Joint QTL mapping and gene expression analysis identify positional candidate genes influencing pork quality traits

Rayner González-Prendes1, Raquel Quintanilla2, Angela Cánovas1, Arianna Manunza1, Tainã Figueiredo Cardoso1,3, Jordi Jordana4, José Luis Noguera2, Ramona N. Pena5 & Marcel Amills1

Meat quality traits have an increasing importance in the pig industry because of their strong impact on consumer acceptance. Here, we have combined phenotypic and microarray expression data to map loci with potential effects on five meat quality traits recorded in the *longissimus dorsi* (LD) and *gluteus medius* (GM) muscles of 350 Duroc pigs, i.e. pH at 24 hours post-mortem (pH24), electric conductivity (CE) and muscle redness (a*), lightness (L*) and yellowness (b*). We have found significant genome-wide associations for CE of LD on SSC4 (~104 Mb), SSC5 (~15 Mb) and SSC13 (~137 Mb), while several additional regions were significantly associated with meat quality traits at the chromosome-wide level. There was a low positional concordance between the associations found for LD and GM traits, a feature that reflects the existence of differences in the genetic determinism of meat quality phenotypes in these two muscles. The performance of an eQTL search for SNPs mapping to the regions associated with meat quality traits demonstrated that the GM a* SSC3 and pH24 SSC17 QTL display positional concordance with cis-eQTL regulating the expression of several genes with a potential role on muscle metabolism.

The physicochemical properties of the porcine muscle and its post-mortem maturation determine the organoleptic properties of fresh meat and cured products and, consequently, their acceptance by consumers1. The genetic determinism of electrical conductivity, acidity and color, which have been often used as predictors of meat quality, has been explored by performing genome-wide association studies (GWAS) in F2 populations2–4 as well as in purebred pigs5,6. An important limitation of using F2 intercrosses in GWAS studies is that they are not representative of the purebred populations that constitute the selection nuclei of breeding companies. On the other hand, certain breeds, such as Large White, have been strongly introgressed with Asian alleles that do not segregate in other European porcine populations7.

In a previous study, we measured electrical conductivity at 24 hours (CE), pH at 24 hours (pH24) and color (lightness or L*, redness or a*, and yellowness or b*) in *gluteus medius* (GM) and *longissimus dorsi* (LD) samples from 350 Duroc pigs (Lipgen population)8. Performance of a genome scan with 105 microsatellites revealed that the QTL maps for these two muscles were quite different8. Indeed, the only QTL that remained significant at the genome-wide level were those associated with GM a*, on *Sus scrofa* chromosome 13 (SSC13, 84 cM), and GM b* (SSC15, 108 cM). Unfortunately, the confidence intervals of these QTL were quite large due to the poor resolution of the microsatellite-based analysis. Moreover, we may have missed many QTL due to the relatively large spacing between markers. In the current work, we aimed to circumvent these limitations by employing a GWAS approach to identify meat quality QTL in the Lipgen population mentioned above. Taking advantage that microarray measurements of gene expression in the GM muscle were available for 104 Lipgen pigs, we have

---

1Center for Research in Agricultural Genomics (CSIC-IRTA-UAB-UB), Campus Universitat Autònoma de Barcelona, Bellaterra 08193, Spain. 2Animal Breeding and Genetics Program, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Torre Marimon, Caldes de Montbui 08140, Spain. 3CAPES Foundation, Ministry of Education of Brazil, Brasilia D. F., Zip Code 70.040-020, Brazil. 4Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain. 5Department of Animal Science, University of Lleida - Agrotecnio Center, Lleida 25198, Spain. Correspondence and requests for materials should be addressed to M.A. (email: marcel.amills@uab.cat)
performed an additional analysis where we have investigated the co-localization between GM QTL and expression QTL in cis (cis-eQTL).

**Materials and Methods**

**Ethics approval.** The manipulation of Duroc pigs followed Spanish national guidelines and it was approved by the Ethical Committee of Institut de Recerca i Tecnologia Agroalimentàries (IRTA).

**Measurement of phenotypic and expression data.** Phenotypic records were collected in a commercial Duroc line of 350 barrows distributed in five half-sib families (Lipgen population). A detailed description of the management conditions of this commercial line has been previously reported. Meat quality analyses were performed 24 h after slaughter at the IRTA-Centre of Food Technology by using 200 g samples of the LD and GM muscles. Electrical conductivity was estimated with a Pork Quality Meter (Intek GmbH) while pH_hc was measured with a pH-meter equipment with a Xerolyte electrode (Crison). Meat L*, a* and b* color parameters were determined with a Minolta Chroma-Meter CR-200 (Konica Minolta) equipment (light source C and aperture 2). Microarray expression data of GM samples from 104 Duroc pigs were obtained in a previous study (data can be found in the Gene Expression Omnibus public repository, accession number: GSE19275) based on the use of GeneChip Porcine Genomic arrays (Affymetrix, Inc., Santa Clara, CA). A detailed description of the techniques and methods used to perform the RNA purification and microarray hybridization steps can be found in Canovas et al. Briefly, GM samples from 104 pigs were grinded in liquid nitrogen and homogenized with a mechanical rotor. Total RNA was purified with an acid phenol protocol and it was subsequently used as a template to synthesize double stranded cDNA with the One Cycle cDNA Synthesis Kit (Affymetrix, Inc.). cRNAs were purified with the GeneChip Sample Cleanup Module (Affymetrix, Inc.), fragmented and added to a hybridisation cocktail. The GeneChip Porcine Genome Array was equilibrated to room temperature and prehybridised with 1 × hybridisation buffer at 45 °C for 10 min. The hybridisation cocktail was heated to 99 °C for 5 min in a heat block and cooled to 45 °C for 5 min. Subsequently, a hybridization step was carried out at 45 °C for 16 hours. GeneChips were washed and labeled with streptavidin phycoerythrin in a Fluidics Station 450 (Affymetrix, Inc) and they were scanned in an Agilent G3000 GeneArray Scanner (Agilent Technologies, Inc.). The “Affy” and “Sympleaffy” packages from the Bioconductor project were employed to establish a set of quality control metrics to assess the quality of RNA samples and the efficiencies of the labelling and hybridisation steps. Data pre-processing and normalization were carried out with the BRB-ArrayTools software version 3.7.1. Genes displaying more than 20% of expression values over ±1.5 times the median expression of all arrays were retained for further analysis.

**Genome-wide association analysis for meat quality and expression data.** Genotyping was performed with the Porcine SNP60 BeadChip (Illumina, San Diego, CA) which contains 62,163 single nucleotide polymorphisms (SNPs). Quality genotyping analyses were carried out with the GenomeStudio software (Illumina), as previously reported. We removed SNPs (a) mapping to the X chromosome, (b) with a rate of missing genotypes higher than 5%, (c) that did not conform Hardy-Weinberg expectations (threshold set at a P-value ≤ 0.001), (d) that had a minor allele frequency below 0.05, (e) that had a GenCall score < 0.15, (f) that had a call rate < 95% or (g) that could not be mapped to the pig genome (Sus scrofa 10.2 assembly). After filtering the raw data, a GWAS was carried out with 36,710 SNPs. Single-SNP association analyses were performed with the Genome-wide Efficient Mixed-Model Association (GEMMA) software under an additive genetic model that included the genomic kinship matrix to account for relatedness. The statistical model assumed in this analysis was:

\[ y_{ijklm} = \mu + batch_j + \beta weight_k + \delta g_{ijkl} + e_{ijklm} \]  

(1)

where \( y_{ijklmn} \) is the vector of phenotypic observations i.e. pH_hc, CE, L*, a* and b* measured at the GM and LD muscles of the \( i \)th individual; \( \mu \) is the population mean of each trait; \( batch \) is a systematic effect of the \( j \)th fattening batch, with 4 categories; \( \beta \) is the regression coefficient on the covariate weight at slaughter (weight_k); \( g \) is the SNP allelic effect, estimated as a regression coefficient on the corresponding g genotype (values −1, 0, 1) of the \( l \)th SNP; and \( e_{ijklm} \) is the residual effect. The statistical relevance of the systematic environmental sources of variation and the covariates included in the model were previously reported by Gallardo et al. Correction for multiple testing was implemented with a false discovery rate approach.

Microarray data were available exclusively for GM muscle samples. Following the strategy employed in the Genotype-Tissue Expression (GTEx) pilot analysis, we primarily searched for cis-eQTL because they are expected to have larger effects than their trans-counterparts. We used two different strategies: **Analysis 1**, we retrieved 12 genes localized within GM QTL regions and we looked for cis-eQTL that might regulate their expression and **Analysis 2**, we made a search for cis-eQTL at a whole genome scale and we analyzed if there was a positional concordance between GWAS signals and cis-eQTL identified in this way. This second strategy made possible to identify cis-eQTL that might be located in the vicinity of GWAS signals. Genes corresponding to each probe included in the GeneChip Porcine Genomic array (Affymetrix, Inc., Santa Clara, CA) were identified in the BioMart database. The statistical model assumed in this analysis was:

\[ y_{ijklm} = \mu + batch_j + lab_k + \delta g_{ijkl} + e_{ijklm} \]  

(2)

where \( y_{ijklm} \) is the vector that defines the expression of each gene in the GM muscle of the \( i \)th individual; \( \mu \) is the mean expression of each gene in the population; \( batch \) and \( lab \) are the systematic effects i.e. \( batch \), of fattening (with 4 categories) and \( lab \), (microarray data were generated in two different laboratories); \( g \) is the SNP allelic effect estimated as a regression coefficient on the corresponding g genotype (values −1, 0, 1) of the \( l \)th SNP; and
et al. previously reported by Larzul gluteus profundus null h2 increased proportion of pale, soft and exudative (PSE) meat25. Electrical stunning induces a more rapid pH fall increase the incidence of dark, firm and dry (DFD) meat, while a short lairage time has been associated with an marker, ~133 Mb) detected by these authors8. genes involved in muscle tissue development, cell proliferation and migration and muscle contraction. differences in the gene expression patterns of the LD and GM muscles, a feature that was especially prominent for SSC5 and SSC13 (Table 2). The SSC4, 104 megabase (Mb) region, lies close to a previously reported QTL for CE of LD and GM muscles of Duroc pigs explained by SNP markers (h2) and its standard error (SE).

Results and Discussion
The SNPs arrayed in the Porcine SNP60 BeadChip explain a limited amount of the phenotypic variance of meat quality traits. By using the GEMMA software, we have estimated the proportion of phenotypic variance explained by the 36,710 SNPs (h2) genotyped with the Porcine SNP60 BeadChip (Table 1). In general, estimates of h2 ranged from low to moderate and differed between muscles. Discrepancies in the genealogic heritability (h2) estimates of meat quality traits recorded in different skeletal muscle samples were previously reported by Larzul et al.20. In this way, these authors found h2 of 0.03 and 0.23 for L* measured in the gluteus profundus and longissimus muscles, respectively. Similarly, the h2 values of pH14 measured in 4 different muscles oscillated between 0.17 (longissimus) and 0.39 (biceps femoris)20. When Gallardo et al.8 performed a QTL scan for meat quality traits in the Lipgen population, they also found that QTL maps differed markedly amongst traits recorded in the GM and LD muscles. As a whole, these results suggest that there are muscle-specific factors that modulate the genetic determinism of meat quality traits. Indeed, Quintanilla et al.21 identified remarkable differences in the gene expression patterns of the LD and GM muscles, a feature that was especially prominent for genes involved in muscle tissue development, cell proliferation and migration and muscle contraction.

Several h2 values obtained by us were comparable to genealogic heritabilities estimated for porcine meat quality traits in previous studies. For instance Gjerlaug-Enger et al.22 reported heritabilities for a* of 0.43 and 0.46 in Duroc and Landrace pigs, respectively. Similarly, Van Wijk et al.23 and Gjerlaug-Enger et al.24 described heritabilities of 0.11 (crossbred pigs) and from 0.12 (Landrace) to 0.27 (Duroc) for pH14. More unexpected were the null h2 values obtained in the current work for traits such as b* (in GM) and L* (in both muscles). We attribute these null heritabilities to our inability to detect genetic variants that may have small effects or that segregate at very low frequencies24.

Environmental variables may also obscure the contribution of genetic factors. Indeed, meat quality traits can be affected by poor on-farm handling, mixing of unfamiliar animals and high pig density and long travel distance during transportation25. Such events may increase the stress of the swine brought to the abattoir and, consequently, they may have negative consequences on meat quality25. At the abattoir, extended lairage time can increase the incidence of dark, firm and dry (DFD) meat, while a short lairage time has been associated with an increased proportion of pale, soft and exudative (PSE) meat25. Electrical stunning induces a more rapid pH fall early post mortem and an inferior water-holding capacity than CO2 stunning, while an accelerated chilling may have negative consequences on meat tenderness and water-holding capacity25. In summary, all these factors, and others that are not mentioned, can have a strong impact on the post-mortem pH, electrical conductivity and color of pig meat and “dilate” the contribution of polygenes25.

Genome-wide and chromosome-wide associations with meat quality traits in Duroc pigs. At the genome-wide level, we found significant associations between CE of LD and three genomic regions on SSC4, SSC5 and SSC13 (Table 2). The SSC4, 104 megabase (Mb) region, lies close to a previously reported QTL for CE identified by Cepica et al.26. We also found positional concordance between the SSC13 (137.0 Mb) region associated with LD CE and a semimembranosus CE QTL reported by Evans et al.27. At the chromosome-wide level, a coincidence was detected between a* QTL on SSC3 (50–57 Mb, Table 3) and a QTL for the same trait reported by Li et al.28 on SSC3 (35 Mb). Overall, our results confirm the existence of differences in the genetic determinism of meat quality traits recorded in the GM and LD muscles. The only exception was a region on SSC5 that significantly affected CE in both LD and GM muscles (Table 3). When we compared these data with the set of QTL previously reported by Gallardo et al.8 in the same Lipgen population we found one coincidence i.e. the GWAS signal identified on SSC4 (132 Mb) for CE in LD overlapped the confidence interval of a LD CE QTL (S0097 marker, ~133 Mb) detected by these authors8.

In general the positional coincidence between GWAS signals detected by us and those reported in previous studies was weak, indicating that the majority of associations reported in the current work are new. For instance, when we compared our a*, b* and pH14 data with those described in six additional GWAS studies4,6,29–32 we only found one positional coincidence between the SSC10 (70.6 Mb) genomic region associated with LD a* in the Lipgen population.
population (Table 3) and the SSC10 (72.8 Mb) region identified by Ma et al. as associated with the same trait in the\nseminembranosus muscle of White Duroc × Erhualian F₂ pigs.

The level of coincidence of trait-associated regions between these six GWAS for a*, b* and pH₂₄ traits was also quite low. Only about 20% of the regions identified as significantly associated with any of these phenotypes were shared between two studies or more, indicating that the majority of associations are population-specific. These shared regions were: (a*) SSC4 (80–85 Mb), SSC6 (17–22 Mb), SSC7 (31–32 Mb), SSC12 (58–63 Mb), SSC15 (133–136 Mb), and (b*) SSC15 (129–133 Mb); and (pH₂₄) SSC3 (15–19 Mb), SSC5 (133–136 Mb). This latter region on SSC15 (133–136 Mb) appeared to be associated with a*, b*, pH₂₄, shear force and cook loss in many independent studies but not in ours. Interestingly, this SSC15 region contains the protein kinase AMP-activated non-catalytic subunit gamma 3 (PRKAG3) gene, whose polymorphism has causal effects on muscle glycogen depletion, a parameter that can have a strong influence on meat quality traits.

Besides technical and methodological reasons, a probable cause for the lack of positional concordance between GWAS studies would be genetic heterogeneity. Indeed, Yang et al. performed a GWAS for blood lipid traits in 2,400 Laiwu, Erhualian and Duroc × Landrace × Yorkshire pigs and they identified a total of 22 QTL. Notably, only six regions were identified in more than one population, and 16 were detected in a single population.

Positional concordance between cis-eQTL for genes expressed in the GM muscle and QTL for GM traits. In general, eQTL are highly enriched in variants with causal effects on phenotypic variation and they can provide valuable information about candidate genes to be further investigated. Integrative analyses of QTL and eQTL data have been performed in pigs, making possible to combine the power of recombination with

| Trait | SSC | N  | SNP            | Location (Mb) | P-value | q-value | δ ± SE | A1  | MAF |
|-------|-----|----|----------------|---------------|---------|---------|-------|-----|-----|
| LD CE | 4   | 4  | H3GA0013593    | 104.2–104.8   | 6.19E-06 | 0.04    | 0.28 ± 0.06 | A   | 0.39 |
|       | 4   | 5  | ASGA0024711    | 15.4          | 2.46E-06  | 0.04    | −0.32 ± 0.07 | G   | 0.18 |
|       | 13  | 1  | ALGA0027807    | 137.0         | 7.34E-06 | 0.04    | 0.27 ± 0.06 | A   | 0.39 |

Table 2. Genomic regions significantly associated at the genome-wide level with meat quality traits in Duroc pigs. LD: longissimus dorsi muscle, CE: Electrical conductivity at 24 hours post-mortem, pH₂₄: pH at 24 hours post-mortem; a*: Minolta redness; L*: Minolta lightness, N: Number of SNPs significantly associated with the trait under study, Location (Mb): region containing SNPs significantly associated with the trait under study, P-value: nominal P-value, q-value: q-value calculated with a false discovery rate approach, δ: allelic effect and its standard error (SE), A1: minority allele, MAF: frequency of the minority allele.

| Trait  | SSC | N  | SNP            | Location (Mb) | P-value | q-value | δ ± SE | A1  | MAF |
|--------|-----|----|----------------|---------------|---------|---------|-------|-----|-----|
| LD CE  | 4   | 9  | ALGA0026686    | 93.5–98.8     | 1.54E-05 | 0.01    | −0.28 ± 0.06 | G   | 0.50 |
|        | 32  | 1  | H3GA0013593    | 104.2–107.1   | 6.19E-06 | 0.01    | −0.28 ± 0.06 | A   | 0.39 |
|        | 1   | 11 | ASGA0024711    | 131.0         | 2.04E-04  | 0.02    | −0.26 ± 0.07 | G   | 0.17 |
|        | 5   | 11 | ASGA0024564    | 13.0–14.7     | 3.15E-05  | 0.03    | −0.37 ± 0.09 | A   | 0.39 |
| GM CE  | 5   | 5  | ASGA0024711    | 14.4–16.1     | 2.46E-06  | 0.004   | −0.32 ± 0.07 | G   | 0.18 |
| LD pH₂₄| 16  | 3  | MARCO086782    | 6.0–6.4       | 5.27E-04  | 0.05    | 0.08 ± 0.02 | G   | 0.09 |
|        | 2   | 11 | MARCO089269    | 17.3–18.5     | 5.09E-04  | 0.05    | −0.06 ± 0.02 | G   | 0.19 |
|        | 10  | 1  | ASGA0091353    | 20.9–29.5     | 4.01E-04  | 0.05    | 0.05 ± 0.02 | G   | 0.41 |
| GM pH₂₄| 17  | 2  | MARCO038923    | 14.2–16.4     | 9.11E-05  | 0.04    | −0.06 ± 0.02 | A   | 0.48 |
|        | 5   | 1  | MARCO101162    | 53.1–57.2     | 2.70E-04  | 0.04    | 0.07 ± 0.02 | G   | 0.29 |
|        | 3   | 1  | H3GA0049744    | 64.5–65.3     | 1.81E-04  | 0.04    | −0.06 ± 0.02 | G   | 0.38 |
| LD a*  | 10  | 1  | ALGA00113811   | 70.6          | 2.99E-05  | 0.04    | 0.46 ± 0.11 | A   | 0.36 |
| GM a*  | 3   | 3  | H3GA0009494    | 16.6–17.0     | 7.85E-05  | 0.01    | 0.70 ± 0.17 | A   | 0.16 |
|        | 27  | 1  | H3GA0009489    | 50.2–57.2     | 1.27E-04  | 0.01    | 0.65 ± 0.17 | A   | 0.18 |
|        | 4   | 2  | ALGA0021059    | 119.7–119.9   | 7.85E-04  | 0.04    | 0.48 ± 0.14 | A   | 0.24 |
|        | 4   | 1  | ALGA0021078    | 120.0–120.4   | 7.85E-04  | 0.04    | 0.48 ± 0.14 | A   | 0.24 |
| GM L*  | 16  | 1  | MARCO073433    | 3.5           | 3.45E-05  | 0.04    | 1.23 ± 0.29 | C   | 0.24 |

Table 3. Genomic regions associated at the chromosome-wide level with meat quality traits in Duroc pigs. GM: gluteus medius muscle, LD: longissimus dorsi muscle, CE: Electrical conductivity at 24 hours post-mortem, pH₂₄: pH at 24 hours post-mortem; a*: Minolta redness; L*: Minolta lightness, N: Number of SNPs significantly associated with the trait under study, Location (Mb): region containing SNPs significantly associated with the trait under study, P-value: nominal P-value, q-value: q-value calculated with a false discovery rate approach, δ: allelic effect and its standard error (SE), A1: minority allele, MAF: frequency of the minority allele.
cans39. Interestingly, there are evidences that galactosidase 
enzymes, (cAMP), a secondary messenger that can have broad effects on muscle metabolism41. Indeed, AMPc is an activa-
tor of the cAMP-dependent protein kinase, a molecule involved in the phosphorylation of enzymes that promote 
expression studies in order to identify promising candidate genes35. For instance, multiple associations between 
SNPs mapping to porcine chromosomes 4 and 6 and meat quality traits have been detected36. Through an eQTL 
approach, it was possible to identify several genes on SSCA (ZNF704, IMPA1 and OXSR1) and SSC6 (IHH1D1, 
SIGLEC10, TBCB, LOC100518735, KIF1B, LOC100514845) whose variation is concomitantly associated with 
gene expression and phenotype data36. Similarly, Ma et al.36 used a genetical genomics approach to demonstrate 
that a splice mutation in the PHKG1 gene is the causal mutation for a glycolytic potential QTL mapping to SSC3.

We have used this integrative strategy to identify potential candidate genes for meat quality traits in a dataset 
of 12 loci that mapped to GM QTL regions (Analysis 1). In doing so, we have detected 3 cis-eQTLs (Table 4) that 
co-localize with three chromosome-wide QTLs. One of them maps to SSC3 (16.6–17.0 Mb) and displays associ-
ations with a* (Fig. 1a); while the other two are located on SSC17 (53.1–57.2; 64.5–65.3) and show significant 
associations with a* (Fig. 1a); while the other two are located on SSC17 (53.1–57.2; 64.5–65.3) and show significant 
associations with a* and pH24 (Fig. 2b, c). Interestingly, two of these three cis-regulated genes encode lysosomal 
enzymes, i.e. cathepsin A (CTSA) and glucuronidase β (GUSB), that might be released during the post-mortem 
maturity of meat37,38. Cathepsin A is a lysosomal serine protease that can also protect galactosidase 
from intralysosomal proteolysis 38, while glucuronidase 
β is mainly involved in the degradation of glycosaminogly-
cans39. Interestingly, there are evidences that galactosidase β and glucuronidase β might affect the degradation of 
the collagen mucopolysaccharide, thus having a potential impact on meat ultrastructural properties38.

In Analysis 2, we have identified three additional cis-eQTL that map near to the SSC3 QTL for a* and 
the SSC17 QTL for pH24 (Table 5). The ADCY3 locus, that co-localizes with the SSC3 QTL for a* (Fig. 2a), 
encodes an adenylyl cyclase catalysing the conversion of ATP into cyclic adenosine-3′,5′-monophosphate 
(cAMP), a secondary messenger that can have broad effects on muscle metabolism41. Indeed, AMPc is an activa-
tor of the AMP-dependent protein kinase, a molecule involved in the phosphorylation of enzymes that promote 
the conversion of glycogen into glucose42. Noteworthy, the amount of glycogen stored in the muscle determines 
the post-mortem production of lactic acid, a molecule that has strong effects on meat color. Another eQTL of 
interest is the one influencing the mRNA levels of the secretory leukocyte peptidase inhibitor (SLPI) gene. This 
cis-eQTL co-localizes with the SSC17 QTL for GM pH24 (Fig. 2b). The SLPI gene encodes a serine-protease that 
inhibits protein-degrading enzymes with strong effects on meat tenderization i.e. when the skeletal muscle is 
being degraded and transformed into meat, SLPI attenuates muscle proteinolysis by binding to proteases and ren-
dering them inactive42. Finally, the co-localization of the IGKC cis-eQTL and the SSC3 QTL for a* (Fig. 2c) does 
not have an obvious biological interpretation because this gene is mainly related with humoral immunity.

Conclusions

We have detected genome-wide and chromosome-wide significant QTL for meat quality traits recorded in a 
Duroc commercial line with a population size that was moderate but comparable to the ones used in other porcine 
GWAS43–45. The limited positional concordance between the set of QTL detected by us and those reported 
by other authors in purebred populations suggests the existence of a significant amount of genetic heterogeneity
Figure 1. Cis-eQTL (left panel) for the GUSB (1a), CTSA (1b) and FAM210B (1c) genes which map to QTL regions associated with meat quality traits recorded in the gluteus medius muscle (right panel). The x-axis represents chromosome length (Mb), and the y-axis shows the $-\log_{10}(P\text{-value})$ of the associations found. The horizontal line indicates the threshold of significance ($q\text{-value} \leq 0.05$). The vertical line depicts the genomic location of the GUSB, CTSA and FAM210B genes.
Figure 2. Co-localization of cis-eQTL (left panel) for the ADCY3 (2a), SLP1 (2b) and IGKC (2c) genes and QTL for meat quality traits recorded in the gluteus medius muscle (right panel). The x-axis represents chromosome length (Mb), and the y-axis shows the –log10 (P-value) of the associations found. The horizontal line indicates the threshold of significance (q-value ≤ 0.05). The vertical line depicts the genomic location of the ADCY3, SLP1 and IGKC genes.
for meat quality traits in porcine breeds. We have found remarkable differences between the QTL maps for the LD and GM muscles, suggesting that meat quality is determined to a great extent by genetic factors that are muscle-specific. Finally, we have observed a number of cis-eQTL that co-localize with meat quality QTL regions. Several of these cis-eQTL regulate the expression of genes which may play important roles in muscle physiology and post-mortem meat maturation. Sequencing of the regulatory regions of these loci might be useful to uncover the identity of the causal mutations explaining the existence of these QTLs.

References

1. Ciobanu, D. C., Lonergan, S. M. & Huff-Lonergan, E. J. Genetics of meat and carcass traits in The Genetics of the Pig. 2nd edn. (ed. Rothschild, M. F. & Ruivinsky, A.) 356 (CABI, 2011).
2. Luo, W. et al. Genome-wide association analysis of meat quality traits in a porcine Large White × Minzhu intercross population. Int J Biol Sci. 8, 580–595 (2012).
3. Nonneman, D. et al. Genome-wide association of meat quality traits and tenderness in swine. J Anim. Sci. 91, 4043–4050 (2013).
4. Ma, J. et al. Genome-wide association study of meat quality traits in a White Duroc × Erhualian F2 intercross and Chinese Sutai pigs. PloS One 8, e64047 (2013).
5. Becker, D., Wimmers, K., Luther, H., Hofer, A. & Leeb, T. A Genome-wide association study to detect QTL for commercially important traits in Swiss Large White boars. PloS One 8, e5995 (2013).
6. Sanchez, M. P. et al. Genome-wide association study of production traits in a commercial population of Large White pigs: evidence of haplotypes affecting meat quality. Genet Sel Evol. 46, 12 (2014).
7. Fang, M. & Andersson, L. Mitochondrial diversity in European and Chinese pigs is consistent with population expansions that occurred prior to domestication. Proc. Biol. Sci. 273, 1803–1810 (2006).
8. Gallardo, D. et al. Quantitative trait loci analysis of a Duroc commercial population highlights differences in the genetic determination of meat quality traits at two different muscles. Anim. Genet. 43, 800–804 (2012).
9. Gallardo, D. et al. Polymorphism of the pig acetyl-coenzyme A carboxylase α gene is associated with fatty acid composition in a Duroc commercial line. Anim. Genet. 40, 410–417 (2009).
10. Canovas, A., Quintanilla, R., Amills, M. & Pena, R. N. Muscle transcriptomic profiles in pigs with divergent phenotypes for fatness traits. BMC Genomics 11, 372 (2010).
11. Chomczyński, P. & Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156–159 (1987).
12. Gentleman, R. C. et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 5, R80 (2004).
13. Xu, X., Zhao, Y. & Simon, R. Gene set expression comparison kit for BRB-Array tools. Bioinformatics 24, 137–139 (2008).
14. Manuzza, A. et al. A genome-wide association analysis for porcine serum lipid traits reveals the existence of age-specific genetic determinants. BMC Genomics 15, 758 (2014).
15. Zhou, X. & Stephens, M. Genome-wide efficient mixed-model analysis for association studies. Nat. Genet. 44, 821–824 (2012).
16. Casellas, J. et al. Bayes factor analyses of heritability for serum and muscle lipid traits in Duroc pigs. J. Anim. Sci. 88, 2246–2254 (2010).
17. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B 57, 289–300 (1995).
18. GTEx Consortium. Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. Science 348, 648–660 (2015).
19. Smedley, D. et al. The BioMart community portal: an innovative alternative to large centralized data repositories. Nucleic Acids Res. 43, W589–598 (2015).
20. Larzul, C. et al. Selection for reduced muscle glycolytic potential in Large White pigs. II. Correlated responses in meat quality and muscle compositional traits. Genet. Sel. Evol. 31, 61–76 (1999).
21. Quintanilla, R., Pena, R. N., Canovas, A. & Amills. M. Differential gene expression profile between two porcine skeletal muscles: longissimus dorsi and gluteus medius. In Book of abstracts of the 32th International Conference on Animal Genetics (2010).
22. Gjerlaug-Enger, E., Aass, L., Ødegard, J. & Vangen, O. Genetic parameters of meat quality traits in two pig breeds measured by rapid methods. Anim. Genet. 4, 1832–1843 (2010).
23. Van Wijk, H. J. et al. Genetic parameters for carcass composition and pork quality estimated in a commercial production chain. J. Anim. Sci. 83, 324–333 (2005).
24. Zuk, O., Hechter, E., Sunyaev, S. & Lander, E. The mystery of missing heritability: Genetic interactions create phantom heritability. Proc. Natl. Acad. Sci. USA 109, 1193–1198 (2012).
25. Culp, K. & Anderssen, H. J. Factors of significance for pork quality—A review. Meat Sci. 64, 219–237 (2003).
26. Cepica, S. et al. Linkage and QTL mapping for sus scrofa chromosome 4. J. Anim. Breed. Genet. 120, 28–37 (2003).
27. Evans, G. J. et al. Identification of quantitative trait loci for production traits in commercial pig populations. Genetics 164, 621–627 (2003).
28. Li, H. D. et al. Quantitative trait loci analysis of swine meat quality traits. J. Anim. Sci. 88, 2904–2912 (2010).
29. Bernal Rubio, Y. L. et al. Implementing meta-analysis from genome-wide association studies for pork quality traits. J. Anim. Sci. 93, 5607–5617 (2015).
30. Ponsukulil, S., Murani, E., Trakooljul, N., Schwerin, M. & Wimmers, K. Discovery of candidate genes for muscle traits based on GWAS supported by eQTL analysis. Int. J. Biol. Sci. 10, 327–337 (2014).
31. Liu, X. et al. Genome-wide association analyses for meat quality traits in Chinese Erhualian pigs and a Western Duroc × (Landrace × Yorkshire) commercial population. Genet. Sel. Evol. 47, 44 (2015).
32. Zhang, C. et al. Genome-wide association studies (GWAS) identify a QTL close to PRKAG3 affecting meat pH and colour in crossbred pigs. BMC Genetics 16, 33 (2015).
33. Milan, D. et al. A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. Science 288, 1248–1251 (2000).
34. Yang, H. et al. Genome-wide association analysis for blood lipid traits measured in three pig populations reveals a substantial level of genetic heterogeneity. PloS One 10, e0131667 (2015).
35. Ernst, C. W. & Steibel, J. P. Molecular advances in QTL discovery and application in pig breeding. Trends Genet. 29, 215–224 (2013).
36. Ma, J. et al. A Splice mutation in the PHKG1 gene causes high glycogen content and low meat quality in pig skeletal muscle. PLoS Genet. 10, e1004710 (2014).
37. Dutton, R. T. & Lawrie, R. A. Release of lysosomal enzymes during post-mortem conditioning and their relationship to tenderness. Int. J. Food Sci. Technol. 4, 43–50 (2007).
38. Hiraoka, M. Cathepsin A/protective protein: an unusual lysosomal multifunctional protein. Cell. Mol. Life Sci. 56, 894–907 (1999).
39. Naz, H. et al. Human α-glucuronidase: structure, function, and application in enzyme replacement therapy. Rejuvenation Res. 16, 352–363 (2013).
40. Chang, H. J., Xu, X. L., Zhou, G. H., Li, C. B. & Huang, M. Effects of characteristics changes of collagen on meat physicochemical properties of beef semitendinosus muscle during ultrasonic processing. Food Bioprocess. Technol. 5, 285–297 (2009).
41. Hong, S. et al. et al. Upregulation of adenylate cyclase 3 (ADCY3) increases the tumorigenic potential of cells by activating the CREB pathway. Oncotarget 4, 1791–1803 (2013).
42. Urso, L. et al. Alterations in mRNA expression and protein products following spinal cord injury in humans. J. Physiol. 579, 877–892 (2007).
43. Ramayo-Caldas, Y. et al. et al. Genome-wide association study for intramuscular fatty acid composition in an Iberian × Landrace cross. J. Anim. Sci. 90, 2883–2893 (2012).
44. Fontanesi, L. et al. et al. A genome wide association study for backfat thickness in Italian Large White pigs highlights new regions affecting fat deposition including neuronal genes. BMC Genomics 13, 583 (2012).
45. Becker, D., Wimmers, K., Luther, H., Hofer, A. & Leeb, T. A genome wide association study to detect QTL for commercially important traits in Swiss Large White boars. PLoS One 8, e55951 (2013).

Acknowledgements
The authors are indebted to Selección Batallé S.A. for providing the animal material. We gratefully acknowledge to J. Reixach (Selección Batallé), I. Diaz (IRTA) and J. Soler (IRTA) for their collaboration in the experimental protocols. Thanks to Anna Mercadé and Anna Castelló for their technical support. This research was partially funded with projects AGL2013-48742-C2-1-R and AGL2013-48742-C2-2-R, awarded by the Spanish Ministry of Economy and Competitiveness (MINECO). We also acknowledge the support of the Spanish Ministry of Economy and Competitiveness for the Center of Excellence Severo Ochoa 2016–2019 (SEV-2015-0533) grant awarded to the Center for Research in Agricultural Genomics. Rayner Gonzalez-Prendes was funded by a FPU Ph.D. grant from the Spanish Ministerio de Educación (FP12/00860). Thanks also to the CERCA Programme of the Generalitat de Catalunya.

Author Contributions
R.Q., M.A., J.L.N., A.M. and J.J. conceived the study and designed the experiment; R.Q. and J.L.N. produced the animal material and collected the phenotypic data; T.F. contributed to molecular tasks; R.G.-P. carried out the genome-wide association analyses for meat quality phenotypes and expression data; R.N.P. and A.C. contributed to the analysis of microarray data; R.G.-P. and M.A. wrote the manuscript. All authors helped to draft the manuscript and read and approved its final version.

Additional Information
Competing financial interests: The authors declare no competing financial interests.

How to cite this article: González-Prendes, R. et al. Joint QTL mapping and gene expression analysis identify positional candidate genes influencing pork quality traits. Sci. Rep. 7, 39830; doi: 10.1038/srep39830 (2017).

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

© The Author(s) 2017