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

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1 Title page: Perilipin genes and production traits in pigs

2

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

5

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19 **Summary**

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

36

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

38 **Introduction**

39 Growth rate and carcass lean content are crucial characteristics for the economic
40 viability of pork production. Selection emphasizing lean content has led to reduce some
41 pork quality attributes, including the intramuscular fat (**IMF**) content. The use of
42 molecular markers may be useful to improve the genetic progress in traits that are
43 difficult and expensive to measure (Dekkers 2004), but also to break down unfavorable
44 genetic correlations between antagonistic traits, such as those between lean growth rate
45 or carcass lean content and IMF content (Ros-Freixedes *et al.* 2012; Ros-Freixedes *et al.*
46 2013). In this scenario, performing association studies with candidate genes related to
47 proteins affecting fat metabolism is of particular interest.

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

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

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

69

70

71 **Materials and methods**

72 *Animals, traits and sample collection*

73 A panel of 20 unrelated pigs from three Italian heavy breeds was used for the
74 SNP screening of *PLIN1* gene, including eight Italian Large White, four Italian Duroc
75 and eight Italian Landrace pigs. A total of 607 Duroc barrows from 88 sires and 348
76 dams were used for the association analyses. These pigs were randomly sampled in seven
77 batches from the same commercial line and performance-tested from 75 d to 210 d of
78 age under commercial conditions (Ros-Freixedes *et al.* 2012). During the test period they
79 had *ad libitum* access to commercial diets. A complete description of the line and of the
80 procedures followed for testing and sample collection is given in Ros-Freixedes *et al.*
81 (2012). The traits recorded included live body weight (BW), backfat thickness, and loin
82 thickness at 120, 180, and 205 d. Backfat and loin thickness was ultrasonically measured
83 at 5 cm off the midline at the position of the last rib (Piglog 105, Herlev, Denmark).
84 After slaughter at 210 days, the carcass weight and length, the carcass backfat and loin
85 thickness, and the ham weight were measured. Carcass backfat and loin thickness at 6
86 cm off the midline between the third and fourth last ribs, together with the carcass lean
87 percentage, were estimated using an on-line ultrasound automatic scanner (AutoFOM,

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

93

94 *Single nucleotide polymorphism discovery and genotyping*

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

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

108 The *JN860199:g.173G>A PLIN1* SNP polymorphism, which was selected for
109 subsequent analyses, was genotyped by PCR-restriction fragment length polymorphism
110 assay. PCR products obtained with the “P2” primer set (**Table S1**) were digested with
111 *Hin1*II (Fermentas, Vilnius, Lithuania) and the resulting products were resolved on
112 polyacrylamide gels. For *PLIN2*, the *GU461317:g.98G>A* SNP, in the 3' UTR region

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

117 The *JN860199:g.173G>A PLIN1* SNP was genotyped by PCR-restriction fragment
118 length polymorphism assay by restricting the “P2” PCR product (**Table S1**) with *Hin1II*
119 (Fermentas, Vilnius, Lithuania). For *PLIN2*, the *GU461317:g.98G>A* SNP was
120 genotyped by High Resolution Melting PCR in a Rotor-Gene™ 6000 (Corbett Research,
121 Mortlake, New South Wales, Australia) following the protocol described in Davoli *et al.*
122 (2011).

123

124 *Association analysis*

125 The additive, dominant, and epistatic effects of the *PLIN* genotypes were
126 estimated independently for each trait using a Bayesian setting, in line with the
127 methodology described in Ros-Freixedes *et al.* (2012). A two-generation pedigree was
128 used for the analyses. In matrix notation, the model used for the i th trait was $\mathbf{y}_i = \mathbf{X}_i\mathbf{b}_i +$
129 $\mathbf{Z}_i\mathbf{a}_i + \mathbf{e}_i$, 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
130 systematic, polygenic, and residual effects, respectively; and \mathbf{X}_i and \mathbf{Z}_i the known
131 incidence matrices that relate \mathbf{b}_i and \mathbf{a}_i with \mathbf{y}_i , respectively. The systematic effects were
132 the batch (7 levels), the age at test as a covariate, and orthogonal coefficients for additive
133 (a), dominance deviation (d) and first-order epistatic effects (aa: additive \times additive; ad:
134 additive \times dominance; da: dominance \times additive; and dd: dominance \times dominance) for
135 *PLIN1* and *PLIN2* SNPs. Pigs in a given batch were contemporaneous pigs tested at the
136 same unit and slaughtered in the same abattoir. The litter effect was not included
137 because, on average, there were less than 2 piglets per litter. The orthogonal coefficients

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

140 The models were solved using Gibbs sampling with the TM software (Legarra *et*
141 *al.* 2008). The traits were assumed to be conditionally normally distributed as
142 $[y_i | \mathbf{b}_i, \mathbf{a}_i, \mathbf{I}\sigma_{ei}^2] \sim N(\mathbf{X}\mathbf{b}_i + \mathbf{Z}\mathbf{a}_i, \mathbf{I}\sigma_{ei}^2)$, where σ_{ei}^2 is the residual variance and \mathbf{I} the
143 appropriate identity matrix. The animal effects conditionally on the additive genetic
144 variance σ_{ai}^2 were assumed multivariate normally distributed with mean zero and
145 variance $\mathbf{A}\sigma_{ai}^2$, where \mathbf{A} was the numerator relationship matrix. The matrix \mathbf{A} was
146 calculated using 1043 animals in the pedigree. Flat priors were used for \mathbf{b}_i while the
147 variance components were set to the values obtained by Ros-Freixedes *et al.* (2013) with
148 data and pedigree from 1996 onwards. Statistical inferences were derived from the
149 samples of the marginal posterior distribution using a unique chain of 500,000 iterations,
150 where the first 100,000 were discarded and one sample out of 100 iterations retained.
151 The additive, dominance, and epistatic effects were assessed by calculating both the
152 probability of each of these components being greater or lower than zero and their
153 highest posterior density interval at 95% of probability (HPD95). Statistics of marginal
154 posterior distributions and the convergence diagnostics were obtained using the BOA
155 package (Smith 2005). Convergence was tested using the Z-criterion of Geweke
156 (Geweke 1992) and visual inspection of convergence plots.

157

158

159 **Results and discussion**

160 *Polymorphisms and sequence variation of PLIN genes*

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

163 in the exons 1, 2, 5, and 8 (data not shown) and five SNP in intronic regions. Seven
164 genomic regions, covering the positions of the ten putative SNP, were subjected to direct
165 sequencing in 20 animals from three Italian heavy pig breeds. A total of 2,437 bp of the
166 pig *PLINI* gene were screened, which covered 1,126 bp of the coding sequence, the
167 complete 183-bp 5' UTR, and 1,128 bp of intronic regions and part of the promoter and
168 3' downstream genomic region, according to the annotation of the Ensembl entry
169 [ENSSSCG00000001844]. The sequencing covered the positions of the putative SNPs
170 detected *in silico*, with the exception of the SNP on exon 8, which was not analyzed due
171 to the unsuccessful amplification of this region. Four SNPs (two intronic and two exonic)
172 out of the ten SNPs discovered *in silico* were detected by sequencing Italian heavy pig
173 breeds (Table 1). The other six polymorphisms identified *in silico* were not detected
174 during the sequencing. The two intronic SNPs were novel and the sequences were
175 reported to GenBank [JN860199; SNP *g.173G>A* and *g.3484C>G*], while the two
176 exonic SNPs, which were detected in our *in silico* analysis, were both synonymous and
177 had been reported before (GenBank: AM931171; SNP *g.4119A>G* and *g.7966T>C*;
178 Vykoukalová *et al.* 2009). The four SNP were in complete linkage disequilibrium in the
179 initial panel of 20 pigs. The intronic *JN860199:g.173G>A* SNP was selected for
180 subsequent analyses because a restriction enzyme was available to analyze this mutation.

181 To assess the association of these mutations with productive parameters, the
182 *PLIN1 JN860199:g.173G>A* and *PLIN2 GU461317:g.98G>A* SNPs were genotyped in
183 a population of 607 Duroc pigs, which had data available on performance, fattening and
184 meat quality traits (Ros-Freixedes *et al.* 2012). The allele frequencies and the distribution
185 genotypes for *PLIN1* and *PLIN2* SNPs are reported in **Table 2**. In both SNPs the alleles
186 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 \times additive effect for BW at 120 d (-0.88 kg, with

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

213 The additive, dominant, and epistatic effects of *PLIN1* *g.173G>A* and *PLIN2*
214 *g.98G>A* SNPs associated to backfat and loin thickness at 120 d, 180 d and 205 d of age
215 are given in **Table 4**. The *PLIN1* *g.173G>A* SNP did not show a clear pattern of
216 association with fatness traits, but results for the *PLIN2* *g.98G>A* SNP indicated that A
217 allele is positively associated to backfat thickness at early ages (+0.17 mm and +0.19
218 mm, at 120 d and at 180 d, respectively, with a probability of being positive of 91% and
219 98%) and negatively to backfat thickness at 205 d (-0.22 mm, with a probability of being
220 positive of 10%). The effect of the *PLIN2* *g.98G>A* SNP on backfat thickness followed
221 a similar pattern as for ADG, with the positive effect of allele A at 120 d vanishing at
222 later ages.

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

236 No evidence was found indicating that meat quality traits (pH and IMF) were
237 additive by *PLIN1* and *PLIN2* SNP, although some minor changes were observed for
238 IMF fatty acid composition (**Table 6**). In particular, allele A at *PLIN1* decreased PUFA
239 (-0.20%) and increased MUFA (0.20%) while allele A at *PLIN2* decreased SFA (-
240 0.24%). Evidence supporting the existence of dominant and epistatic effects associated
241 to carcass and meat quality traits was mostly circumscribed to traits where the additive
242 effects were more evident (carcass length and carcass lean weight), thereby suggesting
243 that the mode of action of *PLIN1* and *PLIN2* on the traits that they are influencing is
244 subjected to complex regulations. As for BW and ADG, the dominant effect associated
245 to lean weight was negative in *PLIN1* (-0.19 kg, with a probability of 2% of being
246 positive) but positive in *PLIN2* (0.41 kg, with 99.9% probability of being positive).
247 These dominant values were around two-fold higher than their respective additives, a
248 result which supports for an underdominant *PLIN1* and overdominant *PLIN2* gene action
249 for lean weight. To assess the stability of the estimates to model over-parameterization,
250 the additive and dominance effects were also estimated ignoring the epistatic effects.
251 The estimates obtained (results not shown), although slightly higher, were in line with
252 those reported with the model that included epistasis, thereby confirming the favourable
253 effects of allele G at *PLIN1* and allele A in *PLIN2* on growth and carcass traits.

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

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

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

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

291

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297

298

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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
<i>JN860199 g.173G>A</i>	1,428	Intron 2	-
<i>JN860199 g.3484C>G</i>	4,739	Intron 2	-
<i>AM931171g.4119A>G</i>	4,856	Exon 3	Synonymous ³
<i>AM931171g.7966T>C</i>	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

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

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		<i>PLIN1</i> (JN860199:g.173G>A)				<i>PLIN2</i> (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

400 **Table 3.** Mean (standard deviation) and additive, dominant, and epistatic effects of *PLIN1* *JN860199:g.173G>A* and *PLIN2*
 401 *GU461317:g.98G>A* polymorphisms associated to live body weight and growth rate at different ages
 402

Trait	Mean (SD)	Additive (a) and dominant (d) effects ¹								Epistatic effects ¹							
		<i>PLIN1, g.173G>A</i>				<i>PLIN2, g.98G>A</i>											
		a ₁	P(>0)	d ₁	P(>0)	a ₂	P(>0)	d ₂	P(>0)	a ₁ a ₂	P(>0)	a ₁ d ₂	P(>0)	d ₁ a ₂	P(>0)	d ₁ d 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

403 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
 404 value being positive. In bold, probabilities above 0.90 or below 0.10.
 405
 406

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

409

Trait	Mean (SD)	Additive (a) and dominant (d) effects ¹								Epistatic effects ¹							
		<i>PLIN1,g.173G>A</i>				<i>PLIN2, g.98G>A</i>											
		a ₁	P(>0)	d ₁	P(>0)	a ₂	P(>0)	d ₂	P(>0)	a ₁ a ₂	P(>0)	a ₁ d ₂	P(>0)	d ₁ a ₂	P(>0)	d ₁ d 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

410

411

412 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
 413 value being positive. In bold, probabilities above 0.90 or below 0.10.

414

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

417

Trait	Mean (SD)	Additive (a) and dominant (d) effects ¹								Epistatic effects ¹							
		<i>PLIN1</i> ,g.173G>A				<i>PLIN2</i> , g.98G>A											
		a ₁	P(>0)	d ₁	P(>0)	a ₂	P(>0)	d ₂	P(>0)	a ₁ a ₂	P(>0)	a ₁ d ₂	P(>0)	d ₁ a ₂	P(>0)	d ₁ d 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

418

419 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
 420 value being positive. In bold, probabilities above 0.90 or below 0.10.

421

422

423 **Table 6.** Mean (standard deviation) and additive, dominant, and epistatic effects for *PLIN1 JN860199:g.173G>A* and *PLIN2*
 424 *U461317:g.98G>A* polymorphisms associated to meat quality traits
 425

Trait ¹	Mean (SD)	Additive (a) and dominant (d) effects ²								Epistatic effects ²							
		<i>PLIN1,g.173G>A</i>				<i>PLIN2, g.98G>A</i>											
		a ₁	P(>0)	d ₁	P(>0)	a ₂	P(>0)	d ₂	P(>0)	a ₁ a ₂	P(>0)	a ₁ d ₂	P(>0)	d ₁ a ₂	P(>0)	d ₁ d 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

426 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:
 427 polyunsaturated fatty acids (C18:2+C18:3+C20:2+C20:4) in muscle *gluteus medius*

428 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
 429 value being positive. In bold, probabilities above 0.90 or below 0.10.

430 **Supplementary information**

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

432

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 AGGGAAGTGTGGTGAGAGG 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

433

434 ¹ Annealing temperature

435 **Table S2.** Quantitative trait loci (QTL) co-localizing with the porcine *PLIN2* mapping
 436 position¹.

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

437

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

440

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