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Associations between muscle gene expression pattern and technological and sensory meat traits highlight new biomarkers for pork quality assessment

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ABSTRACT

Meat quality (MQ) results from complex phenomenon and despite improved knowledge on MQ development, its variability remains high. The identification of biomarkers and the further development of rapid tests would thus be helpful to evaluate MQ in pork industries. Using transcriptomics, the present study aimed at identifying biomarkers of eight pork quality traits: ultimate pH, drip loss, lightness, redness, hue angle, intramuscular fat, shear force and tenderness, based on an experimental design inducing a high variability in MQ. Associations between microarray gene expression and pork traits (n = 50 pigs) highlighted numerous potential biomarkers of MQ. Using quantitative RT-PCR, 113 transcript–trait correlations including 40 of these genes were confirmed ($P<0.05$, $|r|\leq 0.73$), out of which 60 were validated ($P<0.05$, $|r|\leq 0.68$) on complementary experimental data (n = 50). Multiple regression models including 3 to 5 genes explained up to 59% of MQ trait variability. Moreover, functional analysis of correlated-trait genes provided information on the biological phenomena underlying MQ.

1. Introduction

Eating and technological qualities of pork result from interactions between pig genetic background, rearing system, slaughtering conditions and carcass and meat processing (Lebret, 2008; Rosenvold & Andersen, 2003; Sellier & Monin, 1994 for reviews). To date, many factors influencing pig meat quality (MQ) have been identified but its variability remains high and the muscle properties underlying high eating quality are still unclear (Ngapo & Gariépy, 2008). Therefore, the identification of biomarkers and the further development of rapid tests would be helpful for the control and improvement of MQ in pork industries. Transcriptomics which enables high throughput screening of expressed genes in a given tissue is of utmost interest to study the development of complex phenotypic traits determined by genetic × environment interactions such as MQ. Working at gene expression level thus appears highly relevant to improve knowledge on the biological mechanisms underlying MQ and identify biomarkers, i.e. genes whose expression level is associated to MQ traits (Te Pas, Hoekman, & Smits, 2011). MQ biomarkers could thus allow determination of traits whose measurement is difficult or expensive like shear force or sensory tenderness or late compared with the high production rate in meat industry, for example ultimate pH (pHu) and drip loss. Some studies have already been conducted to identify markers of pork traits such as pHu, color parameters, drip loss, shear force, intramuscular fat content, … and improved biological knowledge on their development (Hamill et al., 2012; Lobjois et al., 2008; Ponsuksili, Jonas, et al., 2008; Ponsuksili et al., 2009; Te Pas et al., 2010; Wimmers et al., 2007). However, many of them compared contrasted groups for a given trait instead of considering a range of MQ values. Moreover, they did not include a validation step of potential markers on different samples as those used for identification. The present study aimed at identifying and validating biomarkers of eight technological and sensory pork quality traits, based on an experimental design inducing a high variability in MQ. This was achieved through the use of two pig breeds, Large White and Basque, reared in different production systems. Our objectives were to associate eight technological and sensory traits, i.e. pHu, drip loss, color parameters L* (lightness), a* (redness) and h° (hue angle), intramuscular fat (IMF), shear force and tenderness, to muscle transcriptome profiles in order to identify new biological markers of MQ. Most of these traits are related to technological MQ, however they all indirectly also refer to sensory quality: appearance (drip loss, L*, a*, h°), tenderness (pHu, drip loss, IMF, shear force), and juiciness (pHu, drip loss, IMF) (Huff-Lonergan et al., 2002; Lebret, 2009; Ngapo & Gariépy, 2008). Moreover, a functional analysis of genes involved in transcript–trait associations was undertaken to better understand the biological phenomena underlying MQ. Afterwards, the highest transcript–trait correlations were confirmed on the same data set using RT-PCR. Finally,

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confirmed biomarkers were tested for internal validation on complementary data from the same animal design.

2. Material and methods

2.1. Ethical statement

The experiments were conducted following French guidelines for animal care and use edited by the French Ministries of High Education and Research, and of Agriculture and Fisheries (http://ethique.ipbs.fr/sdv/charteexpeanimale.pdf). All animals were reared and slaughtered in compliance with national regulations and according to procedures approved by the French veterinary Services. Our research unit was a holder of a pig experimentation agreement (No. C-35-275-32) and all the technical and scientific staff involved in the experiments were holder of an individual agreement for experimentation on living animals, delivered by the Veterinary Services of the French Ministry of Agriculture.

2.2. Animals

Two experimental replicates (R1 and R2) were undertaken in two successive years, each including 50 castrated males of pure local Basque breed (B, n = 30) distant from other European pig breeds and leading to high quality pork products (Guéblez, Labroue, & Mercat, 2002; Laval et al., 2000), or pure commercially selected Large White (LW, n = 20) breeds. These pigs were reared in different production systems themselves influencing MQ traits (Guéblez et al., 2002; Lebret, Prunier, et al., 2011). R1 was used for identification and confirmation of expression patterns related to MQ and R2 for internal validation of transcript–trait correlations.

In R1, 20 B littermates issued from 10 litters (6 boars) and 20 LW littermates issued from 10 litters (9 boars) were reared in two different housing systems at INRA experimental farm (Saint-Gilles, France), with 10 pigs of each breed in each system. At the average body weight (BW) of 35 kg, littermates were placed in either a conventional (C) housing system (one pen per breed, totally slatted floor, 1.0 m^2/pig): pigs LWC and BC, or an alternative (A) housing system (one pen per breed, indoor bedding and free access to an outdoor area, 2.4 m^2/pig): pigs LWA and BA. In addition, 10 B castrated males, half-littermates of BC and BA pigs (previous birth batch issued from 9 litters and 6 boars including 4 common boars with BA and BC) were placed at 35 kg BW in an extensive (E) free-range production system (pen of 2.5 ha with a shed, 650 m height) in a farm of the Basque pork chain (Banca, 64430, France): pigs BE.

In order to slaughter the LWC, LWA, BC, BA and BE pigs (n = 10 per group) at similar BW (around 145 kg) and around the same time, and based on the differences in growth rate between breeds and production systems (Alfonso, Mourot, Insausti, Mendizabal, & Arana, 2005; Guéblez et al., 2002), BE pigs were put on experiment 5 months, and BC and BA pigs 3 months before LWC and LWA pigs.

Pigs of C and A systems received standard growing and finishing diets. BE pigs had free access to the natural resources of the extensive pen (grass, acorns and chestnuts) and received a complementary standard growing–finishing diet. All pigs had permanent access to water.

Pigs were slaughtered at the average BW of 145 kg. BC, BA, LWC and LWA pigs were slaughtered at INRA experimental slaughterhouse (Saint-Gilles) in 4 sessions, each including pigs from these 4 groups, on the basis of their BW. BE pigs were slaughtered in a commercial slaughterhouse of the B pork chain (Saint-Jean-Pied de Port, 64220, France) in one session. Pre-slaughter handling and slaughtering conditions were standardized between the 2 slaughterhouses, with similar fasting period (around 36 h) and slaughtering by electrical stunning (350 V) and exsanguination.

The second experimental replicate R2 (n = 50), similar as R1, i.e. including 10 pigs in each of the LWC, LWA, BC, BA and BE groups was undertaken for internal validation of correlations between gene expressions and MQ traits. In R2, BC and BA castrated males littermates were issued from 10 litters and 6 boars, and LWC and LWA were issued from 7 litters and 7 boars. The BE pigs were issued from 9 litters and 7 boars including 5 common boars with BC and BA pigs. Rearing and slaughtering conditions of pigs in R2 replicate were similar as in R1.

2.3. Technological and sensory MQ traits

Several muscle and MQ traits were determined on the *Longissimus* muscle (LM, right loin) of all LW and B pigs in the same laboratory and with similar sampling conditions and analytical methods for both R1 and R2 pigs (Lebret, Damon, et al., 2011). Regarding MQ traits considered for identification of markers, pHu, color coordinates L*, a*, b*, C* and h°, and intramuscular fat (IMF) content was assessed on LM samples taken 24 h after slaughter according to Lebret, Prunier, et al. (2011), with few adaptations: pHu was determined after homogenization of LM sample (last rib) in iodoacetate; meat color (1st lumbar vertebra (LV) level) was determined after 1 h 30 blooming; IMF (chloroform–methanol extract) was determined on minced frozen sample without prior freeze-drying. Drip loss was determined on a 100 ± 10 g LM slice (1st LV level) without prior trimming, between 1 and 3 d p.m. according to Honikel (1998). Meat shear force and sensory tenderness were also determined. Samples of left loin, 8th–12th dorsal vertebrae (DV) level for shear force and right loin, 4th–12th DV level for sensory tenderness, aged up to 4 d p.m. were deboned, vacuum-packed and stored at −20 °C. Maximal shear force (SF) was determined from 10 measurements per sample, according to Honikel (1998). After thawing, 4 × 8 cm samples parallel to fiber axis were vacuum-packed, heated (70 °C, 50 min), cooled at room temperature and cut in 1 cm^2 section for measurement of SF perpendicularly to muscle fibers using a Warner–Bratzler cell fitted on a universal testing machine (Instron France, Guyancourt, France). Sensory tenderness was assessed by a 12 members trained panel, with one sample per breed and housing system at each session. After thawing, roasts were cooked in an oven up to a core temperature of 80 °C (around 55 min). Samples (pieces of roast slices) were scored for tenderness on a scale from 0 (low) to 10 (high); individual panelist scores were averaged for data analyses.

2.4. RNA extraction

Thirty minutes after exsanguination, a LM sample (last rib) was collected on R1 and R2 pigs, immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction (Damon, Wyszynska-Koko, Vincent, Hérault, & Lebret, 2012). Briefly, total RNA was extracted using trizol reagent and purified on silica-based spin-columns. Quality and concentration of total RNA were checked by electrophoresis and UV spectrometry, respectively.

2.5. Loin muscle transcriptome analysis and correlations with MQ traits

Microarray hybridization was undertaken as previously described (Damon et al., 2012) using the specific 15 K pig skeletal muscle microarray (GEO (Gene Express omnibus) accession no. GPL11016; Damon, Hérault, Vincent, Le Roy, & Cherel, 2011). Briefly, the 50 RNA samples from the R1 pigs and the reference (pool of an equal amount of the 50 LM RNA) were labeled with Cy3 and Cy5, respectively (Quick-Amp labeling kit, Agilent Technologies, Santa Clara, USA) and hybridized at 65 °C for 17 h (Agilent SureHyb hybridization chambers). After washing, microarrays were scanned at 5 μm/pixel resolution (scanner G2505B, Agilent) and images were analyzed (Agilent feature extraction software 9.5). These MIAME compliant microarray