Title page: Perilipin genes and production traits in pigs

Relationship between perilipin genes polymorphisms and growth, carcass, and meat quality traits in pigs

S. Gol[1], R. Ros-Freixedes[1], P. Zambonelli[2], M. Tor[1], R.N. Pena[1], S. Braglia[2], M. Zappaterra[2], J. Estany[1] & R. Davoli[2]

[1]Departament de Producció Animal-Agrotecnio Center, Universitat de Lleida, 25198 Lleida, Catalonia, Spain

[2]Department of Agricultural and Food Science (DISTAL), University of Bologna, via Fratelli Rosselli, 107, 42123 Reggio Emilia, Italy

Correspondence

Joan Estany, Department de Producció Animal, Universitat de Lleida, Rovira Roure 191, 25198 Lleida, Spain

Tel: +34. 973 702893;

Fax: +34. 973 702874;

E-mail: jestany@prodan.udl.cat

Summary

The perilipins (**PLIN**) belong to a family of structural proteins that play a role regulating intracellular lipid storage and mobilization. Here, *PLIN1* and *PLIN2* have been evaluated as candidate genes for growth, carcass, and meat quality traits in pigs. A sample of 607 Duroc pigs were genotyped for two single nucleotide polymorphisms, one in intron 2 of the *PLIN1* gene (*JN860199:g.173G>A)* and the other at the 3' untranslated region of the *PLIN2* gene (*GU461317:g.98G>A*). Using a Bayesian approach we have been able to find evidence of additive, dominant, and epistatic associations of the *PLIN1* and *PLIN2* polymorphisms with early growth rate and carcass length. However, the major effects were produced by the dominant A allele at the *PLIN2* polymorphism, which also affected the carcass lean weight. Thus, pigs carrying an additional copy of the A allele at the *g.98G>A PLIN2* polymorphism had a probability of at least 98% of producing carcasses with heavier lean weight (+0.41 kg) and ham weight (+0.10 kg). The results obtained indicate that the *PLIN2* polymorphism could be a useful marker for lean growth. In particular, it may help to reduce the undesired negative correlated response in lean weight to selection for increased intramuscular fat content, a common scenario in some Duroc lines involved in the production of high quality pork products.

Key Words:, body weight; Duroc pigs; fat; genetic markers; lean growth

Introduction

Growth rate and carcass lean content are crucial characteristics for the economic viability of pork production. Selection emphasizing lean content has led to reduce some pork quality attributes, including the intramuscular fat (**IMF**) content. The use of molecular markers may be useful to improve the genetic progress in traits that are difficult and expensive to measure (Dekkers 2004), but also to break down unfavorable genetic correlations between antagonistic traits, such as those between lean growth rate or carcass lean content and IMF content (Ros-Freixedes *et al.* 2012; Ros-Freixedes *et al.* 2013). In this scenario, performing association studies with candidate genes related to proteins affecting fat metabolism is of particular interest.

The perilipins (**PLIN**) belong to a family of structural proteins that coat intracellular lipids into cytosolic droplets (Kimmel *et al.* 2010), where they regulate intracellular lipid storage and mobilization by fine-tuning the activity of lipases (Bickel *et al.* 2009). The composition of PLIN changes as lipid droplets enlarge and mature. Perilipin 2 (**PLIN2**) is the most prominent PLIN protein in most adult cell types and in immature adipocytes. In contrast, the large central mature lipid droplets of mature adipocytes are largely coated by perilipin 1 (**PLIN1**). Recently, PLIN1 and PLIN2 have been shown to co-localize in the skeletal muscle of pigs (Gandolfi *et al.* 2011).

Mutations in the *PLIN* genes have been associated to body fat mass in mice (Saha *et al.* 2004) and humans (Qi *et al.* 2004; Corella *et al.* 2005; Ruiz *et al.* 2011). So far only two reports in pigs have investigated the association of *PLIN1* and *PLIN2* polymorphisms with a limited number of production traits. In the first report, two synonymous single nucleotide polymorphisms (**SNP**) in exons 3 and 6 of *PLIN1* showed suggestive associations with average daily gain (**ADG**) and backfat thickness in Large White pigs (Vykoukalová *et al.* 2009). In a second study, a 3' untranslated region (**UTR**)

SNP at the *PLIN2* gene (*GU461317:g.98G>A*) was found to be associated to lean growth and content but not to visible intermuscular fat (Davoli *et al.* 2011). The aim of the present study was to further investigate the contribution of *PLIN1* and *PLIN2* genes to a wider range of performance, carcass, and meat quality traits in pigs and, in particular, to confirm whether *PLIN1* and *PLIN2* genotype variants exert a differential effect on lean growth and IMF content.

Materials and methods

Animals, traits and sample collection

A panel of 20 unrelated pigs from three Italian heavy breeds was used for the SNP screening of *PLIN1* gene, including eight Italian Large White, four Italian Duroc and eight Italian Landrace pigs. A total of 607 Duroc barrows from 88 sires and 348 dams were used for the association analyses. These pigs were randomly sampled in seven batches from the same commercial line and performance-tested from 75 d to 210 d of age under commercial conditions (Ros-Freixedes *et al.* 2012). During the test period they had *ad libitum* access to commercial diets. A complete description of the line and of the procedures followed for testing and sample collection is given in Ros-Freixedes *et al.* (2012). The traits recorded included live body weight (BW), backfat thickness, and loin thickness at 120, 180, and 205 d. Backfat and loin thickness was ultrasonically measured at 5 cm off the midline at the position of the last rib (Piglog 105, Herlev, Denmark). After slaughter at 210 days, the carcass weight and length, the carcass backfat and loin thickness, and the ham weight were measured. Carcass backfat and loin thickness at 6 cm off the midline between the third and fourth last ribs, together with the carcass lean percentage, were estimated using an on-line ultrasound automatic scanner (AutoFOM,

SFK-Technology, Herlev, Denmark). After chilling for about 24 h at 2ºC, the pH was measured in the *longissimus dorsi* and in the *semimembranosus* muscles. Samples of at least 50 g of *gluteus medius* muscle and *longissimus dorsi* were taken, immediately vacuum packaged, and stored in deep freeze until required for IMF content and fatty acid determination (Bosch *et al.* 2009).

Single nucleotide polymorphism discovery and genotyping

Genomic DNA was isolated from freeze-dried muscle samples using standard protocols (Sambrook *et al.* 1989). To search for sequence variation in the pig *PLIN1* gene, the genomic, cDNA, and EST sequences available in the GenBank (http://www.ncbi.nlm.nih.gov/Genbank) and in the Ensembl databases (http://www.ensembl.org) were compared for an *in silico* variability analysis. Italian heavy pigs were used to validate the *in silico*-identified SNPs.

Seven primer pairs (**Table S1**) were designed using Primer3 v.0.4.0 software (http://frodo.wi.mit.edu/primer3/) to amplify seven porcine *PLIN1* gene fragments. The PCR products were sequenced on both strands using the BigDye Terminator v3.1 Cycle Sequencing kit (Life Technologies, Grand Island, NY, USA) in an ABI PRISM 3100-Avant Genetic Analyzer (Life Technologies). The sequences obtained were compared by multiple alignments, performed with MEGA software v4.0 (www.megasoftware.net/).

The *JN860199:g.173G>A PLIN1* SNP polymorphism, which was selected for subsequent analyses, was genotyped by PCR-restriction fragment length polymorphism assay. PCR products obtained with the "P2" primer set (**Table S1**) were digested with *Hin*1II (Fermentas, Vilnius, Lithuania) and the resulting products were resolved on polyacrylamide gels. For *PLIN2*, the *GU461317:g.98G>A* SNP, in the 3' UTR region

of the gene, was genotyped by High Resolution Melting PCR in a Rotor-Gene™ 6000 (Corbett Research, Mortlake, New South Wales, Australia) following the protocol described in Davoli *et al.* (2011). The linkage disequilibrium between SNPs was estimated as r^2 using the Haploview software (Barrett 2009).

The *JN860199:g.173G>A PLIN1* SNP was genotyped by PCR-restriction fragment length polymorphism assay by restricting the "P2" PCR product (**Table S1**) with *Hin*1II (Fermentas, Vilnius, Lithuania). For *PLIN2*, the *GU461317:g.98G>A* SNP was genotyped by High Resolution Melting PCR in a Rotor-Gene™ 6000 (Corbett Research, Mortlake, New South Wales, Australia) following the protocol described in Davoli *et al.* (2011).

Association analysis

The additive, dominant, and epistatic effects of the *PLIN* genotypes were estimated independently for each trait using a Bayesian setting, in line with the methodology described in Ros-Freixedes *et al.* (2012). A two-generation pedigree was used for the analyses. In matrix notation, the model used for the *i*th trait was $\mathbf{y}_i = \mathbf{X}_i\mathbf{b}_i + \mathbf{Z}_i\mathbf{a}_i + \mathbf{e}_i$, where $\mathbf{y}_i$ is the vector of observations for trait *i*; $\mathbf{b}_i$, $\mathbf{a}_i$, and $\mathbf{e}_i$ are the vectors of systematic, polygenic, and residual effects, respectively; and $\mathbf{X}_i$ and $\mathbf{Z}_i$ the known incidence matrices that relate $\mathbf{b}_i$ and $\mathbf{a}_i$ with $\mathbf{y}_i$, respectively. The systematic effects were the batch (7 levels), the age at test as a covariate, and orthogonal coefficients for additive (a), dominance deviation (d) and first-order epistatic effects (aa: additive × additive; ad: additive × dominance; da: dominance × additive; and dd: dominance × dominance) for *PLIN1* and *PLIN2* SNPs. Pigs in a given batch were contemporaneous pigs tested at the same unit and slaughtered in the same abattoir. The litter effect was not included because, on average, there were less than 2 piglets per litter. The orthogonal coefficients

for the genetic effects were calculated using the algorithm proposed by Alvarez-Castro & Carlborg (2007).

The models were solved using Gibbs sampling with the TM software (Legarra *et al.* 2008). The traits were assumed to be conditionally normally distributed as $\left[\mathbf{y}_i \middle| \mathbf{b}_{i,}\mathbf{a}_{i,}\mathbf{I}\sigma^2_{ei}\right] \sim \mathrm{N}(\mathbf{Xb}_i + \mathbf{Za}_{i,}\mathbf{I}\sigma^2_{ei})$, where σ^2_{ei} is the residual variance and **I** the appropriate identity matrix. The animal effects conditionally on the additive genetic variance σ^2_{ai} were assumed multivariate normally distributed with mean zero and variance $\mathbf{A}\sigma^2_{ai}$, where **A** was the numerator relationship matrix. The matrix **A** was calculated using 1043 animals in the pedigree. Flat priors were used for $\mathbf{b}_i$ while the variance components were set to the values obtained by Ros-Freixedes *et al.* (2013) with data and pedigree from 1996 onwards. Statistical inferences were derived from the samples of the marginal posterior distribution using a unique chain of 500,000 iterations, where the first 100,000 were discarded and one sample out of 100 iterations retained. The additive, dominance, and epistatic effects were assessed by calculating both the probability of each of these components being greater or lower than zero and their highest posterior density interval at 95% of probability (HPD95). Statistics of marginal posterior distributions and the convergence diagnostics were obtained using the BOA package (Smith 2005). Convergence was tested using the *Z*-criterion of Geweke (Geweke 1992) and visual inspection of convergence plots.

Results and discussion

Polymorphisms and sequence variation of PLIN genes

The *in silico* analysis on publicly available genomic, EST, and cDNA sequences revealed ten SNPs (detected at least twice) within the coding sequence of *PLIN1*, located

in the exons 1, 2, 5, and 8 (data not shown) and five SNP in intronic regions. Seven genomic regions, covering the positions of the ten putative SNP, were subjected to direct sequencing in 20 animals from three Italian heavy pig breeds. A total of 2,437 bp of the pig *PLIN1* gene were screened, which covered 1,126 bp of the coding sequence, the complete 183-bp 5' UTR, and 1,128 bp of intronic regions and part of the promoter and 3' downstream genomic region, according to the annotation of the Ensembl entry [ENSSSCG00000001844]. The sequencing covered the positions of the putative SNPs detected *in silico*, with the exception of the SNP on exon 8, which was not analyzed due to the unsuccessful amplification of this region. Four SNPs (two intronic and two exonic) out of the ten SNPs discovered *in silico* were detected by sequencing Italian heavy pig breeds (Table 1). The other six polymorphisms identified *in silico* were not detected during the sequencing. The two intronic SNPs were novel and the sequences were reported to GenBank [JN860199; SNP *g.173G>A* and *g.3484C>G*], while the two exonic SNPs, which were detected in our *in silico* analysis, were both synonymous and had been reported before (GenBank: AM931171; SNP *g.4119A>G* and *g.7966T>C*; Vykoukalová *et al.* 2009). The four SNP were in complete linkage disequilibrium in the initial panel of 20 pigs. The intronic *JN860199g.173G>A* SNP was selected for subsequent analyses because a restriction enzyme was available to analyze this mutation.

To assess the association of these mutations with productive parameters, the *PLIN1 JN860199:g.173G>A* and *PLIN2 GU461317:g.98G>A* SNPs were genotyped in a population of 607 Duroc pigs, which had data available on performance, fattening and meat quality traits (Ros-Freixedes *et al.* 2012). The allele frequencies and the distribution genotypes for *PLIN1* and *PLIN2* SNPs are reported in **Table 2**. In both SNPs the alleles were segregating at intermediate frequencies, with the G allele being the less frequent in

187 *JN860199:g.173G>A* (minor allele frequencies of 0.38) and alleles G and A showing
188 identical gene frequency for *GU461317:g.98G>A*.
189
190 ## *Effect of PLIN genotypes*
191 The additive, dominant, and epistatic effects of *PLIN1 g.173G>A* and *PLIN2*
192 *g.98G>A* SNPs associated to BW and growth rate at different ages during the fattening
193 period are given in **Table 3.** The substitution of A for G in *PLIN1* showed some evidence
194 of a negative additive effect on BW (-0.66 kg at 120 d and -0.68 kg at 180 d, with a
195 probability of 6% and 10% of being greater than zero, respectively), but a strong
196 evidence of a positive additive effect in *PLIN2*, with values of +0.95 kg, +1.19 kg, and
197 +1.08 kg at 120 d, 180 d and 205 d, respectively, with an associated probability of being
198 greater than zero superior to 95% in the three ages. The substitution effect of A for G for
199 BW was similar at 120 d, 180 d, and 205 d, thereby indicating that the beneficial effect
200 of allele A on BW was due to increased growth at early stages. In concordance, the effect
201 of allele A at *PLIN2* for ADG was evident up to 120 d (+7.26 g/d, with a probability of
202 being positive of 98%) but not thereafter, both from 120 to 180 d (+4.15 g/d) and from
203 180 to 205 d (-0.42 g/d). Consequently, the variance associated to the additive effects of
204 *PLIN2 g.98G>A* SNP (Falconer & Mackay 1996) is able to capture a greater proportion
205 of the additive variance of BW (Ros-Freixedes *et al.* 2013) at 120 d (1.49%) than at 205
206 d (1.12%). Regarding the dominant effects, a negative dominant effect for BW at 120
207 and 180 days in *PLIN1* (-1.04 kg and -1.56 kg, respectively) and a positive dominant
208 effect for BW at 180 days in *PLIN2* (+1.17 kg were observed (**Table 3**). No clear
209 evidence of epistasis between *PLIN1* and *PLIN2* SNPs was observed for BW and ADG,
210 with the exception of an additive × additive effect for BW at 120 d (-0.88 kg, with

associated probability of being positive of 6%) and for ADG up to 120 d (-7.94 g/d, with associated probability of being positive of 4%).

The additive, dominant, and epistatic effects of *PLIN1 g.173G>A* and *PLIN2 g.98G>A* SNPs associated to backfat and loin thickness at 120 d, 180 d and 205 d of age are given in **Table 4**. The *PLIN1 g.173G>A* SNP did not show a clear pattern of association with fatness traits, but results for the *PLIN2 g.98G>A* SNP indicated that A allele is positively associated to backfat thickness at early ages (+0.17 mm and +0.19 mm, at 120 d and at 180 d, respectively, with a probability of being positive of 91% and 98%) and negatively to backfat thickness at 205 d (-0.22 mm, with a probability of being positive of 10%). The effect of the *PLIN2 g.98G>A* SNP on backfat thickness followed a similar pattern as for ADG, with the positive effect of allele A at 120 d vanishing at later ages.

In agreement with these results, no strong evidence of association of *PLIN1* and *PLIN2* SNPs with carcass backfat thickness, and carcass loin thickness was observed (**Table 5**). However, allele G at *PLIN1* and allele A at *PLIN2* had some beneficial effects on other carcass traits. Thus, pigs carrying an additional copy of allele G at *PLIN1* and allele A at *PLIN2* had longer carcasses (+0.62 cm and +0.43 cm, with a probability of being positive greater than 96% and 99%, respectively) and, more interestingly, those carrying allele A at *PLIN2* showed a higher carcass lean weight (+0.41 kg, with a probability of being positive of 99.9%). This latter effect should be interpreted as a result of a moderate but favorable change in both carcass weight (+0.58 kg), mostly as a consequence of increased growth rate at early ages, and carcass lean percentage (+0.23). As a result, the *PLIN2 g.98G>A* SNP reached to explain 0.59% of the additive variance of lean weight. Moreover, a positive effect of allele A at *PLIN2* on ham weight was also detected (0.10 kg, with a probability of being positive of 94%).

No evidence was found indicating that meat quality traits (pH and IMF) were additive by *PLIN1* and *PLIN2* SNP, although some minor changes were observed for IMF fatty acid composition (**Table 6**). In particular, allele A at *PLIN1* decreased PUFA (-0.20%) and increased MUFA (0.20%) while allele A at *PLIN2* decreased SFA (-0.24%). Evidence supporting the existence of dominant and epistatic effects associated to carcass and meat quality traits was mostly circumscribed to traits where the additive effects were more evident (carcass length and carcass lean weight), thereby suggesting that the mode of action of *PLIN1* and *PLIN2* on the traits that they are influencing is subjected to complex regulations. As for BW and ADG, the dominant effect associated to lean weight was negative in *PLIN1* (-0.19 kg, with a probability of 2% of being positive) but positive in *PLIN2* (0.41 kg, with 99.9% probability of being positive). These dominant values were around two-fold higher than their respective additives, a result which supports for an underdominant *PLIN1* and overdominant *PLIN2* gene action for lean weight. To assess the stability of the estimates to model over-parameterization, the additive and dominance effects were also estimated ignoring the epistatic effects. The estimates obtained (results not shown), although slightly higher, were in line with those reported with the model that included epistatis, thereby confirming the favourable effects of allele G at *PLIN1* and allele A in *PLIN2* on growth and carcass traits.

Our findings are consistent with the results in Vykoukalová *et al.* (2009), who found suggestive associations of the two exonic *PLIN1* SNP with ADG in Large White pigs, and, particularly, with those in Davoli *et al.* (2011), who reported a favorable effect of allele A at *PLIN2* on ADG, feed conversion ratio, lean cuts, and ham weight estimated breeding values in Italian Duroc. The five members of the *PLIN* family have been studied in depth in humans and model animals. Most reports have focused on *PLIN1*, the main perilipin protein in mature adipocytes, particularly in relation to BW and obesity-related

phenotypes (Smith & Ordovas 2012), but results do not show a consistent trend across them. It must be taken into account that, depending on the energy state of the organism, *PLIN1* either limits lipase access to stored triglycerides (in the fed state) or facilitates hormonally stimulated lipolysis (in the fasted state). This dual activity is illustrated by the fact that both *PLIN1*-null and *PLIN1*-overexpressing mice are protected from diet-induced obesity (Saha *et al.* 2004). In our pig population, mutations in the *PLIN1* did not correlate with growth or fat deposition traits. This indicates that genes other than *PLIN1* are the main players of fat deposition in pig, or that other mutations outside the transcribed sequence, for instance in the 5' or 3' regulatory regions, might have a more relevant effect over the expression of the gene. In contrast, only few reports in humans and mice have focused on *PLIN2* gene. Our results indicate that allele A at the *PLIN2 g.98G>A* SNP has beneficial effects on early growth, lean growth and prime retail cuts. In agreement with this, the genomic position of *PLIN2* on chromosome 1 co-localizes with quantitative trait loci for ADG (Liu *et al.* 2007), BW at birth (Guo *et al.* 2008), and daily feed intake (Kim *et al.* 2000) (**Supplementary Table S2).** Of the five PLIN proteins, PLIN2 and 3 are by far the most prominent in human skeletal muscle (Gjelstad *et al.* 2012), with PLIN2 accounting for >60% of total perilipin content. It has been shown that PLIN2 is also the main perilipin in pig muscle (Gandolfi *et al.* 2012). Therefore, it is not surprising that *PLIN2* is related to growth and lean weight, as perilipins regulate not the deposition of fat *per se*, but more importantly, the accessibility of lipases to the stored fats in response to the energy demands of the cells.

Our results indicate that *PLIN2 g.98G>A* SNP could be a useful marker for lean growth, which is a relevant trait for the pig industry in general, very interested in fast-growing lean animals. Although results are encouraging for Duroc, further association studies are needed to confirm whether this polymorphism similarly affects other pig

breeds. However, it is in this breed where it can be of particular interest. Duroc lines are the most used in premium quality markets, where pigs are raised to heavy weights and IMF becomes a key trait. In such scenario it is very convenient to find selection criteria addressed to reduce the undesired negatively correlated response on BW to selection for IMF.

Acknowledgements

This work was partially funded by national projects AGL2009-09779 and AGL2012-33529 (Ministry of Education and Innovation, Spain) and AGER-HEPIGET grant N. 2011-0279 (Italy). S. Gol is recipient of a PhD scholarship from MINECO, Spain (BES-2014-FPU13/04975).

References

Alvarez-Castro J.M., Carlborg O. (2007) A unified model for functional and statistical epistasis and its application in quantitative trait loci analysis. *Genetics*, **176**, 1151–1167.

Barret J.C. (2009). *Haploview: Visualization and analysis of SNP genotype data.* Cold Spring Harb. Protoc.

Beeckmann P., Schröffel J., Moser G., Bartenschlager H., Reiner G., Geldermann H. (2003). Linkage and QTL mapping for Sus scrofa chromosome 1. *J. Anim Breed Genet.*, **120**, 1-10.

Bickel P.E., Tansey J.T., Welte M.A. (2009). PAT proteins, an ancient family of lipid droplet proteins that regulate cellular lipid stores. *Biochim. Biophys. Acta*., **1791**, 419- 440.

311 Bosch L., Tor M. Reixach J., Estany J. (2009). Estimating intramuscular fat content
312 and fatty acid composition in live and post-mortem samples in pigs. *Meat Sci*. **82**,
313 432-437.
314 Corella D., Sorli Q.L., Godoy J.V., Portoles D., Coltell O. (2005). Obese subjects
315 carrying the 11482G>A polymorphism at the perilipin locus are resistant to weight
316 loss after dietary energy restriction. *J. Clin. Endocrinol. Metab*. **90**, 5121-5126.

317 Davoli R., Gandolfi G., Braglia S., Comella M. Zambonelli P., Buttazzoni L. (2011).
318 New SNP of the porcine perilipin 2 (PLIN2) gene, association with carcass traits and
319 expression analysis in skeletal muscle. *Mol. Biol. Rep*. **38**, 1575-1583.

320 Dekkers J.C. (2004). Commercial application of marker- and gene-assisted selection in
321 livestock: Strategies and lessons. *J. Anim. Sci*. **82** E-Suppl, E313-328.

322 Falconer S., Mackay F.C. (1996). *Introduction to Quantitave Genetics*. Pearson
323 Prentice Hall. Harlow, England.

324 Gandolfi G., Mazzoni M., Zambonelli P., Lalatta-Costerbosa G., Tronca A., Russo V.
325 (2011). Perilipin 1 and perilipin 2 protein localization and gene expression study in
326 skeletal muscles of european cross-breed pigs with different intramuscular fat
327 contents. *Meat Sci*. **88**, 631-637.

328 Geweke J. 1992. Evaluating the accuracy of sampling-based approached to the
329 calculation of posterior moments (with discussion). In: Bernardo J. M.,Berger J.
330 O., Dawid A.P., Smith A.F.M., editors, *Bayesian statistics.* Oxford Univ.
331 Press, Oxford, UK. 69–193.

Gjelstad I., Haugen M.F., Gulseth H., Norheim L.F., Jans A., Bakke S.S. (2012). Expression of perilipins in human skeletal muscle in vitro and in vivo in relation to diet, exercise and energy balance. *Arch. Physiol. Biochem*. **118**, 22-30.

Guo Y.M., Lee G.J., Archibald A.L., Haley C.S. (2008). Quantitative trait loci for production traits in pigs: A combined analysis of two Meishan x Large White populations. *Anim. Genet*. **39**, 486-495.

Kim K.S., Larsen N., Short T., Plastow G., Rothschild M.F. (2000). A missense variant of the porcine melanocortin-4 receptor (MC4R) gene is associated with fatness, growth, and feed intake traits. *Mamm. Genome*. **11**, 131-135.

Kimmel A.R., Brasaemle D.L., McAndrews-Hill M., Sztalryd C., Londos C. (2010). Adoption of PERILIPIN as a unifying nomenclature for the mammalian PAT-family of intracellular lipid storage droplet proteins. *J. Lipid Res*. **51**, 468-471.

Legarra A., Varona L., Maturana E.L. (2008). TM Threshold Model. Accessed Set.20, 2013. http://snp.toulouse.inra.fr/alegarra/manualtm.pdf

Liu G., Jennen D.G., Tholen E., Juengst H., Kleinwachter T., Holker M. (2007). A genome scan reveals QTL for growth, fatness, leanness and meat quality in a duroc-pietrain resource population. *Anim. Genet*. **38**, 241-252.

Qi L., Shen H., Larson I., Schaefer E.J., Greenberg A.S., Tregouet D.A. (2004). Gender-specific association of a perilipin gene haplotype with obesity risk in a white population. *Obes. Res*. **12**, 1758-1765.

Ros-Freixedes R., Reixach J., Bosch L., Tor M., Estany J. (2013). Response to selection for decreased backfat thickness at restrained intramuscular fat content in Duroc pigs. *J. Anim. Sci*. **91**, 3514-21.

355 Ros-Freixedes R., Reixach J., Bosch L., Tor M., Estany J. (2012). Expected genetic
356 response for oleic acid content in pork. *J. Anim. Sci*. **90**, 4230-4238.

357 Ruiz J.R., Larrarte E., Margareto J., Ares R., Alkorta P., Labayen I. (2011).
358 Preliminary findings on the role of PLIN1 polymorphisms on body composition and
359 energy metabolism response to energy restriction in obese women. *Br. J. Nutr*. **106**,
360 486-490.

361 Russo V., Buttazzoni L., Baiocco C., Davoli R., Costa N., Schivazappa C. Virgili R.
362 (2000). Heritability of muscular cathepsin B activity in Italian large white pigs. *J.*
363 *Anim. Breed. Genet*. **117**, 37-42.

364 Saha P.K., Kojima H., Martinez-Botas J. Sunehag A.L., Chan A.L. (2004). Metabolic
365 adaptations in the absence of perilipin: Increased beta-oxidation and decreased
366 hepatic glucose production associated with peripheral insulin resistance but normal
367 glucose tolerance in perilipin-null mice. *J. Biol. Chem*. **279**, 35150-35158.

368 Sambrook J., Fritschi E.F., Maniatis T. (1989). *Molecular cloning: a laboratory*
369 *manual*, Cold Spring Harbor Laboratory Press, New York.

370 Smith B.J. (2005). Bayesian Output Analysis Program (BOA). Version 1.1.5. The
371 University of Iowa. Acceded Set. 20, 2013. http://www.public-health.uiowa.edu/boa.

372 Smith C.E., Ordovás J.M. (2012). Update on perilipin polymorphisms and obesity.
373 *Nutr. Rev*. **70**, 611-621.

374 Tansey J.T., Huml A.M., Vogt R. Davis K.E., Jones J.M., Fraser K.A. (2003).
375 Functional studies on native and mutated forms of perilipins. A role in protein kinase
376 A-mediated lipolysis of triacylglycerols. *J. Biol. Chem*. **278**, 8401-8406.

377 Tao X., Jihong Y., Li G., Bin F., Yi Z., Xiaodong C. (2008). Cloning, chromosome
378 mapping and expression pattern of porcine PLIN and M6PRBP1 genes. *Genet. Sel.*
379 *Evol*. **40**, 215-226.

380 Vykoukalová Z., Knoll A. Cepica S. (2009). Porcine perilipin (PLIN) gene: structure,
381 polymorphism and association study in large white pigs. *Czech Journal of Animal*
382 *Science*. **54**, 359-364.

383 **Table 1.** Single nucleotide polymorphisms (SNP) detected by sequencing the porcine

384 *PLIN1* gene in Italian heavy pigs.

SNP[1]	Gene position [2]	Gene location	Amino acid change
JN860199 g.173G>A	1,428	Intron 2	-
JN860199 g.3484C>G	4,739	Intron 2	-
AM931171g.4119A>G	4,856	Exon 3	Synonymous[3]
AM931171g.7966T>C	8,703	Exon 6	Synonymous[3]

385

386 [1] GenBank accession number is indicated.

387

388 [2] Position from the start codon as referred to the entry
389 [Ensembl:ENSSSCG00000001844; assembly Sscrofa10.2: chromosome 7;
390 60,126,614:60,139,897:-1].

391

392 [3] These SNPs are also reported by Vykoukalová *et al.* 2009

393

Table 2. Number of pigs (N), frequency of the allele G (f (G)), and number of pigs per *PLIN1* and *PLIN2* genotypes by batch.

		PLIN1 (*JN860199:g.173G>A*)				*PLIN2* (*GU461317:g.98G>A*)			
	N	f(G)	GG	AG	AA	f(G)	GG	AG	AA
Batch 1	108	0.51	36	38	34	0.49	23	60	25
Batch 2	102	0.51	31	42	29	0.37	16	44	42
Batch 3	66	0.35	13	20	33	0.50	15	36	15
Batch 4	69	0.33	6	34	29	0.43	16	27	26
Batch 5	84	0.26	6	32	46	0.60	31	39	14
Batch 6	95	0.31	8	42	45	0.61	37	42	16
Batch 7	83	0.32	8	37	38	0.48	19	42	22
Total	607	0.38	108	245	254	0.50	157	290	160

Table 3. Mean (standard deviation) and additive, dominant, and epistatic effects of *PLIN1 JN860199:g.173G>A* and *PLIN2 GU461317:g.98G>A* polymorphisms associated to live body weight and growth rate at different ages

		Additive (a) and dominant (d) effects[1]								Epistatic effects[1]							
		PLIN1,g.173G>A				*PLIN2, g.98G>A*											
Trait	Mean (SD)	a_1	P(>0)	d_1	P(>0)	a_2	P(>0)	d_2	P(>0)	a_1a_2	P(>0)	a_1d_2	P(>0)	d_1a_2	P(>0)	d_1d_2	P(>0)
Body weight, kg																	
120 d	61.28 (12.13)	-0.66	**0.06**	-1.04	**0.05**	0.95	**0.99**	0.77	0.89	-0.88	**0.06**	0.47	0.71	-0.51	0.29	1.35	0.86
180 d	107.32 (11.01)	-0.68	**0.10**	-1.56	**0.03**	1.19	**0.98**	1.17	**0.94**	-0.78	0.14	0.64	0.73	0.13	0.55	0.59	0.65
205 d	122.15 (11.33)	-0.42	0.27	-0.51	0.29	1.08	**0.96**	1.03	0.87	-1.01	0.12	0.19	0.56	0.46	0.63	0.18	0.55
Daily gain, g/d																	
0-120 d	500.77 (80.94)	-4.76	**0.09**	-6.93	**0.09**	7.26	**0.98**	5.51	0.86	-7.94	**0.04**	4.70	0.76	-4.59	0.27	12.04	0.88
120-180 d	766.88 (112.88)	-1.95	0.38	-6.83	0.29	4.15	0.74	4.37	0.69	2.26	0.60	1.10	0.54	15.38	0.87	-10.22	0.30
180-205 d	596.23 (193.43)	5.72	0.70	22.65	**0.94**	-0.42	0.48	-9.57	0.48	-8.23	0.28	-3.27	0.41	20.03	0.82	-22.91	0.24

[1] The numbers 1 and 2 refers to *PLIN1* and *PLIN2*, respectively, with the additive effects expressed as A-G; P (>0): Posterior probability of a value being positive. In bold, probabilities above 0.90 or below 0.10.

Table 4. Mean (standard deviation) and additive, dominant, and epistatic effects of *PLIN1 JN860199:g.173G>A* and *PLIN2 U461317:g.98G>A* polymorphisms associated to backfat and loin thickness at different ages.

		Additive (a) and dominant (d) effects[1]								Epistatic effects[1]							
		PLIN1,g.173G>A				*PLIN2, g.98G>A*											
Trait	Mean (SD)	a_1	P(>0)	d_1	P(>0)	a_2	P(>0)	d_2	P(>0)	a_1a_2	P(>0)	a_1d_2	P(>0)	d_1a_2	P(>0)	d_1d_2	P(>0)
Backfat thickness, mm																	
120 d	11.05 (2.72)	-0.07	0.29	-0.18	0.17	0.17	**0.91**	-0.07	0.33	-0.23	**0.07**	0.03	0.55	-0.14	0.29	0.59	**0.95**
180 d	17.76 (3.74)	-0.06	0.27	-0.15	0.14	0.19	**0.98**	-0.10	0.31	-0.76	0.16	0.54	0.69	0.15	0.56	0.79	0.68
205 d	20.66 (4.15)	0.01	0.52	-0.24	0.16	-0.22	**0.10**	-0.03	0.46	-0.41	**0.03**	0.06	0.58	0.12	0.63	0.05	0.54
Loin thickness, mm																	
120 d	40.38 (3.25)	0.33	**0.92**	-0.40	0.15	-0.42	**0.04**	-0.59	**0.04**	0.07	0.59	-0.23	0.31	-0.91	**0.04**	0.31	0.66
180 d	45.04 (3.97)	0.26	0.85	-0.56	0.20	-0.05	0.41	-0.63	**0.03**	0.23	0.75	1.51	**0.93**	0.49	0.82	-0.42	0.28
205 d	48.57 (4.49)	0.00	0.51	0.11	0.61	0.02	0.52	-0.08	0.42	-0.46	0.09	-0.33	0.25	-0.47	0.19	0.31	0.65

[1] The numbers 1 and 2 refers to *PLIN1* and *PLIN2*, respectively, with the additive effects expressed as A-G; P (>0): Posterior probability of a value being positive. In bold, probabilities above 0.90 or below 0.10.

Table 5. Mean (standard deviation) and additive, dominant, and epistatic effects of *PLIN1 JN860199:g.173G>A* and *PLIN2 U461317:g.98G>A* polymorphisms associated to carcass traits.

Trait	Mean (SD)	Additive (a) and dominant (d) effects[1]								Epistatic effects[1]							
		PLIN1,g.173G>A				*PLIN2, g.98G>A*											
		a_1	P(>0)	d_1	P(>0)	a_2	P(>0)	d_2	P(>0)	a_1a_2	P(>0)	a_1d_2	P(>0)	d_1a_2	P(>0)	d_1d_2	P(>0)
Carcass weight, kg	93.69 (9.28)	-0.20	0.36	0.41	0.70	0.58	0.86	-0.95	0.11	1.09	**0.94**	0.19	0.57	-0.07	0.47	-0.50	0.38
Carcass backfat, mm	22.59 (3.68)	-0.09	0.33	0.02	0.52	-0.15	0.24	0.10	0.65	0.32	0.88	0.41	0.85	0.19	0.69	-0.21	0.36
Carcass loin, mm	45.25 (7.23)	0.23	0.69	-0.19	0.39	0.28	0.73	-0.52	0.22	0.58	0.83	0.69	0.78	-0.74	0.22	-0.70	0.31
Carcass lean, %	43.77 (4.96)	0.08	0.62	-0.01	0.50	0.23	0.80	-0.47	0.11	-0.17	0.32	-0.20	0.36	-0.14	0.41	0.20	0.59
Carcass length, cm	86.58 (2.96)	-0.62	**0.04**	0.81	**>0.99**	0.42	**0.99**	-0.82	**<0.01**	0.92	**0.98**	-0.22	0.24	-0.45	0.11	-0.14	0.39
Lean weight, kg	40.73 (5.29)	0.07	0.85	0.19	**0.98**	0.41	**>0.99**	-0.72	**<0.01**	0.30	**>0.99**	-0.11	0.20	-0.37	**<0.01**	-0.06	0.38
Ham weight, kg	12.09 (1.16)	0.00	0.51	-0.04	0.34	0.10	**0.94**	-0.05	0.28	0.09	0.86	0.20	**0.95**	-0.04	0.39	-0.10	0.28

[1] The numbers 1 and 2 refers to *PLIN1* and *PLIN2*, respectively, with the additive effects expressed as A-G; P (>0): Posterior probability of a value being positive. In bold, probabilities above 0.90 or below 0.10.

Table 6. Mean (standard deviation) and additive, dominant, and epistatic effects for *PLIN1 JN860199:g.173G>A* and *PLIN2 U461317:g.98G>A* polymorphisms associated to meat quality traits

		Additive (a) and dominant (d) effects[2]								Epistatic effects[2]							
		PLIN1,g.173G>A				*PLIN2, g.98G>A*											
Trait[1]	Mean (SD)	a_1	P(>0)	d_1	P(>0)	a_2	P(>0)	d_2	P(>0)	a_1a_2	P(>0)	a_1d_2	P(>0)	d_1a_2	P(>0)	d_1d_2	P(>0)
pH24 LM	5.71 (0.25)	0.00	0.58	0.01	0.61	-0.01	0.23	0.02	0.86	-0.01	0.24	0.03	**0.90**	0.00	0.47	-0.03	0.20
pH24 SM	5.72 (0.25)	0.01	0.79	0.00	0.52	0.00	0.43	0.03	**0.92**	-0.02	0.12	0.00	0.57	0.01	0.61	-0.03	0.22
IMF, %	4.50 (1.66)	0.10	0.85	-0.07	0.32	0.04	0.67	0.06	0.67	-0.16	0.11	0.05	0.59	0.11	0.70	0.18	0.73
SFA, %	34.99 (3.68)	0.01	0.53	0.01	0.53	-0.24	**0.04**	0.07	0.66	-0.15	0.19	-0.22	0.19	-0.08	0.40	-0.08	0.41
MUFA, %	50.54 (3.11)	0.20	**0.94**	-0.05	0.40	0.30	0.99	-0.17	0.17	0.04	0.59	-0.15	0.29	-0.06	0.42	0.74	**0.98**
PUFA, %	14.47 (2.75)	-0.20	**0.06**	0.04	0.59	-0.06	0.32	0.10	0.73	0.12	0.77	0.40	**0.95**	0.15	0.71	-0.60	**0.05**
pH24 LM	5.71 (0.25)	0.00	0.58	0.01	0.61	-0.01	0.23	0.02	0.86	-0.01	0.24	0.03	**0.90**	0.00	0.47	-0.03	0.20

[1] IMF: intramuscular fat; SFA: saturated fatty acids (C14:0+C16:0+C18:0); MUFA: monounsaturated fatty acids (16:1+C18:1+C20:1); PUFA: polyunsaturated fatty acids (C18:2+C18:3+C20:2+C20:4) in muscle *gluteus medius*

[2] The numbers 1 and 2 refers to *PLIN1* and *PLIN2*, respectively, with the additive effects expressed as A-G; P (>0): Posterior probability of a value being positive. In bold, probabilities above 0.90 or below 0.10.

Supplementary information

Table S1. Primers used for single nucleotide polymorphism discovery in *PLIN1* gene.

Primer	Sequence (5'-3')	Gene regions	Product size (bp)	Ta [1]
P1	F GTCAAATAACCATAGCAACCAAC R ATTCCCAGAAGACCCTAACC	partial promoter; exon 1, partial Intron 1	253	61
P2	F AGGGAACTGATGGTGAGAGG R TCCGCAAGAAGGAGTGAGG	partial intron 1; exon 2, partial intron 2	306	60
P3	F AGAGCCAAGGTTGTGACCAG R CAGGCAGTGAACGAGCAAG	partial intron 2; exon 3, partial intron 3	415	61
P4	F ATCTGCACGCCTGACTCC R TGGTGGCCTCTTGGTAATTC	partial intron 4; exon 5; partial intron 5	375	60
P5	F CGGGATGACCACTTTCTAACC R GCTCAGGGCAGACACTCAC	partial intron 5; exon 6	289	60
P6	F AGGTGCTGTGAAGTCAGTGG R TGTTCCAGGGTGAGGTGAAG	partial intron 6; exon 7; partial intron 7	368	61
P7	F GGATAGTGAGGAGGGGAAGG R CAGGAGACTGGGGAAGGAG	partial intron 7; exon 8; 3'downstream genomic region	431	63

[1] Annealing temperature

435 **Table S2.** Quantitative trait loci (QTL) co-localizing with the porcine *PLIN2* mapping
436 position[1].

QTL trait	QTL (cM)	Reference [2]
PLIN2 (SSC1q2.3-2.7; 227.3 Mb on SSC assembly 10.2)		
Abdominal fat	107.6	Geldermann *et al.* (2010)
Adipocyte diameter	94.3-122.6	Geldermann *et al.* (2003)
Average daily gain	3.0-140.5	Liu *et al.* (2007)
Average daily gain	42.36-134.76	Onteru *et al.* (2013)
Average daily gain	49.4-79.4	Rückert & Bennewitz (2010)
Average daily gain	73-140.5	Harmegnies *et al.* (2006)
Average daily gain	100.8-118.5	Mohrmann *et al.* (2006)
Average daily gain	127.1-140.5	Evans *et al.* (2003)
Backfat thickness	80.0-110.5	Liu *et al.* (2007)
Body weight at birth	16.4-132	Guo *et al* (2008)
Daily feed intake	78.7-79.4	Kim *et al.* (2000)
Ham weight	94.3-122.6	Geldermann *et al.* (2003)
Lean meat percentage	94.3-122.6	Geldermann *et al.* (2003)
pH48 hours post mortem (loin)	102.9-119.5	Thomsen *et al.* (2004)

437

438 [1] Source: animal genome gbrowse (http://www.animalgenome.org/cgi-
439 bin/gbrowse/pig/), accessed on 22-11-2014.

440

441 [2] References
442
443 Evans G.J., Giuffra E., Sanchez A., Kerje S., Davalos G., Vidal O., Illán S., Noguera
444 J.L., Varona L., Velander I., Southwood O.I., de Koning D.J., Haley C.S., Plastow
445 G.S.,Andersson L. (2003). Identification of quantitative trait loci for production traits
446 in commercial pig populations. *Genetics*. **164**, 621-627.
447
448 Geldermann H., Cepica S., Stratil A., Bartenschlager H., Preuss S. (2010). Genome-
449 wide mapping of quantitative trait loci for fatness, fat cell characteristics and fat
450 metabolism in three porcine F2 crosses. *Genet Sel Evol*. **28**, 31-42.
451
452 Geldermann H., Müller E., Moser G., Reiner G., Bartenschlager H., Cepica S., Stratil
453 A., Kuryl J., Moran C., Davoli R., Brunsch C. (2003). Genome-wide linkage and QTL
454 mapping in porcine F2 families generated from Pietrain, Meishan and Wild Boar
455 crosses. *J. Anim Breed Genet*. **164**, 621-627.

Guo Y.M., Lee G.J., Archibald A.L., Haley C.S. (2008). Quantitative trait loci for production traits in pigs: A combined analysis of two Meishan x Large White populations. *Anim. Genet*. **39**, 486-495.

Harmegnies N., Davin F., De Smet F., Buys N., Georges M., Coppieters W. (2006). Results of a whole-genome quantitative trait locus scan for growth, carcass composition and meat quality in a porcine four-way cross. *Animal genetics*. **37**, 543-553

Kim K.S., Larsen N., Short T., Plastow G., Rothschild M.F. (2000). A missense variant of the porcine melanocortin-4 receptor (MC4R) gene is associated with fatness, growth, and feed intake traits. *Mamm. Genome*. **11**, 131-135.

Liu G., Jennen D.G., Tholen E., Juengst H., Kleinwachter T., Holker M. (2007). A genome scan reveals QTL for growth, fatness, leanness and meat quality in a duroc-pietrain resource population. *Anim. Genet*. **38**, 241-252.

Mohrmann M., Roehe R., Knap P.W., Looft H., Plastow G.S., Kalm E (2006). Quantitative trait loci associated with AutoFOM grading characteristics, carcass cuts and chemical body composition during growth of Sus scrofa. *Anim. Genet*. **37**, 435-443.

Onteru S.K., Gorbach D.M., Young J.M., Garrick D. J., Dekkers J.C. M., Rothschild M.F. (2013). Whole Genome Association Studies of Residual Feed Intake and Related Traits in the Pig. *PloS one*. **8**, 1756-1773.

Rückert C., Bennewitz J. (2010). Joint QTL analysis of three connected F2-crosses in pigs. *Genetics, selection, evolution* **42**, 40-48.

Thomsen H., Lee H.K., Rothschild M.F., Malek M., Dekkers J.C. (2004). Characterization of quantitative trait loci for growth and meat quality in a cross between commercial breeds of swine. *J. Anim. Sci*. **82**, 2213-2228.