies to identify genetic loci influencing teat number. Through multiple association analyses, *BRINP3*, *LIN52*, *ABCD4*, and *UBE3B* were identified as functional candidate genes regulating Danish Landrace and Large White pigs. Some of these genes were also found to be associated with carcass traits, suggesting that a connection between teat number and body composition exists. Valuable insights into the genetics of teat number were provided by these findings, offering important information for breeding pigs with improved maternal traits.

Key words: candidate gene, genome-wide association study, pig, teat number

Abbreviations: GWAS, genome-wide association studies; iWGS, imputed whole-genome sequence; LDLA, linkage disequilibrium and linkage analysis; TTN, total teat number; PheWAS, phenome-wide association studies; SNP, single nucleotide polymorphism; TWAS, transcriptome-wide association studies

Introduction

The teat number is an important morphological and reproductive trait in pig production, and it is one of the key target traits in modern breeding programs. Functioning teats are responsible for milk release, which contains essential nutrients and serves as a vital energy source for the growth and development of piglets. The teat number reflects the lactation capacity of the sow. Additionally, previous research suggested that sows with more teats tended to have a higher litter size (Kim et al., 2005). Although some studies suggested that there was no correlation between teat number and litter size (Zhang et al., 2000), the teat number determines the number of piglets a sow can nurse and has significant potential for improvement.

The number of teats is greatly influenced by the genetic background of the pig breed. The mean value of teat

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number for Dutch Landrace pigs (n = 275,513) was 15.3, Dutch Large White pigs (n = 1,166) 15.79 (Deng et al., 2024), and Norwegian Landrace pigs (n = 313,475) 15.84 (van Son et al., 2019). For Erhualian pigs (n = 320), the mean value of teat number was 20.38 (Tang et al., 2017), and that of Tibet pigs (n = 654) was only 10.85 (Zhang et al., 2009).

Danish breeders have successively utilized the best linear unbiased prediction and Genomic Selection methods to continuously improve the litter size traits of Danish Landrace and Large White pigs. Currently, the average number of live piglets per litter for both breeds had exceeded 16. However, the selection for teat number in Danish Landrace and Large White pigs was limited to the phenotypic culling method of "selecting and retaining sows with 7 pairs or more" of teats. This had resulted in an average teat number of approximately 14 per sow, which is lower than the average number of live piglets born per litter. Therefore, there is a greater emphasis on selecting sows based on their teat number, as the number of normal teats provided by the sow per piglet is closely related to piglet survival rate (Alexopoulos et al., 2018).

This study aims to identify loci and candidate genes associated with teat number in Danish Landrace and Large White pigs using genome-wide association studies (**GWAS**), transcriptome-wide association studies (**TWAS**) and phenome-wide association studies (**PheWAS**). These findings could provide insights for the improvement of teat number traits in Danish pigs.

Materials and Methods

Ethical approval and consent to participate

All animal experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals prepared by the Institutional Animal Welfare and Ethics Committee of Nanjing Agricultural University, Nanjing, China [certification no: SYXK (Su) 2022-0031].

Animals and data collection

In this study, the teat number of 491 Danish Landrace sows and 777 Danish Large White sows were recorded from Guizhou Zhiyuan Breeding Co., Ltd. Additionally, the teat number of 270 Danish Large White sows were recorded from Henan Huayang Kongzhai Pig Genetic Technology Co., Ltd. And the hair follicle tissue samples were collected from all pigs (n = 1,538). All phenotypic records were collected at birth.

Genotyping and quality control

Genomic DNA was extracted from ear tissue samples using Megi Universal Nucleic Acid Extraction Kit. Quantification and qualification of DNA were performed using a NanoDrop 2000 with the standard: OD260/280 ranging from 1.7 to 2.1, and concentration exceeding 50 ng/μL. Genotyping was performed using the Compass Porcine 50K Breeding Beadchip (Tianjing, China) containing 51,315 SNPs across the whole genome. The physical positions of all single nucleotide polymorphisms (**SNPs**) were updated to the *Sus scrofa 11.1* build (*Sscrofa11.1*) of the pig reference genome. Quality control was carried out using PLINK v1.9 software (Chang et al., 2015). Data with SNP call rates < 90% were removed. SNPs with genotype-missing rates > 0.1 and minor allele frequency (**MAF**) < 0.05 were removed. Only SNPs on autosomal chromosomes were retained. After quality control, 419 Danish Landrace pigs with 35,237 eligible SNPs and 1,047 Danish Large White pigs with 33,347 eligible SNPs were used for further analysis.

Genetic parameters estimation

The heritability values of left (**LTN**), right (**RTN**), and total teat number (**TTN**) for Danish Landrace and Large White pigs were estimated using the HIBLUP (v1.1.0) software (Yin et al., 2023). The single-trait model was as follows:

$y_{ij} = \mu + b_i + a_j + e_{ij}$ (model 1),

where y_{ij} represents LTN, RTN, or TTN, μ is the mean value of phenotype, b_i is the fixed effects (different farms were considered as the fixed effects in Danish Large White pigs, no fixed effect was considered in Danish Landrace pigs), a_j is the random additive effect, e_{ij} is random residual. This study assumed that $a_j \sim N\left(0,\ G\sigma_a^2\right)$ and $e_{ij} \sim N\left(0, I\sigma_e^2\right)$, G is genotype matrix, σ_a^2 is the additive genetic variance, σ_e^2 is the residual variance, I is the identity matrix. The formula of heritability is as follows:

$$h^2 = \sigma_a^2 / \left(\sigma_a^2 + \sigma_e^2\right).$$

Imputation

The whole-genome resequencing data of 1,602 pigs of multiple breeds was from the PigGTEX project (Teng et al., 2024). Haplotype phasing of the reference panel (Browning and Browning, 2007) and the imputation of the SNP-chip data to the whole-genome density (Browning et al., 2018) were performed using the Beagle software (version 5.2) with the default parameters. SNPs with dosage R-squared (DR^2) values < 0.9 and MAF values < 0.05 were removed. After quality control, 491 Danish Landrace pigs with 5,889,264 SNPs and 1,047 Danish Large White pigs with 5,516,377 SNPs were retained for further analysis.

GWAS

GWAS was performed in each breed population using SNP-chip data and iWGS data. The univariate linear mixed model implemented in LDAK v5.2 software (Speed et al., 2012), testing the association between individual markers and teat number. The model is as follows:

$y = Xa + Wb + u + e$ (model 2),

where y represents phenotype value, a is the fixed effects (same as model 1), b represents the substitution effect of the alleles, u is the random additive effect following $N\ (0,\ K\ \sigma_a^2)$ distribution, K is the genomic kinship matrix calculated with the LDAK-Thin algorithm (Speed et al., 2012) under the default parameters (prune threshold = 0.98, window size = 100 kb). X and W are the incidence matrices.

The genome-wide significance threshold was set to $0.05/N$ based on adopting the Bonferroni correction method, and $1/N$ was set as the suggestive threshold, where N represents the number of filtered SNPs (35,237 SNPs in Landrace pigs, 33,347 SNPs in Large White pigs) in SNP-chip data. For iWGS data, due to the overly stringent genome-wide significance threshold using associated with Bonferroni correction, N represents the number of effective SNPs (162,540 SNPs in Landrace pigs, 144,381 SNPs in Large White pigs), which was estimated with PLINK v1.9 software using the "--indep-pairwise 50 5 0.2" parameter. The proportion of phenotypic

Table 1. Descriptive statistics and heritability for teat number in Danish Landrace and Large White pigs

Breed	N[a]	Trait	Min[b]	Max[c]	Mean[d] ± SD [e]	CV[f] (%)	h^2 ± SE[g]
Landrace	491	LTN	5	8	7.14 ± 0.47	6.53	0.193 ± 0.06
		RTN	6	9	7.17 ± 0.50	6.96	0.064 ± 0.05
		TTN	11	17	14.31 ± 0.78	5.48	0.221 ± 0.07
Large White	1047	LTN	4	9	6.99 ± 0.47	6.74	0.149 ± 0.04
		RTN	6	9	7.14 ± 0.51	7.16	0.135 ± 0.04
		TTN	10	17	14.12 ± 0.80	5.63	0.219 ± 0.05

[a]Number of individuals with phenotypic records.
[b]Minimum of phenotype.
[c]Maximum of phenotype.
[d]Mean of phenotype.
[e]Standard deviation.
[f]Coefficient of variation.
[g]Heritability ± standard error.

variance explained (**PVE**) by the SNP additive effects was calculated as follows:

$$\mathrm{PVE} = \frac{2 \times b^2 \times \mathrm{MAF} \times (1 - \mathrm{MAF})}{\sigma_p^2} \times 100\%,$$

where b is the estimate of allele substitution effect, and σ_p^2 represents the phenotypic variance.

Linkage disequilibrium and linkage analysis

linkage disequilibrium and linkage analysis (**LDLA**) is a fine-mapping method that utilizes both linkage and linkage disequilibrium information simultaneously (Zhang et al., 2012). Haplotypes were constructed using the Beagle software. The pseudomarker software (Gertz et al., 2014) was used to dynamically cluster the haplotypes into clusters. Each haplotype at a marker locus is labeled with the clustered haplotype cluster. Haplotypes within a cluster are considered to originate from the same ancestor. Linear regression analysis was employed to assess the association between haplotypes and teat number. The model is as follows:

$y = Xa + Wh + e$ (model 3),

where the definitions of y, a, e, X, and W are the same as those in model 2. h represents the random effect of haplotypes. This study assumed that $u \sim N\left(0, H\sigma_h^2\right)$, H is kinship matrix, σ_h^2 is the additive genetic variance.

Meta-analysis

The results of GWAS from 2 populations were merged for meta-analysis using METAL software (Willer et al., 2010). The Z-value method (Le et al., 2017) is utilized, whereby P-value and effect sizes are standardized into Z-value with the number of individuals serving as weights. The model is as follows:

$Z = \frac{\sum_i \phi^{-1}\left(\frac{P_i}{2}\right) \times \mathrm{sign}(\Delta_i) \times \sqrt{N_i}}{\sqrt{\sum_i N_i}}$ (model 4),

where ϕ^{-1} is inverse normal transformation, P_i is the P-value of SNP_i, Δ_i is the sign of the effect size, N_i is the sample size. The threshold was calculated using the number of SNPs pruned in Landrace pigs.

TWAS

TWAS was performed using the TWAS-server website from the PigGTEX project (Zhang et al., 2023). Due to the lack of transcriptome data for tissues directly related to teat number, such as mammary primordia, transcriptome data from embryonic and placental tissues (which were associated with reproduction and embryonic development) were used in this study. The MetaXcan statistical method was employed. The significance threshold was also set to $1/N$ based on adopting the Bonferroni correction method, where N represents the number of predicted genes.

PheWAS

PheWAS query for candidate genes was conducted using the PigBiobank website from the PigGTEX project (Zeng et al., 2024). For each gene, the top 30 associated items were selected from the query results. The occurrence frequency and the most significant P-value of each trait were recorded among these top 30 items. The heat maps were visualized using the Pheatmap R package.

Function of candidate genes

The Ensembl BioMart tool (https://asia.ensembl.org; release 111—January 2024) was used to identify genes within the QTL confidence interval from GWAS. PubMed (https://pubmed.ncbi.nlm.nih.gov/) was used to search the literature for functional annotation of the candidate genes from GWAS and TWAS.

Results

Descriptive statistics and heritability estimates

The maximum, minimum, mean, standard error, and coefficient of variation values for each teat number trait are presented in Table 1. The variability within the population is observed for the 3 types of teat number in both pig breeds, which facilitates the identification of loci and candidate genes. The heritability values of 3 traits in Landrace pigs were 0.193, 0.064, and 0.221, respectively, while in Large White pigs were 0.149, 0.135, and 0.219, respectively, indicating that the teat number trait exhibits moderate heritability. The heritability of TTN is higher than that of LTN and RTN in both breeds of pigs.

The test of population stratification

Principal component analysis (**PCA**) revealed a clear distinction between various pig breeds (Fig. 1). There was no population stratification observed in Landrace pigs. In Large White pigs, no evidence of population stratification was observed on

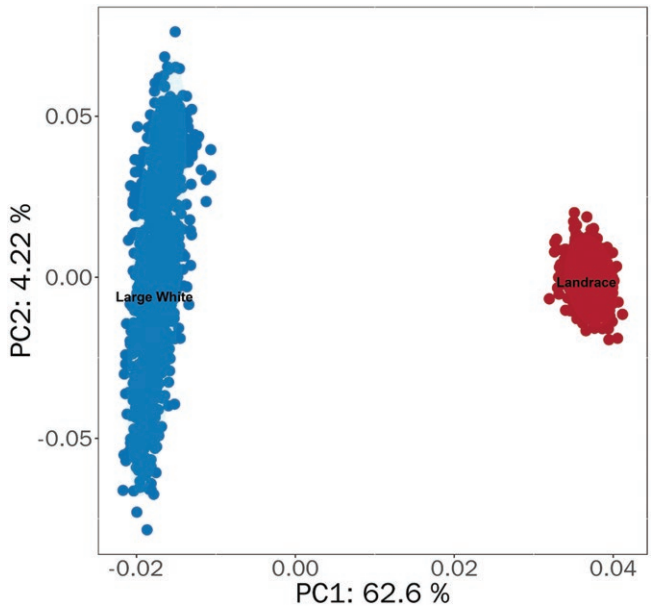

Figure 1. The top 2 principal component analysis plot of population structure. Note: PC1 and PC2 represent principal component 1 and 2 respectively. The blue dots represent Danish Large White pigs, and the red bots represent Danish Landrace pigs.

the first principal component (PC1), which explained 73.31% of the variance. However, on the second principal component (PC2), accounting for 4.94% of the variance, there was dispersion within the population. Considered the relatively smaller variance explained by PC2, differences in the PC2 dimension were disregarded. The PCA for the Large White pigs was also performed, indicating no population stratification within Yorkshire pigs (Fig. S1).

The accuracy of genotype imputation

To evaluate the accuracy of the imputation, 5% of SNPs were randomly removed and imputed again in SNP-chip data. The allele concordance rate of these 5% SNPs was used as the criteria for evaluating the accuracy of the imputation. The average concordance rates were 98.34% and 98.93% in Landrace pigs and Large White pigs, respectively.

GWAS

The λ values for the GWAS of Landrace and Large White pigs were closed to 1. Additionally, the observed *P*-values from the quantile–quantile (Q-Q) plots show no noticeable deviation from the expected *P*-values (Figs. S4 and S5), suggesting the absence of population stratification, thus mitigating the risk of result inflation.

In Landrace pigs, 2 SNPs (rs703074937 and rs81427863) significantly associated with TTN were identified on SSC10 using GWAS based on SNP-chip data (Fig. 2a, Table 2), each explaining 3.85% of the phenotypic variance. Based on iWGS data, 30 SNPs located in 2 QTLs on SSC10 (Fig. 2b, Table 3), spanning from 3.59 and 3.68 Mb (rs338129333) and at 5.16 Mb (rs318931827), respectively, were associated with TTN. The most significant SNPs within the 2 QTLs explained 5.14% and 5.02% of the phenotypic variance respectively. However, no significant signals were identified for LTN and RTN in the results of GWAS either by the SNP-chip data or iWGS data (Figure S2).

In Large White pigs, 3 SNPs associated with both RTN and TTN were identified on SSC7 using GWAS based on SNP-chip data, which located within the regions of 91.30 Mb (7_91308348), 96.10 Mb (rs80820713), and 96.25 Mb (rs80904324) (Fig. 2c, Fig. S3, Table 2), explaining proportions of phenotypic variance ranging from 2.25% to 4.44%. Based on iWGS data, 79 and 119 SNPs located within the region of 94.94 to 97.58 Mb were significantly associated with RTN and TTN respectively (Fig. 2d, Fig. S3, Table 3). The most significantly associated SNPs within this QTL for RTN and TTN are located at 97.27 Mb (rs342113326) and 97.29 Mb to 97.35 Mb (rs345161260), respectively, both explaining 4.46% of the phenotypic variance. Similarly, no SNPs associated with LTN were identified.

Fine mapping through the integration of LDLA and meta-analysis for TTN

Based on haplotype-associated LDLA, the candidate confidence interval of QTL for TTN was calculated as the $-\log_{10}P$ value of the most significant loci minus 2 (Karim et al., 2011). The QTL confidence interval for TTN was determined to be 2.89 to 5.43 Mb on SSC10 in Landrace pigs (Fig. 3a, Table 4), 96.00 to 97.95 Mb on SSC7 in Large White pigs (Fig. 3b, Table 4).

Meta-analysis was performed to refine the QTL candidate interval, combining data from multiple populations to increase sample size. The multi-population meta-analysis, combining results from Landrace and Large White pigs, identified 155 SNPs significantly associated with TTN on SSC7 (Fig. 3c). However, the significant signals observed on SSC10 in Landrace pigs disappeared, indicating potential breed-specific differences. The QTL confidence interval was determined to be 97.15 to 97.58Mb on SSC7 using the $-\log_{10}P$ value of the most significant loci minus 2 (Fig. 3d, Table 4). This confidence interval was a subset of the region identified by LDLA for TTN in Large White pigs. Therefore, the intersection of the candidate intervals from LDLA and meta-analysis was taken as the final QTL candidate interval.

Within the QTL candidate interval on SSC10 identified by GWAS in Landrace pigs, 2 protein-coding genes were identified. There were 10 protein-coding genes in the QTL candidate interval determined by LDLA in Large White pigs and meta-analysis (Fig. 3d, Table 4).

TWAS

In Landrace pigs, 6 genes (Embryo: *ENSSSCG00000010078*, *UBE3B*, and *SPPL3*; Placenta: *RPUSD3*, *TRPV4*, and *ENSSSCG00000011567*) associated with TTN were identified on SSC13 and SSC14 (Fig. 4a). In Large White pigs, *NPC2* was associated with TTN based on transcriptome data of embryo tissue (Fig. 4b). However, the genes within the candidate QTL regions identified by GWAS were absent from the candidate genes related to TTN in the TWAS. The reason for this situation was that the TWAS-server did not predict the expression levels of genes within the candidate QTL regions identified by GWAS, except for *ABCD4*, along with the tissue specificity of gene expression.

PheWAS

To validate the results from GWAS and TWAS, PheWAS was conducted on the identified candidate genes. The results show that the association *P*-values between all genes and traits fall below 1×10^{-4} after the top 30 items. Therefore, this study

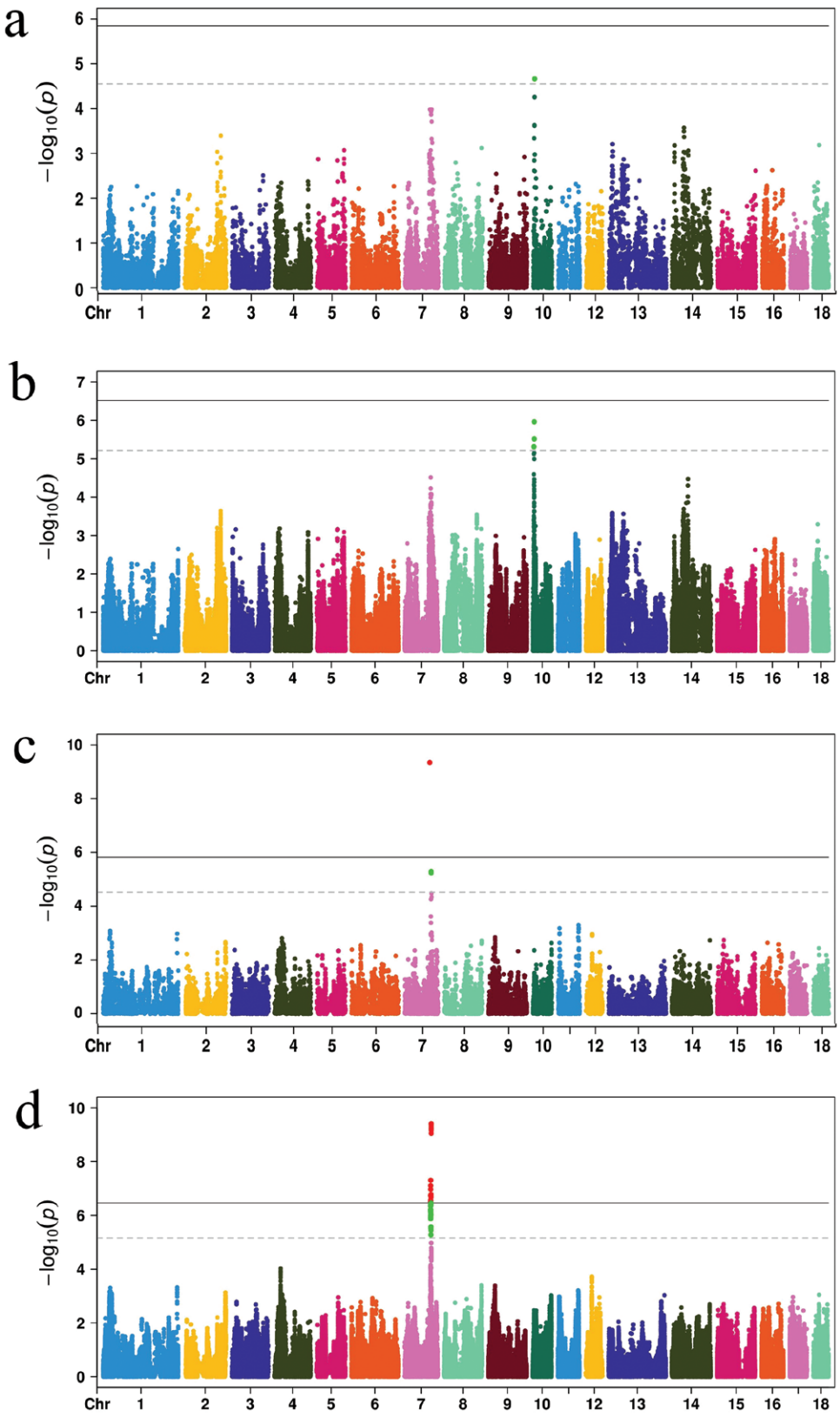

Figure 2. Manhattan plots of genome-wide association studies for the TTN in (a and b) Danish Landrace and (c and d) Large White pigs. Note: The results in (a) and (c) were based on single nucleotide polymorphism-chip data analysis, while those in (b) and (d) were based on imputed whole-genome sequencing data analysis.

only presents the top 30 associated items. Among the top 30 associated items in PheWAS, nearly all candidate genes (all genes except for *ENSSSCG00000057756*) had at least 1 item associated with teat number in pigs. Interestingly, a considerable number of the candidate genes for TTN were also associated with carcass traits, such as backfat thickness,

Table 2. GWAS results for the teat number based on SNP-chip data in Danish Landrace and Large White pigs

Breed	Trait	Chr[a]	SNP	Position (bp)	*P*-value[b]	PVE[c] (%)	Nearest gene
Landrace	TTN	10	rs703074937	5,138,276	2.18E−05	3.85	*KCTD3*
		10	rs81427863	5,150,788	2.18E−05	3.85	*KCTD3*
Large White	RNT	7	7-91308348	91,308,348	3.68E−10	4.26	*PIGH*
		7	rs341936668	95,374,332	8.38E−06	2.25	*RGS6*
		7	rs80820713	96,101,509	9.14E−06	2.29	*DPF3*
		7	rs80904324	96,254,563	1.02E−05	2.26	*DPF3*
	TTN	7	7_91308348	91,308,348	4.56E−10	4.44	*PIGH*
		7	rs80820713	96,101,509	5.20E−06	2.53	*DPF3*
		7	rs80904324	96,254,563	5.79E−06	2.50	*DPF3*

[a]*Sus scrofa* chromosome.
[b]*P*-value according to the Wald test.
[c]Phenotypic variation explained.

Table 3. GWAS results for the teat number based on iWGS data in Danish Landrace and Large White pigs

Breed	Trait	Chr	Lead SNP	Position of lead SNP (bp)	*P*-value	PVE (%)	Nearest gene
Landrace	TTN	10	rs338129333	3,598,494	4.82E−06	5.14	*BRINP3*
		10	rs318931827	5,162,509	1.09E−06	5.02	*KCTD3*
Large White	RTN	7	rs342113326	97,271,001	1.23E−10	4.46	*COQ6, ENTPD5*
	TTN	7	rs345161260	97,294,953	3.63E−10	4.46	*ENTPD5*

loin eye depth, and carcass length. That suggested a potential association of genetic framework between the teat number and carcass traits. Additionally, some of the candidate genes identified in the TWAS were associated with reproductive traits, such as total number of born, number born of healthy pigs, and total litter weight of piglets born alive, likely due to the selection of embryonic and placental tissues for the study (Fig. 5, Fig. 6).

Candidate genes

In this study, multiple methods were employed to identify candidate genes associated with teat number. First, in PheWAS, the candidate genes for TTN were selected based on the occurrence frequency of the genes associated with teat number being greater than 4 and the most significant *P*-value below 10^{-8}. *LIN52*, *ABCD4*, *BBOF1*, and *ALDH6A1* were identified as candidate genes for TTN. Then, all genes identified in GWAS and TWAS were integrated and their functions were annotated. Considering comprehensively integration of the above methods, *BRINP3*, *LIN52*, *ABCD4*, and *UBE3B* were identified as functional candidate genes for TTN.

Discussion

Most studies suggest that the heritability of TTN estimated by GBLUP is moderate. It was reported that the heritability of TTN in Landrace pigs (n = 1,550) to be 0.37 (Lopes et al., 2014). Duroc pigs in the United States (n = 3,331) and Canada (n = 2,025) were 0.19 and 0.34, respectively (Zhuang et al., 2020). While Hong et al. estimated the heritability of Landrace pigs (n = 2,068) to be 0.22 (Hong et al., 2021). In this study, the heritability values estimated for Danish Landrace and Large White pigs were 0.221 and 0.219, respectively, which was also close to moderate heritability level. The heritability of LTN and RTN in this study is lower than TTN, which was consistent with previous research (Li et al., 2021). This difference might be related to the phenotypic distribution of specific populations.

Compared to previous studies and QTL database (https://www.animalgenome.org/cgi-bin/QTLdb/SS/index [accessed on March 31, 2025]), the QTL on SSC7, identified in the results of GWAS for RTN and TTN in Large White pigs and meta-analysis for TTN in this study, overlap with several reported QTLs (Tan et al., 2017; Zhuang et al., 2020; Bovo et al., 2021; Li et al., 2023). However, in our previous study, a significant QTL associated with TTN was discovered on SSC5 in Dutch Large White pigs (Deng et al., 2024). Additionally, the QTL on SSC10 identified from GWAS and LDLA was a novel candidate QTL for TTN in Landrace pigs. All of these findings suggested breed-specific for the teat number trait.

GWAS, TWAS, and PheWAS are all effective methods for detecting associations between genes and traits. GWAS and TWAS can identify associations between variants, genes, and phenotypes in a forward manner, while PheWAS can identify phenotypes associated with specific genes in a reverse manner. Additionally, PheWAS provides insights into cross-phenotypic associations and gene pleiotropy (Robinson et al., 2018). A previous study combined GWAS and PheWAS to investigate the associations among 6 *GRAMD* family genes in cattle and assess genotype–phenotype associations in other species. The results revealed groups of traits sharing a common genetic basis, suggesting that the *GRAMD* gene family has the potential to enhance cattle productivity, health, and robustness. That highlighted the importance of understanding the relationships between SNPs, genes, traits, and their categories (Kunej et al., 2024). In this study, we used PheWAS to validate in reverse the associations between genes and traits identified in GWAS and TWAS, obtaining supportive results. Furthermore, consistent with the findings of the previous study, we

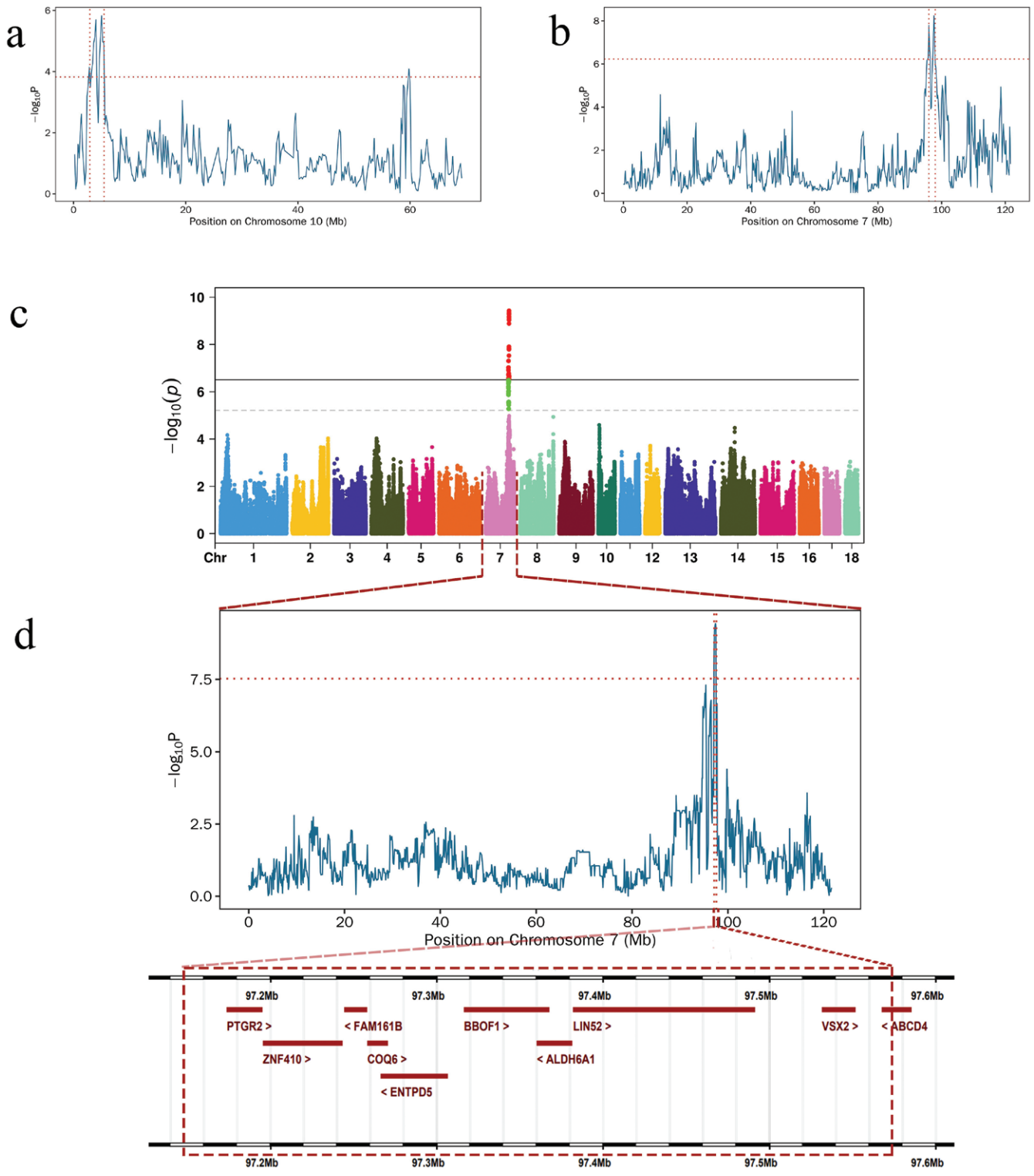

Figure 3. QTL mapping for the TTN in Danish Landrace and Large White pigs. (a) The QTL mapping on SSC10 for TTN using LDLA in Landrace pigs. The threshold was calculated as $-log_{10}P$-value of the most significant loci minus 2. (b) The QTL mapping on SSC7 for TTN using LDLA in Large White pigs. (c) Manhattan plot and Q-Q plot of meta-analysis for TTN across 2 breeds. (d) The QTL mapping on SSC7 for TTN using meta-analysis across 2 breeds. The method of threshold calculation was same as (a). The protein-coding genes within the QTL confidence interval were shown in the red box.

found that genes associated with teat number exhibit a certain degree of pleiotropy. This also suggests a potential relationship between teat number and carcass traits, particularly backfat thickness and carcass length.

Most of the studies suggest that *VRTN* and *ABCD4* on SSC7 are the most likely functional genes (van Son et al., 2019; Hong et al., 2021). However, in this study, only *ABCD4* was included within the QTL confidence interval. Additionally, the most significant SNP in the results of GWAS in Large White pigs and meta-analysis was located on *ENTPD5* and *LIN52* respectively. *ENTPD5* has been reported to be associated with protein glycosylation and ATP hydrolysis

Table 4. Protein-coding genes for TTN in the QTL regions identified through LDLA and meta-analysis

Method	Breed	Chr	QTL region (Mb)	Genes[a]
LDLA	Landrace	10	2.89 to 5.43	***BRINP3***, *ENSSSCG00000057756*
	Large White	7	96.00 to 97.95	*NPC2*, *ISCA2*, *ENSSSCG00000031305*, *AREL1*, *FCF1*, *DPF3*, *ENSSSCG00000028159*, *ZFYVE1*, *RBM25*, *PSEN1*, *PAPLN*, *NUMB*, *ENSSSCG00000021676*, *ENSSSCG00000062678*, *ENSSSCG00000056471*, *ENSSSCG00000002349*, *ACOT6*, *DNAL1*, *MIDEAS*, *PTGR2*, *ZNF410*, *FAM161B*, *COQ6*, *ENTPD5*, *BBOF1*, *ALDH6A1*, *LIN52*, *VSX2*, *ABCD4*, *VRTN*, *SYNDIG1L*, *RIOX1*, *PNMA1*, *LTBP2*
Meta-analysis	—	7	97.15 to 97.58	*PTGR2*, *ZNF410*, *FAM161B*, *COQ6*, *ENTPD5*, *BBOF1*, *ALDH6A1*, ***LIN52***, *VSX2*, ***ABCD4***

The bold genes indicate functional candidate genes identified through functional annotation.
[a]The genes indicated in bold were identified as potential functional candidate genes for the corresponding trait under analysis based on literature support.

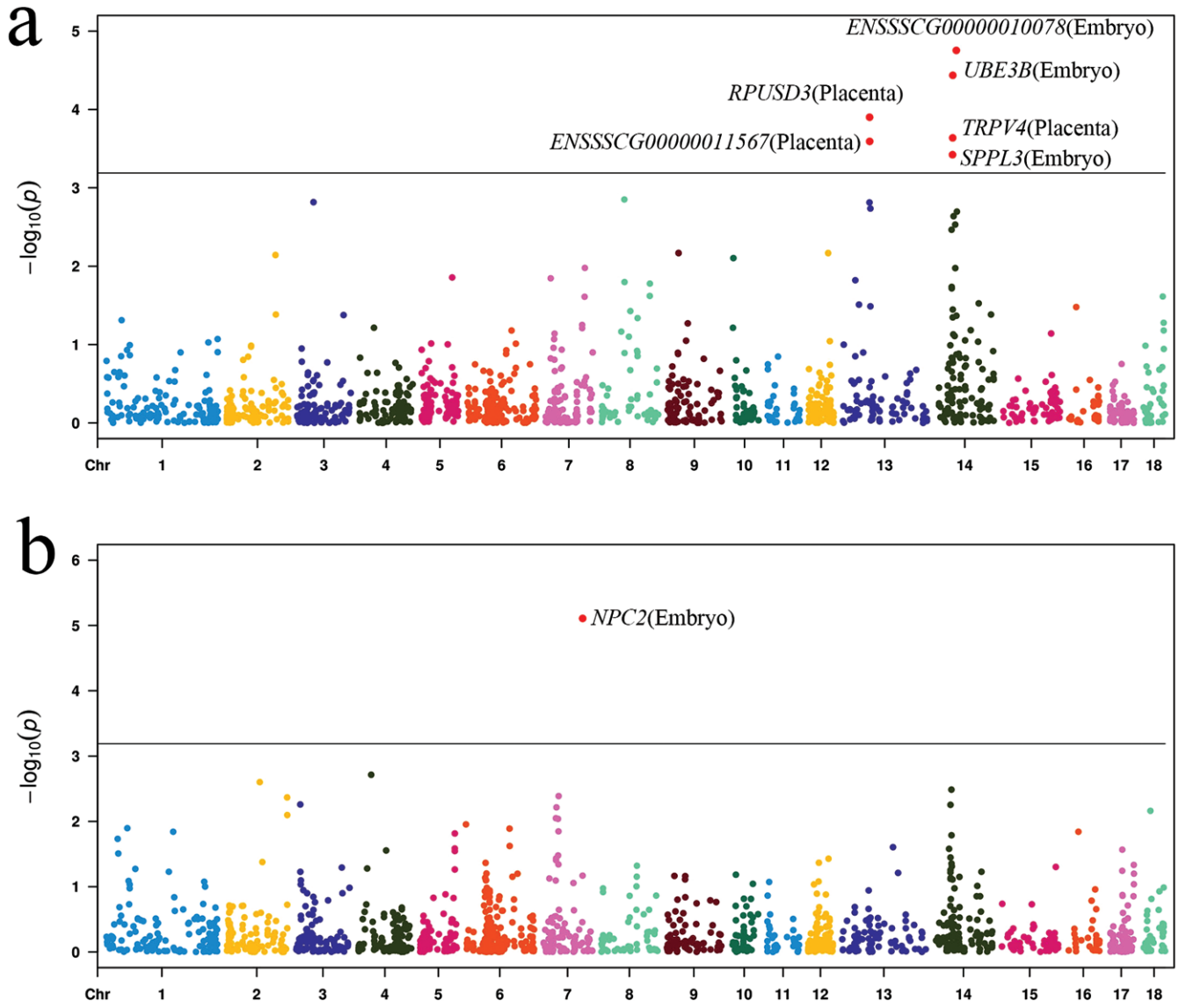

Figure 4. Manhattan plots of TWAS based on embryo and placenta tissues for the TTN in Danish Landrace (a) and Large White (b) pigs.

(de Campos et al., 2021). However, the role of *ENTPD5* in influencing teat number and the genetic mechanisms remain to be further studied. *LIN52* was involved in regulating the transcription process of cell apoptosis (Reddien et al., 2007). Additionally, it forms complexes with multiple genes that regulate the expression of mitosis-related genes during the cell

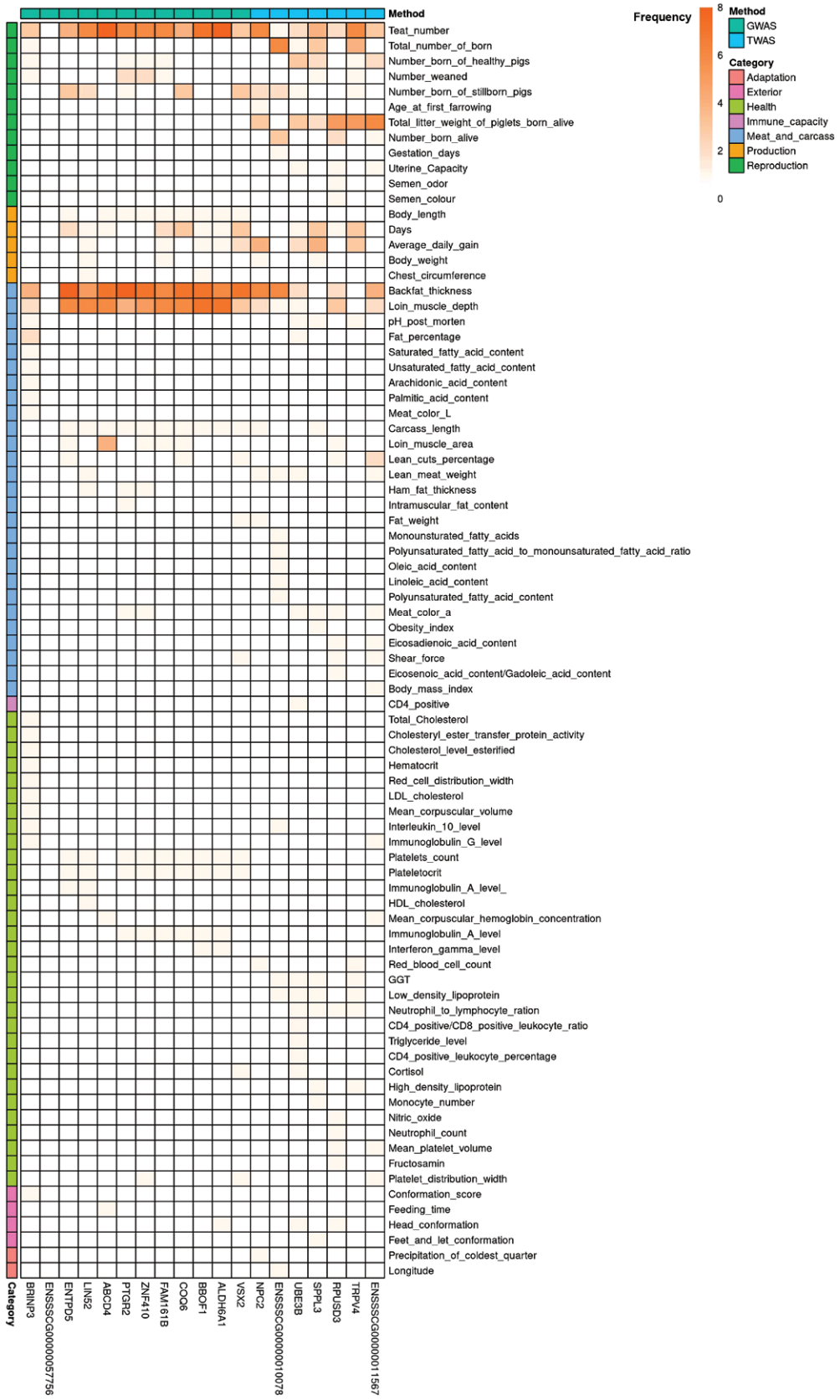

Figure 5. Heat map of the occurrence frequency each trait among top 30 associated items in phenome-wide association studies.

cycle (Sadasivam et al., 2012). The previous study identified differential lncRNA and miRNA target genes in ovarian and testicular tissues (Ma et al., 2020), and the genes related to mammary gland development such as the Wnt and Gata families (Robinson, 2007), including *LIN52*, indicating a close association of *LIN52* with gonadal development. Therefore,

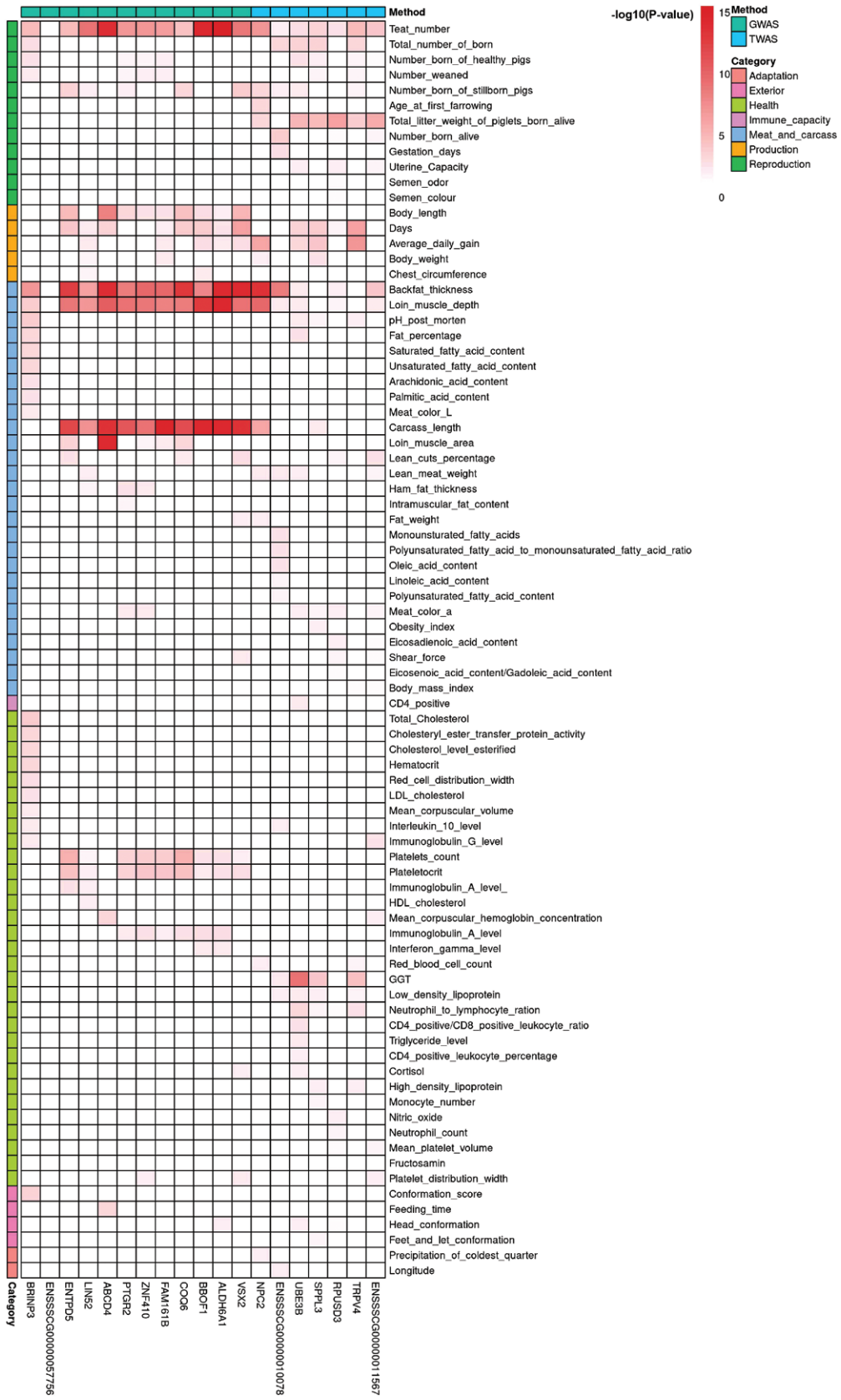

Figure 6. Heat map of the most significant *P*-value each trait among top 30 associated items in phenome-wide association studies.

LIN52 might be a functional gene influencing teat formation. *UBE3B* encodes a member of the E3 ubiquitin-conjugating enzyme family. It had been reported that *UBE3B* plays a key role in the growth and metastasis of breast tumors (Wang et al., 2023, 2024). *BRINP3* was identified as a candidate gene for TTN in Landrace pigs. It had been reported to be

associated with the reproductive ability of Korean cattle, and also noted in horses that *BRINP3* played an important role in reproductive performance, which was under selection pressure.

Conclusion

In summary, this study combined multiple association analyses, including GWAS, TWAS, and PheWAS, to identify 3 key genetic markers (rs338129333, rs318931827, and rs345161260) and 3 novel functional candidate genes (*BRINP3*, *LIN52*, and *UBE3B*) regulating teat number (TTN) in Danish Landrace and Large White pigs. Additionally, genes associated with TTN in PheWAS showed strong associations with backfat thickness and carcass length, suggesting a potential genetic association between teat number and carcass traits. This research lays the foundation for identifying genetic loci that regulate teat number in Danish pigs and advances their molecular breeding.

Supplementary Data

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

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Conflict of interest statement. The authors declare that they have no competing interests.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Zijian Qiu (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing), Yanzhen Yin (Conceptualization, Data curation, Formal analysis, Investigation, Validation, Writing—review & editing), Ruihua Huang (Funding acquisition, Investigation, Project administration, Resources), Jin Zhou (Investigation, Validation, Writing—review & editing), Qian Liu (Data curation, Formal analysis, Supervision, Visualization, Writing—review & editing), Kaiyue Liu (Formal analysis, Visualization), Chenxi Liu (Investigation, Visualization), Pinghua Li (Conceptualization, Funding acquisition, Investigation, Resources, Software, Writing—review & editing), and Qingbo Zhao (Methodology, Supervision, Writing—review & editing)

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