Polymorphism of the pig 2,4-dienoyl CoA reductase 1 (DECR1) and its association with carcass and meat quality traits

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ABSTRACT: We characterized the nearly complete coding sequence of the pig 2,4-dienoyl CoA reductase 1 (DECR1) gene, which encodes an enzyme involved in the β-oxidation of polyunsaturated fatty enoyl-CoA esters and maps on a linoleic QTL located on Chromosome 4. Sequencing of a 937-bp fragment encompassing exons 2 and 10 revealed the existence of two missense SNP at exon 2 (C181 → G181) and exon 5 (C458 → G458). These two SNP are associated with Val (C) → Leu (G) and Ser (C) → Thr (G) conservative AA replacements at positions 61 and 153 of the DECR1 protein, respectively. Moreover, DECR1 genotyping in a representative sample of 184 pigs from the Large White, Piétrain, Iberian, Duroc, and Landrace breeds demonstrated the existence of disequilibrium linkage between these two SNP (Haplotype 1: C181C458; Haplotype 2: G181G458). An association analysis between DECR1 genotype and growth, carcass, and meat quality traits in a highly selected Landrace population (n = 470) revealed differences among genotypes for isocitrate dehydrogenase activity (highest posterior density [HPD] of 90%), longissimus thoracis pH (HPD of 95%), lightness (HPD of 90 to 95%), and redness (HPD of 95%). Because these associations were not consistently found in the three available genotype comparisons, we believe that exon 2 and 5 polymorphisms at the DECR1 gene might be in linkage disequilibrium with the true causal mutation influencing isocitrate dehydrogenase activity and muscle color and pH.

Key Words: Carcass Traits, Fatty Acids, Meat Quality, Pig, Quantitative Trait Loci, Reductase

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Introduction

Meat tenderness and flavor are greatly affected by the proportion and composition of i.m. fat (Rosenvold and Andersen, 2003). The inclusion of CLA in the diet alters 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 s.c. adipose tissue was mapped on pig 4q1.2 (interval 71 to 86 cM) in an Iberian × Landrace cross (Pérez-Enciso et al., 2000; Clop et al., 2003). The Iberian allele was associated with a 1.5% decrease in linoleic acid content, and the QTL explained as much as 40% of the phenotypic differences observed between these two commercial breeds. This finding prompted the characterization of candidate genes that might be involved in the metabolism of this fatty acid.

2,4-Dienoyl CoA reductase 1 (DECR1) is a nuclear-encoded mitochondrial enzyme that 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). The transcription unit of the human DECR1 gene includes 10 exons and nine introns of variable size that span 30 kb (Helander et al., 1997). The deficiency of this enzyme in human has been associated with the presence of 2-trans-4-cis-decadienoylcarnitine, a metabolite produced by incomplete oxidation of the linoleic acid in urine and blood of the affected patients (Roe et al., 1990).

The chromosomal location of the pig DECR1 gene, which coincides with the linoleic QTL previously described by Pérez-Enciso et al. (2000), and the crucial role of this enzyme in the β-oxidation of PUFA, made
evident the need for a more detailed characterization of the molecular features of this gene in the pig. The main objectives of our work were to identify polymorphisms in the DECR1 coding sequence and to investigate whether they are associated with phenotypic variation in 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 F1 generation of 470 individuals. An average of 94 offspring was obtained from each male. In the herd, boars and gilts were performance tested. Both sexes were penned in groups of 10 to 12 animals separated according to their sex, and during the whole test period, they had ad libitum access to a cereal-based commercial diet (13.4 MJ/kg of ME, 17.5% CP, 1% lysine; as-fed basis). Weight and backfat thickness were recorded at 156, 171, and 178 d of age. Backfat thickness was measured with an Accu-Gage Lean Meater (Renco Corp., North Minneapolis, MN) as the average of two ultrasonic measurements taken on each side of the spinal column, 5 cm from the middorsal line at the position of the last rib. The F0 and F1 individuals were genotyped for the ryanodine receptor 1 (Ryr1) gene according to Fujii et al. (1991).

Pigs were slaughtered when 180.0 d old (SD = 4.9 d) and weighing 104.6 kg (SD = 11.1 kg) live weight. Phenotypic records of the following traits were obtained: carcass weight, carcass length, fat thickness in the cervical region and at the last-rib level, and weight of the right and left hams, shoulders, cutlet, ribs, and bacon. Fat thickness was measured with a Fat-O-Meater (SFK Technology, Herlev, Denmark). The meat quality traits analyzed were pH and electrical conductivity determined at 45 min and 24 h after slaughter in the semimebranosus and the longissimus thoracis muscles. The pH was measured using a portable meter equipped with a xerolyte electrode (Crison, Barcelona, Spain), and electrical conductivity was measured using a Pork Quality Meter (Intek GmbH, Munich, Germany). Muscle color parameters in the CIELAB space, lightness ($L^*$), redness ($a^*$), and yellowness ($b^*$) (CIE, 1976), were quantified in duplicate with a Minolta Chroma-Meter CR-200 (Konica Minolta, Madrid, Spain) at 24 h postmortem on the exposed cut surface of the muscle. Moreover, we analyzed the chemical composition of the muscle by measuring fat, CP, OM, and DM content from semimebranosus muscle samples (AOAC, 1990).

Muscle samples for biochemical analyses were obtained at 24 h postmortem at the last-rib level. Samples for ELISA and enzyme activity analyses were taken from the longissimus thoracis core. They were frozen in liquid N and stored at −80°C until analysis. Samples for determination of heme pigment content were vacuum-packed and stored at −20°C until analysis.

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

Genomic DNA and RNA Extraction and cDNA Synthesis

Four hundred microliters of Tris-EDTA buffer (10 mM Tris-HCl, pH = 8, 1 mM EDTA) was added to 0.4 mL of blood, and this mixture was centrifuged at 13,000 × g for 30 s. The supernatant was discarded and this washing step was repeated four or five times until a white pellet was obtained. Subsequently, cells were resuspended in 0.4 mL of lysis buffer K (50 mM KCl, 10 mM Tris-HCl, 0.5% Tween 20, and 0.1 mg/mL of proteinase K) and incubated for 5 h at 56°C. Genomic DNA was phenol-chloroform extracted and precipitated with 25 μL of 2 M NaCl and two volumes of ethanol. The genomic DNA pellet was centrifuged at 13,000 × g for 10 min, washed with 70% ethanol, and resuspended in 100 μL of Tris-EDTA.

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 DECR1 cDNA

We amplified 937 bp of the pig DECR1 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 to 3 μL of the reverse transcription reaction, and 0.5 U Taq DNA polymerase (Ecogen S.R.L., Barcelona, Spain) in a final volume of 20 μL. The thermal profile comprised 35 cycles of 94°C for 1 min, 63°C for 2 min, and 72°C for 3 min. The amplified product was sequenced forward and reverse in 10 individuals with the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA). Sequencing reactions were analyzed in a capillary 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 DECRI gene were ENOIL-EXO2-5 and ENOIL-EXO2-3, 5’-CAG 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 MgCl2, 100 μM dNTP, 0.5 μM of each primer, 30 (exon 2) or 60 ng (exon 5) of genomic DNA, and 0.5 (exon 2) or 0.75 U (exon 5) of Taq DNA polymerase (Ecogen S.R.L.) in a final 25- (exon 2) or 30-μL (exon 5) volume. The amplification of the second exon involved one denaturation step at 94°C for 1.5 min, 35 cycles at 94°C for 1.5 min, 58°C for 2 min, and 72°C for 2.5 min, and a final extension step of 72°C for 20 min. The thermal profile of the exon 5 PCR comprised 30 cycles of 94°C for 1 min, 61°C for 1 min, and 72°C 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, Cerdanyola del Vallès, Spain) and typed with the SnaPshot ddNTP Primer Extension kit (Applied Biosystems). The primers used in this typing procedure were SNAp2-DECR; 5’-CCA CCA AAT ACT TTT CAA GGA AAA-3’ (exon 2), and SNAp5-DECRI; 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), Piétrain (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 was as follows:

\[ y = X\beta + Z_1u + Z_2p + e \]

where \( \beta \) = the systematic effects (two sex effects, two Ryrl configuration effects and three DECRI genotype configurations), \( u \) = the vector of additive genetic effects, \( p \) = the litter effects, \( e \) = the residual vector, and \( X, Z_1, Z_2 \) are the incidence matrices that link phenotypic data with systematic, genetic, and permanent environmental effects, respectively. The likelihood of data is the following multivariate normal distribution:

\[ f(y|\beta, u, p, \sigma^2) = MVN(X\beta + Z_1u + Z_2p, \sigma^2 I) \]

Prior distributions of \( \beta \) were assumed flat between a range of possible values to ensure that the posterior distribution was proper. Prior distributions of the additive \( (u) \) and litter effects \( (p) \) are the following multivariate normal distributions:

\[ f(u|\sigma^2_u) = MVN(0, \sigma^2_u I) \]

\[ f(p|\sigma^2_p) = MVN(0, \sigma^2_p I) \]

where \( \sigma^2_u \) and \( \sigma^2_p \) = the additive and litter variance, respectively. Prior distribution for \( \sigma^2_u, \sigma^2_p, \) and \( \sigma^2 \) 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 location parameters \( (sex, Ryrl, \) and \( DECRI \) configuration effects) were univariate normal distributions, and the posterior conditional distributions of the variance components were inverted \( \chi^2 \). 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 Garcia-Cortés et al. (1998). All iterations of the analysis were used to compute posterior means and SD invoking the ergodic property of the chain (Gilks et al., 1996). By using this approach, all of the available information from the output of the Gibbs sampler could be considered.

The Bayesian approach considers the marginal posterior distribution of the differences between haplotypes assuming that several tests are carried out jointly; however, we did not perform any correction for multiple testing for different traits. Nevertheless, in this case, the posterior probability over (or below) zero is the probability of a difference bigger (or lower) than zero given the data, and it can be understood as a false discovery rate. For this reason, and under the assumption of Gaussian marginal posterior distributions, differences whose highest posterior density at 95% does not include the zero value have a false discovery rate lower than 2.5%, and differences whose highest posterior density at 90% does not include the zero value have a false discovery rate lower than 5%.

Results and Discussion

We have amplified and sequenced a 937-bp amplicon including the near complete coding sequence of the pig DECRI 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 DECRI orthologous sequences, respectively. The alignment of 10 DECRI sequences from pigs belonging to diverse breeds confirmed the existence of one G → C polymorphism at exon 2 (position 181 of the coding sequence), previously described by Clop et al. (2002). Moreover, we found a second G → C polymorphism at exon 5 (position 458 of the coding sequence). The exon 2 and 5 polymorphisms are associated with
Val (C) → Leu (G) and Ser (C) → Thr (G) conservative AA replacements at positions 61 and 153 of the DECR1 protein, respectively. We examined the allelic frequencies of both mutations. Our results indicate the existence of two segregating DECR1 haplotypes in the Landrace, Duroc, Large White, Pietrain, and Iberian pig breeds: Haplotype 1 (H₁:C₁₈₁–C₄₅₈) and Haplotype 2 (H₂:G₁₈₁–G₄₅₈; Table 1). According to the mapping data independently reported by Pérez-Enciso et al. (2000) and Davoli et al. (2002), the pig DECR1 gene is located within the interval of a linoleic QTL on 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 DECR1 coding sequence, we have performed an association analysis in a two-generation Landrace pedigree that included 76 founders and 470 offspring and for which growth, fatness, and meat quality records were available. The genotyping of the Ryr₁ gene in the F₀ revealed that the five males and 56 females were NN, whereas 14 and one females were Nn and nn, respectively. In the offspring, 423 and 47 F₁ pigs were NN and Nn, respectively.

We did not find any substantial differences among DECR1 haplotypes with regard to growth and carcass traits (Table 2) after integrating out the nuisance parameters (variance components, additive, litter, sex, and Ryr₁ effects) by the Bayesian analysis through the Gibbs Sampler. Associations with a posterior probability of a difference bigger than zero over 90% were found for cutlet and shoulder weight. A few meat quality traits, including ICDH (H₂H₂ < H₁H₁; posterior probability of a difference > 0 is > 90%), longissimus thoracis muscle pH at 24 h (H₂H₂ < H₁H₁; posterior probability of a difference > 0 is > 95%), L* value (H₂H₂ < H₁H₁ and H₂H₂ < H₁H₁; posterior probabilities of a difference > 0 are > 90 and > 95%, respectively), and a* value (H₂H₂ < H₁H₁ and H₂H₂ < H₁H₁; posterior probability of a difference > 0 is > 95%) showed relevant associations (Table 3). In Bayesian analysis, the prior distributions have an important role in the posterior inference. However, in this case, the prior information for the systematic effects is vague with null or very low influence in the posterior distributions. Moreover, the inference was focused in the systematic effects, considering as nuisance parameters the rest of unknowns of the model. For this reason, the posterior inference is robust.

The DECR1 enzyme has a key role in the β-oxidation of PUFA and, as a consequence, quantitative variations of its activity or expression might affect fatty acid composition, especially linoleic content and meat quality. In this framework, the simultaneous association of the DECR1 genotype with longissimus thoracis muscle pH at 24 h and color is particularly interesting because the

**Table 1. Frequencies of the DECR1 haplotypes (H₁: C₁₈₁–C₄₅₈; H₂: G₁₈₁–G₄₅₈) in diverse pig breeds**

<table>
<thead>
<tr>
<th>Breed</th>
<th>N</th>
<th>H₁H₁</th>
<th>H₁H₂</th>
<th>H₂H₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duroc</td>
<td>31</td>
<td>0.097</td>
<td>0.710</td>
<td>0.193</td>
</tr>
<tr>
<td>Iberian</td>
<td>22</td>
<td>0.500</td>
<td>0.363</td>
<td>0.137</td>
</tr>
<tr>
<td>Large White</td>
<td>27</td>
<td>0.593</td>
<td>0.370</td>
<td>0.037</td>
</tr>
<tr>
<td>Landrace</td>
<td>76</td>
<td>0.079</td>
<td>0.369</td>
<td>0.552</td>
</tr>
<tr>
<td>Pietrain</td>
<td>28</td>
<td>0.107</td>
<td>0.643</td>
<td>0.250</td>
</tr>
</tbody>
</table>

**Table 2. Association between pig DECR1 haplotypes (H₁: C₁₈₁–C₄₅₈; H₂: G₁₈₁–G₄₅₈) and phenotypic variation of growth and carcass traits in a Landrace outbred population**

<table>
<thead>
<tr>
<th>Trait</th>
<th>H₁H₁ – H₁H₂</th>
<th>H₁H₁ – H₂H₂</th>
<th>H₁H₂ – H₂H₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live weight, kg</td>
<td>1.0 (2.0)</td>
<td>2.3 (2.1)</td>
<td>1.3 (1.1)</td>
</tr>
<tr>
<td>156 d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>171 d</td>
<td>2.0 (2.4)</td>
<td>3.0 (2.5)</td>
<td>1.0 (1.3)</td>
</tr>
<tr>
<td>178 d</td>
<td>1.7 (2.3)</td>
<td>2.6 (2.5)</td>
<td>0.9 (1.4)</td>
</tr>
<tr>
<td>Backfat thickness, mm</td>
<td>-0.15 (0.27)</td>
<td>0.03 (0.29)</td>
<td>0.19 (0.15)</td>
</tr>
<tr>
<td>156 d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>171 d</td>
<td>0.22 (0.28)</td>
<td>0.22 (0.30)</td>
<td>0.00 (0.15)</td>
</tr>
<tr>
<td>178 d</td>
<td>0.25 (0.32)</td>
<td>0.34 (0.35)</td>
<td>0.09 (0.19)</td>
</tr>
<tr>
<td>Carcass length, m</td>
<td>0.38 (0.72)</td>
<td>0.23 (0.76)</td>
<td>-0.15 (0.40)</td>
</tr>
<tr>
<td>Carcass weight, kg</td>
<td>1.0 (1.9)</td>
<td>0.9 (2.0)</td>
<td>0.0 (1.0)</td>
</tr>
<tr>
<td>Backfat thickness, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical</td>
<td>0.14 (0.10)</td>
<td>0.10 (0.11)</td>
<td>-0.03 (0.06)</td>
</tr>
<tr>
<td>Last-rib level</td>
<td>-0.03 (0.08)</td>
<td>0.04 (0.08)</td>
<td>0.07 (0.04)</td>
</tr>
<tr>
<td>Ham weight, kg</td>
<td>0.05 (0.19)</td>
<td>0.07 (0.19)</td>
<td>0.03 (0.11)</td>
</tr>
<tr>
<td>Shoulder weight, kg</td>
<td>0.072 (0.07)</td>
<td>0.12 (0.07)†</td>
<td>0.06 (0.04)</td>
</tr>
<tr>
<td>Cutlet weight, kg</td>
<td>0.15 (0.12)</td>
<td>0.23 (0.13)†</td>
<td>0.07 (0.07)</td>
</tr>
<tr>
<td>Ribs weight, kg</td>
<td>-0.02 (0.15)</td>
<td>-0.07 (0.17)</td>
<td>-0.05 (0.10)</td>
</tr>
<tr>
<td>Bacon weight, kg</td>
<td>0.05 (0.09)</td>
<td>0.11 (0.09)</td>
<td>0.06 (0.05)</td>
</tr>
</tbody>
</table>

*Standard deviations of the differences among genotypes are indicated in parentheses. The number of F₁ individuals for each of the genotypes was: H₁H₁ (n = 29); H₁H₂ (n = 204); and H₂H₂ (n = 237).
†Null difference not included in the highest posterior density of 90%.
oxidation of the hemo muscular pigments, which partly explain muscle redness, is highly dependent on pH kinetics. Moreover, the manipulation of the SFA and PUFA ratio by adding CLA to the diet affects meat color, muscle conductivity, and ultimate pH 24 h postmortem (D’Souza and Mullan, 2002; Tischendorf et al., 2002). The association between the DECR1 genotype and ICDH is also suggestive from a physiological point of view because both enzymes are functionally related. In this way, isocitrate dehydrogenase 1 is involved in the production of cytosolic NADPH, which is required for the activity of several reductases, such as DECR1, and for fatty acid biosynthesis (Shechter et al., 2003). Moreover, isocitrate dehydrogenase 1 regulates the β-oxidation pathway by modulating the levels of acid phytanic, a known agonist to peroxisome proliferator-activated receptors, which are acutely involved in fatty acid degradation (Shechter et al., 2003).

The associations between the polymorphism of the DECR1 gene and meat redness and pH might be explained by the fact that the two allelic variants we have detected are associated with a differential DECR1 enzymatic activity. In fact, the genomic location of this gene coincides with a QTL influencing the linoleic content, the fatty acid double-bond index, and the peroxidability index (Pérez Enciso et al., 2000; Clop et al., 2003). However, we do not favor this interpretation for two reasons. First, the two AA replacements identified at positions 61 (Val/Leu) and 153 (Ser/Thr) are conservative and, in principle, they are not expected to involve a dramatic change on the biochemical properties of DECR1. Second, the associations we have found are scarce and none of them are consistently found in the three available genotype comparisons. As a consequence, the more straightforward explanation would be that the DECR1 polymorphisms we have found are not the causal mutations of the associations. Exon 2 and 5 polymorphisms might be in linkage disequilibrium with the true causal mutation influencing ICDH and muscle color and pH, which may lie in another region of the DECR1 gene or even in a neighboring locus.

Implications

We have characterized two haplotypes in the 2,4-dienoyl CoA reductase 1 gene that are associated with meat color, isocitrate dehydrogenase activity, and pH in a Landrace purebred population. The identification of single nucleotide polymorphisms in the genes governing lipid biosynthesis and degradation will be essential
for understanding the genetic basis of these metabolic processes.

Literature Cited


