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Genetic variability of transcript abundance in pig skeletal muscle at slaughter: Relationships with meat quality traits[1]

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ABSTRACT: A family structured population of 325 pigs (females and barrows) was produced as an intercross between 2 commercial sire lines and was subjected to a systematic transcriptome analysis of LM samples obtained shortly after slaughter. Additionally, measurements of meat quality traits of fresh and cooked loin were gathered from the same animals. The transcriptome analysis was achieved by microarray hybridization, using a custom repertoire of 15,000 6mer DNA probes targeting transcripts expressed in growing pig skeletal muscle. These data allowed us to estimate the heritability of expression abundance for each of the quantified RNA species. The abundance of 9,765 RNA was estimated as heritable with a false discovery rate of 5%, from which 1,174 were deemed as highly heritable ($h^2 > 0.50$). We also observed a large number of transcripts whose LM expression abundance is genetically correlated with 4 meat quality traits: the loin pH measured at 45 min postmortem (pH45), 253 transcripts; the loin cooking loss (CL), 134 transcripts; the cooked loin shear force (SFc), 184 transcripts; and the loin color redness (a*) value, 190 transcripts. Heritable and meat quality genetically correlated transcripts showed an over-representation of biological processes involved in the induction of apoptosis (genetically correlated with CL), complement activation (genetically correlated with SFc), glucose metabolism (genetically correlated with a*), and cation channel activity (genetically correlated with pH45). Overall, the biological functions highlighted in the highly heritable transcripts and the lack of transcript that would be genetically correlated with LM glycolytic potential suggest that the genetic variability of the LM postmortem transcriptome is focused on muscle tissue response to postmortem ischemia and reflects more distantly the antemortem muscle physiology. Because of the contrasting distributions of the genetic correlations between LM RNA concentrations and the different meat quality traits studied, indirect selection strategies of meat quality traits based on measurements of selected LM RNA species could be only proposed for a subset of the analyzed meat characteristics (pH45, SFc, a*, CL). A substantial improvement in the efficiency of selection for these meat quality traits could result from measuring muscle RNA concentrations on selection candidates, if the same genetic parameters can be verified using in vivo-sampled muscles.

Key words: gene expression, indirect selection, loin meat quality, longissimus muscle, *Sus scrofa*, transcriptome analysis

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INTRODUCTION

Quantification of each of the RNA species found in animal cells or transcriptome analysis developed as a follow-up of genome sequencing and array technology. Transcriptome analysis initially allowed an in-depth biological description of cells or tissues that were exposed to well-defined treatments, using differential expression experiments with a limited number of samples. Further improvements in technology standardization and throughput now allow for a large number of samples to be reliably processed. Population-scale transcriptome analysis can, therefore, be used to assess the full extent

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of the variation in transcript concentrations in a given cell type or tissue among individuals in a population.

Variations in transcript abundance can drive or accompany variations in livestock production phenotypes, as mediating responses to environmental challenges or the translation of genetic variability. Meat qualitative traits are contributed by the physiology of corresponding skeletal muscles, from development (Wimmers et al., 2007) until conversion from muscle to meat (Davoli and Braglia, 2007; Ponsuksili et al., 2008). Transcriptome analysis of skeletal muscle thus has the potential to uncover variations in the abundance of selected transcripts, which could be seen and used as intermediate outcomes of the genetic variation that contributes to meat quality traits. Indirect selection strategies based on these intermediate molecular phenotypes might prove valuable for the genetic improvement of meat quality traits. Opportunities for using those molecular phenotypes in breeding applications ultimately depends on the genetic parameters for these traits, defined as skeletal muscle RNA concentrations, heritabilities ($\mathbf{h^2}$), and genetic correlations ($\mathbf{r_G}$) with meat quality traits of economic value. Here, we report a population-scale analysis of transcript abundance in pig skeletal muscle, which enabled the estimation of genetic parameters for these molecular phenotypes.

MATERIALS AND METHODS

Procedures and slaughtering facilities were approved by the French Veterinary Services.

Experimental Population

The 325 pigs (females and barrows) used in the gene expression analyses are part of a larger F_2 resource population of 1,000 animals which was setup within the framework of a QTL detection program. These animals were produced as a second generation intercross between 2 commercial sire lines (FH016, Pietrain type line, and FH019, synthetic line from Hampshire, Duroc, and Large-White founders, France-Hybrides, St Jean de Braye, France).

The 325 animals considered for LM transcriptome analysis were produced as 4 half-sib families, using 4 F_1 males and 15 F_1 females, where each F_1 male was mated with the same group of 3 to 4 full-sib F_1 females for 2 to 3 successive litters, thus purposely generating large-size full-sib families. All F_2 animals were genotyped as homozygous wild type genotypes NN and rn+rn+ with regard to the HAL and RN loci, respectively (Otsu et al., 1992; Milan et al., 2000).

All F_2 animals were raised on the same farm and slaughtered in the same abattoir. All pigs were killed at 110 kg of BW according to standard procedures for commercial slaughtering (Orleans Viandes, Fleury-les-Aubrais, France). Half-carcasses were chilled at −12°C for 4 h postmortem, then stored at 3°C, and cut at 24 h postmortem.

Meat Quality Measurements

Loin meat quality measurements are briefly described because they have been already detailed previously (Laville et al., 2007). The summary statistics of the meat quality traits as recorded in the whole resource population are provided in Table 1.

Loin Processing and Measurements. The loins were sliced at 30 h postmortem, chops were stored at 4°C for 2 d, then grilled in a dry oven at 240°C for 30 min. Drip loss (**DL**) was calculated as 100 × (1 − weight after storage/weight before storage). Cooking loss (**CL**) of the LM was calculated as 100 × (1 − weight after cooking/weight after storage).

Warner-Bratzler Shear Force Measurements. For both the raw (**SFr**) and the cooked (**SFc**) meat shear force measurements, 10 cylinders of 1 cm in diameter were sheared in a Warner-Bratzler cell. The maximal force recorded during shearing (at rupture time) was used as the cylinder shear force value and expressed in Newtons.

Loin Color Components and pH. Indicators of lightness (L*), redness (a*), and yellowness (b*) were recorded from each rib eye surface within 2 h after loin slicing.

The pH at 45 min in LM (**pH45**) was measured in a 1:10 (wt/wt) dispersion of LM in a 5 m*M* sodium io-

Table 1. Summary statistics for loin meat quality traits[1]

Loin quality trait	Abbreviation	Unit	n	μ	σ	Range
pH 45-min	pH45	pH unit	840	6.55	0.18	5.45 to 7.40
pH 24-h	pH24	pH unit	1,023	5.71	0.20	5.37 to 6.73
Glycolytic potential	GP	μmol/g	805	159.40	24.97	70.30 to 276.80
Intramuscular fat	IMF	g/100 g	804	2.24	0.66	0.79 to 4.92
Color CIE L*	L*	CIE u	852	49.63	3.05	37.92 to 57.54
Color CIE a*	a*	CIE u	852	7.86	1.22	4.64 to 13.00
Color CIE b*	b*	CIE u	852	5.12	1.30	0.48 to 9.30
Shear force, cooked	SFc	N	753	33.65	1.16	17.94 to 55.48
Shear force, raw	SFr	N	753	35.34	1.19	17.92 to 61.81
Cook loss	CL	%	739	26.46	2.58	15.01 to 38.52
Drip loss	DL	%	731	1.72	0.95	0.06 to 5.63

[1]L* = lightness; a* = redness; b* = yellowness.

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doacetate/150 m*M* potassium chloride buffer. Loin ultimate pH (**pH24**) was recorded at 24 h postmortem in LM at the last rib level on half-carcasses before cutting.

Intramuscular Fat Content. Total lipids were extracted according to the method outlined by Folch et al. (1957). The lipid content of fresh tissue (g/100 g) was obtained by considering DM content determined from the weight of minced LM tissues.

Glycolytic Potential. One gram of LM was homogenized into 10 mL of 0.55 *M* perchloric acid. The glycolytic potential (**GP**) was then calculated according to Monin and Sellier (1985) as GP = 2([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate]. The GP was expressed as micromoles of lactate equivalent per gram of wet tissue.

Microarray Design and Annotation

A specific repertoire of 15,198 6mer DNA probes was set up to maximize the information content and gene coverage in analysis of pig skeletal muscle transcriptome at slaughter (Damon et al., 2011). Briefly, we used consensus contig sequences from SIGENAE pig transcript assembly (SIGENAE v8, http://www.sigenae.fr/) selected from previous analyses of LM transcriptome (Lobjois et al., 2008). Thirty-three thousand contig sequences were submitted to eARRAY online software v4.5 (Agilent Technologies, http://earray.chem.agilent.com/earray) to design one to two 60-mer oligo probes per contig sequence (average of 1.33 probe per contig). Analysis of test LM RNA samples using all designed probes allowed for the final selection of 15,198 informative probes, while maximizing gene coverage when analyzing LM samples. Microarrays were manufactured by in situ synthesis on glass slides using an Agilent 15K (8-plex) microarray format (Agilent Technologies France, Massy, France). This repertoire was deposited in NCBI GEO online resource (http://www.ncbi.nlm.nih.gov/geo/) under reference GPL11016.

The identification of transcripts targeted by each of the 60-mer probes was conducted from probe and contig sequence homology searches using known pig transcripts (Ensembl Sscrofa9, http://www.ensembl.org/Sus_scrofa/; NCBI *Sus Scrofa* RefSeq, http://www.ncbi.nlm.nih.gov/RefSeq/) or the transcriptomes of related species (*Bos taurus*, *Equus caballus*, *Homo sapiens*). Annotation was based on similarity and quality criteria established by Casel et al. (2009; i.e., at least 18 consecutive base pairs within 60-mer probe sequence and 85% of homology). Overall, we validated at least 1 reference annotation for 12,939 probes (85%) corresponding to 9,169 unique genes.

RNA Extraction and Microarray Hybridization

Ten-gram samples from the LM were collected 20 min after stunning and bleeding and snap frozen in liquid N. Total RNA was extracted by crushing the frozen tissue in Trizol reagent (Invitrogen, Cergy-Pontoise, France).

Total RNA (350 ng) from each animal was labeled individually with Cy3 using the Low RNA Input Linear Amplification Kit PLUS, One-Color (ref 5188–5339, Agilent Technologies France). A single 1-color labeled sample was hybridized (65°C, 17 h) per array in Agilent's SureHyb Hybridization Chambers containing 300 ng of Cy3-labeled cRNA sample. Microarrays were scanned at 5 μm/pixel resolution using the Agilent DNA Microarray Scanner G2505B, and images were analyzed with Agilent Feature Extraction Software (Version 9.5), using the GE2-v5_95_Feb07 FE extraction protocol. The raw intensities from the 15,198 probes were log-transformed and then centered within sample by subtraction of the sample median value across all probes. The microarray data have been deposited in the NCBI GEO and are accessible through GEO Series accession number GSE28714 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE28714).

Functional Analysis

The functional analysis of transcript lists of interest was undertaken by the analysis of the enrichment for specific Gene Ontology (**GO**) terms using the Database for Annotation, Visualization and Integrated Discovery (**DAVID**; Huang et al., 2009a,b) as implemented in the online software (http://david.abcc.ncifcrf.gov/). The annotated probes for both the background reference and the lists of interest were matched to their human orthologs using official human Entrez gene ID. Analyses were performed using the GO-FAT terms of DAVID. The background included 12,063 probes, representing 8,638 ortholog human genes. The functional annotation clustering of the enriched GO terms was performed with a similarity score (kappa) of 0.75 and a multiple linkage threshold of 0.50. Cluster enrichment scores are geometrical means of *P*-values for each GO term included in the cluster and expressed on a negative logarithmic scale. Clusters were selected using enrichment score cutoffs of 1.30 and 1.10 for highly heritable transcripts and meat quality trait correlated transcripts, respectively.

Genetic Parameters Estimation

For each hybridization probe, we conducted a univariate variance analysis and independent bivariate analyses combining RNA quantification measurement with each of the meat quality traits under study. We analyzed the data with a mixed model of variance including a random polygenic effect fitted under an animal model, a fixed effect for slaughter batch, and a fixed effect for sex (intramuscular fat model). The chop weight was used as a covariate for the CL and SFc models. A fixed effect fitting hybridization batch (15 levels) was used for RNA quantification traits. A

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REML methodology was applied to estimate the variance components, corresponding genetic parameters, and their SE, using the software package ASREML 2.0 (Gilmour et al., 2006). The pedigree used to set up the animal model included 3 generation of ancestors in addition to the phenotyped animals. The meat quality phenotypes from all NN rn+rn+ genotype animals of the entire F_2 resource population were used in bivariate analyses when available (731 to 1,023 records per trait, as reported in Table 1). Variance component estimates were considered in the results only when model log-likelihood converged.

Proportion of Heritable Transcripts

The probability of estimating a nonzero heritability in the absence of genetic variability was determined empirically from the distribution of 3,000 estimates produced using 3,000 independent phenotype data sets of 325 records simulated without genetic variation. A false discovery rate (**FDR**) was calculated using the software package R/qvalue (http://cran.r-project.org/web/packages/qvalue/index.html) according to the methodology proposed by Storey and Tibshirani (2003).

RESULTS

Estimates of Heritability for RNA Quantification Abundance

From univariate analyses, we estimated narrow sense heritability values as significantly different from zero with a FDR of 5%, corresponding to a threshold of 0.051 when applied to this result set, for 9,765 probes over 15,198 probes analyzed. The median value of these significant estimates of additive genetic variation is 0.236 (the median of SE estimates is 0.211), including heritability estimates greater than 0.50 for 1,174 probes targeting 1,146 contigs. The distribution of the 9,765 significant heritability estimates is presented in Figure 1. Those 9,765 probes represent 65% of probes, 9,219 unique design contig sequences, and were annotated as products from 6,000 unique genes. We have verified that the heritability estimates presented here were not substantially correlated with either the average expression level ($r = -0.11$) or the expression level SD ($r = 0.09$), suggesting that our observations are not the result of a scaling effect.

Biological Processes Represented in Highly Heritable LM Transcripts

To study genes associated with highly heritable probe signals into clusters of related functional terms, a functional annotation clustering was performed with 1,174 probes, corresponding to 911 annotated transcripts that were transcribed from 843 unique genes. Nine clusters were found to be significant with an enrichment score greater than 1.30 (Table 2). The lists of genes included within each cluster are listed in supplemental file 1 (available in the online version of this paper). The highlighted GO terms show an over-representation of GO terms associated with the regulation of Rho protein signal transduction (cluster 1), the induction, regulation, and process of apoptosis (clusters 2, 4, and 8), calcium transport and metal ion binding (clusters 3 and 6), focal adhesion and adherent cell junctions (clusters 5 and 7) and glucose metabolism (cluster 9).

Genetic Correlations Between RNA Concentrations and Meat Quality Traits

Estimation of the genetic correlation (r_G) between expression and meat phenotype was determined only for the 9,765 probes that showed a significant genetic variability ($h^2 > 0.051$). The limited number of animals available per molecular trait ($n = 325$) makes it possible to only broadly estimate the genetic correlations between RNA concentrations and meat quality traits, with the SE of r_G estimates ranging from a median SE of 0.48 (a*) to a median SE of 0.60 (pH45; Table 3).

The uncertainty of those estimates is reduced for the most heritable molecular traits, ranging from a me-

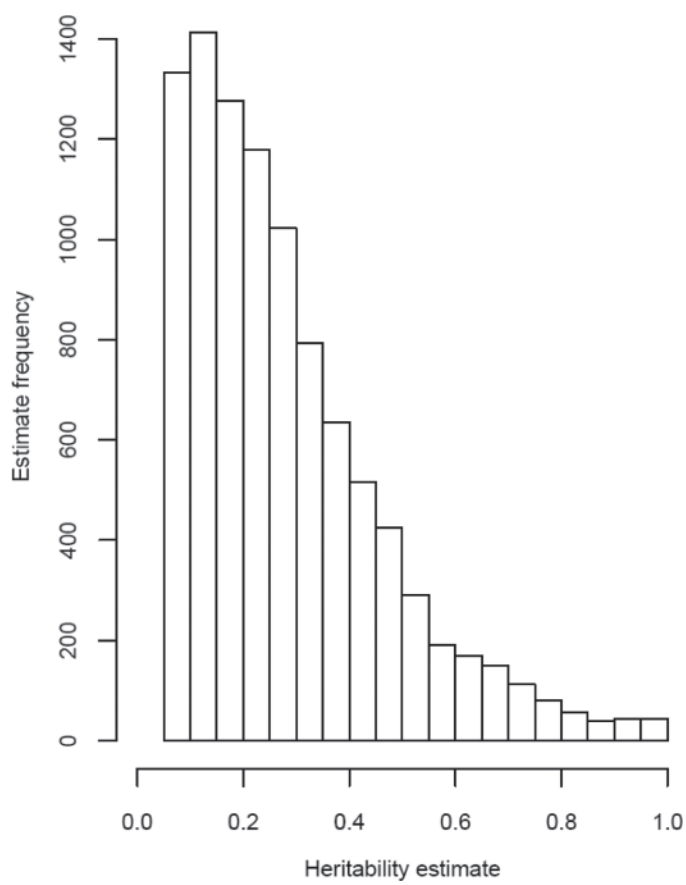

Figure 1. Distribution of heritability estimates for transcript abundance in postmortem LM, as measured using 9,765 probes ($h^2 > 0$ under a false discovery rate of 5%).

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Table 2. Functional annotation clustering of highly heritable transcripts ($h^2 > 0.50$)

Cluster	ES[1]	n_{GO}[2]	Cluster-specific GO term[3]	n_G[4]	*P*-value[5]
1	1.99	3	GO:0035023~regulation of Rho protein signal transduction	13	0.009
2	1.95	3	GO:0043065~positive regulation of apoptosis	39	0.013
3	1.58	3	GO:0051924~regulation of calcium ion transport	9	0.037
4	1.57	3	GO:0006917~induction of apoptosis	29	0.037
5	1.52	5	GO:0005925~focal adhesion	13	0.028
6	1.52	3	GO:0046872~metal ion binding	203	0.031
7	1.38	3	GO:0005924~cell-substrate adherens junction	14	0.019
8	1.32	4	GO:0006915~apoptosis	44	0.096
9	1.30	3	GO:0006006~glucose metabolic process	18	0.048

[1]Cluster enrichment score (ES).
[2]Number of Gene Ontology (GO) terms (n_{GO}) associated with this cluster.
[3]Identity of the lowest hierarchical level and most specific GO term.
[4]Number of different genes (n_G) associated with the specific GO term.
[5]Modified Fisher's exact test *P*-value.

dian SE of 0.37 (a*) to a median SE of 0.44 (pH45), when considering only those probes where h^2 in bivariate analyses is greater than 0.50 (929 to 1,509 probes, Table 3). We summarized in Table 3 the properties of the distribution of the r_G estimates for these highly heritable transcript levels (interquartile range and median value), further restricted to the r_G estimates with an SE less than 0.50 to focus on the most interpretable results. We illustrated contrasting examples of the distributions of these sets of genetic correlation estimates for 3 different traits (pH45, GP, a*) in Figure 2. Larger numbers of genetically correlated transcripts ($|r_G| > 0.70$) were observed for the measurements of pH45, SFc, a*, and CL than regarding other meat quality traits. In contrast, the measurements of DL, GP, and pH24 show a substantial genetic correlation with very few muscle transcript levels.

Functional Analysis of Transcripts Genetically Correlated with Meat Quality Traits

The list of genes assigned to transcripts that were quantified as heritable traits ($h^2 > 0.50$) and were genetically correlated ($|r_G| > 0.70$) with pH45, SFc, a*, and CL were characterized for functional enrichment in GO terms using the microarray repertoire as background. The clusters of GO terms enriched in the correlated gene lists for each of these 4 traits established using an enrichment scores cutoff of 1.10 are presented in Table 4. For each cluster, the most specific GO term explicit definition and the enrichment *P*-value associated with this term are shown. The lists of genes included within each cluster are listed in supplemental file 2 (available in the online version of this paper).

Table 3. Distribution and SE of genetic correlation (r_G) estimates between LM transcript concentrations and meat quality traits

		$0 < SE\ r_G < 1$		h^2 [RNA] > 0.5, SE r_G < 0.5						
		All r_G values		All r_G values				$\|r_G\| > 0.7$		$\|r_G\| > 0.9$
MQ trait[1]	h^2[2]	n[3]	SE r_G[4]	n[3]	IQR r_G[5]	Q2 r_G[5]	SE r_G[4]	n[3]	SE r_G[4]	n[3]
pH45	0.12	7,489	0.60	929	1.07	−0.11	0.43	253	0.39	55
pH24	0.21	8,775	0.51	1,140	0.37	−0.07	0.39	4	0.27	0
GP	0.22	8,987	0.52	1,164	0.24	0.02	0.40	0	—	0
IMF	0.11	7,386	0.59	1,001	0.32	−0.04	0.44	31	0.41	6
L*	0.20	7,760	0.54	1,139	0.51	−0.11	0.41	66	0.35	9
a*	0.44	7,303	0.48	1,171	0.56	0.22	0.37	190	0.25	44
b*	0.24	8,608	0.55	1,171	0.41	0.03	0.43	44	0.31	7
SFc	0.26	8,175	0.56	1,381	0.58	0.25	0.44	184	0.32	81
SFr	0.43	8,311	0.49	1,509	0.60	0.05	0.38	36	0.28	3
DL	0.22	8,346	0.55	1,452	0.28	0.04	0.43	12	0.34	1
CL	0.21	8,030	0.53	1,428	0.67	0.13	0.42	134	0.35	14

[1]Meat quality (MQ) traits are LM pH 45 min postmortem (pH45); loin 24-h pH (pH24); glycolytic potential (GP); intramuscular fat (IMF); loin color variables L* (lightness), a* (redness) and b* (yellowness); shear force on cooked (SFc) or raw (SFr) loin; loin drip loss (DL); and loin cooking loss (CL).
[2]Median value of the meat quality trait heritability estimate in bivariate analyses.
[3]Number of probes meeting heading-specified conditions on h^2, SE r_G, and r_G, reported for all converged bivariate analyses of heritable transcripts levels.
[4]Median value of the genetic correlation estimates SE.
[5]Interquartile range (IQR) and median value (Q2) of genetic correlation estimates (r_G) distribution.

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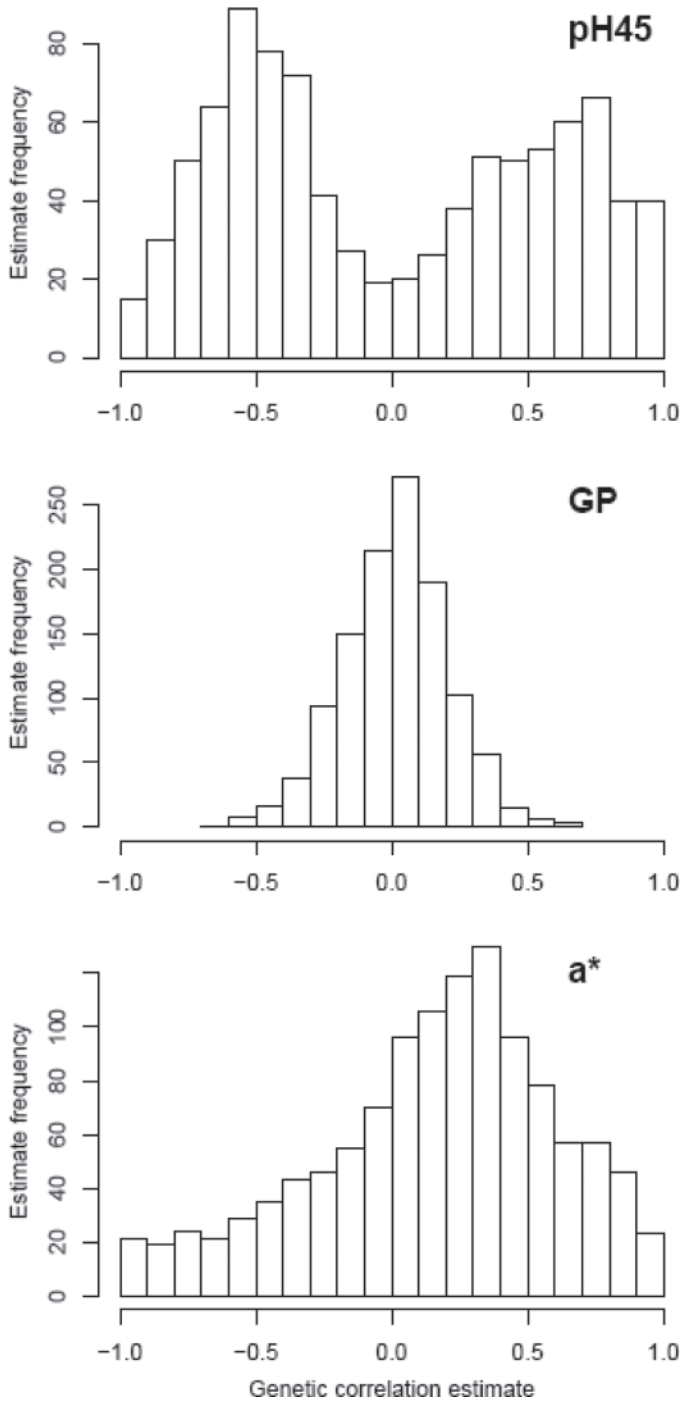

Figure 2. Distribution of genetic correlation estimates between heritable transcripts ($h^2 > 0.50$, SE $r_G < 0.50$) and LM pH 45 min postmortem (pH45), LM glycolytic potential (GP), and loin color redness (a*). r_G = genetic correlation.

For CL, 3 clusters highlight the genetic variability of transcripts involved in the induction of apoptosis and regulation of fatty acid oxidation. Transcript levels from genes known to be involved in complement activation and protein polymerization were observed as genetically correlated with SFc. The clusters associated with transcripts genetically correlated with a* are focused on glucose metabolism and neoglucogenesis. The transcripts that are genetically correlated with pH45 highlight 2-cell compartments, the lumen of intracellular organelles and the membrane of cytoplasmic vesicles, whereas the voltage-dependent cation channel activity is highlighted as a biological process.

DISCUSSION

Genetic Variability of Transcript Abundance in Pig Postmortem LM

Our results document that 65% of the postmortem LM RNA concentration measurements, as assayed by hybridization on a muscle-specific array, are genetically determined because essentially all probes were informative (Damon et al., 2011). The limited number of full- and half-sib families included in this experimental population may generate a sampling variance in estimates. Nonetheless, the SE of the heritability estimates for these molecular traits do support our interpretation of the actual genetic variability of LM transcriptome, even if the individual transcript heritability values were only broadly assessed.

The observed genetic variability in LM transcript concentration is consistent with other reports of genetic variability in transcript abundance in a variety of organisms. Studies in yeast have shown that more than 60% of the transcripts whose abundance was measured in segregating populations could be characterized with heritabilities greater than 0.69 (Brem and Kruglyak, 2005). The available data from human tissues show that the relative concentrations of respectively 59 and 71% of transcripts, as measured in total blood or adipose tissue respectively, are significantly heritable, with an average heritability estimate of 0.30 for those heritable transcripts (Emilsson et al., 2008). Data from studies performed in mice document a median value of 0.14 for heritability estimated among all of the possible transcripts quantification levels measured in adipose tissues (Petretto et al., 2006).

Functional Interpretation of Transcriptome Genetic Variability

Apoptosis induction has been proposed as an essential mechanism involved in the transformation of muscle in meat, contributing to the tenderization of meat through an early triggering activity of caspases (Herrera-Mendez et al., 2006; Ouali et al., 2006). Variations in the regulation of apoptosis have been further suggested as an underlying cause of differential tenderness in bovine meat by the observation of differences in the concentrations of proteins from the inner mitochondrial membranes of muscle samples taken as early as 10 min postexsanguination (Laville et al., 2009). Therefore, the observation of large individual and genetic variation in the induction of the apoptosis processes in postmortem LM samples can be seen as a consequence of the exsanguination-induced muscle ischemia. The selection of a muscle sampling point at 20 min after stunning

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Table 4. Functional analysis of heritable transcripts that were genetically correlated with meat quality traits

Cluster	ES[1]	n_{GO}[2]	Cluster-specific GO term[3]	*P*-value[4]
			Loin cooking loss (CL, 79 genes)	
1	1.79	3	GO:0043065~positive regulation of apoptosis	0.015
2	1.59	3	GO:0042981~regulation of apoptosis	0.024
3	1.23	4	GO:0046320~regulation of fatty acid oxidation	0.036
			Cooked loin shear force (SFc, 110 genes)	
1	1.59	21	GO:0006958~complement activation, classical pathway	0.002
2	1.15	5	GO:0032271~regulation of protein polymerization	0.031
			Loin redness a* (119 genes)	
1	2.23	3	GO:0006006~glucose metabolic process	0.002
2	1.62	6	GO:0006111~regulation of gluconeogenesis	0.002
3	1.5	14	GO:0006739~NADP metabolic process	0.008
4	1.26	6	GO:0030072~peptide hormone secretion	0.045
			pH45 min LM (122 genes)	
1	1.45	3	GO:0070013~intracellular organelle lumen	0.051
2	1.27	3	GO:0030659~cytoplasmic vesicle membrane	0.049
3	1.13	12	GO:0022843~voltage-gated cation channel activity	0.060

[1]Cluster enrichment score (ES).

[2]Number of different Gene Ontology (GO) terms associated with this cluster.

[3]Identity of the lowest hierarchical level and most specific GO term. NADP = NAD phosphate.

[4]Modified Fisher's exact test *P*-value.

may have highlighted individual variation in the early and unbuffered response to a sudden challenge.

Proportion of Transcripts Genetically Correlated with Meat Quality Traits

The genetic correlation between 2 traits is thought to reflect either genetic linkage between loci affecting each trait or pleiotropic effects of the same loci on both traits. Our population design maximizes long-range genetic linkage effects in the F_2 generation. Therefore, this mechanism may have substantially inflated some of our estimates of the genetic correlations between molecular and meat quality traits in this population. The accuracy of the genetic correlation estimates proved to be severely limiting for interpretation because of the limited size of our data set. However, we propose a cautious interpretation of the entire distribution of the genetic correlations estimates for each meat quality trait that we restricted to the most heritable RNA concentrations.

The large differences we observed between the distributions of the genetic correlation estimates among the different meat quality traits was not expected, considering the genome-wide relevance of the repertoire of probes used and the existence of genetic variability in all of the meat quality traits studied. A naïve assumption may have been to identify, among the large number of heritable transcripts, some transcripts that would be genetically correlated with each of the meat quality traits. This would be expected if postmortem muscle transcriptome was patterning the whole muscle physiology. In this respect, the lack of RNA species showing substantial genetic correlations with the meat pH24 and the glycolytic potential (traits nonetheless determined by a sizable genetic variation, h^2 of GP = 0.22) brings into question the dynamics of phenotype construction and the persistence of its footprint on gene expression profiles over time. To explain this observation, one may simply hypothesize that the expression of genes involved in the genetic control of muscle glycogen stores depletion and recovery during antemortem period does not persist, or at least does not dominate the patterns of postmortem gene expression that we analyzed. But this hypothesis does not account for the extent of our observation that all of the genes contributing to the control of glycogen storage and mobilization are well represented in the array used for transcriptome analysis, are transcribed in LM postmortem, and some even showed substantial genetic variation in LM mRNA content (as those reported for their genetic correlation with meat redness a*). An alternative hypothesis, therefore, would be that the gene expression levels are essentially constrained by homeostatic regulatory mechanisms, which over time are buffering all sources of variation, including the genetic variability, in this case between the antemortem and postmortem time points. Gene expression buffering has been recognized as an essential control mechanism to dampen the consequences of transcription noise (Fraser et al., 2004; Raser and O'Shea, 2004) and proposed as an essential function of microRNA (Wu et al., 2009). This latter hypothesis would support a relative independence between the genetic variability of antemortem muscle transcription (the genome expression profile that one may suppose is responsible for mediating or accompanying variation in the control of antemortem glycogen stores) and the genetic variability of postmortem transcription profiles that we evaluated in this work, reflecting muscle reaction to ischemia. Finally, this independence would support the observed disconnection between the genetic variability of the muscle glycolytic potential or the ultimate pH, and the genetic variability of postmortem transcript abundance.

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We observed that the genetic variability of the postmortem LM transcriptome is linked with the genetic variability of a postmortem muscle physiology measurement (pH45) and distantly assessed measurements of meat color (a*) and loin cooking (SFc, CL). The transcription profiles may subsequently better reflect muscle physiology contemporary of the RNA sampling point, beside its translation into remote consequences that affect meat consumption. A recent study reported significant phenotypic associations between postmortem LM transcriptome analysis and loin color redness a* (Te Pas et al., 2010). Our data suggest that the associations reported by these authors may result from a genetic determinism with pleiotropic consequences on the transcriptome and loin redness.

Functional Interpretation of Transcripts Genetically Correlated with Meat Quality Traits

Cooking Loss. The functional analysis of transcripts that show a substantial genetic correlation with loin cooking losses highlights apoptosis as an overrepresented cell function in this group of genes. The first steps of apoptosis involve the condensation of the cytoplasm, cell shrinking, and result in the expansion of extracellular space (Bortner and Cidlowski, 2007). The genetic correlations that we observed may thus result from a genetic variability that drives the dynamics of apoptosis induction postmortem and subsequently affects the water-holding capacity of meat during cooking.

Cooked Loin Shear Force. Complement activation has been described as an essential component of skeletal muscle ischemia-reperfusion injury (Weiser et al., 1996) that is thought to be mediated through the action of membrane-complement attack complexes (Kyriakides et al., 1999). More specifically, the identified transcripts of C1S and C1R complement subunits are encoding essential proteases of this complex (Davis et al., 2008), and genetic correlation of their expression levels with meat shear force would be consistent with the role generally accepted for postmortem proteases activity in meat tenderness variation in pigs (Melody et al., 2004).

Loin Color a*. Loin color redness principally results from oxymyoglobin concentration and oxidative status (Gorelik and Kanner, 2001). We identified the transcript concentration of glycolysis enzymes as genetically correlated with loin color redness a*, which suggests that the regulation of glycolysis kinetics or its extent may drive the muscle oxidative capacity with regard to oxymyoglobin.

pH45. The voltage-gated cation channel activity and cation channel complex cell component point to the excitation-contraction coupling mechanism, which operates through the release of free Ca^{2+} from sarcoplasmic reticulum stores. Contraction mobilizes the available energy and results in increased glycolysis in the case of hypoxic tissues, leading to acidosis where resulting pyruvate is metabolized into lactate. The RYR1 mutation (Fujii et al., 1991) does constitute, by itself, an example of genetic variability with documented pleiotropic effects on both calcium channel activity in skeletal muscle and pH45 (Larzul et al., 1997; Sellier, 1998). However, the genetic variability observed here in pH45 and transcription is independent of the RYR1 polymorphism because the animals used in the present study were not carrying the RYR1 mutated allele (see methods). This observation illustrates that the same set of functions can be affected by different polymorphisms leading to similar effects, and that the genetic variability for the pH45 trait in a HAL NN (wild-type) population could be mediated nonetheless through similar biological pathways as those affected by the RYR1 mutation. Malignant hyperthermia susceptibility (**MHS**) is a genetic condition in human and pig resulting from the same or additional mutations in the *RYR1* gene (Fujii et al., 1991; Manning et al., 1998). Malignant hyperthermia susceptibility in humans can also result from a mutation in the α 1S subunit of the voltage-gated calcium channel CACNA1S (Monnier et al., 1997). We identified as genetically correlated with pH45, the LM concentration of the CACNA1A and CACNA1G transcripts, encoding 2 paralogs of CACNA1S.

Use of RNA Measurements for Genetic Improvement of Meat Quality

Alternative selection criteria are to be considered relative to their heritability and genetic correlation with traits belonging to breeding goals. For measurements taken on the same set of animals relative to selection candidates, an alternative selection criteria with an heritability h'^2 and a genetic correlation r_G with a selected trait of heritability h^2 would have to verify $r_G * h' > h$ to support a potential benefit in increased accuracy of breeding value estimation (Searle, 1965). In the case of selection for improvement of a meat quality trait with a typical heritability of 0.25, an illustration of a competitive alternative selection criterion could be the quantification of a specific RNA species in a given tissue sample associated with an heritability of at least 0.50 and a genetic correlation of at least 0.70 with the selected meat quality trait. These conditions are met by a large number of LM transcript levels for pH45, a*, SFc, and CL. These traits would therefore be candidates for the application of indirect selection strategies based on measurements of RNA concentrations in postmortem LM.

A substantial improvement in the accuracy of breeding values estimation may be at hand if informative RNA species such as those identified in this work for selected meat quality traits could be measured in skeletal muscle from selection candidates themselves. Compared with the measurements of the same trait in

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relatives, measurements on selection candidates would allow an improvement in the selection response by 50% when compared with the systematic measurement of 2 full-sibs, or 100% when compared with the systematic measurement of 4 half-sibs, for a typical meat quality trait with a heritability of 0.25. Improvement in the selection response would result from increased accuracy of breeding values estimated from an animal itself vs. phenotypes of relatives (Falconer and Mackay, 1996).

Taken together, our data support the likelihood of heritable LM transcripts that are genetically correlated with some of the pig meat quality traits. It would be of significant practical interest for applications in breeding programs to re-estimate these genetic parameters using RNA quantification measurements acquired from in vivo-sampled muscles.

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