ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

doi: 10.1111/j.1365-2052.2010.02098.x

Transcriptome analysis to identify differential gene expression affecting meat quality in heavy Italian pigs

C. Gorni*, C. Garino*,†,1, S. Iacuaniello*,†, B. Castiglioni‡, A. Stella*,‡, G. L. Restelli†, G. Pagnacco† and P. Mariani*

*Parco Tecnologico Padano, 26900 Lodi, Italy. †Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare, Università Degli Studi di Milano, 20134 Milano, Italy. ‡Istituto di Biologia e Biotecnologia Agraria, CNR, 20133 Milano, Italy

Summary

Suppressive subtractive hybridization (SSH) was used to analyse the muscle transcriptome and identify genes affecting meat quality within an Italian pig population of Large White and Landrace purebred individuals. Seven phenotypes were recorded at slaughter: dorsal fat thickness, ham fat thickness, ham fat coverage, muscle compactness, marbling, meat colour and colour uniformity. Two subtractive libraries were created from longissimus dorsi tissue of selected pigs with extreme phenotypes for meat quality. Eighty-four differentially expressed ESTs were identified, which showed homology to expressed pig sequences and/or to genomic pig sequences produced within the pig genome project. Sixty-eight sequences were mapped on the pig genome, and most of these sequences co-localized with the same chromosomal positions as QTLs that have been previously identified for meat quality. Thirty sequences, including eight matching known genes previously related to muscle metabolic pathways, were selected to statistically validate their differential expression. Association analysis and *t*-test results indicated that 28 ESTs of the 30 analysed were associated with phenotypes investigated here and have significant differential expression levels ($P \leq 0.05$) between the two tails of the phenotypic distribution.

Keywords heavy pig, meat quality, skeletal muscle, Suppression subtractive hybridization.

Introduction

Pig breeding is an important resource for most European Countries, and genetic selection of pigs for economically important traits is an integral part of the survival and success of commercial pig production. Commercial breeds and their hybrids used to produce ham and fresh meat are usually referred to 'heavy pigs' and 'lighter pigs', respectively. Eight per cent of the European pigs are reared in Italy (DEFRA, 2006), and around 90% of meat is transformed into different types of ham, most of which are in the Italian traditional products list (http://www.prodotti-tipici-italiani.com).

Address for correspondence

C. Gorni, Parco Tecnologico Padano, Via Einstein snc, Cascina Codazza, 26900 Lodi, Italy.
E-mail: chiara.gorni@tecnoparco.org

[1]Present address: DiSCAFF and Drug & Food Biotechnology Center, Università degli Studi del Piemonte Orientale, 28100 Novara, Italy.

Accepted for publication 16 May 2010

As the industry requires heavy cuts and mature meat for the production of processed meat, pigs for this sector are selected to maximize production and meat quality traits such as lean cut weight, ham fat thickness and curing loss after salting (Russo 1986). Meat quality traits can either be measured through objective procedures for carcass composition (backfat, loin muscle area or depth) and quality (pH, lipid content, texture, water holding capacity) or they can also be classified by trained staff following subjective observations (marbling, tenderness, texture, colour). Carcass and meat quality traits are complex traits usually affected by many genes, and their heritability is often moderate to low (Sellier *et al.* 1998).

However, research focused on genetic improvement of pork quality demonstrates the potential importance of allelic variation of single genes (Davoli & Braglia 2008), such as G to A transition at intron 3–3072 of *IGF2* (*insulin-like growth factor 2*), a gene for muscle growth (Van Laere *et al.* 2003), and the Arg200Gln substitution in the *PRKAG3* (*protein kinase adenosine monophosphate-activated gamma(3)-subunit*) gene that affects glycogen content in skeletal muscle (Milan *et al.* 2000b).

Despite the increasing economic importance of meat quality and the number of QTLs and positional candidate genes already known (Rothschild *et al.* 2007), the exploitation of the opportunities for using a marker-assisted selection plan is limited by the lack of knowledge of the number and interaction of genes affecting the meat qualitative characteristics (Dekkers 2004).

Recently, the focus of research has moved to the analysis of the skeletal muscle transcriptome and comparing the gene expression profile in the muscles of different quality, using expression microarrays (Bai *et al.* 2003; Lobjois *et al.* 2008; Ponsuksili *et al.* 2008) and cDNA libraries (Davoli *et al.* 2002; Yao *et al.* 2002; Kim *et al.* 2006b). These tools provide information on networks of expressed genes in muscle tissue and increase the knowledge of the biological pathways controlling meat quality traits.

The aim of this study was to investigate the skeletal muscle transcriptome in heavy pigs (Large White and Landrace) used for ham production to identify transcription profiles that may lead to information that can be used in animal selection. Here, we report the results of the differentially expressed loci, their genome mapping and association between their expression and meat quality traits.

Materials and methods

Animals

One hundred purebred pigs of Large White (70 animals) and Landrace (30 animals) were sampled at the abattoir; these animals were selected to minimize the genetic relationships within the population. In this population, 23 females and 47 castrated males were of the Large White breed and 7 females and 23 castrated males were of the Landrace breed. Animals had been reared in the same conditions and butchered in the same slaughterhouse at the age of 10 months and at an average weight of 170 kg.

Samples of the skeletal muscle tissue (longissimus dorsi) were taken from animals and cut into pieces not exceeding 5 mm in thickness. These were stored in RNAlater® (SIGMA) according to the manufacturer's instructions.

Phenotypic data

Phenotypic data were collected for each pig at slaughter for the following seven linear traits: dorsal fat thickness, ham fat thickness, ham fat coverage, muscle compactness, marbling, meat colour and colour uniformity. Dorsal fat thickness (DFT) and ham fat thickness (HFT) were objectively scored (mm), while ham fat coverage, muscle compactness, marbling, meat colour uniformity and meat colour were subjective and visually scored by trained staff as linear points with values between 1 and 5 (Table 1).

Table 1 Summary statistics of the phenotypic traits measured.

Trait	Abbreviation	Mean[1]	SD	Min[2]	Max[3]
Dorsal fat thickness	DFT	45.66	9.50	20	65
Ham fat thickness	HFT	32.64	9.78	10	60
Fat coverage[4]	FC	3.09	0.75	1	5
Muscle compactness[5]	MC	1.86	0.95	1	4
Marbling[6]	MARB	1.45	0.62	1	3
Colour uniformity[7]	COLOUR-U	1.66	0.74	1	4
Colour[8]	COLOUR	2.7	0.46	1	3

[1]Average value of phenotypic score in the population.
[2]Minimum value of phenotypic score in the population.
[3]Maximum value of phenotypic score in the population.
[4]Subjective fat coverage score, with 1 = devoid of fat, 2 = practically devoid, 3 = excellent, 4 = slightly abundant, 5 = overly abundant.
[5]Subjective muscle compactness score, with 1 = excellent, 2 = good, 3 = medium, 4 = poor, 5 = bad.
[6]Subjective marbling score, with 1 = excellent (not visible), 2 = low visible, 3 = moderately visible, 4 = visible, 5 = extremely visible.
[7]Subjective colour uniformity score, with 1 = excellent (uniform), 2 = slightly not uniform, 3 = moderately not uniform, 4 = not uniform, 5 = highly not uniform.
[8]Subjective colour score, with 1 = pale, 2 = slightly pale, 3 = normal, 4 = slightly dark, 5 = dark.

Statistical analysis

Statistical analyses were performed using the SAS/STAT software (SAS Institute Inc.). First, a principal component analysis (PCA) was performed on the seven previously defined phenotypes, and then multivariate analysis was performed on the first two principal components to adjust for sex effect and sampling day. A new 'meat quality' index was calculated for each individual, combining the residuals for the first and the second principal component genetic index. Finally, 32 animals picked from the extreme tails (named 'negative' and 'positive') of the distribution, as calculated from the seven adjusted phenotypes, were selected for molecular and association analyses.

RNA extraction and suppression subtractive hybridization

mRNA of the animals in the tails of the trait distribution described elsewhere was extracted from the skeletal muscle tissue samples using a Poly(A) Pure Kit (Ambion), and both quality and quantity were evaluated using the 2100 BioAnalyzer (Agilent). Two mRNA pools were created from the 16 animals at the extreme tails of the phenotypic distribution to normalize for individual differences. Suppression subtractive hybridization (SSH, Diatchenko *et al.* 1996) was performed using a PCR Select cDNA Subtraction kit (Clontech) and Advantage cDNA polymerase mix (Clontech) as described by the manufacturer.

Forward and reverse subtractions were carried out using 2 μg of the pooled mRNA samples. A control experiment was included following the manufacturer's instructions.

Libraries analysis

The two SSH cDNA populations were cloned into the pGEM-T Easy vector and transformed into *E. coli* JM109 (Promega). Following overnight growth on selective media, colonies were picked and incubated at 37 °C in 5 ml LB medium. DNA was extracted, and plasmids containing inserts were amplified by PCR using T7 and SP6 primers under the following conditions: 95 °C for 3 min followed by 35 cycles at 94 °C for 30 s, 56 °C for 30 s, 72 °C for 1 min and a final cycle at 72 °C for 10 min. PCR products were analysed on 2% agarose gels and sequenced.

Sequences were analysed using the MAP algorithm (Huang 1994) to evaluate redundancy and background of the libraries, by identifying the presence of multiple-copy inserts or inserts present in both libraries, respectively. The Basic Local Alignment Search Tool (BLAST; Altschul *et al.* 1990) was used to identify sequence homology of each differentially expressed template with publicly available pig genome data in different partially overlapping databases: GenBank (EST and Nucleotide nr/nt), Genbank Genome Pig (SScrofa5, release July 2008), ENSEMBL (SScrofa9, release September 2009), DFGI Pig gene index release 13.0 (7 March 2008; http://compbio.dfci.harvard.edu/tgi/tgipage.html) and genomic sequences from the Pig Genome survey sequences available at NARGP (http://www.animalgenome.org/blast/).

The sequence homology was considered 'true' only if: (i) *e*-value was higher than −10, (ii) more than 80% of the sequence length was homologous to the target and (iii) the homology percentage was higher than 90% for pig sequences and higher than 75% for non-pig sequences.

In-silico comparative and physical mapping

To identify the chromosomal position of the ESTs identified in this study, the following analyses were performed:

1. The Ensembl (SScrofa9, release September 2009; http://www.ensembl.org/Sus_scrofa/Info/Index) database was searched to identify sequences already integrated in the genome assembly.
2. Available localizations were retrieved from pig syntenic maps available at INRA (https://www.lgc.toulouse.inra.fr/pig/compare/SSC.htm) and from pre-existing comparative mapping data (Rogatcheva *et al.* 2008 and references therein).
3. If the porcine localization was not known, an *in-silico* pig-human comparative mapping approach was applied. Sequences homologous to human sequences were mapped to the human genome at Ensembl (http://www.ensembl.org/Homo_sapiens/index.html). The data obtained were integrated into comparative maps available at PigQTL database (Hu & Reecy 2007; http://www.animalgenome.org/QTLdb/pig.html) to predict the putative sequence localization on pig genome.
4. In addition, ESTs selected for association analysis were physically mapped using the INRA-University of Minnesota 7000rad porcine radiation hybrid panel (IMpRH, Yerle *et al.* 1998). Primer sets corresponding to the target sequences were designed (Table S2) and tested against mouse, hamster and pig DNA in 20-μl reactions containing 20 ng of genomic DNA, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 50 ng each primer, 1 U Taq polymerase and 1× reaction buffer (Applied Biosystems). DNA was amplified under the following PCR thermal profile: 95 °C for 3 min followed by 40 cycles at 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min and a final cycle at 72 °C for 10 min. Amplification products were visualized after electrophoresis through ethidium bromide–stained 2% agarose gels. Primers that did not amplify a pig-specific fragment were redesigned and retested. Primers that generated pig-specific PCR products were used to type, in duplicate, the IMpRH panel. The IMpRH database (Milan *et al.* 2000a; http://imprh.toulouse.inra.fr/) was used to assess map positions.

Real-time PCR

Differential expression of selected ESTs was verified by quantitative RT-PCR (RT-qPCR) on the 32 animals previously selected. Transcripts from each individual pig were amplified in triplicate and detected using the EXPRESS SYBR GreenER qPCR Supermix with ROX (Invitrogen), and the primer pairs were described in Table S2. RT-qPCR was performed using an 7500 Fast Real-Time PCR System (Applied Biosystems) in a 10-μl reaction volume with 200 μM of each primer set. The PCR conditions were as follows: 52 °C 2 min., 94 °C 3 min, then 45 cycles of 94 °C 15 s, 60 °C 1 min. Dissociation curve analysis was run to ensure the absence of non-specific PCR products. Samples that did not include reverse transcriptase were included as negative controls to monitor DNA contamination, and four blank samples were added as qPCR negative controls.

geNorm algorithm (Vandesompele *et al.* 2002) was used to determine the most stable reference genes from a set of six candidate reference genes (*ACTB*, *GAPDH*, *AK1*, *PPIA*, *HPRT*, *B2M*) tested on 32 pig samples analysed.

RT-qPCR data were analysed using QBASE^{PLUS} software (Biogazelle). PCR efficiency E can be calculated from the slope of a serial dilution of representative templates (standard curve), and inter-run calibration was used to detect and correct inter-run variation. 'Target-specific amplification efficiency' and 'scale to minimum' parameters were used to obtain a calibrated normalized relative quantity of expression (Hellemans *et al.* 2007) for each EST in each sample.

Association analysis

Associations between the normalized expression values and the phenotypic traits were tested using the general linear model procedure (SAS procedure GLM; SAS Institute Inc.), including the fixed effects of sex and slaughter day and the random effect of litter.

Differences in gene expression average between the two groups (16 animals each) were evaluated using a *t*-test and were considered statistically significant at $P \leq 0.05$.

Results

Suppression subtractive hybridization and sequence homology

SSH was used to characterize differential gene expression between two groups of 16 pigs (11 castrated males and 5 females) each, selected from the extreme tails of the distribution of seven adjusted meat quality–related phenotypes (see Materials and methods).

Following SSH, the forward and reverse subtracted cDNA populations were cloned and two subtracted libraries representing differentially expressed genes were created. The first library was identified as 'positive', because inserts are specific for individuals in the positive tail of the distribution, while the second library was named 'negative' because the inserts are specific for individuals in the negative tail of the distribution.

A total of 200 clones (100 for each library) were randomly picked, and DNA sequence was determined. Among the 200 clones, 43 were false positive (no insert), 16 were artefacts (RsaI restriction site within the sequence), 27 gave low-quality sequence (contained long adenine stretches), 26 were replicates (library redundancy) and 4 were present in both libraries (library background). In summary, library screening identified 84 inserts represented in only one of the two libraries, 52 inserts from the positive and 32 from the negative library (Table S1). Average length of library inserts was 483 nucleotides, ranging from 92 to 793 base pairs.

The 84 cDNA sequences were submitted to the Genbank EST database (accession numbers from EV825881 to EV825966). Sixty-eight of the ESTs identified were homologous to the pig genome assembly (SScrofa9/SScrofa5), while sixteen match to unassembled sequences produced by the pig genome project (http://www.sanger.ac.uk/Projects/S_scrofa/, http://www.ncbi.nlm.nih.gov/Traces) (Table S1). Of the 68 ESTs homologous to the pig genome assembly, eight match with known genes (Table 2).

ESTs functional annotation

The eight ESTs showing homology to known genes (Table 2) are involved in different metabolic pathways, and GO analysis (Kanehisa & Goto 2000) placed these ESTs into important molecular/biological functional groups, including cytochrome-c oxidase activity (GO:004129), motor activity (GO:0003774), ATP binding (GO:0005524) and intracellular signalling cascade (GO:0007242). Among the ESTs identified, *Cytochrome oxidase II* and *III* (*COXII*, *COXIII*) and *Myosin heavy polypeptide 1 skeletal muscle 2×* (*MYH1*) are involved in oxidative metabolism and muscle composition (Toniolo *et al.* 2004; Fernandez *et al.* 2008). *ATPase H^+ transporting lysosomal accessory protein 1* (*ATP6AP1*, Jansen *et al.* 1998), *Kalirin Rho GEF kinase* (*KALRN*, Mains *et al.* 1999), *Sorting nexin 13* (*SNX13*, Worby & Dixon 2002) and *Arginine and glutamate rich 1* (*ARGLU1*, Olsen *et al.* 2006) code for proteins playing an important role in intracellular trafficking and signalling, whereas *Myosin regulatory light chain 2* (*MYL2*) is involved in muscular ATPase activity (Katoch & Soni 1999) and contractile properties of skeletal muscle fibres (Bozzo *et al.* 2005).

These eight ESTs, plus 22 additional sequences selected across chromosomes, were physically mapped using a radiation hybrid panel, and their expression was investigated by quantitative real-time PCR.

ESTs physical and in-silico mapping

The 84 ESTs were mapped to chromosomes by radiation hybrid mapping using the IMpRH panel or using comparative map information and alignment with the Ensembl genome. Using this approach, 68 SSH sequences could be localized on the pig genome. Two sequences (EV825888, EV825927) were homologous to mitochondrial DNA genes, whereas the remaining 66 were distributed across pig chromosomes (Fig. S1). Screening the PigQTL database and bibliography for previously identified QTLs showed that most of the ESTs were located within or close to previously identified QTL regions affecting meat quality traits (Table S1).

RT-qPCR and association analysis

Thirty SSH sequences matching genes (8 sequences), ESTs (10 sequences) or pig genome assembly sequences (12 sequences) were selected to perform RT-qPCR for the validation of their differential expression. The expression level of ESTs analysed was quantified based on the Ct values obtained for the cDNA of the animals in the positive and negative tails of the trait distribution. geNorm analysis indicated that *Cyclophilin A* (*PPIA*) and *beta-2-microglobulin* (*B2M*) were the optimal reference genes to normalize expression results.

After data normalization, the animals in the two groups of the population distribution tails were classified according to the seven different phenotypes. Association analysis and *t*-test results indicated that 28 of the 30 ESTs investigated were associated with the phenotypes investigated and were

Table 2 Summary of 30 ESTs selected for validation, phenotypic traits associated to their differential expression and QTLs previously described at the same chromosome positions.

Genebank acc. no.	SSc	Map position	Acc. number of homologous gene or nucleotide sequence[1]	Gene symbol	Differential expression $P \leq 0.05$	QTL previously identified	Reference
EV825927[2]	mtDNA	mtDNA	AF304200; *Cytochrome oxidase II*	*COXII*	Ham Fat Coverage$^-$	–	–
EV825888[2]	mtDNA	mtDNA	AF304202; *Cytochrome oxidase III*	*COXIII*	–	–	–
EV825962[3]	1	SW2185-SW2432	AC026770[4]	–	Dorsal Fat Thickness$^+$ Ham Fat Thickness$^+$	BFT	Kim *et al.* 2006a; de Koning *et al.* 2003;
EV825892[2]	1	SW2432-SW2166	–	–	Marbling$^-$	MARBL	Malek *et al.* 2001a;
EV825925[2]	1	SWR817-SW2416	–	–	Meat Colour$^-$	MCOLOR	Liu *et al.* 2007;
EV825956[2]	1	SW970-SWR982	AL136980[4]	–	Ham Fat Coverage$^-$	BFT	Malek *et al.* 2001b; Liu *et al.* 2007;
EV825947[3]	1	SW970-SWR982	–	–	Muscle Compactness$^+$	BFT	Malek *et al.* 2001b; Liu *et al.* 2007;
EV825944[2]	1	SW970-SWR982	–	–	Dorsal Fat Thickness$^-$	BFT	Malek *et al.* 2001b; Liu *et al.* 2007;
EV825952[3]	1	SW1462-SW1311	AL353806[4]	–	Ham Fat Thickness$^+$	HFATP HAMP LEANP	Karlskov-Mortensen *et al.* 2006; Liu *et al.* 2007;
EV825948[3]	3	S0397-S0002	–	–	Ham Fat Coverage$^+$	HFT	Harmegnies *et al.* 2006;
EV825913[3]	4	SWR153-telomer	NT_032977[4]	–	Meat Colour$^+$	MCOLOR	Malek *et al.* 2001a; Ovilo *et al.* 2002;
EV825935[2]	6	SW1067-SW782	AC011518[4]	–	Dorsal Fat Thickness$^-$	HAMWT	van Wijk *et al.* 2006;
EV825930[3]	6	SW1059-S0031	–	–	Marbling$^+$	MARBL	van Wijk *et al.* 2006;
EV825932[3]	7	SW1614-SWR2152	AC137561[4]	–	Meat Colour$^+$	MCOLOR	Ovilo *et al.* 2002;
EV825951[3]	7	SW2537-SW2446	CT867964	–	Ham Fat Thickness$^+$	BFT	Kim *et al.* 2006a;
EV825960[3]	8	SW2410-S0353	–	–	Meat Colour Uniformity$^+$	MCOLOR	van Wijk *et al.* 2006;
EV825953[3]	9	SWR915-S0019	NM_015132[4]; *Sorting Nexin 13*	*SNX13*	Ham Fat Thickness$^+$	BFT	Kim *et al.* 2006a;
EV825950[3]	9	SW2116-SW749	–	–	–	MCOLOR	Edwards *et al.* 2008;
EV825965[3]	11	SW1465-telomer	BC030508[4]; *Arginine and Glutamate rich 1*	*ARGLU1*	Ham Fat Thickness$^+$	FP	Edwards *et al.* 2008;
EV825939[3]	12	SWR1021-telomer	AB025262; *Myosin, heavy polypeptide 1, skeletal muscle 2x*	*MYH1*	Ham Fat Coverage$^+$	HAMWT	Milan *et al.* 2002; Hu *et al.* 2008;
EV825917[2]	13	SW344-SW864	–	–	Ham Fat Thickness$^-$	BFT	Nezer *et al.* 2002;
EV825903[3]	13	SWR2189-S0075	NG_012742[4]; *Kalirin, Rho GEF kinase*	*KALRN*	Marbling$^+$	BFT	de Koning *et al.* 2003;
EV825942[2]	14	S0058-SW2508	AF513016; *Myosin light chain 2*	*MYL2*	Muscle Compactness$^-$	BFT	Kim *et al.* 2006a;
EV825931[2]	14	SW1425-SW210	–	–	Dorsal Fat Thickness$^-$	BFT	Kim *et al.* 2006a;
EV825912[2]	14	SW1082-SW1552	–	–	Dorsal Fat Thickness$^-$	BFT BFSHO	Dragos-Wendrich *et al.* 2003;
EV825904[2]	14	SW1804-SW1333	–	–	Meat Colour Uniformity$^-$	MCOLOR	de Koning *et al.* 2001;
EV825938[3]	15	SW964-S0369	–	–	Ham Fat Thickness$^+$ Ham Fat Coverage$^+$	HFT	Harmegnies *et al.* 2006;

Table 2 Continued

Genebank acc. no.	SSc	Map position	Acc. number of homologous gene or nucleotide sequence[1]	Gene symbol	Differential expression $P \leq 0.05$	QTL previously identified	Reference
EV825936[2]	16	SW1454-S0026	AC114321[4]	–	Ham Fat Thickness$^-$	BFT	Liu *et al.* 2008;
EV825922[2]	18	SW2540-SW1984	AK236871	–	Dorsal Fat Thickness$^-$	BFT FP	Edwards *et al.* 2008; Dragos-Wendrich *et al.* 2003;
EV825934[2]	X	SW2588-telomer	BC104511[5]; *ATPase, H+ transporting, lysosomal accessory protein 1*	*ATP6AP1*	Dorsal Fat Thickness$^-$	FW FP	Milan *et al.* 2002; Rohrer *et al.* 2006;

[1]Genebank EST and Nucleotide collection nr/nd; homology >90% if swine; >75% if other species.
[2]From negative library.
[3]From positive library
[4]Human.
[5]Bovine.
All accession numbers refer to NCBI databases.
$^+$Over-expressed in the positive tail of the phenotypic distribution.
$^-$Over-expressed in the negative tail of the phenotypic distribution.
BFSHO, fat thickness at shoulder; BFT, back fat or back fat thickness; FP, fat percentage; FW, fat weight; HAMP, ham percentage; HAMWT, ham weight; HFATP, ham fat percentage; HFT, ham fat thickness; LEANP, lean meat percentage; LFW, leaf fat weight; MARBL, marbling; MCOLOR, meat colour.

differentially expressed ($P \leq 0.05$) between the two groups of pigs (Table 2; Figs 1 and S2).

In detail, six ESTs (EV825952, EV825951, EV825953, EV825965, EV825917, EV825936) were associated with ham fat thickness, six ESTs (EV825935, EV825931, EV825912, EV825922, EV825934, EV825944) with dorsal fat thickness, four ESTs (EV825927, EV825956, EV825948, EV825939) with ham fat coverage, three ESTs (EV825892, EV825930, EV825903) with marbling, and five ESTs with meat colour (EV825925, EV825913, EV825932) or meat colour uniformity (EV825960, EV825904). EV825947 and EV825942 were associated with muscle compactness, EV825962 was associated with both dorsal fat thickness and ham fat thickness, and EV825938 was associated with both ham fat thickness and ham fat coverage. Two ESTs (EV825888 and EV825950) were associated with any of the seven phenotypes analysed. Differential expression was confirmed for all 28 ESTs associated with the phenotypes. In particular, the expression of ESTs that match *SNX13* (EV825953), *ARGLU1* (EV825965), *MYH1* (EV825939) and *KALRN* (EV825903) was high (3-fold, 6-fold, 3-fold, 4-fold up-regulated, respectively) in the positive tail of the phenotypic distributions. Conversely, *COXIII* (EV825927), *ATP6AP1* (EV825934) and *MYL2* (EV825942) were up-regulated (3-fold, 3-fold, 6-fold, respectively) in the negative phenotypic groups. The expression level of *COXII* (EV825888) appeared to be very similar between the two groups of animals analysed for all phenotypes considered.

Discussion

Transcriptome analysis and sequence homology

To investigate variations in the pig transcriptome involved in meat quality and muscle metabolic pathways, different tissues and individuals coming from different breeds and crosses are often analysed (Bai *et al.* 2003; Xu *et al.* 2003; Kim *et al.* 2005a,b; Cagnazzo *et al.* 2006; Lobjois *et al.* 2008). In this study, a different approach was used. Seven phenotypes related to meat quality were recorded from a slaughtered commercial population. To minimize the number of animals to be analysed, these phenotypes were combined to create an aggregated 'meat index', and 32 animals belonging to the extreme tails (16 animals in the positive tail and 16 animals in the negative tail) of the Gaussian distribution of the population were selected to identify ESTs differentially expressed between the two groups using the SSH experiment.

The majority of ESTs identified by SSH were neither annotated nor identified in previous microarray studies, demonstrating the complementary nature of the techniques (Bai *et al.* 2003; Lobjois *et al.* 2008). Although genome-wide gene profiling studies using microarrays provide direct information on the relative levels of gene expression, the

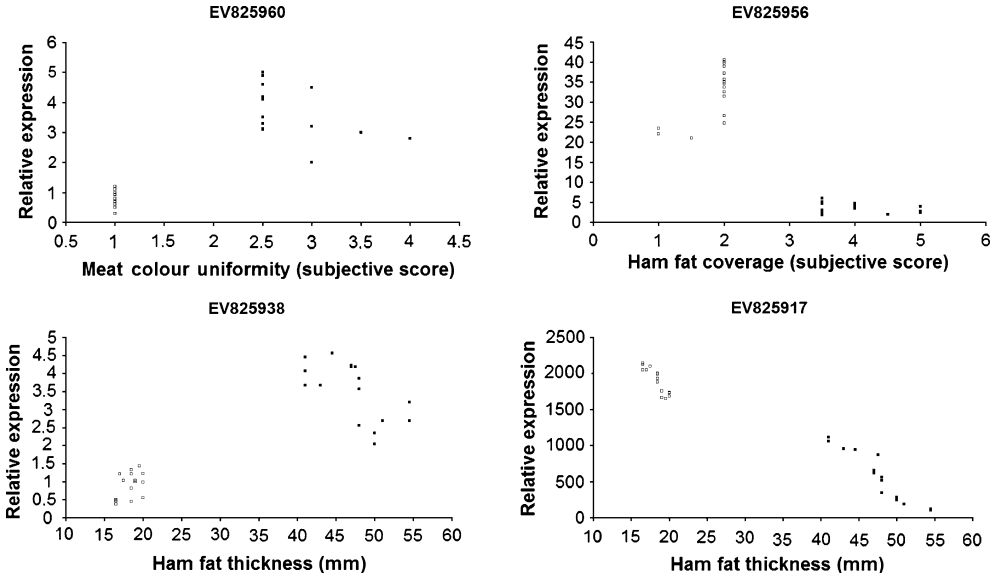

Figure 1 Plots of the RT-qPCR expression data (*y*-axis) of 4 representative ETSs versus phenotypic values (*x*-axis) of the associated traits. Messenger RNA (mRNA) levels are represented as calibrated normalized relative quantity of expression.

SSH technique approach is able to discover differentially expressed genes that have not been previously annotated (Cao *et al.* 2004). This study reported that only 9.5% of the identified ESTs showed homology with annotated genes.

Mapping and QTLs for meat quality

Physical and *in-silico* mapping, using the IMpH and SScrofa9 or SScrofa5 pig genome assembly, located two sequences (EV825888 and EV825927) on the pig mitochondrial genome and 66 ESTs on the pig genome. As shown in Fig. S1, these ESTs are not randomly distributed across chromosomes, and no sequences were mapped on SSc5 or SSc17. Conversely, 11 ESTs mapped to SSc1, and interestingly, this chromosome has previously been reported to harbour genes that play an important role in pig meat quality (Liu *et al.* 2007).

Preliminary analysis demonstrated that the use of aggregate 'meat index' was not optimal to validate the differentially expressed ESTs (data not shown). In fact, the 'meat index' was calculated by combining the first two principal components of the variability of each phenotype, and it is not directly related to any of the original biological traits. For this reason, the animals in the two groups of the population distribution tails were re-classified according to the original seven phenotypes prior to association analysis between EST expression and phenotypes, and validation of the relative expression by RT-qPCR. Based on this analysis, a differential expression confirmation rate of 90% was obtained, and 28 ESTs were indicated as potential candidate genes for the QTLs. In addition, for all confirmed ESTs except EV825927 that were mapped on mtDNA, QTLs for the same phenotypic traits considered in this study, or for related traits (e.g. fatness), have been previously indentified (Table 2). As an example, we reported that differential expression of EV825962 affects both dorsal fat thickness and ham fat thickness traits. In the same chromosome region on SSc1, SW2185–SW2432, a QTL for backfat thickness was identified (de Koning *et al.* 2003; Kim *et al.* 2006a). The differential expression of EV825930 confirms the presence of a QTL for marbling in SSc6 SW1059–S0031, as reported by van Wijk *et al.* (2006). EV825938 was mapped to the same region where Harmegnies *et al.* (2006) reported a QTL for ham fat thickness. This EST was here associated with ham fat thickness phenotype and was differentially expressed in the two groups of pig analysed.

Variation expression results of the 28 ESTs associated with the seven phenotypic traits were compared to the SSH original library data to identify the specific library (positive or negative) where the ESTs have been picked up. This analysis confirmed that 14 ESTs isolated from the negative library (Table 2) were over-expressed in the negative tail of the phenotypic distributions, while the remaining 14 ESTs isolated from the positive library were up-regulated in the positive tail of the phenotypic distributions. This evidence indicates that the SSH approach used to analyse our pig population is robust for identifying differentially expressed genes affecting meat quality phenotypes.

EST functional annotation and differential expression

A large number of the sequences identified in this study did not match annotated ESTs; only eight of the 84 ESTs differentially represented between the two libraries were able to be linked to known biological mechanisms, pathways and functions in which they might be involved.

Gene Ontology (GO) annotation, not available for *ARGLU1*, indicates that seven sequences (*ATP6AP1*, *COXII*, *COXIII*, *KALRN*, *MYL2*, *MYH1*) are involved in ATP and NADH binding, oxidative phosphorylation, intracellular signal trafficking and cytoskeleton activity. The same physiological pathways were also reported in microarray studies for meat tenderness (Lobjois *et al.* 2008) and water holding capacity of pig muscle (Ponsuksili *et al.* 2008). In addition, RT-qPCR analysis indicated that the ESTs homologous to *SNX13*, *MYH1*, *KALRN* were up-regulated in animals within the positive tail of the phenotypic distributions.

Sorting Nexin 13 (*SNX13*) encodes a protein in the family of peripheral membrane proteins that are involved in vesicular trafficking. The protein PX domain has been shown to bind to phosphatidylinositol 3-monophosphate (PtdIns3P), which has a regulatory function in a variety of sorting processes and regulates endocytic trafficking and degradation of the epidermal growth factor receptor (Zheng *et al.* 2006). Nevertheless, the relationship between this gene or protein and meat quality traits was not previously investigated. The role of the *Kalirin* gene in muscle tissue is not yet clear, but it is associated with the cytoskeleton and intracellular signalling mechanisms; this association suggests that this gene may be involved in post-mortem cell modifications.

The protein coded by the *myosin heavy polypeptide IIx* gene (*MYH1*) is one of the isoforms of the muscle myosin heavy chain (MyHC) and is an intermediate between oxidative type I and glycolytic type IIb fibres (Chang *et al.* 2003). The data presented here indicated the presence of a positive correlation between *MYH1* over-expression and ham fat coverage. The same positive correlations between *MYH1* expression and meat colour, pH value, marbling and backfat were previously reported by Hu *et al.* (2008). The data here presented indicated that ESTs homologous to *COXIII*, *ATP6AP1* and *MYL2* were up-regulated in animals classified in the negative tail of the phenotypic distributions.

The role of mitochondrion metabolism in meat quality was first reported (Eikelenboom & van den Bergh 1973), but recent studies have indicated that mitochondrial gene variations (Fernandez *et al.* 2008), rather than mitochondrial gene expression (Luo *et al.* 2008; Wyllie *et al.* 2008), can affect meat quality traits. In this study, we reported a negative correlation between over-expression of *COXIII* and phenotypes affecting meat quality. This negative correlation was also reported by Ponsuksili *et al.* (2008). Increased oxidative phosphorylation in post-mortem muscle tissues could promote the production of lactic acid and pH decline, thus influencing water holding capacity (Rosenvold & Andersen 2003). The negative correlation between high expression levels of the *ATP6AP1* and meat quality can be related to its function in proton (H^+) and calcium (Ca^{2+}) transport. Control of ion transport is important to prevent cellular acidification and decrease in pH (Küchenmeister *et al.* 1999), which is related to post-mortem maturation of meat. The third gene, *MYL2*, encodes the myosin regulatory light chain associated with myosin slow heavy chain. Expression microarray studies by Shmueli *et al.* (2003) and Yanai *et al.* (2005) indicated that this gene is highly expressed in heart and muscle tissues; however, its role in adult skeletal muscle has not been investigated. The involvement of *MYL2* in cardiac hypertrophy (Ramirez & Padron 2004) and calcium-binding capacity suggests that this gene may be involved in meat-related traits.

Conclusions

SSH was used to identify differentially expressed sequences in a commercial population of purebred pigs selected for meat quality traits. Comparative mapping analysis showed that these ESTs co-localize with chromosomal regions where QTLs for meat quality traits have been previously identified. Thirty of the 84 ESTs identified by the SSH experiment were physically mapped, and their differential expression validated for the meat quality phenotypes was investigated. Interestingly, differential expression was confirmed for 90% of the ESTs considered, and ESTs were associated with phenotypic traits for which QTLs were previously identified at the same chromosomal positions. In addition, eight of the 30 ESTs analysed matched to known genes, while the remaining 22 sequences are previously identified pig ESTs (2 sequences) or novel pig ESTs (20 sequences).

Further investigation of the functions of the novel differentially expressed sequences identified in this study will add fundamental information to allow a better understanding of muscle metabolism and control of variation in porcine meat quality.

Acknowledgements

We are grateful to Mr. Levoni and the staff of the slaughterhouse (Mantua, Italy) for collecting samples and phenotypic data. The IMpRH panel for physical mapping of the sequences identified was kindly provided by Dr. M. Yerle, INRA, Toulouse, France. We are grateful to Dr. John L. Williams for valuable discussions. This work received financial support by the Italian Ministry of Education, University and Scientific Research (FIRB MIUR RBNE01MMHS).

References

Altschul S.F., Gish W., Miller W., Myers E.W. & Lipman D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology* **215,** 403–10.

Bai Q., McGillivray C., da Costa N., Dornan S., Evans G., Stear M.J. & Chang K.C. (2003) Development of a porcine skeletal muscle cDNA microarray: analysis of differential transcript expression in phenotypically distinct muscles. *BMC Genomics* **4,** 8.

Beeckmann P., Schroffel J., Moser G., Bartenschlager H., Reiner G. & Geldermann H. (2003) Linkage and QTL mapping for *Sus scrofa* chromosome 3. *Journal of Animal Breeding and Genetics* **120,** 20–7.

Bozzo C., Spolaore B., Toniolo L., Stevens L., Bastide B., Cieniewski-Bernard C., Fontana A., Mounier Y. & Reggiani C. (2005) Nerve influence on myosin light chain phosphorylation in slow and fast skeletal muscles. *The FEBS Journal* **272,** 5771–85.

Cagnazzo M., te Pas M.F., Priem J., de Wit A.A., Pool M.H., Davoli R. & Russo V. (2006) Comparison of prenatal muscle tissue expression profiles of two pig breeds differing in muscle characteristics. *Journal of Animal Science* **84,** 1–10.

Cao W., Epstein C., Liu H. *et al.* (2004) Comparing gene discovery from Affymetrix GeneChip microarrays and Clontech PCR-select cDNA subtraction: a case study. *BMC Genomics* **5,** 26.

Cepica S., Bartenschlager H. & Geldermann H. (2007) Mapping of QTL on chromosome X for fat deposition, muscling and growth traits in a wild boar x Meishan F_2 family using a high-density gene map. *Animal Genetics* **38,** 634.

Chang K.C., Da Costa N., Blackley R., Southwood O., Evans G., Plastow G., Wood J.D. & Richardson R.I. (2003) Relationships of myosin heavy chain fibre types to meat quality traits in traditional and modern pigs. *Meat Science* **64,** 93–103.

Davoli R. & Braglia S. (2008) Molecular approaches in pig breeding to improve meat quality. *Briefings in Functional Genomics and Proteomics* **6,** 313–321.

Davoli R., Fontanesi L., Zambonelli P. *et al.* (2002) Isolation of porcine expressed sequence tags for the construction of a first genomic transcript map of the skeletal muscle in pig. *Animal Genetics* **33,** 3–18.

Department for Environment Food and Rural Affairs (DEFRA). National Statistics 2006. www.statistics.gov.uk.

Dekkers J.C.M. (2004) Commercial application of marker-and gene-assisted selection in livestock: strategies and lessons. *Journal of Animal Science* **82,** E313.

Diatchenko L., Lau Y.F., Campbell A.P. *et al.* (1996) Suppression subtractive hybridization: a method for generating differentially regulated or tissue-specific cDNA probes and libraries. *Proceedings of the National Academy of Sciences of the United States of America* **93,** 6025–30.

Dragos-Wendrich M., Moser G., Bartenschlager H., Reiner G. & Geldermann H. (2003) Linkage and QTL mapping for Sus scrofa chromosome 11. *Journal of Animal Breeding and Genetics* **120,** 89–94.

Edwards D.B., Ernst C.W., Raney N.E., Doumit M.E., Hoge M.D. & Bates R.O. (2008) Quantitative trait locus mapping in an F2 Duroc x Pietrain resource population: II. Carcass and meat quality traits. *Journal of Animal Science* **86,** 254–66.

Eikelenboom G. & van den Bergh S.G. (1973) Mitochondrial metabolism in stress-susceptible pigs. *Journal of Animal Science* **37,** 692–6.

Evans G.J., Giuffra E., Sanchez A., Kerje S., Davalos G., Vidal O., Illan S., Noguera J.L., Varona L. & Velander I. (2003) Identification of quantitative trait loci for production traits in commercial pig populations. *Genetics* **164,** 621.

Fernandez A.I., Alves E., Fernandez A., de Pedro E., Lopez-Garcia M.A., Ovilo C., Rodriguez M.C. & Silio L. (2008) Mitochondrial genome polymorphisms associated with longissimus muscle composition in Iberian pigs. *Journal of Animal Science* **86,** 1283–90.

Harmegnies N., Davin F., De Smet S., Buys N., Georges M. & Coppieters W. (2006) Results of a whole-genome quantitative trait locus scan for growth, carcass composition and meat quality in a porcine four-way cross. *Animal Genetics* **37,** 543–53.

Hellemans J., Mortier G., De Paepe A., Speleman F. & Vandesompele J. (2007) qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome biology* **8,** R19.

Hu Z.L. & Reecy J.M. (2007) Animal QTLdb: beyond a repository. A public platform for QTL comparisons and integration with diverse types of structural genomic information. *Mammalian Genome* **18,** 1–4.

Hu H., Wang J., Zhu R., Guo J. & Wu Y. (2008) Effect of myosin heavy chain composition of muscles on meat quality in Laiwu pigs and Duroc. *Science in China. Series C, Life Sciences* **51,** 127–32.

Huang X. (1994) On global sequence alignment. *Computer Applications in the Biosciences* **10,** 227–35.

Jansen E.J., Holthuis J.C., McGrouther C., Burbach J.P. & Martens G.J. (1998) Intracellular trafficking of the vacuolar H+-ATPase accessory subunit Ac45. *Journal of Cell Science* **111**(Pt 20), 2999–3006.

Kanehisa M. & Goto S. (2000) KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Research* **28,** 27–30.

Karlskov-Mortensen P., Bruun C.S., Braunschweig M.H., Sawera M., Markljung E., Enfält A.C., Hedebro-Velander I., Josell Å., Lindahl G. & Lundström K. (2006) Genome-wide identification of quantitative trait loci in a cross between Hampshire and Landrace I: carcass traits. *Animal Genetics* **37,** 156–62.

Katoch S.S. & Soni A. (1999) Changes in myosin ATPase activity in skeletal muscles of rat during cold stress. *Indian Journal of Biochemistry and Biophysics* **36,** 204–6.

Kim J.J., Rothschild M.F., Beever J., Rodriguez-Zas S. & Dekkers J.C. (2005a) Joint analysis of two breed cross populations in pigs to improve detection and characterization of quantitative trait loci. *Journal of Animal Science* **83,** 1229–40.

Kim J.J., Zhao H., Thomsen H., Rothschild M.F. & Dekkers J.C. (2005b) Combined line-cross and half-sib QTL analysis of crosses between outbred lines. *Genetical Research* **85,** 235–48.

Kim C.W., Hong Y.H., Yun S.I. *et al.* (2006a) Use of microsatellite markers to detect quantitative trait loci in Yorkshire pigs. *The Journal of Reproduction and Development* **52,** 229–37.

Kim T.H., Kim N.S., Lim D. *et al.* (2006b) Generation and analysis of large-scale expressed sequence tags (ESTs) from a full-length enriched cDNA library of porcine backfat tissue. *BMC Genomics* **7,** 36.

de Koning D.J., Harlizius B., Rattink A.P., Groenen M.A., Brascamp E.W. & van Arendonk J.A. (2001) Detection and characterization of quantitative trait loci for meat quality traits in pigs. *Journal of Animal Science* **79,** 2812–9.

de Koning D.J., Pong-Wong R., Varona L., Evans G.J., Giuffra E., Sanchez A., Plastow G., Noguera J.L., Andersson L. & Haley C.S. (2003) Full pedigree quantitative trait locus analysis in commercial pigs using variance components. *Journal of Animal Science* **81,** 2155.

Küchenmeister U., Nürnberg K., Fiedler I., Kuhn G., Nürnberg G. & Ender K. (1999) Cell injury and meat quality of pig in the time period post mortem from two genotypes susceptible or resistant to

malignant hyperthermia. *European Food Research and Technology* **209,** 97–103.

Lee S.S., Chen Y., Moran C., Cepica S., Reiner G., Bartenschlager H., Moser G. & Geldermann H. (2003) Linkage and QTL mapping for *Sus scrofa* chromosome 2. *Journal of Animal Breeding and Genetics* **120,** 11–9.

Liu G., Jennen D.G., Tholen E. *et al.* (2007) A genome scan reveals QTL for growth, fatness, leanness and meat quality in a Duroc-Pietrain resource population. *Animal Genetics* **38,** 241–52.

Liu G., Kim J.J., Jonas E., Wimmers K., Ponsuksili S., Murani E., Phatsara C., Tholen E., Juengst H. & Tesfaye D. (2008) Combined line-cross and half-sib QTL analysis in Duroc–Pietrain population. *Mammalian Genome* **19,** 429–38.

Lobjois V., Liaubet L., SanCristobal M., Glenisson J., Feve K., Ralliéres J., Le Roy P., Milan D., Cherel P. & Hatey F. (2008) A muscle transcriptome analysis identifies positional candidate genes for a complex trait in pig. *Animal Genetics* **39,** 147–62.

Luo G.F., Yu T.Y., Wen X.H., Li Y. & Yang G.S. (2008) Alteration of mitochondrial oxidative capacity during porcine preadipocyte differentiation and in response to leptin. *Molecular and Cellular Biochemistry* **307,** 83–91.

Mains R.E., Alam M.R., Johnson R.C., Darlington D.N., Back N., Hand T.A. & Eipper B.A. (1999) Kalirin, a multifunctional PAM COOH-terminal domain interactor protein, affects cytoskeletal organization and ACTH secretion from AtT-20 cells. *Journal of Biological Chemistry* **274,** 2929–37.

Malek M., Dekkers J.C., Lee H.K., Baas T.J., Prusa K., Huff-Lonergan E. & Rothschild M.F. (2001a) A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. II. Meat and muscle composition. *Mammalian Genome* **12,** 637–45.

Malek M., Dekkers J.C., Lee H.K., Baas T.J. & Rothschild M.F. (2001b) A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. I. Growth and body composition. *Mammalian Genome* **12,** 630–6.

Milan D., Hawken R., Cabau C. *et al.* (2000a) IMpRH server: an RH mapping server available on the Web. *Bioinformatics* **16,** 558–9.

Milan D., Jeon J.T., Looft C., Amarger V., Robic A., Thelander M., Rogel-Gaillard C., Paul S., Iannuccelli N. & Rask L. (2000b) A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. *Science* **288,** 1248.

Milan D., Bidanel J.P., Iannuccelli N., Riquet J., Amigues Y., Gruand J., Le Roy P., Renard C. & Chevalet C. (2002) Detection of quantitative trait loci for carcass composition traits in pigs. *Genetics, Selection, Evolution* **34,** 705–28.

Nezer C., Moreau L., Wagenaar D. & Georges M. (2002) Results of a whole genome scan targeting QTL for growth and carcass traits in a Pietrain x Large White intercross. *Genetics, Selection, Evolution* **34,** 371–87.

Olsen J.V., Blagoev B., Gnad F., Macek B., Kumar C., Mortensen P. & Mann M. (2006) Global, *in vivo*, and site-specific phosphorylation dynamics in signaling networks. *Cell* **127,** 635–48.

Ovilo C., Clop A., Noguera J.L. *et al.* (2002) Quantitative trait locus mapping for meat quality traits in an Iberian x Landrace F_2 pig population. *Journal of Animal Science* **80,** 2801–8.

Ponsuksili S., Jonas E., Murani E., Phatsara C., Srikanchai T., Walz C., Schwerin M., Schellander K. & Wimmers K. (2008) Trait correlated expression combined with expression QTL analysis reveals biological pathways and candidate genes affecting water holding capacity of muscle. *BMC Genomics* **9,** 367.

Ramirez C.D. & Padron R. (2004) Familial hypertrophic cardiomyopathy: genes, mutations and animal models. A review. *Investigacion Clinica* **45,** 69–99.

Rogatcheva M.B., Chen K., Larkin D.M., Meyers S.N., Marron B.M., He W., Schook L.B. & Beever J.E. (2008) Piggy-BACing the human genome I: constructing a porcine BAC physical map through comparative genomics. *Animal Biotechnology* **19,** 28–42.

Rohrer G.A., Thallman R.M., Shackelford S., Wheeler T. & Koohmaraie M. (2006) A genome scan for loci affecting pork quality in a Duroc-Landrace F population. *Animal Genetics* **37,** 17–27.

Rosenvold K. & Andersen H.J. (2003) Factors of significance for pork quality – a review. *Meat Science* **64,** 219–37.

Rothschild M.F., Hu Z.L. & Jiang Z. (2007) Advances in QTL mapping in pigs. *International Journal of Biological Sciences* **3,** 192–7.

Russo V. (1986) Selezione e incrocio per il miglioramento quanti-qualitativo della produzione suinicola. *Suinicoltura* **27,** 28.

Sellier P. (1998) Genetics of meat and carcass traits. In. *The Genetics of the Pig* (Ed. by Rothschild M.F., Ruvinsky A.), pp. 463–493, CAB International, Cambridge (GBR).

Shmueli O., Horn-Saban S., Chalifa-Caspi V., Shmoish M., Ophir R., Benjamin-Rodrig H., Safran M., Domany E. & Lancet D. (2003) GeneNote: whole genome expression profiles in normal human tissues. *Comptes Rendus Biologies* **326,** 1067–72.

Thomsen H., Lee H.K., Rothschild M.F., Malek M. & Dekkers J.C.M. (2004) Characterization of quantitative trait loci for growth and meat quality in a cross between commercial breeds of swine. *Journal of Animal Science* **82,** 2213.

Toniolo L., Patruno M., Maccatrozzo L., Pellegrino M.A., Canepari M., Rossi R., D'Antona G., Bottinelli R., Reggiani C. & Mascarello F. (2004) Fast fibres in a large animal: fibre types, contractile properties and myosin expression in pig skeletal muscles. *Journal of Experimental Biology* **207,** 1875–86.

Van Laere A.S., Nguyen M., Braunschweig M., Nezer C., Collette C., Moreau L., Archibald A.L., Haley C.S., Buys N. & Tally M. (2003) A regulatory mutation in *IGF2* causes a major QTL effect on muscle growth in the pig. *Nature* **425,** 832–6.

Vandesompele J., De Preter K., Pattyn F., Poppe B., Van Roy N., De Paepe A. & Speleman F. (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome biology* **3,** research 0034.1-0034.11.

van Wijk H.J., Dibbits B., Baron E.E., Brings A.D., Harlizius B., Groenen M.A., Knol E.F. & Bovenhuis H. (2006) Identification of quantitative trait loci for carcass composition and pork quality traits in a commercial finishing cross. *Journal of Animal Science* **84,** 789–99.

Wimmers K., Fiedler I., Hardge T., Murani E., Schellander K. & Ponsuksili S. (2006) QTL for microstructural and biophysical muscle properties and body composition in pigs. *BMC Genetics* **7,** 15.

Worby C.A. & Dixon J.E. (2002) Sorting out the cellular functions of sorting nexins. *Nature Reviews. Molecular Cell Biology* **3,** 919–31.

Wyllie C.J., Sargent C.A. & Affara N.A. (2008) Identifying and locating genes of possible commercial and biological importance within the pig mitochondrial genome. *Proceedings of XVI Plant and Animal Genome Conference, San Diego, USA.*, P569.

Xu D.Q., Zhang Y.B., Xiong Y.Z., Gui J.F., Jiang S.W. & Su Y.H. (2003) Construction of forward and reverse subtracted cDNA libraries between muscle tissue of Meishan and Landrace pigs. *Yi Chuan Xue Bao* **30,** 668–72.

Yanai I., Benjamin H., Shmoish M., Chalifa-Caspi V., Shklar M., Ophir R., Bar-Even A., Horn-Saban S., Safran M. & Domany E. (2005) Genome-wide midrange transcription profiles reveal expression level relationships in human tissue specification. *Bioinformatics* **21,** 650–9.

Yao J., Coussens P.M., Saama P., Suchyta S. & Ernst C.W. (2002) Generation of expressed sequence tags from a normalized porcine skeletal muscle cDNA library. *Animal Biotechnology* **13,** 211–22.

Yerle M., Pinton P., Robic A. *et al.* (1998) Construction of a whole-genome radiation hybrid panel for high-resolution gene mapping in pigs. *Cytogenetics and Cell Genetics* **82,** 182–8.

Yue G., Schroffel J., Moser G., Bartenschlager H., Reiner G. & Geldermann H. (2003) Linkage and QTL mapping for *Sus scrofa* chromosome 12. *Journal of Animal Breeding and Genetics* **120,** 95–102.

Zheng B., Tang T., Tang N., Kudlicka K., Ohtsubo K., Ma P., Marth J.D., Farquhar M.G. & Lehtonen E. (2006) Essential role of RGS-PX1/sorting nexin 13 in mouse development and regulation of endocytosis dynamics. *Proceedings of the National Academy of Sciences* **103,** 16776.

Supporting Information

Additional supporting information may be found in the online version of this article.

Figure S1 Chromosome position of ESTs identified in this study.

Figure S2 Plots of the RT-qPCR expression data (*y*-axis) of the 30 selected ETSs vs. phenotypic values (*x*-axis) of the associated traits.

Table S1 Summary of 84 ESTs, identified in differential expression libraries.

Table S2 Primer pairs and annealing temperature used for physical mapping and differential expression validation by RT-qPCR of 30 selected ESTs.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.