Animal 18 (2024) 101043

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Genomic architecture of carcass and pork traits and their association with immune capacity

T. Jové-Juncà [a], D. Crespo-Piazuelo [a], O. González-Rodríguez [a], M. Pascual [a], C. Hernández-Banqué [a], J. Reixach [b], R. Quintanilla [a], M. Ballester [a,*]

[a] Animal Breeding and Genetics Program, IRTA, Torre Marimon, 08140 Caldes de Montbui, Barcelona, Spain
[b] Selección Batallé S.A., Av. dels Segadors s/n, 17421 Riudarenes, Girona, Spain

ARTICLE INFO

Article history:
Received 25 May 2023
Revised 16 November 2023
Accepted 20 November 2023
Available online 28 November 2023

Keywords:
Carcass quality
Genetic correlations
Immunity
pH
Pig

ABSTRACT

Carcass and pork traits have traditionally been considered of prime importance in pig breeding programmes. However, the changing conditions in modern farming, coupled with antimicrobial resistance issues, are raising the importance of health and robustness-related traits. Here, we explore the genetic architecture of carcass and pork traits and their relationship with immunity phenotypes in a commercial Duroc pig population. A total of nine traits related to fatness, lean content and meat pH were measured at slaughter (~190 d of age) in 378 pigs previously phenotyped (~70 d of age) for 36 immunity-related traits, including plasma concentrations of immunoglobulins, acute-phase proteins, leukocytes subpopulations and phagocytosis. Our study showed medium to high heritabilities and strong genetic correlations between fatness, lean content and meat pH at 24 h postmortem. Genetic correlations were found between carcass and pork traits and white blood cells. pH showed strong positive genetic correlations with leukocytes and eosinophils, and strong negative genetic correlations with haemoglobin, haematocrit and cytotoxic T cell proportion. In addition, genome-wide association studies (**GWASs**) pointed out four significantly associated genomic regions for lean meat percentages in different muscles, ham fat, backfat thickness, and semimembranosus pH at 24 h. The functional annotation of genes located in these regions reported a total of 14 candidate genes, with *BGN*, *DPP10*, *LEPR*, *LEPROT*, *PDE4B* and *SLC6A8* being the strongest candidates. After performing an expression GWAS for the expression of these genes in muscle, two signals were detected in *cis* for the *BGN* and *SLC6A8* genes. Our results indicate a genetic relationship between carcass fatness, lean content and meat pH with a variety of immunity-related traits that should be considered to improve immunocompetence without impairing production traits.

Implications

Nowadays, pig industry is facing new challenges due to the emergence of antibiotic resistance and society demands for healthier livestock products. The incorporation of health-related traits in breeding programmes will contribute to obtain more healthy/robust pig populations. However, little is known about the impact of these traits on productive efficiency and meat quality. This research demonstrated a genetic association between meat and carcass phenotypes with immunity-related traits to be considered in selection programmes to improve simultaneous selection for immunocompetence without impairing meat quality.

Introduction

Pork is currently the second most consumed meat worldwide, representing one-third of global meat consumption (FAO, 2022). As such, great importance and selection pressure have been applied to meat production and quality phenotypes determining consumer acceptance (Davoli & Braglia, 2007). Growth and fattening are major determinants of yield and meat quality measured at slaughter by traits such as carcass weight, fat thickness or lean meat percentage. Medium heritability values have been reported for these carcass phenotypes (Déru et al., 2020; Khanal et al., 2019; Zhou et al., 2021), and a number of quantitative trait loci (**QTL**) have been already described in the literature. For instance, QTLs for lean meat percentages were detected in *Sus scrofa* chromosome (**SSC**)1, SSC2, SSC4, SSC6 and SSC16 (Óvilo et al., 2022; Wimmers et al., 2006; Zhou et al., 2021), and genes such as *PIK32A* or *IGF2* have been described as strong candidate genes.

* Corresponding author.
E-mail address: maria.ballester@irta.cat (M. Ballester).

https://doi.org/10.1016/j.animal.2023.101043
1751-7311/

The mutation IGF2-intron3-G3072A of the *IGF2* gene has been described to have a major impact in backfat thickness across several populations (Estellé et al., 2005; Jungerius et al., 2004).

The semimembranosus pH measured postmortem is an important meat quality phenotype (Lee et al., 2000) that depends on the rapid descent of pH in skeletal muscle after slaughter due to the accumulation of lactic acid derived from fermentation processes (Rosenvold & Andersen, 2003). Although semimembranosus pH is known to be greatly affected by environmental factors such as preslaughter stress (Gonzalez-Rivas et al., 2020), multiple candidate genes have been previously detected for postmortem pH, the most prominent being *RYR1* (also known as the halothane gene), whose mutation c.1843C > T generates pale, soft and exudative meats (Fujii et al., 1991).

Besides the ever-present interest of the swine industry in improving production and quality traits, the growing interest of selecting for healthier livestock has become more prominent in recent years (Phocas et al., 2016). The persistence and spread of endemic and emerging pathogens jointly with the need to reduce the use of antimicrobials in swine production make it advisable to introduce health-related traits in breeding programmes. Immunity traits have been considered biologically relevant parameters to measure immunocompetence (Visscher et al., 2002). Previous studies have determined the genetic determinism of immunity traits in healthy animals showing medium to high heritabilities and describing a number of candidate genes (Ballester et al., 2020 and 2023). However, the genetic association of these health-related phenotypes with production and quality traits has been poorly explored. Considering the interplay between immunological and metabolic processes (Mathis and Shoelson, 2011; Saravia et al., 2020), it becomes necessary to determine the genetic correlation between health and production traits before establishing a breeding programme including immunity/health-related traits among the selection objectives.

In this study, we aimed to deepen in the genetic architecture of nine meat and carcass phenotypes in a commercial Duroc population, as well as determine their genetic association with immune capacity and other health-related traits.

Material and methods

Animal material

Our study departed of a commercial Duroc from Selección Batallé consisting of 378 animals (184 males and 194 females) distributed in six batches of 63 ± 6 individuals each. Each batch corresponded to a group of contemporary animals born and raised at the same time (within a week period) and fattened in the same farm with identical feeding and management conditions. The whole population was found from 22 boars and 132 sows. Two to four animals were selected from each litter, balancing gender when possible. The animals were slaughtered at an average weight of 129 kg, ageing between 181 and 228 days. The animals were fed ad libitum with a commercial cereal-based diet and were apparently healthy.

Phenotypes

After slaughter, the hot carcass weight was measured. Carcass lean meat percentage and also lean meat percentage of the main retails of the carcass (ham, loin and shoulder) were estimated by an on-line ultrasound automatic scanner (AutoFOM, Frontmatec Group, Kolding, Denmark). The carcass lean percentage was estimated on the basis of measurements of 16 ultrasonic transducers that ultrasonically scans the carcass every 5 mm. The same equipment also gave an estimation of the backfat thickness and loin thickness at 6 cm off the midline between the third and fourth last ribs and a backfat thickness in the ham. pH at 24 h postmortem was measured in the semimembranosus muscle (ham) following the procedure described by Gallardo et al. (2012).

Immunity-related traits were measured from blood extracted at 60 ± 8 days of age. Samples were collected via the external jugular vein into vacutainer tubes with or without anti-coagulants (Sangüesa S.A., Spain). Saliva and hair samples were also collected. Classical haematological parameters, stress parameters, and immunological parameters such as immunoglobulins, phagocytic capacity, acute-phase proteins, peripheral blood proportions of lymphocytes populations and nitric oxide were measured from samples as described by Ballester et al. (2020 and 2023).

Descriptive statistics were measured for each tested phenotype. Normality was measured using Shapiro-Wilk test, and logarithm transformation to reach normal distribution of residuals was applied when needed. Impact of cofactors sex, batch (gathering the environmental seasonal and farm effects) and day of lab analysis within batch (for immunity traits) was tested using the lm() and anova() functions in R. Sex and batch effects were found to be significant in almost all phenotypes and included in subsequent analyses, while the date of analysis only impacted traits related to phagocytic capacity.

Finally, pairwise phenotypic correlations between the residuals (after correction for sex and batch effects) of all analysed phenotypes were calculated using Pearson's method. Statistical analyses were performed using R software version 4.0.4 (R Core Team, 2021) unless stated otherwise. R packages corrplot (Wei, 2021) and data.table (Srinivasan, 2021) were used to reduce computing time.

Single nucleotide polymorphism genotyping

Genomic DNA was extracted from blood samples using NucleoSpin® Blood kit (Macherey-Nagel, Germany). DNA concentration and purity were measured in a Nanodrop ND-1000 spectrophotometer. Animals were genotyped with the GGPSNP70 chip (Illumina, San Diego, CA) using the Infinium HD Assay Ultra protocol (Illumina). Raw data were filtered using Plink v1.90b3.42 (Purcell, 2007) to remove those single nucleotide polymorphisms (**SNPs**) with a minor allele frequency of less than 5% and a missing genotype data of more than 10%. SNPs that did not map to the porcine reference genome (Sscrofa11.1 assembly) were also excluded. A total of 42 641 SNPs remained after filtering.

Estimation of genetic parameters

Genetic parameters were estimated by restricted maximum likelihood using the BLUPF90 software package (Misztal et al., 2014). First, variance components for each trait were estimated under an univariant mixed animal model as follows:

$$Y = X\beta + Zu + e$$

where Y is the vector of phenotypes of all individuals; β is the vector of systematic (fixed) effects on the trait including sex (two levels) and batch (six levels); u is the vector of animal's genetic additive (random) effects; X and Z are the corresponding incidence matrices for β and u; and e is the vector of random residual terms. The assumed distribution of additive genetic effects was $\mathbf{u} \sim N(0, A\boldsymbol{\sigma}_u^2)$, where A is the numerator relationship matrix computed on the basis of pedigree and $\boldsymbol{\sigma}_u^2$ is the additive genetic variance; random errors were distributed as $e \sim N(0, I\boldsymbol{\sigma}_e^2)$. Estimated heritabilities ($\mathbf{h^2}$) for all the analysed traits were obtained from the variance components ($\boldsymbol{h}^2 = \boldsymbol{\sigma}_u^2/(\boldsymbol{\sigma}_u^2 + \boldsymbol{\sigma}_e^2)$). The SEs of the heritability estimates were also computed.

Additionally, also h^2 estimates using GBLUP, thus considering the genomic relationship matrix (***G***) instead of ***A*** to depict the kinship between animals, were computed. We obtained similar results but larger SEs in the additive genetic variance estimates. In order to keep consistency with previous studies in this population (Ballester et al., 2020 and 2023), only parameters estimated on the basis of pedigree information will be shown.

Subsequently, pairwise genetic correlations across carcass and meat quality traits as well as between quality and immune-related traits were estimated in a two-trait animal model described as follows:

$$\begin{bmatrix} \boldsymbol{Y}_{t1} \\ \boldsymbol{Y}_{t2} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X}_{t1} & 0 \\ 0 & \boldsymbol{X}_{t2} \end{bmatrix} \begin{bmatrix} \boldsymbol{\beta}_{t1} \\ \boldsymbol{\beta}_{t2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z}_{t1} & 0 \\ 0 & \boldsymbol{Z}_{t2} \end{bmatrix} \begin{bmatrix} \boldsymbol{u}_{t1} \\ \boldsymbol{u}_{t2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{e}_{t1} \\ \boldsymbol{e}_{t2} \end{bmatrix}$$

where $\boldsymbol{Y_{t1}}$ and $\boldsymbol{Y_{t2}}$ are the vectors of phenotypic observations for trait 1 and trait 2, respectively; $\boldsymbol{\beta_{t1}}$ and $\boldsymbol{\beta_{t2}}$ are the vectors of systematic (fixed) effects on each trait, sex and batch for all traits plus date of laboratory analysis (within batch) for phagocytosis phenotypes, and $\boldsymbol{X_{t1}}$ and $\boldsymbol{X_{t2}}$ the correspondent incidence matrices; $\boldsymbol{u_{t1}}$ and $\boldsymbol{u_{t2}}$ are the vectors of animal genetic additive effects on trait 1 or trait 2 (random effects), and $\boldsymbol{Z_{t1}}$ and $\boldsymbol{Z_{t2}}$ the corresponding incidence matrices; finally $\boldsymbol{e_{t1}}$ and $\boldsymbol{e_{t2}}$ are the vectors of residual errors for each trait. The (co)variance matrix of random genetic effects was defined as:

$$Var\begin{bmatrix} \boldsymbol{u}_{t1} \\ \boldsymbol{u}_{t2} \end{bmatrix} = \begin{bmatrix} \boldsymbol{A}\sigma^2_{u1} & \boldsymbol{A}\sigma_{u1,u2} \\ \boldsymbol{A}\sigma_{u1,u2} & \boldsymbol{A}\sigma^2_{u1} \end{bmatrix} = \boldsymbol{A} \otimes \begin{bmatrix} \sigma^2_{u1} & \sigma_{u1,u2} \\ \sigma_{u1,u2} & \sigma^2_{u1} \end{bmatrix}$$

where σ^2_{u1} and σ^2_{u2} are the additive genetic variance of traits 1 and 2, respectively, $\sigma_{u1,u2}$ is the genetic covariance between the traits, and A is the numerator relationship matrix as defined above. Estimation of the (co)variance components of each pairwise analysis was also performed by restricted maximum likelihood using the BGF90 package. Genetic correlations between traits were obtained as $r_g = \left(\frac{\sigma_{u1,u2}}{\sigma_{u1} \hat{A} \cdot \sigma_{u2}}\right)$, and the SE of the genetic correlation estimates were also computed.

Genome-wide association studies

Genome-wide association studies (**GWASs**) for the nine traits related to production and meat quality were carried out with the 42,641 filtered SNPs using GCTA 1.93.2 software (Yang et al., 2011) with the fastGWA modality. The model used for the GWAS was as follows:

$$\boldsymbol{Y} = \boldsymbol{X\beta} + \boldsymbol{Zg} + \boldsymbol{S}_l a_l + \boldsymbol{e}$$

where *Y* stands for the vector of phenotypic observations for each of the nine analysed traits; β corresponds to the vector of systematic effects sex and batch as described above; *g* is the vector of infinitesimal genetic effects of each individual, with distribution $\boldsymbol{g} \sim N(0, \mathbf{G}\boldsymbol{\sigma}^2_{\boldsymbol{g}})$, where **G** is the genomic relationship matrix calculated using the filtered autosomal SNPs based on the methodology of Yang et al. (2011) and $\boldsymbol{\sigma}^2_{\boldsymbol{g}}$ is the additive genetic variance; $\boldsymbol{S_l}$ is the vector of genotypes (coded as 0, 1, 2) of each individual for the l^{th} SNP, and $\boldsymbol{a_l}$ is the allele substitution effect of the l^{th} SNP on the trait under study. A multiple testing correction was performed using the False Discovery Rate (**FDR**) method (Hochberg & Benjamini, 1990) to minimise type-I errors through the p.adjust function of R base. The significant association threshold was set at FDR $\leq$ 0.05. Significant SNPs were analysed with Variant Effect Predictor software to determine their genetic location (e.g. upstream of a transcript, in coding sequence, in non-coding RNA, in regulatory regions) and possible functional consequences (McLaren et al., 2016).

Candidate genes

Significant variants were considered part of the same associated QTL region if they were less than 1 Mb apart from each other based on linkage disequilibrium of our population. Only regions with two or more significant variants were retained as putative QTL. Biomart software (Durinck et al., 2009) was used to extract genes located inside significant regions leaving 1 Mb downstream/upstream of genomic intervals using the Ensembl Genes 106 Database of Sscrofa11.1 reference assembly. QTLs found were then cross-referenced with the animal QTLdb (Hu et al., 2022).

Functional characterisation of genes was done with the ClueGO v2.5.8 plug-in of Cytoscape v3.9.1 using Gene Ontology (**GO**) terms and considering the human homologues of the detected genes (Bindea et al., 2009; Shannon et al., 2003). In addition, Mouse Genome Database (Blake et al., 2021) and Genecards (Safran et al., 2021) were used to identify gene functions affecting the analysed phenotypes.

Whole-genome and RNA sequencing data

Whole-genome and muscle RNA sequencing data from 100 individuals of our Duroc population (Crespo-Piazuelo et al., 2023) were used to identify regulatory variants. As described in Crespo-Piazuelo et al. (2023), DNA samples were sequenced using NovaSeq6000 platform to a minimum read depth of 10X. DNA sequences were mapped against the reference genome (Sscrofa11.1 assembly) with BWA-MEM/0.7.17 (Li, 2013). Genetic variant calling (SNPs and indels) was performed with GATK/4.1.8.0 Haplotype Caller. Genetic variants with a minor allele frequency of less than 5% and missing genotype data of more than 10% were filtered with PLINK/v1.90b3.42 (Chang et al., 2015), leaving a total of 15 385 316 polymorphisms for downstream analysis. RNA sequences were mapped against the reference genome (Sscrofa11.1 assembly) and the latest Ensembl Genes annotation database (101) to date using STAR/v2.5.3a (Dobin et al., 2013) and gene counts were quantified with RSEM/1.3.0 (Li and Dewey, 2011) using default parameters. Counts were normalised by trimmed mean of M values and transformed to cpm using $\log_2$ with edgeR/3.30.3 Bioconductor package (Robinson et al., 2010).

Expression genome-wide association study

An expression GWAS (**eGWAS**) analysis of our candidate genes was performed using skeletal muscle RNA-seq data from 100 individuals of the Duroc population as well as 15 385 316 polymorphisms from their whole-genome sequencing. The same model as explained before for GWAS studies using both sex and batch as fixed cofactors was used. Significant polymorphisms that were located at less than 1 Mb from the associated gene were defined as *cis*-SNPs (GTEx Consortium, 2015), and its functional prediction was assessed with VEP tool on the Ensembl Genes 106 Database.

Results

Descriptive statistics and phenotypic correlations of carcass and meat quality traits

In the present study, we measured a total of nine phenotypes related to meat and carcass quality on 378 individuals belonging to a commercial Duroc pig line. Descriptive statistics for measured traits are shown in Table 1. The phenotypes generally presented low variation as expected from a commercial population selected for production traits. Loin lean meat percentage showed the highest variation (CV = 0.27) among all analysed traits, while

Table 1
Mean, SD and CV of porcine carcass and meat phenotypes.

Trait	ID	Mean	SD	CV
Carcass weight (kg)	CW	98.22	11.48	0.12
Lean meat percentage (%)	LM	40.35	6.72	0.17
Ham lean meat percentage (%)	HLM	59.48	6.79	0.11
Loin lean meat percentage (%)	LLM	37.19	10.19	0.27
Shoulder lean meat percentage (%)	SLM	54.82	6.57	0.12
Backfat thickness at 3rd–4th rib (mm)	BFT	30.99	6.74	0.22
Loin depth at 3rd–4th rib (mm)	LD	45.72	6.76	0.15
Ham fat thickness (mm)	HFT	20.63	4.45	0.22
pH 24 h semimembranosus	pH24	5.70	0.18	0.03

semimembranosus pH showed the least (CV = 0.03). Of all traits related to the localised lean meat percentage, the lowest variation was found for shoulder lean meat percentage (CV = 0.12).

Estimated pairwise phenotypic correlations ($\mathbf{r_p}$) between all analysed traits once corrected for fixed factors are shown in Fig. 1A; the estimated phenotypic correlation coefficients and their SEs are reported in the Supplementary Table S1. Most of these correlations were significant, their confidence intervals at 95% excluding zero, excepting the phenotypic correlation between loin depth and carcass weight. As expected, the two measures of coverage fat (backfat and ham fat thickness) showed a very high correlation coefficient (r_p = 0.95). Similarly, the different lean meat measures were strongly correlated between them (r_p ranging from 0.91 to 0.98) and more moderately with loin depth (r_p from 0.74 to 0.78) while reported strong negative correlations with backfat and ham fat thickness (r_p ranging from −0.97 and −0.86). Carcass weight showed low to moderate correlations either negative with lean percentage or positive with fatness traits. Finally, the semimembranosus pH showed significant but generally low phenotypic correlations with carcass traits (Fig. 1A).

Genetic determinism and genetic correlations of carcass and meat quality traits

The genetic determinism of the analysed traits was assessed by estimating their heritability (Table 2). Medium to high heritability estimates were obtained for all carcass traits, ranging from 0.50 to 0.66. Among them, carcass weight was the most heritable trait,

Table 2
Heritabilities (h^2) estimates for porcine carcass and meat traits plus the SE and confidence interval (CI).

Trait	h^2	SE	CI
Carcass weight (kg)	0.656	0.170	[0.32,0.99]
Lean meat percentage (%)	0.643	0.182	[0.29,1.00]
Ham lean meat percentage (%)	0.569	0.178	[0.22,0.92]
Loin lean meat percentage (%)	0.642	0.181	[0.29,1.00]
Shoulder lean meat percentage (%)	0.504	0.171	[0.17,0.84]
Backfat thickness at 3rd–4th rib (mm)	0.615	0.180	[0.26,0.97]
Loin depth at 3rd–4th rib (mm)	0.640	0.164	[0.32,0.96]
Ham fat thickness (mm)	0.524	0.178	[0.18,0.87]
pH 24 h semimembranosus	0.348	0.158	[0.038,0.66]

whereas the semimembranosus pH showed a lower but still significant heritability (h^2 = 0.35). The h2 estimate SEs ranged between 0.16 and 0.18, thus conducting to wide confidence intervals for these parameters, but not encompassing zero in any case.

To estimate the genetic relationships between these traits, pairwise genetic correlations ($\mathbf{r_g}$) were calculated; Fig. 1B shows the map of estimated genetic correlations, whereas the genetic correlation coefficients and their SEs can be shown in the Supplementary Table S2. The genetic association pattern showed in Fig. 1B was close to those depicted by phenotypic correlations. The limited sample size did not allow obtaining a high accuracy in the estimated correlation coefficients, the SEs ranging between 0.0025 and 0.22. However, most of the estimated correlations were certainly relevant and different from zero. This way, strong positive genetic associations (r_g > 0.9) were found between the lean meat

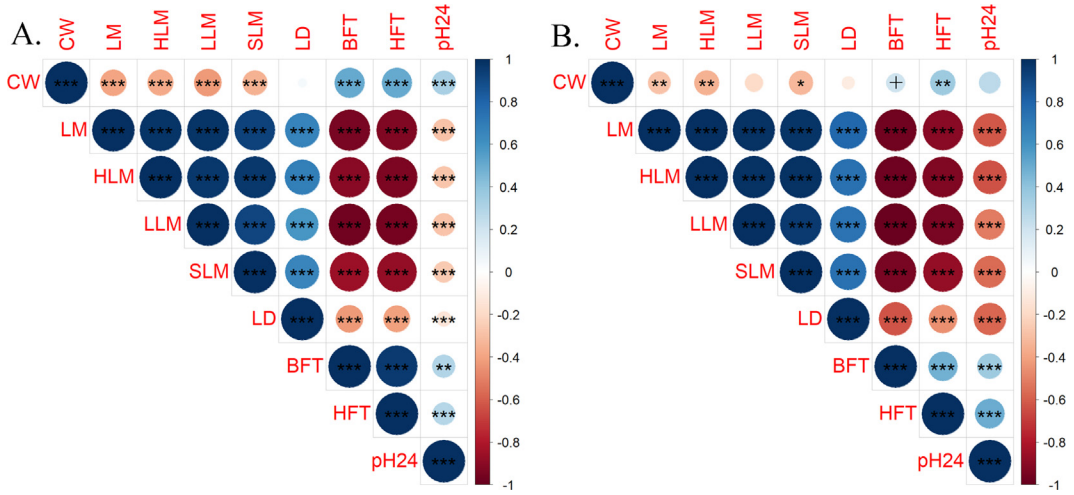

Fig. 1. Heatmap depicting correlations of the residuals between porcine carcass and meat traits: carcass weight (CW), lean meat percentage (LM), ham lean meat percentage (HLM), loin lean meat percentage (LLM), shoulder lean meat percentage (SLM), backfat thickness (BFT), loin depth (LD), ham fat thickness (HFT) and pH 24 h semimembranosus (pH24). **A**. Pairwise phenotypic correlations. **B**. Pairwise genetic correlations. Blue represents positive correlations while red represents negative correlations. Circle size and colour intensity are proportional to absolute value of correlation coefficients. Significance of correlation is marked with symbols: $^{+}P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

percentage measured at different points. Also, a strong genetic antagonism between lean meat percentage in different areas and subcutaneous fat measures (backfat thickness and ham fat thickness) was observed, with r_g estimates ranging from −0.99 to −0.88. The loin depth had stronger associations at genetic level than at phenotypic level, showing rg estimates ∼0.7 with lean content and ∼−0.9 with fatness. Conversely, the semimembranosus pH at 24 h postmortem appeared as negatively associated at genetic level with lean percentages (r_g from −0.64 to −0.52) and positively with fatness traits (r_g 0.49 and 0.36).

Genetic association between quality and immunity traits

Genetic correlations of the analysed carcass and meat quality phenotypes with a plethora of traits related with health and immune capacity were estimated. The map of estimated genetic relationships between carcass and immunity traits is depicted in Fig. 2, whereas the estimated genetic correlation coefficients and their SEs are provided in the Supplementary Table S2. The SEs of estimated genetic correlations between immunity and carcass traits were generally high, ranging from 0.14 to 0.5. Only the most relevant and significantly different from zero genetic correlations will be presented hereafter.

The genetic associations with immunity and hemogram parameters showed a consistent relationship between the number of white blood cells and carcass traits. Total leukocytes and their subtype counts (lymphocyte, eosinophil and neutrophil counts) showed positive genetic correlations with lean content measures and neutrophile counts presented negative correlations with ham fat thickness. These carcass phenotypes had also relevant genetic associations with the relative abundance of some lymphocyte T subpopulations. Conversely to leukocyte counts, the concentration of γδ T cells correlated negatively with lean meat content measures (r_g = −0.49 to −0.53) and positively with fat measures (r_g = 0.46 and r_g = 0.51), whereas the relative proportion among PBMCs (peripheral blood mononuclear cells) of memory T cells showed exactly the opposite pattern, i.e. positive genetic correlations with lean content measures (r_g from 0.43 to 0.58) and negative association with fatness traits (r_g = −0.55 and −0.48). Carcass weight showed lower genetic correlations with immunity and hemogram parameters than carcass fat and lean scores, with the exception of a strong negative correlation with platelet counts (r_g = −0.75). Finally, it is worth noting that no genetic associations seem to exist between carcass traits and other immunity and health phenotypes such as plasma concentrations of Ig, acute-phase proteins, erythrocytes count or haemoglobin concentrations.

Semimembranosus pH was the trait most genetically correlated with immunity and haematological traits measured at young age. To be remarked, the intramuscular pH at 24 h postmortem showed strong genetic antagonism with the cortisol concentration in hair (r_g = −0.61), the haematocrit and haemoglobin concentration (r_g = −0.74 and −0.80, respectively) and with the relative abundance among peripheral blood mononuclear cells (**PBMCs**) of cytotoxic T cells (r_g = −0.71). Despite less strong, the semimembranosus pH also showed positive genetic associations with leukocyte counts (r_g = 0.64).

Genomic regions and candidate genes associated with carcass quality traits

GWAS reported a total of 32 significantly associated SNPs (FDR < 0.05) for seven traits located at four chromosomal regions on pig chromosomes SSC6, SSC14, SSC15 and SSCX (Table 3). Manhattan plots of the GWAS results for the traits with significant associations are displayed in Fig. 3. All the associated SNPs are shown in Supplementary Table S3, and their predicted consequences are shown in Supplementary Table S4. No significant variants were found for loin depth and carcass weight.

The most significant association was found for the semimembranosus pH24 in SSC6 at 146.54–146.62 Mb, with the most associated SNPs (rs81392507 and rs319373989) found in intron 2 of the *PDE4B* gene.

Regarding carcass fatness and lean content, two significant pleiotropic genomic regions were found in SSC15 and SSCX. The region between positions 21.05 and 21.31 Mb in SSC15 was associated with all measures of fat thickness and lean content but shoulder lean meat percentage was suggestive (*P*-value = 2.24×10^{-5}; FDR = 0.0502). The most significant SNP found in this region was rs322842057 located inside intron 1 of the *DPP10* gene. In SSCX, the region between positions 122.70 and 125.68 Mb was associated with all lean meat percentages and fat thickness traits. Top significant variants were found inside the 5′UTR region of *MTCP1*, intronic regions of *SLC6A8*, *PDZD4* and *L1CAM*, upstream of ENSSSG00000012792 and downstream of *HCFC1*. A region in SSC14, between positions 131.31 and 131.57 Mb, with rs80996847 as the most significant SNP located in an intergenic region, was associated with the shoulder lean meat percentage.

A functional analysis was conducted using ClueGO (Bindea et al., 2009) to identify candidate genes functionally associated with the significant traits. For the region in SSC6 associated with pH24, we identified five candidate genes related to the following biological functions: adenylate cyclase-activating adrenergic receptor signalling pathway (*PDE4B*), leptin receptor activity (*LEPR, LEPROT*), regulation of clathrin-dependent endocytosis (*DNAJC6*), and regulation of membrane protein ectodomain proteolysis (*NRDC*).

For the pleiotropic region in SSCX associated with all traits but pH24, we identified several candidate genes related to axonogenesis, cytosolic calcium transporting and semaphoring signalling, mesenchymal cell proliferation, and blood coagulation (*PLXNA3*, *FLNA*, *PLXNB3* and *BGN*), and muscle energy metabolism (*SLC6A8*). In SSC15, the candidate gene *DPP10* related with the regulation of potassium ion transmembrane transport was identified.

In the SSC14 genomic region associated with shoulder lean meat percentage, three different genes were detected, *ATE1* related to proteasomal protein catabolic process, AK4 related to nucleoside triphosphate adenylate kinase activity, and *FGFR2* related to fibroblast growth factor receptor signalling pathway.

Genomic regions associated with candidate genes expression in muscle and putative regulatory polymorphisms

A recent study has described a significant enrichment between cis-eQTLs and complex traits, including meat and carcass traits (Teng et al., 2022). eGWAS analyses were performed for those candidate genes expressed in muscle to identify regulatory polymorphisms. A total of five eQTL were detected for four out of 11 candidate genes analysed (Table 4). Two of the five eQTLs were detected in *cis* for the candidate genes *BGN* and *SLC6A8* associated with all lean meat percentages and fat thickness traits. The top polymorphisms, i.e., the most significantly associated polymorphisms, for *BGN* gene expression, were detected upstream of the gene. For *SLC6A8*, the top polymorphism (rs345048026) was located inside intron 1. Animals were then classified according to genotypes of the top significant expression-associated polymorphisms. For the top polymorphism of *BGN* (rs1108637659), animals with the AA genotype showed a higher expression as compared to animals with the other two genotypes (GA and GG). AA homozygous individuals showed also higher lean meat percentages and lower fat thickness in comparison with GA and GG individuals. An opposite pattern was observed for the top polymorphism (rs345048026) associated with the expression of

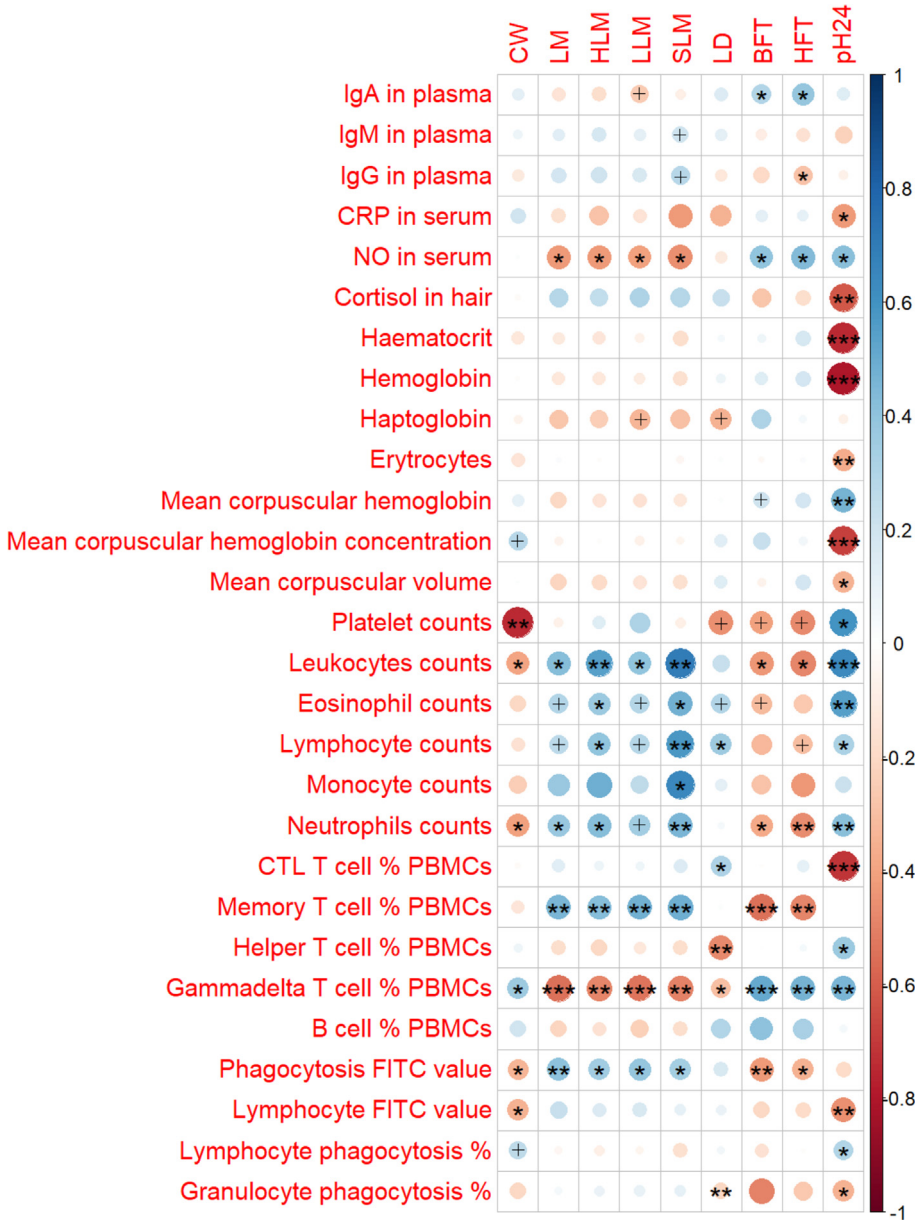

Fig. 2. Heatmap depicting genetic correlations between immunological traits (rows) and carcass and meat quality traits (columns) in pigs. Colour blue represents positive correlations while red represents negative correlations. Size of the circle and colour intensity are proportional to absolute value of correlation coefficients. Abbreviations: CW = Carcass weight; LM = Lean meat percentage; HLM = Ham lean meat percentage; LLM = Loin lean meat percentage; SLM = Shoulder lean meat percentage; BFT = Backfat thickness; LD = Loin depth; HFT = Ham fat thickness; pH24 = pH 24 h semimembranosus; Ig = Immunoglobulin; CRP = C reactive protein; NO = Nitric oxide; CTL = Cytotoxic T cells; FITC = Fluorescein isothiocyanate. Significance of correlation is marked with symbols: $^{+}P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Table 3
Description of the regions significantly associated with porcine carcass and meat phenotypes according to the genome-wide association study (GWAS).

Region	Chr	Start (Mb)	End (Mb)	n° of SNP in region	Top SNP	MAF	*P*-value	FDR	Trait	Functional candidates
1	6	146.54	146.62	4	rs81392507; rs319373989	0.26	3.61E−08	6.33E−04	pH24	*LEPR; LEPROT; DNAJC6; NRDC; PDE4B*
2	14	131.31	131.57	5	rs80996847	0.41	4.06E−06	1.32E−02	SLM	*ATE1; FGFR2; AK4*
3	15	21.05	21.31	4	rs322842057	0.25	8.14E−07	3.54E−03	BFT; LM; HLM; LLM; HFT	*DPP10*
4	X	122.70	125.68	19	rs81000133; rs336426564; rs81473939; rs320982055; rs325378845	0.46	1.38E−07	9.32E−04	BFT; LM; SLM; HFT; HLM; LLM	*BGN; FLNA; PLXNA3; PLNXB3; SLC6A8*

Abbreviations: Chr = Chromosome; SNP = Single nucleotide polymorphism; MAF = Minor allele frequency; FDR = False discovery rate; LM = Lean meat percentage; HLM = Ham lean meat percentage; LLM = Loin lean meat percentage; SLM = Shoulder lean meat percentage; BFT = Backfat thickness; HFT = Ham fat thickness; pH24 = pH 24 h semimembranosus.

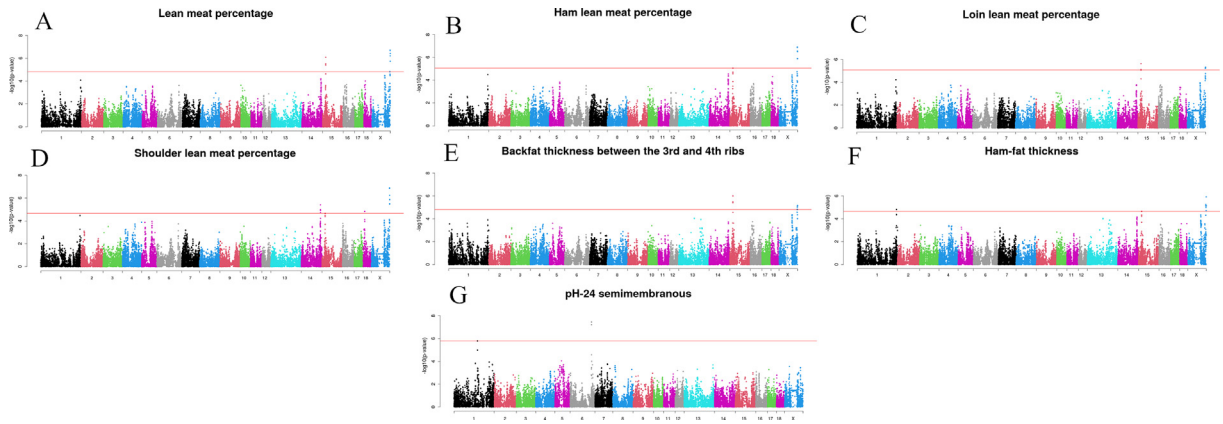

Fig. 3. Manhattan plots depicting significant associations across the whole porcine genome for porcine meat and carcass quality traits: **(A)** Lean meat percentage, **(B)** Ham lean meat percentage, **(C)** Loin lean meat percentage, **(D)** Shoulder lean meat percentage, **(E)** Backfat thickness, **(F)** Ham fat thickness and **(G)** pH 24 h semimembranosus. Red line is located at last significant variant with an FDR < 0.05. Abbreviations: FDR = False Discovery Rate.

Table 4
Description of the expression quantitative trait loci (eQTL) detected for the functional candidate genes for porcine carcass and meat phenotypes expressed in pig muscle.

Gene	SSC	Start (Mb)	End (Mb)	Most significant variant	*P*-value	FDR	n° variants	CIS/TRANS
ENG	11	69.85	69.90	rs339479719	9.26E−10	0.014	3	TRANS
BGN	X	122.92	125.40	rs1108637659	9.95E−11	1.53E−3	34	CIS
SLC6A8	X	124.40	125.17	rs345048026	5.31E−10	8.17E−3	12	CIS
PLXNA3	3	68.01	79.31	Rs3470820030	3.98E−10	0.047	3	TRANS
PLXNA3	7	40.43	40.50	Rs1109534408	9.84E−10	0.015	10	TRANS

Abbreviations: SSC = *Sus scrofa* Chromosome; FDR = False discovery rate.

SLC6A8. Homozygous AA animals showed lower meat and higher fat thickness percentages, and a higher expression of *SLC6A8* compared to their CA and CC counterparts (Fig. 4).

Discussion

In recent years, health and welfare-related traits have begun to be considered in pig breeding schemes to increase resilience, robustness and wellbeing of pigs. Thus, the need to determine the trade-offs and the genetic correlations between health and productive traits has become more apparent, to allow an effective selection of these traits without impairing the performance of the animals. In this study, we have explored the genetic architecture of nine carcass and meat traits in a commercial Duroc population and have estimated genetic correlations between them and 36 health-related traits in order to infer a map of genetic associations.

Estimated genetic parameters denoted an important genetic determinism of the carcass and meat traits in our population, as it has been previously reported in other porcine populations (Déru et al., 2020; Khanal et al., 2019; Zhou et al., 2021). The carcass traits related to lean content and fatness showed high heritability estimates but with wide confidence intervals due to the limited sample size. In any case, estimated heritabilities in our Duroc experimental population were higher than those previously reported in Duroc populations (Eusebi et al., 2017; González-Prendes et al., 2017; Zhou et al., 2021), but in line with former studies in other breeds (Ducos, 1994; Johansson et al., 1987). The semimembranosus pH at 24 h had a more moderated heritability,

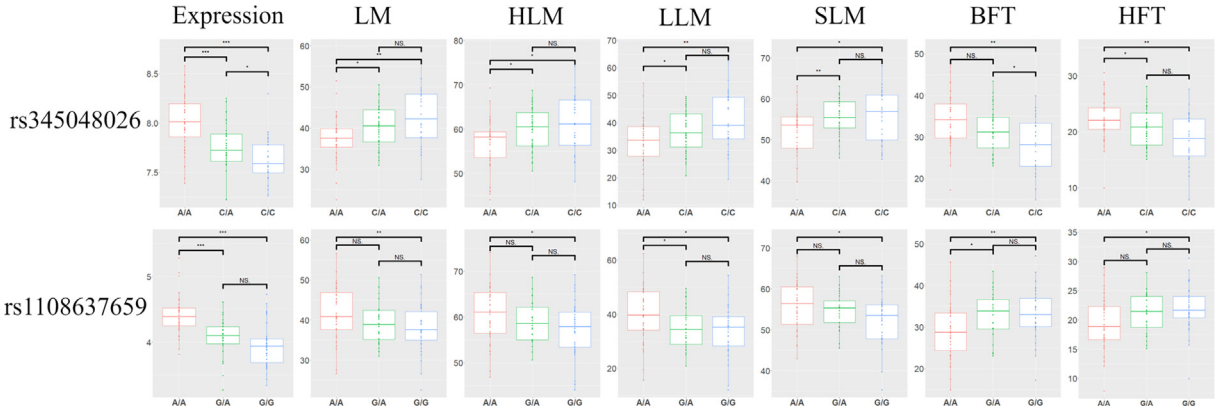

Fig. 4. Boxplots of the individual phenotypes according to the genotypes for the topmost significant porcine cis-eQTLs variants: rs1108637659 and rs345048026 for the quality candidate transcripts *BGN* and *PLXNA3*, respectively. Phenotypes correspond to transcript expression, lean meat percentage (LM), ham lean meat percentage (HLM), loin lean meat percentage (LLM), shoulder lean meat percentage (SLM), backfat thickness (BFT) and ham fat thickness (HFT). Significance levels: NS (non-significant); **P* < 0.05; ***P* < 0.01 and ****P* < 0.001. Abbreviations: eQTL = expression Quantitative Trait Loci.

consistent with increased dependency of environmental and slaughter conditions (Gonzalez-Rivas et al., 2020).

Estimated correlations allowed depicting a map of phenotypic and genetic associations between these carcass traits. Results are in agreement with the strong antagonism between fatness and lean content as detailed in Hoa et al. (2021) and Lebret and Čandek-Potokar (2022) at both phenotypic and genetic levels. Strong positive correlations within lean meat measures were also expected given the uniformity of lean meat percentage across different muscles in this population, as previously reported by (Eusebi et al., 2017). The semimembranosus pH at 24 h seemed to have a positive genetic association with fatness and a negative one with lean content and loin depth, despite these relationships were much weaker at phenotypic level. Multiple major genes related to meat pH and pale, soft and exudative meats meats have been reported to have effects on carcass quality such as the halothane resistance gene (Scheffler & Gerrard, 2007). Our results agree with the lack of the recessive allele for *RYR1* in our population since the recessive allele has been associated with higher carcass yield, lean percentage and susceptibility to acute stress with pH decline and production of pale, soft and exudative pork (Scheffler & Gerrard, 2007). Furthermore, a strong negative association between cortisol concentration in hair and pH at 24 h was observed in our population.

The map of genetic associations with health-related and immunity phenotypes reported consistent associations between carcass traits and white blood cells, including the relative abundance of some T lymphocytes measured at young age. Lean scores correlated positively with the leukocyte counts and the relative proportion among PBMCs of memory T cells, and were negatively associated with γδ T cells. Previous studies in pigs have associated different metabolic profiles according to lean meat or fatness content. Pigs with higher fat deposition showed an increase of the lipogenic profile compared to pigs with higher lean meat content and polyunsaturated fatty acids (Corominas et al., 2013; Poklukar et al., 2020). Metabolic pathways also play an important role in T lymphocyte differentiation and function. While memory T cells display a catabolic signature regulated by the AMP-activated protein kinase pathway and use mitochondrial fatty acid oxidation for survival and development (Howie et al., 2018; Pearce et al., 2009), γδ T cells differentiation relies on the glucose metabolism and glycolysis by mTOR-mediated metabolic pathways (Meng et al., 2021). These observations are in agreement with the opposite correlations between γδ T cells and memory T cells with fatness and lean meat content traits observed in our Duroc animals that appear to be determined by switches in metabolic pathways.

Furthermore, semimembranosus pH showed strong positive correlations with white blood cell counts while having negative genetic associations with haemoglobin, haematocrit and cytotoxic T cell percentage. A previous study indicated that animals with higher pH at 24 h postmortem showed lower drip loss and higher white blood cells and monocyte counts (Koomkrong et al., 2017). Since semimembranosus pH is a vital trait in pork quality assessment, traits showing strong genetic correlations with semimembranosus pH should be checked in genetics to avoid producing pale, soft and exudative or excessively dry meats (Lee et al., 2000).

GWAS studies for the nine meat and carcass traits allowed identifying four QTLs in four chromosomes. We are aware about the modest statistical power of our GWAS derived from the limited sample size of our population, so we do not discard some other genomic regions affecting the analysed traits that may have been discarded by the conservative statistical thresholds.

The pleiotropic regions in SSC15 and SSCX detected for lean meat percentages and for ham and backfat thickness traits were found close to previously reported porcine QTLs for the same phenotypes (Groenen et al., 2012; Kuryl et al., 2003; Cepica et al., 2003). An additional region was found for shoulder lean meat percentage in SSC14, inside a previously found QTL for lean meat percentage reported by (Dragos-Wendrich et al., 2003). It is worth to highlight that two of the detected QTLs were overlapping or very close to genomic regions for immunity and robustness traits (Ballester et al., 2020). In SSC6, the QTL for pH24 was close to QTLs for mean corpuscular volume and mean corpuscular haemoglobin. In SSC14, a QTL colocalised for leucocyte count and shoulder lean meat percentage. In both cases, the involved traits showed significant genetic correlations between them.

The top significant variant in SSC15 was found within the *DPP10* gene. This gene encodes a single-pass type II membrane protein, which has been related to obesity in human (Melén et al., 2010). In SSCX, several candidate genes for fatness and lean phenotypes were found. *FLNA*, which acts as a molecular anchor in Z discs and as such, has an impact on muscle conformation (Mao & Nakamura, 2020). BGN which encodes a member of the *SLRP* family has key roles on collagen upkeep and tendon organisation as well as muscle development and regeneration (Robinson et al., 2017). *BGN* knockout mice have been reported to have conformational effects (IMPC, 2014). Remarkably, a cis-eQTL associated with the expression of BGN was detected, with the most significant variant (rs1108637659) being located upstream of the gene. Animals homozygous for the A allele showed both higher BGN expression and lean meat percentage in different muscles, and lower fatness measures compared to animals carrying the G allele, consistently with the gene function. Therefore, the rs1108637659 polymorphism could be considered a strong candidate that deserves further study. Among the most significant variants of the SSCX QTL, rs81000133 and rs336426564 were found inside intronic regions of the *SLC6A8* gene. The protein encoded by this gene transports creatine across plasmatic membrane. Creatine is used as a high-energy buffer in organs such as the brain or skeletal muscle, and as such has been shown to impact motor functions and muscle metabolism (Bryant Chase et al., 2018). Remarkably, the SNP rs345048026, also located in intron 1 of *SLC6A8*, was identified as the most significant SNP in a cis-eQTL for *SLC6A8* gene expression. This SNP showed a linkage disequilibrium of 0.926 with both rs81000133 and rs336426564. Homozygous animals for the A allele showed both higher expression of *SLC6A8* and fat thickness percentages, jointly with lower meat percentages, than animals carrying the C allele. This result is in agreement with the phenotype of knockout mice for *SLC6A8* which showed a decrease in BW as well as body fat (Baroncelli et al., 2014; Skelton et al., 2011). Among the candidate genes identified in SSC14 for shoulder lean meat, *ATE1* has been reported to affect postnatal weight, body conformation, fat gain and muscle strength in mice studies (Brower & Varshavsky, 2009; Cornachione et al., 2014), while *FGFR2* is involved in fibroblast development (Bonaventure & El Ghouzzi, 2003).

Several candidate genes for semimembranosus pH 24 hours after death were identified in SSC6. *NRDC* is a metalloprotease involved in cell proliferation and migration and has implications in reducing cell growth in certain tissues (Hospital et al., 2002). *LEPR* and *LEPROT*, two different genes controlled by the same promoter, have been related to animal growth (Bailleul et al., 1997). *LEPR* encodes the leptin receptor which controls fat gain and regulates obesity (Bailleul et al., 1997), while *LEPROT* participates in the maturation of Growth Hormone Receptor in the Golgi (Touvier et al., 2009). Both genes are strong candidates for meat parameters in pork, specially *LEPR*, which has been largely reported to affect backfat thickness and intramuscular fat (Cirera et al., 2018; Mackowski et al., 2005; Óvilo et al., 2022; Pérez-Montarelo et al., 2015; Ros-Freixedes et al., 2016; Uemoto et al., 2012). Since fatness traits have high genetic correlations with intramuscular pH, a possible indirect effect of *LEPR* in postmortem pH should be considered. Lastly, *PDE4B* had the most significantly associated variants

for pH at 24 h. This gene plays a role in signal transduction through the concentration of cyclic nucleotides (Enoksson et al., 1998), and has also been related to skeletal muscle atrophy in burns (Balasubramaniam et al., 2018). Remarkably, *PDE4B* is also necessary for the lipopolysaccharide-activated immune response of phagocytic cells (Yang et al., 2018), and has been previously associated with immune-related phenotypes in humans and pigs (Han et al., 2020), including the commercial Duroc population analysed in this study (Crespo-Piazuelo et al., 2021). Therefore, *PDE4B* may be a pleiotropic gene influencing immunity and meat quality traits in pigs.

This study focuses on the genetic basis of nine carcass and pork quality traits as well as their genetic relationships with immunity and health-related traits in a commercial Duroc population. A total of five genes were described for semimembranosus pH, being *LEPR*, *LEPROT* and *PDE4B* the strongest candidates. Other nine genes were highly associated to both carcass lean and fat contents, highlighting *DPP10* and *SLC6A8*. In addition, relevant genetic associations between meat quality traits and several immunity phenotypes were reported. These relationships might pose a challenge for breeding programmes and should be taken into consideration to improve immunocompetence without impairing production traits in pigs.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.101043.

Ethics approval

This study follows the directives of the Spanish Policy for Animal Protection RD 53/2013, in agreement with European Union Directive 2010/63/EU about the correct practices and protection of animals used in experimentation and was approved by the Ethical Committee of the Institut de Recerca i Tecnologia Agroalimentàries (IRTA).

Data and model availability statement

All data generated during this study are included in this publication and its supplementary material. In addition, the raw sequence data for eGWAS have been deposited in the FAANG data portal with the BioProject accession codes PRJEB58030 (https://data.faang.org/dataset/PRJEB58030) and PRJEB58031 (https://data.faang.org/dataset/PRJEB58031). Additional datasets used and analysed during the study are available upon reasonable request to the corresponding author.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

Author ORCIDs

T. Jové-Juncà: https://orcid.org/0000-0002-6436-3279.
D. Crespo-Piazuelo: https://orcid.org/0000-0001-7896-2507.
M. Pascual: https://orcid.org/0000-0002-7233-2632.
C. Hernández-Banqué: https://orcid.org/0000-0003-4962-0086.
J. Reixach: https://orcid.org/0000-0003-0669-7593.
R. Quintanilla: https://orcid.org/0000-0003-3274-3434.
M. Ballester: https://orcid.org/0000-0002-5413-4640.

Author contributions

M.B. and **R.Q.** designed the study. **M.B.** and **J.R.** supervised the generation of the animal material. **M.B., O.G.R., M.P., J.R.** and **R.Q.** performed the sampling. **M.B.** and **O.G.R.** carried out the laboratory analyses. **T.J.J., C.H.B., D.C.P., M.B.,** and **R.Q.** analysed the data. **T.J.J, M.B.** and **R.Q.** interpreted the results and wrote the manuscript. All the authors read and approved the final version of the manuscript.

Declaration of interest

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest.

Acknowledgements

We would like to acknowledge the contribution of the technician staff from both IRTA and Selección Batallé S.A. for their collaboration in farm slaughterhouse and laboratory.

Financial support statement

The study was funded by grants AGL2016-75432-R and PID2020-112677RB-C21 awarded by MCIN/AEI/https://doi.org/10.13039/501100011033. Transcriptomic analysis is part of GENE-SWitCH project (https://www.gene-switch.eu), which is funded by the European Union's Horizon 2020 Research and Innovation Programme under the grant agreement no. 817998. T. Jové-Juncà was funded with an IRTA fellowship (CPI1221) and C. Hernández-Banqué was supported by a FPI grant (PRE2021-097825) granted by the Spanish Ministry of Science and Innovation. The authors are part to a Consolidated Research Group AGAUR, with the reference 2021-SGR-01552.

References

Bailleul, B., Akerblom, I., Strosberg, A.D., 1997. The leptin receptor promoter controls expression of a second distinct protein. Nucleic Acids Research 25, 2752–2758. https://doi.org/10.1093/NAR/25.14.2752.

Balasubramaniam, A., Sheriff, S., Friend, L.A., James, J.H., 2018. Phosphodiesterase 4B knockout prevents skeletal muscle atrophy in rats with burn injury. American Journal of Physiology, Regulatory, Integrative and Comparative Physiology 315, R417–R421. https://doi.org/10.1152/AJPREGU.00042.2018.

Ballester, M., Ramayo-Caldas, Y., González-Rodríguez, O., Pascual, M., Reixach, J., Díaz, M., Blanc, F., López-Serrano, S., Tibau, J., Quintanilla, R., 2020. Genetic parameters and associated genomic regions for global immunocompetence and other health-related traits in pigs. Scientific Reports 10, 18462. https://doi.org/10.1038/s41598-020-75417-7.

Ballester, M., Jové-Juncà, T., Pascual, A., López-Serrano, S., Crespo-Piazuelo, D., Hernández-Banqué, C., González-Rodríguez, O., Ramayo-Caldas, Y., Quintanilla, R., 2023. Genetic architecture of innate and adaptive immune cells in pigs. Frontiers in Immunology 14, 1058346. https://doi.org/10.3389/FIMMU.2023.1058346.

Baroncelli, L., Alessandrì, M.G., Tola, J., Putignano, E., Migliore, M., Amendola, E., Gross, C., Leuzzi, V., Cioni, G., Pizzorusso, T., 2014. A novel mouse model of creatine transporter deficiency. F1000Research 3, 228. https://doi.org/10.12688/F1000RESEARCH.5369.1.

Bindea, G., Mlecnik, B., Hackl, H., Charoentong, P., Tosolini, M., Kirilovsky, A., Fridman, W.H., Pagès, F., Trajanoski, Z., Galon, J., 2009. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics (Oxford, England) 25, 1091–1093. https://doi.org/10.1093/BIOINFORMATICS/BTP101.

Blake, J.A., Baldarelli, R., Kadin, J.A., Richardson, J.E., Smith, C.L., Bult, C.J., 2021. Mouse Genome Database (MGD): Knowledgebase for mouse-human comparative biology. Nucleic Acids Research 49, D981–D987. https://doi.org/10.1093/NAR/GKAA1083.

Bonaventure, J., El Ghouzzi, V., 2003. Molecular and cellular bases of syndromic craniosynostoses. Expert Reviews in Molecular Medicine 5, 1–17. https://doi.org/10.1017/S1462399403005751.

Brower, C.S., Varshavsky, A., 2009. Ablation of arginylation in the mouse N-end rule pathway: loss of fat, higher metabolic rate, damaged spermatogenesis, and neurological perturbations. PloS One 4, e7757.
Bryant Chase, P., Shimizu, T., Wiseman, R.W., Stockebrand, M., Sasani, A., Das, D., Hornig, S., Hermans-Borgmeyer, I., Lake, H.A., Isbrandt, D., Lygate, C.A., Heerschap, A., Neu, A., Choe, C.-U., 2018. A mouse model of creatine transporter deficiency reveals impaired motor function and muscle energy metabolism. Frontiers in Physiology 9, 773. https://doi.org/10.3389/fphys.2018.00773.
Cepica, S., Reiner, G., Bartenschlager, H., Moser, G., 2003. Linkage and QTL mapping for Sus scrofa chromosome X. Journal of Animal Breeding and Genetics 1, 144–151. https://doi.org/10.1046/j.0931-2668.2003.00436.x.
Chang, C.C., Chow, C.C., Tellier, L.C., et al., 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience 4, 7. https://doi.org/10.1186/s13742-015-0047-8.
Cirera, S., Clop, A., Jacobsen, M.J., Guerin, M., Lesnik, P., Jørgensen, C.B., Fredholm, M., Karlskov-Mortensen, P., 2018. A targeted genotyping approach enhances identification of variants in taste receptor and appetite/reward genes of potential functional importance for obesity-related porcine traits. Animal Genetics 49, 110–118. https://doi.org/10.1111/AGE.12641.
Cornachione, A.S., Leite, F.S., Wang, J., Leu, N.A., Kalganov, A., Volgin, D., Han, X., Xu, T., Cheng, Y.S., Yates, J.R.R., Rassier, D.E., Kashina, A., 2014. Arginylation of myosin heavy chain regulates skeletal muscle strength. Cell Reports 8, 470–476. https://doi.org/10.1016/J.CELREP.2014.06.019.
Corominas, J., Ramayo-Caldas, Y., Puig-Oliveras, A., Estellé, J., Castelló, A., Alves, E., Pena, R.N., Ballester, M., Folch, J.M., 2013. Analysis of porcine adipose tissue transcriptome reveals differences in de novo fatty acid synthesis in pigs with divergent muscle fatty acid composition. BMC Genomics 14, 1–14. https://doi.org/10.1186/1471-2164-14-843/FIGURES/4.
Crespo-Piazuelo, D., González-Rodríguez, O., Mongellaz, M., Mercat, M., Bink, M., Huisman, A., Ramayo-Caldas, Y., Sánchez, J., Ballester, M., 2023. Identification of transcriptional regulatory variants in pig duodenum, liver and muscle tissues. GigaScience 12, giad042. https://doi.org/10.1093/gigascience/giad042.
Crespo-Piazuelo, D., Ramayo-Caldas, Y., González-Rodríguez, O., Pascual, M., Quintanilla, R., Ballester, M., 2021. A co-association network analysis reveals putative regulators for health-related traits in pigs. Frontiers in Immunology 12, 784978. https://doi.org/10.3389/fimmu.2021.784978.
Davoli, R., Braglia, S., 2007. Molecular approaches in pig breeding to improve meat quality. Briefings in Functional Genomics & Proteomics 6, 313–321. https://doi.org/10.1093/BFGP/ELM036.
Déru, V., Bouquet, A., Hassenfratz, C., Blanchet, B., Carillier-Jacquin, C., Gilbert, H., 2020. Impact of a high-fibre diet on genetic parameters of production traits in growing pigs. Animal 14, 2236–2245. https://doi.org/10.1017/S1751731120001275.
Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., Gingeras, T.R., 2013. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15–21. https://doi.org/10.1093/bioinformatics/bts635.
Dragos-Wendrich, M., Stratil, A., Hojny, J., Moser, G., Bartenschlager, H., Reiner, G., 2003. Linkage and QTL mapping for Sus scrofa chromosome 18. Journal of Animal Breeding and Genetics 1, 138–143.
Ducos, A., 1994. Paramètres génétiques des caractères de production chez le porc. Mis Au Point Bibliographique, Techni-Porc 17, 35–67.
Durinck, S., Spellman, P.T., Birney, E., Huber, W., 2009. Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. Nature Protocols 4, 1184–1191. https://doi.org/10.1038/NPROT.2009.97.
Enoksson, S., Degerman, E., Hagström-Toft, E., Large, V., Arner, P., 1998. Various phosphodiesterase subtypes mediate the in vivo antilipolytic effect of insulin on adipose tissue and skeletal muscle in man. Diabetologia 41, 560–568. https://doi.org/10.1007/S001250050947.
Estellé, J., Mercadé, A., Noguera, J.L., Pérez-Enciso, M., Óvilo, C., Sánchez, A., Folch, J. M., 2005. Effect of the porcine IGF2-intron3-G3072A substitution in an outbred Large White population and in an Iberian x Landrace cross. Journal of Animal Science 83, 2723–2728. https://doi.org/10.2527/2005.83122723X.
Eusebi, P.G., González-Prendes, R., Quintanilla, R., Tibau, J., Cardoso, T.F., Clop, A., Amills, M., 2017. A genome-wide association analysis for carcass traits in a commercial Duroc pig population. Animal Genetics 48, 466–469. https://doi.org/10.1111/AGE.12545.
FAO, 2022. Organización de las Naciones Unidas para la Alimentación y la Agricultura. FAOSTAT. Retrieved on 5 April 2022 from https://www.fao.org/faostat/es/#data.
Fujii, J., Otsu, K., Zorzato, F., De Leon, S., Khanna, V.K., Weiler, J.E., O'Brien, P.J., Maclennan, D.H., 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science (New York, NY) 253, 448–451. https://doi.org/10.1126/SCIENCE.1862346.
Gallardo, D., Pena, R.N., Quintanilla, R., Ramírez, O., Almuzara, D., Noguera, J.L., Amills, M., 2012. Quantitative trait loci analysis of a Duroc commercial population highlights differences in the genetic determination of meat quality traits at two different muscles. Animal Genetics 43, 800–804. https://doi.org/10.1111/J.1365-2052.2012.02333.X.
González-Prendes, R., Quintanilla, R., Cánovas, A., Manunza, A., Cardoso, T.F., Jordana, J., Noguera, J.L., Pena, R.N., Amills, M., 2017. Joint QTL mapping and gene expression analysis identify positional candidate genes influencing pork quality traits. Scientific Reports 7, 39830. https://doi.org/10.1038/SREP39830.
Gonzalez-Rivas, P.A., Chauhan, S.S., Ha, M., Fegan, N., Dunshea, F.R., Warner, R.D., 2020. Effects of heat stress on animal physiology, metabolism, and meat quality: A review. Meat Science 162,. https://doi.org/10.1016/J.MEATSCI.2019.108025 108025.
Groenen, M.A.M., Archibald, A.L., Uenishi, H., Tuggle, C.K., Takeuchi, Y., Rothschild, M.F., Rogel-Gaillard, C., Park, C., Milan, D., Megens, H.J., Li, S., Larkin, D.M., Kim, H., Frantz, L.A.F., Caccamo, M., Ahn, H., Aken, B.L., Anselmo, A., Anthon, C., Auvil, L., Badaoui, B., Beattie, C.W., Bendixen, C., Berman, D., Blecha, F., Blomberg, J., Bolund, L., Bosse, M., Botti, S., Bujie, Z., Bystrom, B., Capitanu, B., Carvalho-Silva, D., Chardon, P., Chen, C., Cheng, R., Choi, S.H., Chow, W., Clark, R.C., Clee, C., Crooijmans, R.P.M.A., Dawson, H.D., Dehais, P., De Sapio, F., Dibbits, B., Drou, N., Du, Z.Q., Eversole, K., Fadista, J., Fairley, S., Faraut, T., Faulkner, G.J., Fowler, K.E., Fredholm, M., Fritz, E., Gilbert, J.G.R., Giuffra, E., Gorodkin, J., Griffin, D.K., Harrow, J.L., Hayward, A., Howe, K., Hu, Z.L., Humphray, S.J., Hunt, T., Hornshøj, H., Jeon, J.T., Jern, P., Jones, M., Jurka, J., Kanamori, H., Kapetanovic, R., Kim, J., Kim, J.H., Kim, K.W., Kim, T.H., Larson, G., Lee, K., Lee, K.T., Leggett, R., Lewin, H. A., Li, Y., Liu, W., Loveland, J.E., Lu, Y., Lunney, J.K., Ma, J., Madsen, O., Mann, K., Matthews, L., McLaren, S., Morozumi, T., Murtaugh, M.P., Narayan, J., Nguyen, D. T., Ni, P., Oh, S.J., Onteru, S., Panitz, F., Park, E.W., Park, H.S., Pascal, G., Paudel, Y., Perez-Enciso, M., Ramirez-Gonzalez, R., Reecy, J.M., Rodriguez-Zas, S., Rohrer, G. A., Rund, L., Sang, Y., Schachtschneider, K., Schraiber, J.G., Schwartz, J., Scobie, L., Scott, C., Searle, S., Servin, B., Southey, B.R., Sperber, G., Stadler, P., Sweedler, J.V., Tafer, H., Thomsen, B., Wali, R., Wang, J., Wang, J., White, S., Xu, X., Yerle, M., Zhang, G., Zhang, J., Zhang, J., Zhao, S., Rogers, J., Churcher, C., Schook, L.B., 2012. Analyses of pig genomes provide insight into porcine demography and evolution. Nature 491, 393–398. https://doi.org/10.1038/nature11622.
GTEx Consortium, 2015. Human genomics. The Genotype-Tissue Expression (GTex) pilot analysis: multitissue gene regulation in humans. Science 348, 648–660. https://doi.org/10.1126/science.1262110.
Han, X., Ong, J.S., An, J., Hewitt, A.W., Gharahkhani, P., MacGregor, S., 2020. Using Mendelian randomization to evaluate the causal relationship between serum C-reactive protein levels and age-related macular degeneration. European Journal of Epidemiology 35, 139–146. https://doi.org/10.1007/S10654-019-00598-Z.
Hoa, V.B., Seo, H.W., Seong, P.N., Cho, S.H., Kang, S.M., Kim, Y.S., Moon, S.S., Choi, Y. M., Kim, J.H., Seol, K.H., 2021. Back-fat thickness as a primary index reflecting the yield and overall acceptance of pork meat. Animal Science Journal = Nihon Chikusan Gakkaiho 92, e13515.
Hochberg, Y., Benjamini, Y., 1990. More powerful procedures for multiple significance testing. Statistics in Medicine 9, 811–818. https://doi.org/10.1002/SIM.4780090710.
Hospital, V., Nishi, E., Klagsbrun, M., Cohen, P., Seidah, N.G., Prat, A., 2002. The metalloendopeptidase nardilysin (NRDc) is potently inhibited by heparin-binding epidermal growth factor-like growth factor (HB-EGF). The Biochemical Journal 367, 229–238. https://doi.org/10.1042/BJ20020822.
Howie, D., Bokum, A.T., Necula, A.S., Cobbold, S.P., Waldmann, H., 2018. The role of lipid metabolism in T lymphocyte differentiation and survival. Frontiers in Immunology 8, 1949. https://doi.org/10.3389/FIMMU.2017.01949/BIBTEX.
Hu, Z.L., Park, C.A., Reecy, J.M., 2022. Bringing the Animal QTLdb and CorrDB into the future: meeting new challenges and providing updated services. Nucleic Acids Research 50, D956–D961. https://doi.org/10.1093/NAR/GKAB1116.
IMPC, 2014. Obtaining and Loading Phenotype Annotations from the International Mouse Phenotyping Consortium (IMPC) Database. Database Release. Accessed on 12 February 2023 https://www.informatics.jax.org/genes.shtml.
Johansson, K., Andersson, K., Sigvardsson, J., 1987. Evaluation of station testing of pigs III. Genetic parameters for carcass measurements of partially dissected pigs. Acta Agriculturae Scandinavica 37, 120–129.
Jungerius, B.J., Van Laere, A.S., Te Pas, M.F.W., Van Oost, B.A., Andersson, L., Groenen, M.A.M., 2004. The IGF2-intron3-G3072A substitution explains a major imprinted QTL effect on backfat thickness in a Meishan x European white pig intercross. Genetical Research 84, 95–101. https://doi.org/10.1017/S0016672304007098.
Khanal, P., Maltecca, C., Schwab, C., Gray, K., Tiezzi, F., 2019. Genetic parameters of meat quality, carcass composition, and growth traits in commercial swine. Journal of Animal Science 97, 3669–3683. https://doi.org/10.1093/JAS/SKZ247.
Koomkrong, N., Boonkaewwan, C., Laenoi, W., Kayan, A., 2017. Blood haematology, muscle pH and serum cortisol changes in pigs with different levels of drip loss. Asian-Australasian Journal of Animal Bioscience 30, 1751–1755. https://doi.org/10.5713/ajas.17.0037.
Kuryl, J., Pierzchala, M., Hojnu, J., Reiner, G., Bartenschlager, H., Moser, G., 2003. Linkage and QTL mapping for Sus scrofa chromosome 15. Journal of Animal Breeding and Genetics 1, 119–125.
Lebret, B., Čandek-Potokar, M., 2022. Review: Pork quality attributes from farm to fork. Part I. Carcass and fresh meat. Animal 16,. https://doi.org/10.1016/J.ANIMAL.2021.100402 100402.
Lee, S., Norman, J.M., Gunasekaran, S., Van Laack, R.L.J.M., Kim, B.C., Kauffman, R.G., 2000. Use of electrical conductivity to predict water-holding capacity in post-rigor pork. Meat Science 55, 385–389. https://doi.org/10.1016/S0309-1740(99)00166-7.
Li, B., Dewey, C.N., 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bionformatics 12, 323. https://doi.org/10.1186/1471-2105-12-323.
Li, H., 2013. Aling sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv preprint. https://doi.org/10.48550/arXiv.1303.3997.
Mackowski, M., Szymoniak, K., Szydlowski, M., Kamyczek, M., Eckert, R., Rozycki, M., Switonski, M., 2005. Missense mutations in exon 4 of the porcine LEPR gene encoding extracellular domain and their association with fatness traits. Animal Genetics 36, 135–137. https://doi.org/10.1111/J.1365-2052.2005.01247.X.

Mao, Z., Nakamura, F., 2020. Structure and function of Filamin C in the muscle Z-disc. International Journal of Molecular Sciences 21, 2696. https://doi.org/10.3390/ijms21082696.
Mathis, D., Shoelson, S.E., 2011. Immunometabolism: An emerging frontier. Nature Reviews Immunology 11, 81–83. https://doi.org/10.1038/nri2922.
McLaren, W., Gil, L., Hunt, S.E., Riat, H.S., Ritchie, G.R.S., Thormann, A., Flicek, P., Cunningham, F., 2016. The ensembl variant effect predictor. Genome Biology 17, 1–14. https://doi.org/10.1186/S13059-016-0974-4/TABLES/8.
Melén, E., Himes, B.E., Brehm, J.M., Boutaoui, N., Klanderman, B.J., Sylvia, J.S., Lasky-Su, J., 2010. Analyses of shared genetic factors between asthma and obesity in children. The Journal of Allergy and Clinical Immunology 126, 631–637.e8. https://doi.org/10.1016/J.JACI.2010.06.030.
Meng, Z., Cao, G., Yang, Q., Yang, H., Hao, J., Yin, Z., 2021. Metabolic Control of γδ T Cell Function. Infectious Microbes and Diseases 3, 142–148. https://doi.org/10.1097/IM9.0000000000000054.
Misztal, I., S. Tsuruta, D.A.L. Lourenco, I. Aguilar, A. Legarra, and Z. V., 2014. Manual for BLUPF90 family of programs. Accessed on 10 November 2022 http://nce.ads.uga.edu/wiki/lib/exe/fetch.php?media=blupf90_all2.pdf.
Óvilo, C., Trakooljul, N., Núñez, Y., Hadlich, F., Murani, E., Ayuso, M., García-Contreras, C., Vázquez-Gómez, M., Rey, A.I., Garcia, F., García-Casco, J.M., López-Bote, C., Isabel, B., González-Bulnes, A., Wimmers, K., Muñoz, M., 2022. SNP discovery and association study for growth, fatness and meat quality traits in Iberian crossbred pigs. Scientific Reports 12, 16361. https://doi.org/10.1038/S41598-022-20817-0.
Pearce, E.L., Walsh, M.C., Cejas, P.J., Harms, G.M., Shen, H., Wang, L.S., Jones, R.G., Choi, Y., 2009. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature 460, 103–107. https://doi.org/10.1038/NATURE08097.
Pérez-Montarelo, D., Rodríguez, M.C., Fernández, A., Benítez, R., García, F., Silió, L., Fernández, A.I., 2015. Haplotypic diversity of porcine LEP and LEPR genes involved in growth and fatness regulation. Journal of Applied Genetics 56, 525–533. https://doi.org/10.1007/S13353-015-0284-7.
Phocas, F., Belloc, C., Bidanel, J., Delaby, L., Dourmad, J.Y., Dumont, B., Ezanno, P., Fortun-Lamothe, L., Foucras, G., Frappat, B., González-García, E., Hazard, D., Larzul, C., Lubac, S., Mignon-Grasteau, S., Moreno, C.R., Tixier-Boichard, M., Brochard, M., 2016. Review: Towards the agroecological management of ruminants, pigs and poultry through the development of sustainable breeding programmes. II. Breeding strategies. Animal 10, 1760–1769. https://doi.org/10.1017/S1751731116001051.
Poklukar, K., Čandek-Potokar, M., Lukač, N.B., Tomažin, U., Škrlep, M., 2020. Lipid Deposition and Metabolism in Local and Modern Pig Breeds: A Review. Animals 10, 424. https://doi.org/10.3390/ANI10030424.
Purcell, S., 2007. PLINK v1.90b3.42. Accessed on 26 January 2022. http://pngu.mgh.harvard.edu/purcell/plink/.
R Core Team, 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/.
Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140. https://doi.org/10.1093/bioinformatics/btp616.
Robinson, K.A., Sun, M., Barnum, C.E., Weiss, S.N., Huegel, J., Shetye, S.S., Lin, L., Saez, D., Adams, S.M., Iozzo, R.V., Soslowsky, L.J., Birk, D.E., 2017. Decorin and biglycan are necessary for maintaining collagen fibril structure, fiber realignment, and mechanical properties of mature tendons HHS public access. Matrix Biology 64, 81–93. https://doi.org/10.1016/j.matbio.2017.08.004.
Rosenvold, K., Andersen, H.J., 2003. Factors of significance for pork quality-a review. Meat Science 64, 219–237. https://doi.org/10.1016/S0309-1740(02)00186-9.
Ros-Freixedes, R., Gol, S., Pena, R.N., Tor, M., Ibáñez-Escriche, N., Dekkers, J.C.M., Estany, J., 2016. Genome-wide association study singles out SCD and LEPR as the two main loci influencing intramuscular fat content and fatty acid composition in duroc pigs. PloS One 11, e0152496.
Safran, M., Rosen, N., Twik, M., BarShir, R., Stein, T. I., Dahary, D., Fishilevich, S., Lancet, D., 2021. The GeneCards Suite. Practical Guide to Life Science Databases, Accessed on 6 April 2022. https://doi.org/10.1007/978-981-16-5812-9_2.
Saravia, J., Raynor, J.L., Chapman, N.M., Lim, S.A., Chi, H., 2020. Signaling networks in immunometabolism. Cell Research 30, 328–342. https://doi.org/10.1038/s41422-020-0301-1.
Scheffler, T.L., Gerrard, D.E., 2007. Mechanisms controlling pork quality development: The biochemistry controlling postmortem energy metabolism. Meat Science 77, 7–16. https://doi.org/10.1016/J.MEATSCI.2007.04.024.
Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Research 13, 2498–2504. https://doi.org/10.1101/GR.1239303.
Skelton, M.R., Schaefer, T.L., Graham, D.L., deGrauw, T.J., Clark, J.F., Williams, M.T., Vorhees, C.V., 2011. Creatine transporter (CrT; Slc6a8) knockout mice as a model of human CrT deficiency. PloS One 6, e16187.
Srinivasan, M.D., 2021. Extension of 'data.frame'. R package version 1.14.2. Data. Table. Accessed on 5 February 2022. https://cran.r-project.org/package=data.table.
Teng, J., Gao, Y., Yin, H., Bai, Z., Liu, S., Zeng, H., Bai, L., Cai, Z., Zhao, B., Li, X., Xu, Z., Lin, Q., Pan, Z., Yang, W., Yu, X., Guan, D., Hou, Y., Keel, B.N., Fang, L., 2022. A compendium of genetic regulatory effects across pig tissues. BioRxiv 19. https://doi.org/10.1101/2022.11.11.516073.
Touvier, T., Conte-Auriol, F., Briand, O., Cudejko, C., Paumelle, R., Caron, S., Baugé, E., Rouillé, Y., Salles, J.P., Staels, B., Bailleul, B., 2009. LEPROT and LEPROTL1 cooperatively decrease hepatic growth hormone action in mice. The Journal of Clinical Investigation 119, 3830–3838. https://doi.org/10.1172/JCI34997.
Uemoto, Y., Kikuchi, T., Nakano, H., Sato, S., Shibata, T., Kadowaki, H., Katoh, K., Kobayashi, E., Suzuki, K., 2012. Effects of porcine leptin receptor gene polymorphisms on backfat thickness, fat area ratios by image analysis, and serum leptin concentrations in a Duroc purebred population. Animal Science Journal 83, 375–385. https://doi.org/10.1111/J.1740-0929.2011.00963.X.
Visscher, A.H., Janss, L.L., Niewold, T.A., de Greef, K.H., 2002. Disease incidence and immunological traits for the selection of healthy pigs. A review. Veterinary Quaterly 24, 29–34. https://doi.org/10.1080/01652176.2002.9695121.
Wei, T., 2021. Visualization of a Correlation Matrix (Version 0.92). R Package "Corrplot." https://github.com/taiyun/corrplot.
Wimmers, K., Fiedler, I., Hardge, T., Murani, E., Schellander, K., Ponsuksili, S., 2006. QTL for microstructural and biophysical muscle properties and body composition in pigs. BMC Genetics 7, 15. https://doi.org/10.1186/1471-2156-7-15.
Yang, J.-X., Hsiung, T.-C., Weng, F.-C., Ding, S.-L., Wu, C.-P., Conti, M., Chuang, T.-H., Catherine Jin, S.-L., 2018. Synergistic effect of phosphodiesterase 4 inhibitor and serum on migration of endotoxin-stimulated macrophages. Innate Immunity 24, 501–512. https://doi.org/10.1177/1753425918809155.
Yang, J., Lee, S.H., Goddard, M.E., Visscher, P.M., 2011. GCTA: a tool for Genome-wide Complex Trait Analysis. The American Journal of Human Genetics 88, 76–82. https://doi.org/10.1016/j.ajhg.2010.11.011.
Zhou, S., Ding, R., Meng, F., Wang, X., Zhuang, Z., Quan, J., Geng, Q., Wu, J., Zheng, E., Wu, Z., Yang, J., Yang, J., 2021. A meta-analysis of genome-wide association studies for average daily gain and lean meat percentage in two Duroc pig populations. BMC Genomics 22, 12. https://doi.org/10.1186/S12864-020-07288-1.