Títol: Pig *malic enzyme 1* (*ME1*) genotype is associated with backfat thickness and meat quality traits.

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Pig *malic enzyme 1* (*ME1*) genotype is associated with backfat thickness and meat quality traits.[1]

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Abstract

The pig *malic enzyme 1* gene is a candidate for explaining genetic variation at fatness and meat quality traits. In this way, malic enzyme 1 (ME1) provides the NADPH and the acetyl-CoA required in the fatty acid biosynthesis. Moreover, the gene maps on the proximal end of chromosome 1, where a QTL affecting fat deposition has been described.

We have amplified two fragments of 1,457 bp and 1,459 bp corresponding to the complete coding region and the 3' untranslated region (3' UTR) of the pig *ME1* gene. Sequencing of these two fragments in pigs from three different breeds (Landrace, Large White and Piétrain) revealed the existence of five single nucleotide polymorphisms (SNP) in the 3' untranslated region: SNP1 ($C \rightarrow T_{1706}$), SNP2 ($G \rightarrow T_{1762}$), SNP3 ($A \rightarrow C_{1807}$), SNP4 ($C \rightarrow A_{1857}$) and SNP5 ($T \rightarrow A_{1880}$). The genotyping of a two generation pedigree of a selected Landrace population, revealed the existence of three haplotypes: **H1** (C_{1706} G_{1762} A_{1807} C_{1857} A_{1880}), **H2** (C_{1706} G_{1762} A_{1807} C_{1857} T_{1880}) and **H3** (T_{1706} T_{1762} C_{1807} A_{1857} T_{1880}).

Association analyses between *ME1* genotypes and carcass and meat quality traits in a Landrace population showed associations with backfat thickness at 156 d* and 171 d**, fat-o-meter measurement of the loin area*, left cutlet weight*, muscular pH*,** and electric conductivity*, lactate dehydrogenase activity*, organic matter content*, pigmentation* and meat color* and texture* (the null difference between genotypes was located outside the Higher Posterior Densities at 90%* and 95%**).

Keywords: pig malic enzyme 1, fatty acid metabolism, carcass and meat quality traits

Introduction

Malic enzyme (ME) is a ubiquitous tetrameric protein that catalyses the reversible oxidative decarboxylation of L-malate to pyruvate. This reaction, which links the glycolytic pathway and the citric acid cycle, involves the reduction of $NAD(P)^+$ to NAD(P)H. In mammals, two mitochondrial ($NADP^+$ and $NAD(P)^+$ dependent) and one cytosolic ($NADP^+$ dependent) ME isoforms have been identified (Chang and Tong, 2003). Cytosolic ME (ME1) forms part of the tricarboxylate shuttle, which releases acetyl-CoA from the mitochondria to the cytosol. The NADPH and the acetyl-CoA produced in this way might be used in the fatty acid biosynthesis and many other metabolic processes. (Voet and Voet, 1992)

The comparison of the activities of the glucose-6-phosphate dehydrogenase, acetyl-CoA-carboxylase and the ME lipogenic enzymes in the intramuscular tissue of Meishan and growing Large White pigs revealed that ME activity is much higher in the former breed, being one of the major factors influencing intramuscular fat deposition (Mourot and Kouba, 1999).

In the pig, the *ME1* locus has been mapped to chromosome 1 and two transcripts forms have been described, whereas gene structure and polymorphism remain to be characterized (Nunes et al., 1996). Several QTL affecting growth, backfat and other production traits have been positioned on this chromosome. Most of these studies detect a single QTL (Milan et al., 2002; Paszek et al., 1999; Rohrer and Keele, 1998), but the existence of more than one QTL can not be ruled out (Quintanilla et al., 2002; Rohrer, 2000).

The main goal of the current work was to identify new mutations in the coding sequence and the 3'UTR region of the *ME1* gene which might be used as genetic

markers in an association study with several production and meat quality traits recorded in a highly selected Landrace population.

Materials and Methods

Animal material and phenotypical traits

Four hundred and seventy F_1 individuals of a non-inbred maternal Landrace line were obtained by crossing 71 F_0 sows and five F_0 boars. An average of 94 offspring was obtained from each male, being reared under normal intensive conditions at the experimental farm of Nova Genètica. All the animals were typed for the *Ryr1* gene according to Fujii et al. (1991).

During the growth period, weight (W) and backfat thickness (BF) were recorded at 156, 171 and 178 days of age (**W156**, **W171**, **W178** and **BF156**, **BF171**, **BF178**). Pigs were slaughtered at 179 days of age, and the following carcass traits were registered: carcass weight (**CW**) and carcass length (**CL**), as well as the Fat-o-Meter measurement of the carcass (**CaF**) and the loin area (**LoF**), fat thickness in the cervical region (**CeF**) and between the 3rd and 4th ribs (**RiF**), and weight of the left and right hams (**lHW**, **rHW**), shoulders (**lSW**, **rSW**), cutlets (**lCuW**, **rCuW**), ribs (**lRW**, **rRW**) and bacons (**lBW**, **rBW**). Electric conductivity (CE) and pH, measured respectively with a PQM and a Scharlau portable meter equipped with a xerolyt electrode, were both recorded at the *longissimus dorsi* (LD) and the *semimembranosus* (SW) muscles at 45 min and 24 h, yielding eight registers for each individual (**PH45SM**, **PH45LD**, **CE45SM**, **CE45LD**, **PH24SM**, **PH24LD**, **CE24SM**, **CE24LD**).

Chemical composition of the muscle was measured in a *semimembranosus* muscle sample after registering pH (**PHCOMP**) and electric conductivity (**CECOMP**). Samples were homogenized and lyophilized, and subsequently percentage of fat (**FC**), crude protein (**CP**), organic matter (**OM**) and dry matter (**DM**) content were determined (AOAC, 1990).

Mechanical characteristics influencing meat texture were measured with a Texture Analyser TA.TX2 (Stable Micro Systems, Godalming, UK) according to the Warner-Bratzler test (Moller, 1981). Samples from the *longissimus dorsi* muscle were cooked in a water bath at 80 ºC for 1 hour and subsequently they were cooled at room temperature. Six pieces of 2 x 1 x 1 cm were cut following the direction of the muscular fibers and several meat texture traits were recorded. These included shear force (**SF**), which is related with the miofibrillar components of the muscle; maximum shear force (**MSF**), mostly affected by the proportion of connective tissue; total work required to shear the sample (**TWS**); and shear firmness (**SFi**).

Color measurements in the CIELAB space were quantified with a Minolta spectrophotometer at 24 hours post mortem (CIE, 1976). Lightness (**L**), red tendency (**a**), and yellow tendency (**b**) were recorded by duplicate (**L2**, **a2** and **b2**).

Biochemical analyses related with muscle fiber composition and enzymatic activities were performed by using samples of the *longissimus dorsi* core at the last-rib level, which were frozen in liquid nitrogen and stored at -80ºC until they were analyzed. The metabolic profile was determined by measuring the enzymatic activities of the lactate dehydrogenase (**LDH**), in µmol NADH min^{-1}.muscle g^{-1} (Ansay, 1974) and isocitrate dehydrogenase (**ICDH**), in nmol NADPH min^{-1}.muscle g^{-1} (Briand et al., 1981). Quantification of the slow myosin heavy chain (**MHC1**), which

121 is a marker for type I fibers content, was performed with a specific MHC-I monoclonal
122 antibody by the ELISA technique (Picard et al., 1994).

123 Muscle pigmentation (**PIG**) was measured by quantifying the concentration of
124 haem pigment (Hornsey, 1956) expressed in µg of haematin.g^{-1} of muscle and
125 corresponding to *longissimus dorsi* samples which were vacuum-packed and stored
126 at -20ºC until analysis.

127

128 *Identification of polymorphisms in the ME1 cDNA sequence*

129 Total RNA was obtained from the liver samples of twelve pigs belonging to
130 three different breeds (Landrace, Large White and Piétrain). Samples were collected
131 at slaughter and frozen with liquid nitrogen at -196 ºC. They were conserved at -80
132 ºC until processing. Total RNA extraction was performed with the TRIzol reagent
133 (GibcoBRL, Life Technologies, Prat del Llobregat, Spain) as previously described
134 (Amills et al., 2003). Subsequently, RNA was reverse transcribed to cDNA by using
135 the *Thermoscript RT-PCR System* (Invitrogen, Prat del Llobregat, Spain).

136 The *ME1* cDNA was amplified with two set of primers that were designed
137 according to the porcine sequence with GenBank accession no X93016. The first set
138 of primers (ME1.1F 5'- CCA CCT TGC TTC ATC AGT CA -3' and ME1.1R 5'- ATC
139 TGA GAG CGG ACA AAT GC -3') amplified 1,459 bp of the coding region, while the
140 second set (ME1.2F 5'- CCT GAA CCC TCA AAC AAG GA – 3' and ME1.2R 5'- AAG
141 CAT TCT GGA TCT CTT CAA AA -3') amplified 1,457 bp, including the 3' end of the
142 coding region and 1354 bp of the 3' UTR. Amplification reactions were carried out
143 with 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.2 µM of each primer, 1 µl of the cDNA reaction
144 and 0.5 U Taq DNA polymerase in a final 50 µl volume (Ecogen S.R.L, Barcelona
145 08041, Spain). The thermal profile for amplifying the 1,459 bp target was 94 ºC for 5

146 min and 35 cycles of 94 ºC for 1 min, 60 ºC for 1 min and 72 ºC for 1 min; whereas a
147 thermal profile of 94 ºC for 5 min and 35 cycles of 94 ºC for 1 min, 57 ºC for 1 min
148 and 72 ºC for 1 min was used for amplifying the 1,457 bp fragment.

149 The amplified products were sequenced forward and reverse with the Big
150 Dye™ Terminator V3.0 Cycle Sequencing Ready Reaction kit (Applied Biosystems,
151 Warrington, UK)

152

153 *Extraction of genomic DNA and genotyping*

154 Full blood samples were used as a source to isolate pig genomic DNA. Four
155 hundred µl samples were repeatedly washed with 500 µl TE (Tris-HCl 10 mM pH = 8,
156 EDTA 1 mM) and centrifuged at 13,000 g until a white cell pellet was obtained.
157 Peripheral blood mononuclear cells were resuspended in 400 µl buffer K (KCl 50
158 mM, Tris-HCl 10 mM, 0.5% Tween 20) and 40 µg proteinase K, and this mixture was
159 incubated at 56 ºC for five hours. Purification of genomic DNA was carried out by
160 phenol-clorophorm extraction and ethanol precipitation with 25 µl NaCl 2 M and 800
161 µl ice-cold absolute ethanol, followed by a 10 min centrifugation step. The DNA pellet
162 was washed with ethanol 70% and resuspended in 100 µl TE.

163 Genotyping was performed by using the *SnaPshot™ ddNTP Primer Extensión*
164 *kit* (Applied Biosystems, Warrington, UK). Amplification reactions with primers
165 ME1.2F and ME1.2R were carried out in an ABI PRISM 877 Integrated Thermal
166 Cycler (Applied Biosystems, Warrington, UK). The PCR products were subsequently
167 purified with the *ExoSAP-IT kit* (Amersham Biosciences Europe GMBH, Cerdanyola,
168 Spain). Extension reactions were performed in a multiplex format with primers
169 pSNP1: 5' –ATA GCC TAC ATT TCT AAC TCC A- 3', pSNP2: 5' –TTT AAA TAT
170 TGG GAT CTT TTA TAA TGA- 3', pSNP3: 5' –TAA TTG ATA ATT TCC CCT TAA

CAC TCT AAA- 3', pSNP4: 5' –TAA TAA TAT AAT TAG GGT AAA CAT CAC AGT AGA CA- 3' and pSNP5: 5' –ATA TAT ATA TAT AAA TTA TTT TGC TTC ATT TAC TTT CTT G- 3'. We added polidAT tails to the 5' end of the extension primers to make possible the simultaneous electrophoretic analysis of the five amplicons in a single run of the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Warrington, UK).

Statistical analysis

Statistical analysis has been performed for single SNPs and for haplotypes. The assumed model for the phenotypic data of each trait is:

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Z}_1\mathbf{u} + \mathbf{Z}_2\mathbf{p} + \mathbf{e}$$

where **ß** are the systematic effects (sex, *Ryr1* genotype and *ME1* genotype or haplotype), **u** is the vector of additive genetic effects, **p** are the litter effects and **e** is the residual vector, $\mathbf{X}$, $\mathbf{Z_1}$, $\mathbf{Z_2}$, are the incidence matrix that links phenotypic data with systematic, genetic and permanent environmental effects.

The likelihood of data is the following multivariate normal distribution.

$$f\left(\mathbf{y}|\beta,\mathbf{u},\mathbf{p},\sigma_e^2\right) = \mathrm{MVN}\left(\mathbf{X}\beta + \mathbf{Z}\mathbf{u} + \mathbf{Z}\mathbf{p}, \mathbf{I}\sigma_e^2\right)$$

Prior distribution of **β** were assumed flat between a range of possible values to ensure property of the posterior distribution. Prior distribution of the additive (**u**) and litter effects (**p**) are the following multivariate normal distributions:

$$f\left(\mathbf{u}|\sigma_u^2\right) = \mathrm{MVN}\left(0, \mathbf{A}\sigma_u^2\right)$$

$$f\left(p|\sigma_u^2\right) = \mathrm{MVN}\left(0, \mathbf{I}\sigma_p^2\right)$$

where σ_u^2, σ_p^2, σ_e^2 are the additive, litter and residual variance respectively

194 Prior distribution for σ_u^2, σ_p^2 and σ_e^2 were assumed flat between a range of
195 possible values.

196 Bayesian analysis were carried with the Gibbs Sampler algorithm (Geman and
197 Geman, 1984; Gelfand and Smith, 1990; Tanner, 1993) to obtain autocorrelated
198 samples from the joint posterior density and subsequently from the marginal posterior
199 densities of all the unknowns in the model. Specifics on distributions involved can
200 also be found in previous studies (Wang et al., 1993; 1994).

201 The posterior conditional distributions for the locations parameters (sex, *Ryr1*
202 and *ME1* configuration effects) were univariate normal distributions, the posterior
203 distributions of the variance components were inverted chi-squares.

204 The Gibbs sampler analysis was carried out for each analysis through a
205 simple chain of 100,000 iterations, after discarding the first 5,000. The analysis of
206 convergence was calculated using the algorithms of Raftery and Lewis (1992) and
207 García-Cortes et al. (1998). All iterations of the analysis were used to compute
208 posterior means and standard deviations so that all the available information from the
209 output of the Gibbs sampler could be considered.

210

211 **Results and Discussion**

212

213 We have sequenced 2,915 bp including the coding region and most of the 3'
214 UTR region of the *ME1* gene in twelve pigs of three different breeds. Five
215 polymorphisms have been detected in the 3' UTR region. These single nucleotide
216 polymorphisms (SNP) have been named SNP1 ($C \rightarrow T_{1706}$), SNP2 ($G \rightarrow T_{1762}$), SNP3
217 ($A \rightarrow C_{1807}$), SNP4 ($C \rightarrow A_{1857}$) and SNP5 ($T \rightarrow A_{1880}$). These positions are numbered
218 according the porcine sequence with GenBank accession no X93016. Allelic

frequencies of the *ME1* polymorphisms in the Landrace, Large White and Piétrain breeds were calculated by genotyping twenty animals of each breed (see Table 1). Polymorphisms SNP1, SNP2, SNP3 and SNP4 had the same allelic frequencies, a feature that suggested that they might present linkage disequilibrium in each of the three breeds. Genotyping of a two generation Landrace pedigree confirmed this hypothesis, and allowed the identification of two different alleles: C (C_{1706} G_{1762} A_{1807} C_{1857}) and T (T_{1706} T_{1762} C_{1807} A_{1857}). Single nucleotide polymorphism 5 segregated independently from the remaining polymorphisms, a feature that suggests that it might have emerged more recently on an evolutionary scale. Three haplotypes were segregating in this population, being named as **H1** (C_{1706} G_{1762} A_{1807} C_{1857} A_{1880}), **H2** (C_{1706} G_{1762} A_{1807} C_{1857} T_{1880}) and **H3** (T_{1706} T_{1762} C_{1807} A_{1857} T_{1880}).

Association analysis between ME1 SNP1, SNP2, SNP3, SNP4 and SNP5 and carcass and meat quality traits.

Results of the association statistical analysis in the 470 animals of the commercial Landrace population are indicated in Tables 2 and 3. Polymorphisms SNP1, SNP2, SNP3 and SNP4 are shown as alleles C and T.

Close associations between *ME1* genotype and backfat thickness at different ages (**BF156** and **BF171**, see Table 2) have been detected. Genotypes having a T allele have less backfat thickness. The magnitude of the differences between genotypes was age dependent and diminished when the pigs grew older. Thus, while there are significant differences between CC and TT genotypes for backfat thickness at 156 d and at 171 d, these two genotypes do not differ significantly at 178 d. This circumstance might be explained by the fact that fat deposition at the end of the growing period is more dependent on environmental factors, such as diet

composition, than on genetics. This feature might also explain the absence of significant effects in any of the other fatness measures recorded after slaughtering.

Quantitative trait loci affecting backfat thickness have been identified on chromosome 1 (Bidanel et al., 2001; Rohrer, 2000; Rohrer and Keele, 1998). However, these QTL are located distally in the chromosome whereas the pig *ME1* gene maps to p1.2. Moreover, a QTL search in this Landrace population did not reveal the existence of any significant QTL for body composition and meat quality in this region (data not shown). Interestingly, Malek et al. (2001) described a QTL for the tenthrib backfat thickness at chromosome 1, which reached its maximum significance at position 29 cM. This position coincides with the location of the *ME1* gene, a feature that is very suggestive due to the key role of ME1 in fatty acid biosynthesis. Malic enzyme activity also has a strong influence in intramuscular fat content (Mourot and Kouba, 1999). Moreover, high differences in malic enzymatic activity have been found between Landrace and Iberian pigs, two breeds which have major differences in fat deposition (Morales et al., 2002).

We have observed diverse effects of *ME1* genotypes on pH and electric conductivity (**PH45LD**, **CE45LD**, **PH24SM**, **CECOMP**, **PHCOMP**, **PH45SM**, **PH24LD** and **PHCOMP**, see Table 3). Muscle pH and CE are mainly influenced by anaerobic glycogen metabolism that produces lactate (Pearson and Young, 1989). Thus, this association is interesting because pyruvate, which is synthesized in the reaction catalyzed by ME1, can be converted to lactate, by the LDH enzyme, or to oxaloacetate, a precursor of the gluconoegenic pathway. In fact we found associations between *ME1* genotype and **LDH** when comparing CC vs. CT and CC vs. TT genotypes.

Other main effects associated with *ME1* genotype were related to meat color (**a**, **a2**, and **b2**) and pigmentation (**PIG**, see Table 3). Meat color is determined basically by the quantity of muscle pigments (mainly myoglobin) and by the oxidation state of the haemo groups, a feature that depends fundamentally on the pH kinetics. We have detected differences in pigments composition (**PIG**) with regard to *ME1* genotype at SNP5. It is conceivable that malic enzyme may have some biological effect on the total amount of haematin in muscle. However, we can not rule out that the detectable color differences among *ME1* genotypes arose from differences in the oxidation status of haemo groups, a feature that depends on muscle pH.

Association analysis of ME1 haplotypes H1, H2 and H3

Only three of the four possible *ME1* haplotypes segregated in the Landrace population. Interestingly, the association analysis between haplotypes and productive traits confirmed the results described in the previous section, but we also found several new significant associations with conformation traits such as **ICuW**, **ISW** and **rCuW** (see Table 4 and Table 5). According to our results, the H1 haplotype is often related with a lower conformation.

The five SNPs we have found are located in the 3' untranslated region (UTR) of the *ME1* gene. In consequence, they do not involve any amino acid replacement. However, polymorphisms located outside the coding region can affect mRNA half life, expression, or translatability, as previously reported for the human β globin (Bilenoglu et al., 2002) and the mice tumor necrosis factor α gene (Di Marco et al., 2001). Real time PCR quantification of *ME1* mRNA levels in liver and adipose tissue from pigs with different genotypes would be useful in order to investigate if the five 3' UTR

SNPs we have isolated have any association with the amount of *ME1* transcripts, a feature that might pave the way for the performance of functional studies.

Implications

We have characterized five SNPs and three haplotypes in the 3' untranslated region of the pig *ME1* gene. Association analysis between genotypes and growth, carcass and meat quality traits showed detectable effects on backfat thickness and pH. The involvement of ME1 in glucose and lactate metabolism and fatty acid biosynthesis makes evident the interest of characterizing with more detail the functional properties of its allelic variants.

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Table 1. Allelic frequencies of the five single nucleotide polymorphisms (SNP1 to SNP5) located in the 3' UTR of the pig *malic enzyme 1* gene (*ME1*) in three pig breeds.

Polymorphisms		**BREED**		
		Large White (N=20)	**Piétrain** (N=20)	**Landrace** (N=20)
SNP1	**C**	0.25	0.7	0.2
	T	0.75	0.3	0.8
SNP2	**G**	0.25	0.7	0.2
	T	0.75	0.3	0.8
SNP3	**A**	0.25	0.7	0.2
	C	0.75	0.3	0.8
SNP4	**C**	0.25	0.7	0.2
	A	0.75	0.3	0.8
SNP5	**T**	0.95	1	0.9
	A	0.05	0	0.1

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436 Table 2. Association between phenotypic variation of carcass traits and five single nucleotide polymorphisms (SNP) located in the 3'
437 UTR region in the pig *malic enzyme 1* gene. Standard deviations of the differences between genotypes are indicated between
438 parentheses. Single nucleotide polymorphisms 1 to 4 were linked yielding two alleles, C (C_{1706} G_{1762} A_{1807} C_{1857}) and T (T_{1706} T_{1762}
439 C_{1807} A_{1857}), whereas SNP5 segregated independently.
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Traits	**SNP 1 to 4**			**SNP 5**
	CC vs CT	**CC vs TT**	**CT vs TT**	**AT vs TT**
Weight at 156 days (kg)	0.218 (1.102)	-0.166 (2.719)	-0.384 (2.560)	0.243 (1.683)
Backfat at 156 days (mm)	0.263 (0.151)*	0.731 (0.370)*	0.468 (0.348)	0.004 (0.229)
Weight at 171 days (kg)	0.381 (1.281)	-0.310 (3.175)	-0.691 (3.001)	1.574 (1.931)
Back fat at 171 days (mm)	0.304 (0.159)*	0.969 (0.390)**	0.665 (0.367)*	0.187 (0.241)
Weight at 178 days (kg)	-0.016 (1.310)	-1.454 (3.388)	-1.438 (3.246)	1.329 (1.953)
Backfat at 178 days (mm)	0.051 (0.184)	0.588 (0.470)	0.537 (0.444)	-0.240 (0.274)
Carcass weight (kg)	0.590 (1.091)	0.563 (2.701)	-0.027 (2.562)	1.183 (1.552)
Fat-o-Meter measurement of the carcass	0.219 (0.399)	-0.290 (1.037)	-0.509 (0.992)	-0.119 (0.564)
Fat-o-Meter measurement of the loin area	-1.221 (0.729)*	-0.302 (2.008)	0.918 (1.961)	1.969 (1.034)*
Carcass length (m)	-0.144 (0.393)	-0.113 (1.031)	0.031 (0.995)	0.392 (0.585)
Fat thickness in the cervical region (mm)	0.039 (0.058)	-0.131 (0.153)	-0.170 (0.146)	-0.002 (0.086)
Fat thickness between the 3rd and 4th ribs (mm)	-0.012 (0.044)	-0.122 (0.112)	-0.110 (0.107)	0.081 (0.064)
Left ham weight(kg)	0.065 (0.141)	0.101 (0.375)	0.036 (0.362)	-0.023 (0.209)
Left shoulder weight (kg)	0.014 (0.052)	0.185 (0.134)	0.170 (0.129)	-0.002 (0.077)
Left cutlet weight (kg)	-0.086 (0.093)	-0.062 (0.243)	0.023 (0.233)	0.238 (0.138)*
Left ribs weight (kg)	-0.186 (0.138)	-0.229 (0.327)	-0.044 (0.311)	0.151 (0.213)
Left bacon weight (kg)	0.108 (0.071)	0.067 (0.169)	-0.042 (0.157)	0.038 (0.109)
Right ham weight(kg)	0.014 (0.145)	0.118 (0.382)	0.103 (0.368)	-0.012 (0.214)
Right shoulder weight (kg)	0.000 (0.052)	0.079 (0.134)	0.078 (0.129)	0.036 (0.078)
Right cutlet weight (kg)	-0.099 (0.097)	-0.201 (0.245)	-0.101 (0.233)	0.165 (0.146)
Right ribs weight (kg)	-0.152 (0.138)	0.064 (0.300)	0.216 (0.273)	0.175 (0.205)
Right bacon weight (kg)	0.014 (0.078)	-0.080 (0.179)	-0.095 (0.167)	0.004 (0.117

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453 * The null difference is not included in the Highest Posterior Density at 90%

454 ** The null difference is not included in the Highest Posterior Density at 95%

455 Table 3. Association between phenotypic variation of meat quality traits and five single nucleotide polymorphisms (SNP) located in
456 the 3' UTR region in the pig *malic enzyme 1* gene. Standard deviations of the differences between genotypes are indicated between
457 parentheses. Single nucleotide polymorphisms 1 to 4 were linked yielding two alleles, C ($C_{1706}\,G_{1762}\,A_{1807}\,C_{1857}$) and T ($T_{1706}\,T_{1762}$
458 $C_{1807}\,A_{1857}$), whereas SNP5 segregated independently.
459

Traits	SNP 1 to 4			SNP 5
	CC vs CT	CC vs TT	CT vs TT	AT vs TT
PH at the *semimembranosus*, 45' after sacrifice	0.038 (0.037)	0.061 (0.104)	0.024 (0.101)	-0.090 (0.053)*
PH at the *longissimus*, 45' after sacrifice	0.084 (0.034)**	0.009 (0.095)	-0.075 (0.093)	-0.077 (0.049)
CE at the *semimembranosus*, 45' after sacrifice	-0.089 (0.110)	0.339 (0.315)	0.428 (0.308)	-0.087 (0.159)
CE at the *longissimus*, 45' after sacrifice	0.055 (0.061)	0.287 (0.155)*	0.232 (0.148)	-0.032 (0.088)
PH at the *semimembranosus*, 24h after sacrifice	0.033 (0.021)	0.021 (0.059)	-0.011 (0.057)	-0.051 (0.031)
PH at the *longissimus*, 24h after sacrifice	0.016 (0.025)	0.031 (0.069)	0.015 (0.066)	-0.073 (0.037)*
CE at the *semimembranosus*, 24h after sacrifice	-0.087 (0.269)	-0.134 (0.856)	-0.047 (0.834)	-0.186 (0.395)
CE at the *longissimus*, 24h after sacrifice	-0.021 (0.108)	-0.043 (0.362)	-0.022 (0.355)	-0.082 (0.162)
Enzymatic activity of LDH (µmol NADH min^{-1}.muscle $g^{-1)}$	-121.93 (69.798)*	-363.80 (173.25)*	-241.87 (163.75)	44.259 (104.736)
Enzymatic activity of ICDH (nmol NADPH min^{-1}.muscle g^{-1})	-0.067 (0.051)	-0.108 (0.125)	-0.041 (0.117)	-0.001 (0.078)
Slow myosin heavy chain (%)	0.426 (0.503)	0.144 (1.280)	-0.281 (1.209)	-0.625 (0.758)
Pigmentation (µg of haematin.g of muscle $^{-1}$)	-1.129 (0.835)	-4.163 (2.185)*	-3.034 (2.093)	2.227 (1.225)*
Fat in a *semimembranosus* sample (%)	0.447 (0.373)	1.016 (0.862)	0.569 (0.808)	-0.724(0.526)
Dry matter in a *semimembranosus* sample (%)	-0.047 (0.103)	-0.058 (0.254)	-0.012 (0.242)	-0.075 (0.145)
Crude protein in a *semimembranosus* sample (%)	-0.540 (0.353)	-0.714 (0.846)	-0.173 (0.795)	0.585 (0.501)
Organic matter in a *semimembranosus* sample (%)	-0.064 (0.034)*	0.032 (0.087)	0.096 (0.084)	-0.052 (0.048)
PH of the *semimembranosus* sample	0.039 (0.017)*	0.022 (0.046)	-0.017 (0.045)	-0.069 (0.025)**
CE of the *semimembranosus* sample	-0.016 (0.502)	1.437 (1.231)*	1.453 (1.148)*	0.317 (0.755)
Lightness	-0.118 (0.511)	0.134 (1.234)	0.253 (1.174)	0.371 (0.735)
Lightness, duplicate	0.160 (0.505)	-0.420 (1.240)	-0.580 (1.173)	1.045 (0.730)
Redness	-0.509 (0.213)*	-0.928 (0.508)*	-0.419 (0.480)	0.647 (0.306)*
Redness, duplicate	-0.279 (0.210)	-0.584 (0.513)	-0.305 (0.485)	-0.443 (0.309)*
Yellowness	0.091 (0.149)	-0.090 (0.332)	-0.181 (0.307)	0.325 (0.216)
Yellowness, duplicate	0.142 (0.157)	-0.111 (0.354)	-0.254 (0.330)	0.516 (0.231)*
Shear force	-0.023 (0.250)	0.450 (0.753)	0.473 (0.726)	-0.376 (0.347)
Maximum shear force	-0.061 (0.248)	0.427 (0.751)	0.488 (0.725)	-0.430 (0.344)
Total work	-0.117 (2.701)	4.848 (8.015)	4.965 (7.702)	-7.513 (3.757)*
Shear firmness	0.008 (0.018)	0.039 (0.058)	0.031 (0.057)	0.011 (0.026)

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461 ** The null difference is not included in the Highest Posterior Density at 95%

462 Table 4. Association between phenotypic variation of carcass traits and haplotypes in the pig *malic enzyme 1* gene. Standard
463 deviations of the differences between genotypes are indicated between parentheses. Haplotypes include five single nucleotide
464 polymorphisms located in the 3' UTR region: **H1** (C_{1706} G_{1762} A_{1807} C_{1857} A_{1880}), **H2** (C_{1706} G_{1762} A_{1807} C_{1857} T_{1880}) and **H3** (T_{1706} T_{1762}
465 C_{1807} A_{1857} T_{1880}).

Trait	Genotypes									
	H1H2 H1H3	**H1H2 H2H2**	**H1H2 H2H3**	**H1H2 H3H3**	**H1H3 H2H2**	**H1H3 H2H3**	**H1H3 H3H3**	**H2H2 H2H3**	**H2H2 H3H3**	**H2H3 H3H3**
Weight at 156 days (kg)	-2.738 (3.988)	-0.486 (1.817)	-0.058 (1.755)	-0.688 (3.037)	2.252 (3.755)	2.680 (3.628)	2.050 (4.074)	0.428 (1.093)	-0.202 (2.752)	0.405 (2.737)
Backfat at 156 days (mm)	0.247 (0.542)	-0.003 (0.243)	0.255 (0.238)	0.726 (0.413)	-0.250 (0.508)	0.009 (0.491)	0.479 (0.553)	0.258 (0.148)	0.729 (0.376)*	0.470 (0.353)
Weight at 171 days (kg)	-3.550 (4.683)	0.622 (2.096)	1.265 (2.049)	0.228 (3.541)	4.172 (4.399)	4.816 (4.249)	3.778 (4.774)	0.644 (1.279)	-0.393 (3.224)	-1.037 (3.031)
Backfat at 171 days (mm)	-0.419 (0.575)	0.009 (0.255)	0.358 (0.254)	0.957 (0.425)*	0.429 (0.537)	0.777 (0.520)	1.377 (0.583)**	0.349 (0.154)*	0.948 (0.387)**	0.599 (0.365)
Weight at 178 days (kg)	-7.142 (4.798)	-0.063 (2.221)	0.405 (2.177)	-1.776 (3.752)	7.079 (4.468)*	7.547 (4.366)*	0.468 (1.348)	-1.713 (3.373)	-2.181 (3.286)	-3.337 (2.527)
Backfat at 178 days (mm)	-1.382 (0.668)*	-0.535 (0.316)*	-0.389 (0.309)	-0.008 (0.517)	0.847 (0.618)	0.993 (0.602)*	1.373 (0.679)*	0.146 (0.190)	0.526 (0.464)	0.380 (0.450)
Carcass weight (kg)	-4.038 (3.743)	0.104 (1.791)	1.032 (1.832)	0.452 (3.041)	4.142 (3.413)	5.070 (3.354)*	4.490 (3.821)	0.927 (1.143)	0.348 (2.701)	-0.579 (2.579)
Fat-o-Meter measurement of the carcass	-0.291 (1.388)	-0.234 (0.442)	0.023 (0.658)	-0.559 (1.157)	0.057 (1.278)	0.314 (1.263)	-0.269 (1.473)	0.257 (0.415)	-0.325 (1.047)	-0.583 (1.008)
Fat-o-Meter measurement of the loin area	2.094 (2.641)	2.653 (1.159)*	1.220 (1.191)	2.242 (2.207)	0.559 (2.447)	-0.875 (2.437)	0.325 (2.915)	-1.434 (0.750)*	-0.234 (2.015)	1.200 (1.982)
Carcass length (m)	-1.774 (1.456)	0.092 (0.663)	0.052 (0.661)	-0.094 (1.137)	1.866 (1.357)	1.825 (1.332)	1.680 (1.528)	-0.040 (0.408)	-0.186 (1.026)	-0.146 (0.997)
Fat thickness in the cervical region (mm)	0.369 (0.217)*	0.068 (0.097)	0.087 (0.097)	-0.051 (0.168)	-0.300 (0.202)	-0.281 (0.197)	-0.420 (0.225)*	0.019 (0.060)	-0.120 (0.152)	-0.139 (0.148)
Fat thickness between the 3rd and 4th ribs (mm)	0.151 (0.160)	0.117 (0.072)	0.095 (0.072)	0.000 (0.125)	-0.035 (0.148)	-0.057 (0.145)	-0.151 (0.165)	-0.022 (0.045)	-0.116 (0.112)	-0.094 (0.108)
Left ham weight(kg)	-0.523 (0.539)	-0.130 (0.236)	-0.026 (0.235)	-0.049 (0.414)	0.393 (0.493)	0.498 (0.484)	0.474 (0.558)	0.104 (0.146)	0.081 (0.375)	-0.023 (0.366)

Left shoulder weight (kg)	-0.330 (0.190)*	-0.070 (0.088)	-0.032 (0.086)	0.101 (0.148)	0.260 (0.176)	0.297 (0.172)*	0.431 (0.196)*	0.038 (0.053)	0.171 (0.133)	0.133 (0.130)
Left cutlet weight (kg)	-0.655 (0.343)*	0.127 (0.158)	0.079 (0.156)	0.041 (0.268)	-0.782 (0.318)**	-0.734 (0.312)**	0.696 (0.356)*	-0.048 (0.096)	-0.086 (0.241)	-0.038 (0.234)
Left ribs weight (kg)	-0.051 (0.576)	0.186 (0.231)	-0.016 (0.230)	-0.024 (0.370)	0.236 (0.555)	0.035 (0.538)	0.027 (0.564)	-0.201 (0.141)	-0.210 (0.328)	-0.008 (0.310)
Left bacon weight (kg)	-0.107 (0.317)	0.010 (0.122)	0.125 (0.118)	0.056 (0.189)	0.117 (0.298)	0.232 (0.292)	0.163 (0.302)	0.115 (0.070)*	0.047 (0.166)	-0.069 (0.157)
Right ham weight(kg)	-0.530 (0.543)	-0.112 (0.244)	-0.062 (0.422)	0.417 (0.504)	0.468 (0.495)	0.515 (0.569)	0.051 (0.150)	0.098 (0.381)	0.047 (0.371)	-0.240 (0.285)
Right shoulder weight (kg)	0.192 (0.171)	0.049 (0.068)	0.009 (0.069)	-0.007 (0.142)	-0.143 (0.162)	-0.183 (0.163)	-0.199 (0.204)	-0.040 (0.043)	-0.057 (0.130)	-0.017 (0.132)
Right cutlet weight (kg)	-0.877 (0.352)**	0.002 (0.165)	-0.045 (0.161)	-0.229 (0.272)	0.880 (0.316)**	0.832 (0.316)**	0.649 (0.358)*	-0.048 (0.100)	-0.231 (0.244)	-0.183 (0.236)
Right ribs weight (kg)	-0.335 (0.524)	0.152 (0.230)	0.009 (0.224)	0.203 (0.343)	0.487 (0.501)	0.343 (0.482)	0.537 (0.501)	-0.143 (0.146)	0.050 (0.300)	0.194 (0.279)
Right bacon weight (kg)	-0.118 (0.321)	-0.013 (0.125)	0.009 (0.120)	-0.101 (0.200)	0.105 (0.307)	0.127 (0.298)	0.017 (0.311)	0.022 (0.078)	-0.088 (0.180)	-0.110 (0.171)

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468 ** The null difference is not included in the Highest Posterior Density at 95%

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Table 5. Association between phenotypic variation of meat quality traits and haplotypes in the pig *malic enzyme 1* gene. Standard deviations of the differences between genotypes are indicated between parentheses. Haplotypes include five single nucleotide polymorphisms located in the 3' UTR region: **H1** (C_{1706} G_{1762} A_{1807} C_{1857} A_{1880}), **H2** (C_{1706} G_{1762} A_{1807} C_{1857} T_{1880}) and **H3** (T_{1706} T_{1762} C_{1807} A_{1857} T_{1880}).

Trait	Genotypes									
	H1H2 H1H3	**H1H2 H2H2**	**H1H2 H2H3**	**H1H2 H3H3**	**H1H3 H2H2**	**H1H3 H2H3**	**H1H3 H3H3**	**H2H2 H2H3**	**H2H2 H3H3**	**H2H3 H3H3**
PH at the *semimembranosus*, 45' after sacrifice	-0.221 (0.141)*	-0.132 (0.058)*	-0.080 (0.059)	-0.079 (0.114)	0.089 (0.132)	0.141 (0.129)	0.142 (0.152)	0.052 (0.037)	0.000 (0.101)	-0.084 (0.074)
PH at the *longissimus*, 45' after sacrifice	0.038 (0.131)	-0.082 (0.055)	0.005 (0.056)	-0.074 (0.105)	-0.121 (0.122)	-0.034 (0.121)	-0.112 (0.142)	0.087 (0.035)**	0.008 (0.095)	-0.079 (0.093)
CE at the *semimembranosus*, 45' after sacrifice	0.552 (0.426)	0.024 (0.176)	-0.103 (0.179)	0.391 (0.340)	-0.528 (0.407)	-0.655 (0.402)	-0.161 (0.471)	-0.127 (0.111)	0.367 (0.312)	0.494 (0.307)
CE at the *longissimus*, 45' after sacrifice	0.303 (0.221)	0.022 (0.099)	0.059 (0.099)	0.321 (0.173)*	-0.282 (0.210)	-0.244 (0.204)	0.017 (0.231)	0.037 (0.062)	0.299 (0.156)*	0.262 (0.150)*
PH at the *semimembranosus*, 24h after sacrifice	-0.059 (0.081)	-0.066 (0.034)	-0.028 (0.035)	-0.048 (0.064)	-0.007 (0.076)	0.032 (0.074)	0.012 (0.086)	0.038 (0.022)*	0.018 (0.059)	-0.020 (0.057)
PH at the *longissimus*, 24h after sacrifice	-0.142 (0.095)	-0.100 (0.042)**	-0.074 (0.042)*	-0.075 (0.075)	0.042 (0.089)	0.068 (0.087)	0.067 (0.100)	0.026 (0.026)	0.025 (0.068)	-0.001 (0.067)
CE at the *semimembranosus*, 24h after sacrifice	1.587 (0.987)*	0.148 (0.435)	-0.063 (0.425)	0.140 (0.905)	-1.439 (0.932)*	-1.650 (0.918)*	-1.447 (1.125)	-0.211 (0.275)	-0.008 (0.863)	0.203 (0.843)
CE at the *longissimus*, 24h after sacrifice	0.460 (0.409)	0.010 (0.176)	-0.043 (0.174)	0.002 (0.385)	-0.450 (0.387)	-0.504 (0.382)	-0.458 (0.481)	-0.054 (0.110)	-0.008 (0.365)	0.045 (0.359)
Enzymatic activity of LDH (µmol NADH min^{-1}.muscle g^{-1})	-131.116 (255.476)	48.546 (121.952)	-72.495 (119.510)	-319.289 (195.037)*	179.661 (234.807)	58.621 (228.799)	-188.173 (255.358)	-121.041 (72.904)*	-367.835 (172.266)*	-246.794 (166.343)
Enzymatic activity of ICDH (nmol NADPH min^{-1}.muscle g^{-1})	-0.049 (0.183)	0.009 (0.091)	-0.059 (0.089)	-0.102 (0.140)	0.058 (0.167)	-0.010 (0.162)	-0.053 (0.181)	-0.068 (0.054)	-0.112 (0.123)	-0.043 (0.118)
Slow myosin heavy chain (%)	1.138 (1.840)	-0.393 (0.874)	-0.017 (0.867)	-0.292 (1.434)	-1.531 (1.697)	-1.155 (1.650)	-1.430 (1.857)	0.376 (0.519)	0.101 (1.272)	-0.275 (1.233)
Pigmentation (µg of haematin.g of muscle $^{-1}$)	-1.751 (3.084)	2.158 (1.408)	1.084 (1.391)	-2.039 (2.412)	3.909 (2.872)	2.835 (2.812)	-0.288 (3.202)	-1.074 (0.873)	-4.197 (2.167)*	-3.123 (2.114)
Fat in a *semimembranosus* sample (%)	-0.493 (1.287)	-0.958 (0.601)	-0.439 (0.617)	0.030 (0.963)	-0.465 (1.188)	0.054 (1.164)	0.523 (1.223)	0.519 (0.380)	0.988 (0.879)	-0.493 (1.287)

Dry matter in a *semimembranosus* sample (%)	0.048 (0.363)	-0.055 (0.161)	-0.109 (0.160)	-0.111 (0.282)	-0.102 (0.338)	-0.157 (0.331)	-0.159 (0.370)	-0.054 (0.104)	-0.057 (0.256)	0.048 (0.363)
Crude protein in a *semimembranosus* sample (%)	0.242 (1.219)	0.766 (0.569)	0.170 (0.554)	0.073 (0.941)	0.524 (1.122)	-0.072 (1.095)	-0.169 (1.201)	-0.596 (0.358)*	1.219 (0.844)	0.242 (1.219)
Organic matter in a *semimembranosus* sample (%)	-0.014 (0.122)	-0.042 (0.053)	-0.110 (0.053)*	-0.010 (0.095)	-0.028 (0.114)	-0.096 (0.112)	0.004 (0.128)	-0.068 (0.034)*	0.032 (0.086)	-0.014 (0.122)
PH of the *semimembranosus* sample	0.043 (0.063)	-0.069 (0.027)**	-0.030 (0.041)	-0.047 (0.051)	-0.112 (0.060)*	-0.073 (0.059)	-0.091 (0.070)	0.038 (0.017)	0.021 (0.047)	-0.017 (0.046)
CE of the *semimembranosus* sample	-1.592 (1.730)	-0.084 (0.847)	0.000 (0.820)	1.286 (1.369)	1.508 (1.619)	1.592 (1.568)	2.878 (1.794)	0.084 (0.510)	1.370 (1.237)	1.286 (1.160)
Lightness	-1.136 (1.750)	0.136 (0.833)	0.089 (0.820)	0.236 (1.400)	1.272 (1.635)	1.224 (1.601)	1.372 (1.839)	-0.047 (0.530)	0.100 (1.268)	0.148 (1.198)
Lightness, duplicate	-0.169 (1.745)	0.970 (0.825)	1.144 (0.811)	0.523 (1.396)	1.139 (1.638)	1.312 (1.597)	0.691 (1.833)	0.173 (0.521)	-0.448 (1.265)	-0.621 (1.198)
Redness	0.618 (0.728)	0.902 (0.342)**	0.309 (0.336)	0.005 (0.581)	0.284 (0.688)	-0.309 (0.662)	-0.613 (0.755)	-0.593 (0.217)**	-0.897 (0.530)*	-0.303 (0.496)
Redness, duplicate	0.173 (0.727)	0.544 (0.339)	0.230 (0.335)	-0.033 (0.580)	0.371 (0.689)	0.057 (0.667)	-0.206 (0.764)	-0.314 (0.215)	-0.577 (0.528)	-0.263 (0.499)
Yellowness	0.470 (0.489)	0.428 (0.254)	0.480 (0.242)*	0.342 (0.379)	-0.042 (0.452)	0.009 (0.438)	-0.128 (0.484)	0.051 (0.153)	-0.086 (0.340)	-0.137 (0.316)
Yellowness, duplicate	0.626 (0.511)	0.652 (0.270)**	0.749 (0.256)**	0.548 (0.404)	0.026 (0.477)	0.123 (0.458)	-0.078 (0.516)	0.098 (0.162)	0.511 (0.359)	0.270 (0.334)
Shear force	0.347 (0.852)	-0.283 (0.290)	-0.351 (0.383)	0.148 (0.804)	-0.630 (0.797)	-0.698 (0.780)	-0.199 (0.975)	-0.068 (0.257)	0.431 (0.734)	0.499 (0.742)
Maximum shear force	0.348 (0.848)	-0.330 (0.383)	-0.439 (0.381)	0.078 (0.802)	-0.677 (0.793)	-0.787 (0.776)	-0.270 (0.973)	-0.110 (0.255)	0.405 (0.739)	0.517 (0.719)
Total work	5.938 (9.180)	-6.055 (4.193)	-6.794 (4.138)	-1.231 (8.583)	-11.993 (8.573)	-12.733 (8.369)	-7.169 (10.352)	-0.739 (2.768)	4.824 (7.895)	5.564 (7.664)
Shear firmness	0.037 (0.064)	0.018 (0.046)	0.023 (0.029)	0.055 (0.062)	-0.019 (0.060)	-0.014 (0.059)	0.018 (0.076)	0.005 (0.019)	0.037 (0.057)	0.033 (0.056)

* The null difference is not included in the Highest Posterior Density at 90%

** The null difference is not included in the Highest Posterior Density at 95%

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Polymorphism of the pig *2, 4-dienoyl-CoA reductase* gene *(DECR*) and its association with carcass and meat quality traits[1]

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Abstract

We have characterized the near complete coding sequence of the pig 2,4-dienoyl-CoA-reductase (*DECR*) gene, which encodes an enzyme involved in the β-oxidation of polyunsaturated fatty enoyl-CoA esters and maps to a linoleic QTL located at chromosome 4. Sequencing of a 937 bp fragment encompassing exons 2 and 10 revealed the existence of two missense single nucleotide polymorphisms (SNP) at exon 2 (C → G, position 181 in the coding sequence) and exon 5 (C → G, position 458 in the coding sequence). These two SNP are associated with Val (C) → Leu (G) and Ser (C) → Thr (G) conservative amino acid replacements at positions 61 and 153 of the DECR protein, respectively. Moreover, *DECR* genotyping in a representative sample of 184 pigs from the Large White, Pietrain, Iberian, Duroc and Landrace breeds demonstrated the existence of disequilibrium linkage between these two SNP (haplotype 1: $C_{181}C_{458}$, haplotype 2: $G_{181}G_{458}$). The performance of an association analysis between *DECR* genotype and growth, carcass and meat quality traits revealed a few associations with isocitrate dehydrogenase activity (Highest Posterior Density of 90%) and muscular pH (Highest Posterior Density of 95%) and redness (Highest Posterior Density of 95%). Since these associations were not consistently found in the three available genotype comparisons, we believe that exon 2 and 5 polymorphisms at the *DECR* gene might be in linkage disequilibrium with the true causal mutation influencing isocitrate dehydrogenase activity and muscular redness and pH.

Keywords: pig, 2, 4-dienoyl-CoA reductase, fatty acid β-oxidation, carcass and meat quality traits

Introduction

Meat tenderness and flavour are greatly affected by the proportion and composition of intramuscular fat (Rosenvold and Andersen 2003). The inclusion of conjugated linoleic acids in the diet alter carcass composition in pigs by decreasing fat deposition and the ratio of fat to lean tissue (Ostrowska et al., 1999). Recently, a QTL with a significant effect on the percentage of linoleic acid in subcutaneous adipose tissue was mapped to pig 4q1.2 (interval 71-86 cM) in an Iberian x Landrace cross (Perez-Enciso et al., 2000; Clop et al., 2003). The Iberian allele was associated to a 1.5% decrease in linoleic acid content and the QTL explained as much as 40% of the phenotypic differences observed between these two comercial breeds. This finding prompted the characterization of candidate genes which might be involved in the metabolism of this fatty acid.

The 2,4-dienoyl-CoA-reductase (DECR) is a nuclear-encoded mitochondrial enzyme which participates in the β-oxidation pathway by catalyzing the reduction of trans-2-cis-4-dienoyl-CoA to 3-enoyl-CoA (Kunau and Dommes, 1978). This enzyme has a homotetrameric structure and it is mostly expressed in liver, heart, pancreas and kidney. The transcription unit of the human *DECR* gene includes 10 exons and 9 introns of variable size which span 30 kb (Helander et al., 1997). Interestingly, the deficiency of this enzyme in human causes a lethal syndrome characterized by hypocarnitinemia, hyperlysinemia and the presence of 2-trans-4-cis-decadienoylcarnitine in urine and blood of the affected patients (Roe et al., 1990). The presence of this metabolite has been attributed to the incomplete oxidation of the linoleic fatty acid.

The chromosomal location of the pig *DECR* gene, which coincides with the linoleic QTL previously described by Perez-Enciso et al. (2000), and the crucial role of this enzyme in the β-oxidation of polyunsaturated fatty acids, made evident the need of characterizing with more detail the molecular features of this gene in pig. The main objectives of our work were to identify polymorphisms in the *DECR* coding sequence and to investigate if they are associated with phenotypic variation at carcass and meat quality traits.

Materials and Methods

Animal material and recording of phenotypic traits

Five Landrace boars were mated to 71 Landrace sows yielding an F_1 generation of 470 individuals. An average of 94 offspring was obtained from each male. Pigs were bred at the Nova Genètica farm, being fed ad *libitum* and slaughtered at an age of 179,95 ± 4.86 d (104.55 ± 11.05 kg live weight).

Phenotypic records of the folloving growth, fatness and carcass traits were obtained: weight at 156 d, 171 d and 178 d, backfat thickness measured with Fat-o-Meter at 156 d, 171 d and 178 d, carcass weight, carcass length, fat thickness in the cervical region and between the 3rd and 4th ribs, and weight of the right and left hams, shoulders, cutlet, ribs and bacon. The analysed meat quality traits were pH - measured using a Scharlau portable meter equipped with a xerolyt electrode - and electric conductivity (CE) - measured using a Pork Quality Meater - that were determined at 45 min and 24 hours after slaughter in the *semimembranosus* and the *longissimus dorsi* muscles. Muscle colour parameters in the CIELAB space - Lightness (**L**), redness (**a**), and yellowness (**b**) (CIE, 1976)- were quantified by

duplicate with a Minolta spectrophotometer at 24 hours post mortem on the exposed cut surface of the muscle (**L2**, **a2** and **b2**). Moreover, we analysed the chemical composition of the muscle by measuring fat, crude protein, organic matter and dry matter content from *semimebranosus* muscle samples (AOAC, 1990).

Muscle samples for biochemical analyses were obtained at 24 h pm at the last-rib level. Samples for enzyme-linked immunosorbent assay (ELISA) and enzyme activity analyses were taken from the *longissimus dorsi* core. They were frozen in liquid nitrogen and stored at -80 ºC until analysis. Samples for determination of haem pigment content were vacuum-packed and stored at -20 ºC until analysis.

The percentage of slow myosin heavy chain in the muscle was determined with a specific MHC-I monoclonal antibody by the ELISA technique (Picard et al., 1994). The metabolic profile of the muscle was assessed by measuring the lactate dehydrogenase (**LDH**) activity according to Ansay (1974) and the isocitrate dehydrogenase (**ICDH**) activity according to Briand et al. (1981). These activities are expressed as μmol NADH min^{-1}·g $muscle^{-1}$ (**LDH**) and nmol NADPH min^{-1}·g $muscle^{-1}$ (**ICDH**). The concentration of haem pigment (**PIGM**) was determined according to the Hornsey modified method (1956). Results are given in μg of acid haematin per g of muscle

Genomic DNA and RNA extraction and cDNA synthesis

Four hundred μl TE buffer (Tris-HCl 10 mM pH = 8, EDTA 1mM) were added to 0.4 ml blood and this mixture was centrifuged at 13,000 g for 30 sec. The supernatant was discarded and this washing step was repeated 4-5 times until a white pellet was obtained. Subsequently, cells were resuspended in 0.4

ml lysis buffer K (KCl 50 mM, Tris-HCl 10 mM, 0.5% Tween 20, proteinase K) and incubated for 5 hours at 56 ºC. Genomic DNA was phenol-clorophorm extracted and precipitated with 25 µl NaCl 2M and two volumes ethanol. The genomic DNA pellet was centrifuged at 13,000 g for 10 min, washed with ethanol 70% and resuspended in 100 µl of TE.

Total RNA was extracted from ten Piétrain, Vietnamese, Large White, Iberian and Landrace pig liver samples and reverse transcribed to cDNA, as previously described (Amills et al., 2003).

Amplification and sequencing of the pig DECR cDNA

We amplified 937 bp of the pig *DECR* cDNA by using two oligonucleotides ENOIL-EXO2-5; 5'-AGT TTT TCA GTT ATG GGA CAA AAA-3', and DECR-3-CDNA; 5'-GAA CCT TTT GTC TTC CTG ATG AG-3'. The PCR mixture contained 1.5 mM $MgCl_2$, 100 µM dNTP, 0.5 µM of each primer, 2-3 µl of the reverse transcription reaction and 0.5 U Taq DNA polymerase (Ecogen S.R.L., Barcelona, Spain) in a final 20 µl volume. The thermal profile consisted of 35 cycles of 94 ºC for 1 min, 63 ºC for 2 min, and 72 ºC for 3 min. The amplified product was sequenced forward and reverse in ten individuals with the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA). Sequencing reactions were analysed in a capillar electrophoresis device ABI PRISM 310 (Applied Biosystems, Foster City, CA). The primers used in the sequencing reactions were ENOIL-EXO2-5, DECR-3-CDNA, ENOIL-EX3·3; 5'ATT AGG ATG TCC TGC AAC TTT GAT-3' and DECR-FW-EX5; 5'-GTG ATA AAC AAT GCA GCA GG-3'.

Genotyping of the G/C polymorphisms at exons 2 and 5

Primer sequences for amplifying the second exon of the *DECR* gene were ENOIl-EXO2-5 and ENOIL-EXO2-3, 5'-CAC TGA GCA CCT AGG CTG GA-3', whereas primers DECR-FW-EX5 and DECR-REV-EX5; 5'-CTT TCT GTG CTT TAA TTA GTT GC-3' were used for amplifying exon 5. Polymerase chain reactions contained 1.5 mM $MgCl_2$, 100 μM dNTP, 0.5 μM of each primer, 30 ng (exon 2) or 60 ng (exon 5) genomic DNA and 0.5 U (exon 2) or 0.75 U (exon 5) Taq DNA polymerase (Ecogen S.R.L., Barcelona, Spain) in a final 25 μl (exon 2) or 30 μl (exon 5) volume. The amplification of the second exon involved one denaturation step at 94 ºC for 1.5 min, 35 cycles at 94 ºC for 1.5 min, 58 ºC for 2 min and 72 ºC for 2.5 min, and a final extension step of 72 ºC for 20 min. The thermal profile of the exon 5 PCR consisted of 30 cycles of 94 ºC for 1 min, 61 ºC for 1 min and 72 ºC for 1 min. Both polymorphisms were genotyped by primer extension analysis. The PCR products were purified with the ExoSAP-IT kit (Amersham Biosciences Europe GmbH) and typed with the SnaPshot^{TM} ddNTP Primer Extension kit (Applied Biosystems, Foster City, CA). The primers used in this typing procedure were SNAP2-DECR; 5'-CCA CCA AAT ACT TTT CAA GGA AAA-3' (exon 2), and SNAP5-DECR; 5'- CAT TAG GAG AGA GTC TTT CA-3'. The allelic frequencies of the exon 2 and 5 polymorphisms were calculated in a representative sample of 184 pigs from the Large White (N = 27), Pietrain (N = 28), Iberian (N = 22), Duroc (N = 31) and Landrace (N = 76) breeds.

Association analyses with carcass and meat quality traits

The assumed model for the phenotypic data of each trait is:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z}_1\mathbf{u} + \mathbf{Z}_2\mathbf{p} + \mathbf{e}$$

where **ß** are the systematic effects (2 sex effects, 2 *Ryr1* configuration effects and 3 *DECR* genotype configurations), **u** is the vector of additive genetic effects, **p** are the litter effects and **e** is the residual vector, $\mathbf{X}$, $\mathbf{Z_1}$, $\mathbf{Z_2}$, are the incidence matrices that link phenotypic data with systematic, genetic and permanent environmental effects.

The likelihood of data is the following multivariate normal distribution.

$$f\left(\mathbf{y}|\beta,\mathbf{u},\mathbf{p},\sigma_e^2\right)=\mathrm{MVN}\left(\mathbf{X}\beta+\mathbf{Z}\mathbf{u}+\mathbf{Z}\mathbf{p},\mathbf{I}\sigma_e^2\right)$$

Prior distribution of **β** were assumed flat between a range of possible values to ensure property of the posterior distribution. Prior distribution of the additive (**u**) and litter effects (**p**) , are the following multivariate normal distributions:

$$f\left(\mathbf{u}|\sigma_u^2\right)=\mathrm{MVN}\left(0,\mathbf{A}\sigma_u^2\right)$$

$$f\left(p|\sigma_u^2\right)=\mathrm{MVN}\left(0,\mathbf{I}\sigma_p^2\right)$$

where σ_u^2 and σ_p^2 are the additive and litter variance respectively.

Prior distribution for σ_u^2, σ_p^2 and σ_e^2 were assumed flat between a range of possible values.

Bayesian analyses were carried with the Gibbs Sampler algorithm (Geman and Geman, 1984; Gelfand and Smith, 1990; Tanner, 1993) to obtain autocorrelated samples from the joint posterior density and subsequently from the marginal posterior densities of all the unknowns in the model. Specifics on distributions involved can also be found in previous studies (Wang et al., 1993; 1994). The posterior conditional distributions for the locations parameters (sex, *Ryr1* and *DECR* configuration effects) were univariate normal distributions and the posterior distributions of the variance components were inverted chi-

squares. The Gibbs sampler analysis was carried out for each analysis through a simple chain of 100,000 iterations, after discarding the first 5,000. The analysis of convergence was calculated using the algorithms of Raftery and Lewis (1992) and García-Cortes et al. (1998). All iterations of the analysis were used to compute posterior means and standard deviations so that all the available information from the output of the Gibbs sampler could be considered.

Results and Discussion

We have amplified and sequenced a 937 bp amplicon including the near complete coding sequence of the pig *DECR* gene. This cDNA sequence (Genbank accession no AY233130) encompassed exons 2 and 10 and displayed 89% and 83% nucleotide identities with its human and murine *DECR* orthologous sequences, respectively. The alignment of ten *DECR* sequences from pigs belonging to diverse breeds allowed to confirm the existence of one G $\rightarrow$ C polymorphism at exon 2 (position 181 of the coding sequence), previously described by Clop et al. (2002). Moreover, we found a second G $\rightarrow$ C polymorphism at exon 5 (position 458 of the coding sequence). The exon 2 and 5 polymorphisms are associated with a Val (C) $\rightarrow$ Leu (G) and a Ser (C) $\rightarrow$ Thr (G) conservative amino acid replacements at positions 61 and 153 of the DECR protein, respectively. We examined the allelic frequencies of both mutations. Our results indicate the existence of two segregating *DECR* haplotypes in the Landrace, Duroc, Large White, Pietrain and Iberian pig breeds: haplotype 1 (H_1: C_{181} - C_{458}) and haplotype 2 (H_2: G_{181} - G_{458}) (Table 1). According to the mapping data independently reported by Clop et al. (2002) and Davoli et al. (2002), the pig *DECR* gene is located within the interval of a linoleic

QTL at chromosome 4 which was previously described by Pérez-Enciso et al. (2000) and Clop et al. (2003). With the objective of investigating the possible functional role of the two mutations that we have characterized in the pig *DECR* coding sequence, we have performed an association analysis in a two generation Landrace pedigree including 76 founders and 470 offspring for which growth, fatness and meat quality records are available.

We did not find any significant differences among *DECR* haplotypes with regard to growth and carcass traits (Table 2), after integrating out the nuisance parameters (additive, litter, sex and *RYR1* effects) by the Bayesian analysis through the Gibbs Sampler. A few meat quality traits related with ICDH ($H_2H_2 < H_1H_2$, Highest Posterior Density of 90%), longissimus dorsi muscle pH at 24 h ($H_2H_2 < H_1H_2$, Highest Posterior Density of 95%) and a2 ($H_1H_2 < H_2H_2$, Highest Posterior Density of 95%) showed significant associations (Table 3). The DECR enzyme has a key role in the β-oxidation of polyunsaturated fatty acids and, in consequence, quantitative variations on its activity or expression might affect fatty acid composition, specially linoleic content, and meat quality. In this framework, the simultaneous association of the *DECR* genotype with longissimus dorsi muscle pH at 24 h and a2 is particularly interesting, since the oxidation of the hemo muscular pigments, which partly explain muscle redness, is highly dependent on pH kinetics. Moreover, the manipulation of the saturated and polyunsaturated fatty acid ratio by adding conjugated linoleic acid to the diet affects meat colour, muscle conductivity and ultimate pH24 (Tischendorf et al., 2002, D'Souza and Mullan 2002). The association between *DECR* genotype and ICDH is also suggestive from a physiological point of view, since both enzymes are functionally related. In this way, isocitrate dehydrogenase 1 is involved in the production of cytosolic NADPH which

214 is required for the activity of several reductases, such as DECR, and fatty acid
215 biosynthesis (Shechter et al. 2003). Moreover, isocitrate dehydrogenase 1 regulates
216 the β-oxidation pathway by modulating the levels of acid phytanic, a known agonist
217 to peroxisome proliferator-activated receptors which are deeply involved in fatty acid
218 degradation (Shechter et al. 2003).

219 The significant associations between the polymorphism of the *DECR* gene
220 and meat redness and pH might be explained by the fact that the two allelic variants
221 we have detected are associated with a differential DECR enzymatic activity. In fact,
222 the genomic location of this gene coincides with a QTL influencing the linoleic
223 content, the fatty acid double-bond index and the peroxidability index (Pérez Enciso
224 et al. 2000, Clop et al. 2003). However, we do not favour this interpretation by two
225 reasons. First, the two amino acid replacements identified at positions 61 (Val/Leu)
226 and 153 (Ser/Thr) are conservative and, in principle, they are not expected to
227 involve a dramatic change on the biochemical properties of DECR. Second, the
228 associations we have found are scarce and any of them is consistently found in the
229 three available genotype comparisons. In consequence, the more straightforward
230 explanation would be that the *DECR* polymorphisms are not the causal mutations of
231 the associations we have found. Exon 2 and 5 polymorphisms might be in linkage
232 disequilibrium with the true causal mutation influencing ICDH and muscle redness
233 and pH, which may lie in another region of the *DECR* gene or even in a neighboring
234 locus.

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Implications

We have characterized two haplotypes in the *DECR* gene which are associated with meat redness, isocitrate dehydrogenase activity and pH in a Landrace purebred population. The identification of single nucleotide polymorphisms in the genes governing lipid biosynthesis an degradation will be essential for understanding the genetic basis of these metabolic processes.

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Table 1. Frequencies of the *DECR* haplotypes (H_1: C_{181} - C_{458}, H_2: G_{181} - G_{458}) in diverse pig breeds. The sizes of the sampled populations are indicated as N.

Breed	N	H_1H_1	H_1H_2	H_2H_2
Duroc	31	0.097	0.710	0.193
Iberian	22	0.500	0.363	0.137
Large White	27	0.593	0.370	0.037
Landrace	76	0.079	0.369	0.552
Pietrain	28	0.107	0.643	0.250

362 Table 2. Association between pig *DECR* haplotypes (H_1: C_{181} - C_{458}, H_2: G_{181} - G_{458})
363 and phenotypic variation of growth and carcass traits in a Landrace outbred
364 population. Standard deviations of the differences between genotypes are indicated
365 between parentheses.

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Trait	H_1H_1 - H_1H_2	H_1H_1 - H_2H_2	H_1H_2 - H_2H_2
Live weight, kg			
156 d	0.97 (2.04)	2.29 (2.13)	1.32 (1.09)
171 d	2.03 (2.37)	3.04 (2.47)	1.01 (1.29)
178 d	1.73 (2.30)	2.59 (2.50)	0.86 (1.36)
Backfat thickness, mm			
156 d	-0.15(0.27)	0.03 (0.29)	0.19(0.15)
171 d	0.22 (0.28)	0.22 (0.30)	-0.00 (0.15)
178 d	0.25 (0.32)	0.34 (0.35)	0.09 (0.19)
Carcass length, m	0.376 (0.720)	0.229 (0.761)	-0.146 (0.403)
Carcass weight, kg	0.97 (1.88)	0.92 (2.01)	-0.05 (1.02)
Backfat thickness, mm			
cervical	0.137 (0.103)	0.104 (0.111)	-0.033 (0.060)
3^{rd}-4^{th} ribs	-0.032 (0.077)	0.037 (0.082)	0.069 (0.045)
Ham weight, kg			
left	-0.024 (0.256)	-0.000 (0.275)	0.024 (0.146)
right	0.119 (0.261)	0.156 (0.281)	0.037 (0.149)
Shoulder weight, kg			
left	0.057 (0.093)	0.128 (0.101)	0.071 (0.055)
right	0.084 (0.093)	0.122 (0.100)	0.039 (0.054)
Cutlet weight, kg			
left	0.137 (0.165)	0.199 (0.178)	0.062 (0.097)
right	0.177 (0.171)	0.250 (0.186)	0.073 (0.099)
Ribs weight, kg			
left	-0.117 (0.226)	-0.154 (0.242)	-0.038 (0.144)
right	0.069 (0.205)	0.006 (0.221)	-0.063 (0.134)
Bacon weight, kg			
left	0.080 (0.116)	0.120 (0.125)	0.040 (0.074)
right	0.015 (0.124)	0.092 (0.133)	0.077 (0.078)

367

368 * Highest Posterior Density of 90%

369 ** Highest Posterior Density of 95%

370

371

372 Table 3. Association between pig *DECR* haplotypes (H_1: C_{181} - C_{458}, H_2: G_{181} - G_{458})
373 and phenotypic variation of meat quality traits in a Landrace outbred population.
374 Standard deviations of the differences between genotypes are indicated between
375 parentheses.

376

Trait [a]	H_1H_1 - H_1H_2	H_1H_1 - H_2H_2	H_1H_2 - H_2H_2
Semimembranosus pH			
45 min	-0.08(0.07)	-0.07(0.07)	0.01 (0.04)
24 h	0.023 (0.041)	0.018 (0.042)	-0.004 (0.023)
Longissimus dorsi pH			
45 min	-0.027 (0.066)	-0.015 (0.068)	0.013 (0.035)
24 h	0.006 (0.048)	0.059 (0.050)	0.054 (0.027)**
Semimembranosus CE			
45 min	0.355 (0.208)	0.543 (0.210)	0.188 (0.104)
24 h	-0.401 (0.569)	-0.654 (0.581)	-0.254 (0.277)
Longissimus dorsi CE			
45 min	0.036 (0.109)	0.098 (0.113)	0.063 (0.058)
24 h	0.164 (0.239)	0.033 (0.244)	-0.131 (0.113)
LDH, μmol NADH. min^{-1} . muscle g^{-1}	143.34 (120.61)	108.63 (134.33)	-34.72 (72.77)
ICDH, nmol NADP . min^{-1}. muscle g^{-1}	-0.036 (0.090)	0.072 (0.106)	0.109 (0.056)*
PIGM, μg acid haematin . muscle g^{-1}	1.425 (2.584)	2.584 (1.593)	1.158 (0.865)
Slow myosin heavy chain (%)	-0.85 (0.89)	-0.98 (0.99)	-0.13 (0.54)
Fat content (%)	0.55 (0.65)	0.90 (0.69)	0.35 (0.37)
Dry matter content (%)	0.112 (0.189)	0.109 (0.199)	-0.003 (0.104)
Crude protein content (%)	-0.37 (0.63)	-0.81 (0.68)	-0.44 (0.36)
Organic matter content (%)	0.039 (0.063)	0.016 (0.064)	-0.024 (0.033)
Lightness (L)	-1.12 (0.90)	-1.31 (0.93)	-0.19 (0.48)
Lightness (L2) [b]	-1.31 (0.89)	-1.61 (0.92)	-0.30 (0.48)
Redness (a)	0.58 (0.38)	0.55 (0.39)	-0.03 (0.20)
Redness (a2) [b]	0.85 (0.37)**	0.68 (0.39)	-0.17 (0.20)
Yellowness (b)	-0.12 (0.26)	-0.27 (0.27)	-0.15 (0.14)
Yellowness (b2) [b]	-0.04 (0.28)	-0.26 (0.29)	-0.23 (0.15)

377

378 * Highest Posterior Density of 90%

379 ** Highest Posterior Density of 95%

380 [a] LDH: lactate dehydrogenase activity; ICDH: isocitrate dehydrogenase activity;
381 PIGM: concentration of haem pigment; CE: electric conductivity.

382 [b] Minolta Chromameter values for lightness, redness and yellowness were

383 recorded by duplicate.