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Associations of functional candidate genes derived from gene-expression profiles of prenatal porcine muscle tissue with meat quality and muscle deposition

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Summary

Ten genes (*ANK1, bR10D1, CA3, EPOR, HMGA2, MYPN, NME1, PDGFRA, ERC1, TTN*), whose candidacy for meat-quality and carcass traits arises from their differential expression in prenatal muscle development, were examined for association in 1700 performance-tested fattening pigs of commercial purebred and crossbred herds of Duroc, Pietrain, Pietrain × (Landrace × Large White), Duroc × (Landrace × Large White) as well as in an experimental F_2 population based on a reciprocal cross of Duroc and Pietrain. Comparative sequencing revealed polymorphic sites segregating across commercial breeds. Genetic mapping results corresponded to pre-existing assignments to porcine chromosomes or current human–porcine comparative maps. Nine of these genes showed association with meat-quality and carcass traits at a nominal P-value of ≤ 0.05; *PDGFRA* revealed no association reaching the $P \leq 0.05$ threshold. In particular, *HMGA2, CA3, EPOR, NME1* and *TTN* were associated with meat colour, pH and conductivity of loin 24 h postmortem; *CA3* and *MYPN* exhibited association with ham weight and lean content (FOM) respectively at P-values of < 0.003 that correspond to false discovery rates of < 0.05. However, none of the genes showed significant associations for a particular trait across all populations. The study revealed statistical–genetic evidence for association of the functional candidate genes with traits related to meat quality and muscle deposition. The polymorphisms detected are not likely causal, but markers were identified that are in linkage disequilibrium with causal genetic variation within particular populations.

Keywords association, candidate gene, carcass traits, meat quality, myogenesis, pig.

Introduction

It is widely assumed that the number of muscle fibres is determined prenatally in mammals and that genetic factors are major determinants of fibre number. Fibre-type transitions are regularly observed in postnatal muscle. Muscle fibre number and types are important physiological parameters of muscle mass and properties in the live animal (Lefaucheur 2006). Furthermore, they are also key factors of meat-quality parameters, such as shear force, colour, pH and conductivity (Swatland 1973; Lengerken *et al.* 1994; Rehfeldt *et al.* 2004). The earliest embryonic development that is directly relevant to meat quality and quantity is the formation of muscle fibres, which is characterized by two

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developmental stages: (i) the determination and proliferation of myoblasts and (ii) the differentiation and fusion to form multinucleated myofibres. The large number of genes expressed in skeletal muscle at different times of development represents a source of candidate genes that could influence meat quality. Myogenesis depends on the strictly synchronized expression of a number of genes and their interaction with the myogenic regulatory factors (MRFs) playing a key role. The MRF gene family includes the genes *myogenin* (*MYOG*; previously known as *MYF4*), *myogenic determination factor 1* (*MYOD1*; previously known as *MYF3*), *myogenic factor 5* (*MYF5*) and *myogenic factor 6* (*MYF6*; previously known as *MRF4* or *herculin*). These are transcription factors that activate stage-specific expressed genes during myogenesis (for review, see Te Pas & Soumillion 2001). Porcine *MYOG* has been shown to be polymorphic, and its association with muscle mass and growth rate has been proven (Te Pas *et al.* 1999a). Also, *MYF5* exhibits a number of polymorphisms (Stratil & Cepica 1999; Te Pas *et al.* 1999b; Urbanski *et al.* 2006); however, no association with growth, carcass or meat-quality traits has been found. Currently, there are polymorphisms known in *MYOD1* and *MYF6*, but reports about association are mostly negative or not consistent (Ernst *et al.* 1994; Urbansky & Kuryl 2004; Wyszynska-Koko & Kuryl 2004; Wyszynska-Koko *et al.* 2006). To select candidate genes for meat quality, loci with stage- or breed-specific expression during myogenesis have been identified employing microarrays, suppression subtractive hybridization (SSH) and differential-display reverse transcription PCR (DD-RT-PCR) in two breeds, Duroc and Pietrain, which display divergence in meat-quality and muscularity traits. The aim of the present investigation was to determine the association of sequence variation of such selected candidate genes with technological parameters of meat quality and muscularity obtained in commercial pigs at slaughter.

Materials and methods

Animals

Samples were obtained for DNA extraction from approximately 1700 performance-tested fattening pigs of commercial purebred and crossbred herds, Duroc (Du, $N = 125$), Pietrain (Pi, $N = 259$), Pietrain × (Landrace × Large White) (PiF1, $N = 481$) and Duroc × (Landrace × Large White) (DuF1, $N = 626$) as well as an experimental F_2 population based on a reciprocal cross of Duroc and Pietrain (DuPi, $N = 335$). Phenotypic data were obtained at three slaughter houses in the Netherlands and Germany in 2003 and 2004.

Phenotypes

The phenotypic data of animals of the commercial and experimental populations were collected according to the German performance test directives (ZDS, Zentral Verband der Deutschen Schweineproduktion e. V. 2004). The definition and abbreviation of traits, the numbers of records, mean values and standard deviations are shown in Table 1. Meat conductivity and pH were measured using Star-series equipment (Rudolf Matthaeus Company) at 24 h postmortem in M. longissimus dorsi at the 13–14th ribs and in M. semimembranosus. Muscle colour was measured at 24 h postmortem using either OPTO-Star or Minolta sensors, essentially providing brightness measures (reflectance = MC_{OPTO}) and CIELAB parameters (MC_{ML} = lightness, MC_{MA} = redness, MC_{MB} = yellowness) and Japanese colour score (MA_{JC}, 1 = pale to 6 = dark) respectively. Internal muscle reflectance was determined using the fibre optic probe (Swatland 2005). Drip loss was scored based on a bag-method using a size-standardized sample from the longissimus dorsi that was collected at 24 h postmortem. A sample was weighed, suspended in a plastic bag, held at 4 °C and re-weighed 48 h later for water loss (Honikel 1986; Kauffman *et al.* 1986). Drip loss was calculated as a percentage of lost weight based on the starting weight of a sample. Another sample was vacuum-packed and frozen at −20 °C. The meat slice was re-weighed after thawing at 4 °C, and thaw loss was calculated as the difference of the sample weights before and after the treatment. To obtain cook loss, a loin cube was taken from the longissimus dorsi, weighed, placed in a polyethylene bag and incubated in water at 75 °C for 50 min. The bag was then immersed in flowing water at room temperature for 30 min and the solid portion in it was re-weighed. Cook loss was obtained as the difference of the sample weights before and after the treatment. Shear force was measured by the Instron-4310 equipment.

Source of candidate genes and identification of polymorphisms

Expression profiles of embryonic (presumptive) and foetal M. longissimus dorsi were compared between Pietrain and Duroc breeds at 7-key stages of myogenesis (days 14, 21, 35, 49, 63, 77 and 91) employing microarrays, subtractive suppressive hybridization and differential display RT-PCR, and were validated by real-time PCR. The various techniques of expression profiling revealed a total of 584 genes that were temporally regulated during myogenesis and/or differentially expressed between the two breeds (Cagnazzo *et al.* 2006; E. Murani, unpublished data; Te Pas *et al.* 2005; Wimmers *et al.* 2005, 2006a). A shortlist of 46 genes was established based on (i) the consistency of the expression pattern and its reproducibility, (ii) known function of the particular gene (categorized as structural, metabolic, translational, transcriptional, signalling, receptor/endocrine factors, differentiation, proliferation and others) and (iii) the map position, giving preference to those genes located in QTL regions for meat-quality traits. Furthermore, preference was given to breed-specific

Table 1 Data collection in five populations and traits measured with mean and standard deviations.

	Trait	DuF1 (n = 626; number of litters = 101)	Du (n = 125; number of litters = 55)	DuPi (n = 335; number of litters = 43)	Pi (n = 259; number of litters = 64)	PiF1 (n = 481; number of litters = 232)
Number of slaughter days		24	32	33	7	11
Meat colour	OPTO-Star (MC_{OPTO})	–	–	68.7 ± 5.6	72.5 ± 6.5	70.4 ± 8.8
	Minolta a (MC_{MA})	19.66 ± 1.28	18.3 ± 0.89	–	8.75 ± 1.25	–
	Minolta b (MC_{MB})	5.73 ± 0.78	5.43 ± 0.86	–	1.87 ± 1.18	–
	Minolta L (MC_{ML})	54.4 ± 2.9	55.3 ± 3.8	–	45.9 ± 2.9	–
	Japanese colour (MC_{JC})	2.95 ± 0.51	–	–	–	–
Routine meat-quality parameters	pH 24 h p.m. in M.sm. ($pH24_{ham}$)	5.86 ± 0.26	–	5.66 ± 0.14	–	–
	pH 24 h p.m. in M.l.d. ($pH24_{loin}$)	5.81 ± 0.20	5.77 ± 0.23	5.52 ± 0.09	5.52 ± 0.10	5.57 ± 0.11
	Conductivity 24 h p.m. in M.l.d. ($C24_{loin}$; mS/cm)	6.16 ± 2.14	–	2.72 ± 0.72	3.21 ± 0.75	3.41 ± 0.88
Water-binding capacity	Drip loss (Dloss; %)	3.08 ± 1.18	4.20 ± 2.6	2.12 ± 0.93	1.47 ± 0.76	1.94 ± 0.79
	Thaw loss (Tloss; %)	5.32 ± 2.40	4.86 ± 2.47	8.10 ± 1.99	7.50 ± 2.61	8.78 ± 2.61
	Cook loss (Closs; %)	22.1 ± 2.8	27.2 ± 2.7	24.8 ± 2.3	23.8 ± 2.1	25.4 ± 2.1
Technological meat-quality parameters	FOP	40.9 ± 10.2	–	–	–	–
	IMF	1.77 ± 0.76	–	–	–	–
	Loin marbling (MAR)	2.17 ± 0.66	–	–	–	–
	Shearforce (Shforce; *N*)	22.3 ± 4.7	22.0 ± 3.4	34.9 ± 6.2	37.8 ± 6.9	38.1 ± 7.1
Carcass traits	FOM muscle, lean content (FOM)	–	–	83.0 ± 8.4	–	–
	LD (mm)	–	–	63.6 ± 5.3	–	–
	LC (%)	–	–	–	63.6 ± 2.1	–
	Ham weight (HAM; kg)	–	–	16.5 ± 1.3	18.6 ± 1.8	–
	LEA (cm^2)	56.2 ± 5.9[1]	–	51.3 ± 5.4[2]	59.3 ± 6.8[2]	51.9 ± 6.0[2]
	Loin weight (LOIN; kg)	7.42 ± 0.83[3]	–	6.27 ± 0.55[4]	7.02 ± 0.76[4]	–
	Weight at slaughter (kg)[5]	88.2 ± 6.4	89.1 ± 5.4	85.0 ± 5.1	89.8 ± 7.7	92.2 ± 7.5

[1]Hennessey Grading Probe (HGP) loin.
[2]Eye muscle area.
[3]Deboned loin weight.
[4]FOM loin weight.
[5]This trait was not evaluated for association.
FOP, fibre optical probe; IMF, intramuscular fat; LD, loin depth; LC, lean content; LEA, loin eye area.

expressed genes, i.e. genes that are differentially regulated between the breeds, as these have a higher likelihood to represent genetic variation useful in breeding. Genes of the shortlist were screened for polymorphisms by comparative sequencing of PCR fragments of about 400 bp in size in a SNP discovery panel of 20 unrelated animals covering five economically relevant breeds (Duroc, Pietrain, German Landrace, Large White and Dutch Yorkshire). Thirty-six of the 46 genes showed either SNPs or insertions/deletions. To perform association analyses, out of these 10 genes were selected on the basis of an analysis of an independent set of 150 offspring of boars with extreme breeding values for drip loss of Pietrain- and Duroc-based commercial populations (selective genotyping). Chi-squared tests were applied to test for significant differences in genotype frequencies in the extreme groups within the two populations of commercial purebred and crossbred herds of Duroc and Pietrain. Genes were selected that showed either highly significant differences ($P < 0.001$) in genotype frequencies in Pietrain or Duroc or that showed significant differences ($P \leq 0.05$) in both populations.

Mapping

For seven loci, mapping information was available from published porcine genome maps. All loci were genetically mapped using the two-point and multi-point analyses of the CRI-MAP package (v. 2.4; Green *et al.* 1990) together with genotypes of 106 microsatellites from a QTL mapping study in the DuPi population (Liu *et al.* 2007; Table 2).

Genotyping

PCR–RFLPs, PCR–SSCPs, single base extension assays, TaqMan assays and melting curve analysis protocols (Murani *et al.* 2005) have been applied to genotype animals (Table 2).

Association analysis

Association of 10 candidate genes with meat-quality and carcass traits was evaluated using ASREML and taking into account pedigree data including parental and grand-parental generations. Models were fitted to identify other

Table 2 List of candidate genes, their functions, polymorphisms and mapping positions.

Gene	Function	bp sequenced; Acc. No. and details of polymorphisms	Genotyping method	Mapping: Porcine chromosomal assignment; genetic mapping	Mapping: Human chromosomal assignment
ANK1 (*ankyrin 1, erythrocytic*)	Structural	305; DV897570:c.172G>C	TaqMan	[SSC17] SSC17 (*SW335*-13.7-*ANK1*-24.9-*SW840*)	HSA8p11.1
bR10D1 (similar to cDNA FLJ26539 fis)	Unknown (non-coding regulatory RNA?)	429; DQ631863:c.162_164del	SSLP	SSC14 (*S0007*-20.5-*bR10D1*-40.8-*SWC27*)	HSA10q21.1
CA3 (*carbonic anhydrase III, muscle-specific*)	Metalloenzyme catalyses hydration of carbon dioxide	535; NM_001008688:c.1156A>G	SBE	SSC4q11-12[1] SSC4 (*S0227*-35.3-*CA3*-11.9-*S0001*)	HSA8q13-22
EPOR (*erythropoietin receptor*)	Member of the cytokine receptor family	424; AF274305:c.1551C>T	SBE	SSC2q12-q21[2] SSC2 (*SW834*-3.3-*EPOR*-6.5-*S0226*)	HSA19p13.3-13.2
HMGA2 (*high mobility group AT-hook 2*)	Regulation of transcription, cell growth	500; DQ631866:c.84C>A	LC-MCA	[SSC5] SSC5 (*SW1482*-41.3-*HMGA2*-8.6-*S0005*)	HSA12q15
MYPN (*myopalladin*)	Structural protein, sarcomere component	438; AJ560657:c.318T>C	PCR–RFLP	SSC14q26-q29[3] SSC14 (*S0007*-24.8-*MYPN*-38.4-*SWC27*)	HSA10q21.3
NME1 (*non-metastatic cells 1, protein*)	Negative regulation of cell proliferation, cell growth and/or maintenance	859; DQ631864:g.361_363del	SSLP	SSC12p11-p13[4] SSC12 (*SW874*-6.1-*NME1*-41.5-*SW605*)	HSA17q21.3
PDGFRA (*platelet-derived growth factor receptor, α polypeptide*)	Cell proliferation	415; DQ631865:c.94C>T	LC-MCA	SSC8p12[5] SSC8 (*PDGFRA*-5.1-*S0086*)	HSA4q11
ERC1 (formerly known as *RAB6IP2; Rab6-interacting protein 2* [*ELKS*])	Small GTPase-mediated signal transduction	1413; DQ631862:g.1018C>C	LC-MCA	SSC5q11[6] SSC5 (*S0005*-13.7-*RAB6IP2*-21.8-*IGF1*)	HSA12p13
TTN (*titin*)	Muscle structural protein	418; AJ560658:c.137C>T	PCR–RFLP	SSC15q23-q26[3] SSC15 (*SW111*-19.6-*TTN*-19.4-*SW936*)	HSA2q31

[1]Fujishima-Kanaya *et al.* (2004).
[2]Fahrenkrug *et al.* (2000).
[3]Davoli *et al.* (2003).
[4]Jorgensen *et al.* (1997).
[5]Johansson *et al.* (1992).
[6]Ponsuksili *et al.* (2001).

[ ], comparative mapping; SSLP, simple sequence length polymorphism; SBE, single base extension; LC-MCA, LightCycler melting curve analysis; *ANK1* primer/probe set – forward: gcgagaatgtggacgttcgt, reverse: ggcagctgaccaggaagaag; Reporter 1 sequence, cagctcctggtcacc (VIC-labelled); Reporter 2 sequence, cagctcctcgtcacc (FAM-labelled); *CA3* primer – forward: ggcgccccccacagcc, reverse: atgatggacctctagttag; *EPOR* primer – forward: ctcagctgccagctttgag, reverse: tgtgactttctgagcaggatg; *MYPN* primer – forward: gactggtgcatgcattgtaga, reverse: aaacctgcctctccgctta; *TTN* primer – forward: cagagcagtgccaactcttg, reverse: tcaaattgatattctgggcagtt.

significant environmental and genetic effects apart from the candidate gene genotypes by stepwise elimination of non-significant effects. Analyses were carried out separately for each of the populations: Duroc, Pietrain, Pietrain × F_1, Duroc × F_1 and Duroc × Pietrain and each trait using the following basic model:

$$Y_{ijkl} = \mu + \mathrm{SEX}_i + \mathrm{SLADATE}_j + \mathrm{GENE}_k + \mathrm{litter}_{ijkl} + \mathrm{animal}_{ijkl} + e_{ijkl}$$

comprising the fixed effects of SEX = gender, SLADATE = day of slaughter, GENE = genotype at a candidate gene and the random effects of 'animal' and 'litter' [variance–covariance structure: random effect 'animal': $n \sim 0$, $A\sigma^2 a$ (A = relationship matrix); random effect 'litter': $n \sim 0$, $I\sigma^2$litter (I = identity matrix); random effect error: $n \sim 0$, $I\sigma^2 e$; cov(animal, litter) = cov(animal, e) = cov(litter, e) = 0]. For the populations DuF1 and DuPi the fixed effect of 'LINE' – which means the two reciprocal crosses within the DuPi and a total of three lines of Duroc × (Landrace × Large White) – was added to the model. For carcass traits (Table 1) also 'slaughter weight' was considered as covariable in the model. Tests of model effects comprising Wald's *F*-test were conducted.

Results and discussion

An association study was performed on 10 genes, whose candidacy for meat-quality and carcass traits arise from their prenatal differential expression, their known function and/or their mapping to QTL regions, using samples and phenotypic records of animals of the commercial and experimental populations. New polymorphic sites segregating among commercial breeds were detected. Most polymorphisms were situated in the 3′-UTR (Table 2). All the SNPs detected in coding regions were synonymous. Fahrenkrug *et al.* (2002) detected one SNP per 184 bp in porcine ESTs, while Jungerius *et al.* (2003) found one SNP per 108 bp in coding and non-coding porcine genomic sequences. Here, we found one SNP in more than 350 bp, though we covered non-coding regions of the genes, which are known to be more variable than coding regions. The lower molecular diversity might be a result of selection pressure as the analysed candidate genes were implicated in muscularity and meat quality, which are the main targets for selection in the commercial pig breeds. Divergence in the numbers might be also due to the differences in the panel of animals used to detect polymorphisms. We focused on a small number of commercially relevant breeds. Most of the polymorphisms detected here were found to be segregating in the commercial breeds. Allele frequencies observed in five commercial populations are shown in Table 3. Genetic mapping results corresponded to pre-existing chromosomal assignments or current human–porcine comparative maps (Table 2). All genes tested, expect *PDGFRA*, showed association at $P \le 0.05$ with meat-quality and quantity traits totalling to 49 associations. Seven associations remain significant when taking into account for multiple testing, with a nominal *P*-value of 0.003 corresponding to a false discovery rate of $Q = 0.05$. In particular, *HMGA2*, *CA3*, *EPOR*, *NME1* and *TTN* were associated with meat colour, pH and conductivity of loin 24 h postmortem; *CA3* and *MYPN* exhibited association with ham weight and lean content (FOM) respectively at $P < 0.003$, i.e. $Q < 0.05$. However, none of the genes showed significant associations for a particular trait across all populations (Table 4). No significant association was found in purebred Duroc. Gene effects (at $P \le 0.05$) are detailed in Table 5.

Ankyrin 1 (*ANK1*) belongs to a family of proteins that link the integral membrane proteins to the underlying spectrin-actin cytoskeleton and plays key roles in activities such as cell motility, activation, proliferation, contact and the maintenance of specialized membrane domains. Multiple isoforms of ankyrin with different affinities for various target proteins are expressed in a tissue-specific,

Table 3 Allele frequencies of 10 selected genes.

Locus (allele[1])	DuF1	Du	DuPi	Pi	PiF1
ANK1 (C)	0.388	0.440	0.310	0.173	0.223
bR10D1 (del)	0.407[2]	0.597	0.215	0.083	0.113[2]
CA3 (A)	0.440	0.085	0.108[2]	0.279	0.484[2]
EPOR (C)	0.767[2]	0.976	0.657	0.277	0.512[2]
HMGA2 (A)	0.632	0.356[2]	0.717[2]	1.00	0.762[2]
MYPN (C)	0.499[2]	0.576	0.196[2]	Not genotyped	Not genotyped
NME1 (del)	0.190	0.220	0.213[2]	0.481	0.373
PDGFRA (C)	0.729[2]	1.0	0.596	0.107	0.437[2]
ERC1 (C)	0.726[2]	0.996	0.677	0.455[2]	0.502[2]
TTN (C)	Not genotyped	Not genotyped	0.285[2]	0.460	0.645[2]

[1]Alleles given here correspond to alleles designated '1' in Table 5; see Table 2 for full details of polymorphisms.
[2]Deviation from Hardy–Weinberg equilibrium ($P < 0.05$, Pearson χ^2-test).

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Table 4 Summary of associations per gene, per population, per trait (results reported for $P \leq 0.05$; *P*-values in parentheses).

Genes	Populations DuF1	DuPi	Pi	PiF1
ANK1	Dloss (0.03) $pH24_{loin}$ (0.006) Closs (0.03) IMF (0.04)		MC_{MA} (0.05)	Tloss (0.006) Shforce (0.01)
bR10D1	FOP (0.05) Shforce (0.03)	LEA (0.005) FOM (0.008)	MC_{OPTO} (0.03) Tloss (0.04)	Shforce (0.009)
CA3	IMF (0.005)	Tloss (0.02) HAM (0.0008)	$C24_{loin}$ (0.006)	
EPOR	IMF (0.05)	Closs (0.03) $pH24_{loin}$ (0.0002) FOM (0.009) HAM (0.0005)	HAM (0.008)	Closs (0.04) Shforce (0.03)
HMGA2	$pH24_{loin}$ (0.04) Shforce (0.01) MC_{ML} (0.0004) MC_{MB} (0.004)			
MYPN	LEA[1] (0.03)	$pH24_{ham}$ (0.05) LD (0.04) FOM (0.0001) LEA (0.03)		
NME1		LD (0.02) LOIN (0.01)		$C24_{loin}$ (0.001)
ERC1	MC_{JC} (0.05)	MC_{OPTO} (0.04) HAM (0.02)	MC_{ML} (0.02) MC_{OPTO} (0.04) LOIN (0.03)	LEA (0.02)
TTN		$C24_{loin}$ (0.002)	LC (0.05) LEA (0.03)	MC_{OPTO} (0.03)

[1]HGP loin.

developmentally regulated manner. *ANK1*, the prototype of this family, was first discovered in the erythrocytes, but since has also been found in brain and muscles. Mutations in erythrocytic *ANK1* have been associated with hereditary spherocytosis (Nakanishi *et al.* 2001). *ANK1* showed significant association with traits related to water-binding capacity, drip loss, thaw loss, cook loss and pH in PiF1 and DuF1 (Tables 4 and 5). A significant association with IMF was found in DuF1 (Tables 4 and 5). In PiF1, *ANK1* is associated with shear force (Tables 4 and 5). However, the position of the protein in the cell may not be related to a role in these traits. The results indicate that effects seen here were due to linkage and association with a QTL segregating in the white breeds rather than the Du and Pi lines covered by this study. No effects were found in the DuPi, which is consistent with our finding that no QTL for meat-quality and carcass traits segregate close to the position of *ANK1* in the DuPi (Liu *et al.* 2007). The proximal region of SSC17, where *ANK1* is located, exhibits a QTL for juiciness (Malek *et al.* 2001).

The EST *bR10D1* (*FLJ26539*) mapped to SSC14 in accordance with the human–porcine comparative map and genetic mapping. The position of *bR10D1* fell in the confidence interval of pH and meat-colour QTL reported by de Koning *et al.* (2001). In a F_2 resource population based on Duroc and Berlin Miniature Pig (DuMi), QTL were identified for muscle-fibre traits (Wimmers *et al.* 2006b). The function of *FLJ26539* is unknown, however, it is highly conserved between human, mouse and chicken (http://ecrbrowser.dcode.org). The intron-less *FLJ26539* is located close to *BICC1*, a gene encoding an RNA-binding protein that is active in regulating gene expression during embryonic development. *FLJ26539* may represent a novel exon of *BICC1* or a non-coding RNA, regulating the expression of the *BICC1* gene. This is supported by the high level of sequence conservation of *FLJ26539* and the expression pattern of *bR10D1*. The locus *bR10D1* exhibited significant effects on shear force in DuF1 and PiF1, on muscularity in DuPi and on meat colour and thaw loss in Pi (Tables 4 and 5). Allelic gene effects were not additive or dominant, partly due to the distribution of the alleles.

Carbonic anhydrase III (*CA3*) is a member of a multigene family that encodes carbonic anhydrase isozymes, a class of metalloenzymes that catalyse the reversible hydration of carbon dioxide. The expression of the *CA3* gene is strictly tissue-specific and present at high levels in skeletal muscle.

Table 5 Results of association ($P \leq 0.05$) analysis for traits related to meat quality, including routine and technological parameters and water-binding capacity as well as carcass traits with number of animals and lsmeans per genotype.

	Gene	Population	Number of animals[2]			Trait	*P*-value*	Estimated mean per genotype[2]		
			11	12	22			11	12	22
Meat colour	*ANK1*	Pi	3	79	164	MC_{MA}	0.05	9.02 ± 1.00	9.07 ± 1.20	8.62 ± 1.25
	bR10D1	Pi	–	20	222	MC_{OPTO}	0.03		74.1 ± 6.9	72.1 ± 6.5
	HMGA2	DuF1	118	163	33	MC_{MB}	0.004	5.63 ± 0.77	5.72 ± 0.78	6.04 ± 0.82
		DuF1	119	163	33	**MC_{ML}**	**0.0004**	**53.9 ± 2.8**	**53.9 ± 2.5**	**55.9 ± 3.0**
	ERC1	Pi	37	147	59	MC_{ML}	0.02	47.1 ± 2.0	46.1 ± 2.7	45.0 ± 3.2
		Pi	37	147	59	MC_{OPTO}	0.04	69.6 ± 5.1	72.0 ± 6.2	74.0 ± 7.3
		DuPi	145	156	28	MC_{OPTO}	0.04	69.6 ± 5.5	68.4 ± 5.7	65.0 ± 4.3
		DuF1	168	181	7	MC_{JC}	0.05	2.92 ± 0.53	2.97 ± 0.48	2.71 ± 0.64
	TTN	PiF1	157	302	19	MC_{OPTO}	0.03	69.8 ± 9.4	70.5 ± 8.7	73.1 ± 6.6
Routine meat-quality parameters	*ANK1*	DuF1	42	174	144	$PH24_{loin}$	0.006	5.83 ± 0.18	5.77 ± 0.17	5.82 ± 0.18
	CA3	Pi	16	101	125	$C24_{loin}$	0.006	3.08 ± 0.77	3.11 ± 0.75	3.27 ± 0.75
	EPOR	DuPi	132	160	32	**$PH24_{lion}$**	**0.0002**	**5.51 ± 0.09**	**5.52 ± 0.09**	**5.58 ± 0.12**
	HMGA2	DuF1	116	160	33	$PH24_{loin}$	0.04	5.79 ± 0.14	5.82 ± 0.19	5.73 ± 0.14
	MYPN	DuPi	5	110	191	$PH24_{ham}$	0.05	5.66 ± 0.10	5.69 ± 0.18	5.65 ± 0.12
	NME1	PiF1	57	191	166	**$C24_{loin}$**	**0.001**	**3.23 ± 0.89**	**3.55 ± 0.95**	**3.30 ± 0.84**
	TTN	DuPi	4	157	145	**$C24_{loin}$**	**0.002**	**5.68 ± 3.15**	**2.82 ± 0.82**	**2.67 ± 0.68**
Water-binding capacity	*ANK1*	DuF1	33	136	130	Closs	0.03	21.7 ± 2.8	22.6 ± 2.9	21.7 ± 2.7
		DuF1	33	136	130	Dloss	0.03	2.82 ± 0.99	3.26 ± 1.28	2.94 ± 1.09
		PiF1	23	159	262	Tloss	0.006	10.55 ± 3.34	8.64 ± 2.55	8.72 ± 2.50
	bR10D1	Pi	–	20	222	Tloss	0.04		7.68 ± 2.99	7.53 ± 2.53
	CA3	DuPi	–	251	66	Tloss	0.02		7.53 ± 1.99	8.27 ± 1.97
	EPOR	DuPi	129	156	32	Closs	0.03	24.9 ± 2.2	24.7 ± 2.3	25.4 ± 2.4
		PiF1	107	224	94	Closs	0.04	25.8 ± 2.1	25.2 ± 2.1	25.4 ± 2.1
Technological meat-quality parameters	*ANK1*	DuF1	33	133	130	IMF	0.04	1.50 ± 0.56	1.71 ± 0.70	1.75 ± 0.70
		PiF1	23	161	271	Shforce	0.01	39.6 ± 8.1	36.9 ± 6.0	38.6 ± 7.5
	bR10D1	DuF1	27	211	95	FOP	0.05	36.8 ± 9.4	41.5 ± 10.0	40.0 ± 10.0
		DuF1	22	187	88	Shforce	0.03	25.8 ± 4.7	22.5 ± 4.6	21.1 ± 4.8
		PiF1	1	94	324	Shforce	0.009	24.2	39.2 ± 7.64	37.6 ± 6.9
	CA3	DuF1	34	149	104	IMF	0.005	1.96 ± 0.86	1.74 ± 0.66	1.62 ± 0.69
	EPOR	DuF1	151	140	–	IMF	0.05	1.65 ± 0.68	1.83 ± 0.72	
		PiF1	107	223	93	Shforce	0.03	38.1 ± 6.7	37.9 ± 7.1	37.7 ± 7.2
	HMGA2	DuF1	110	140	29	Shforce	0.01	22.1 ± 4.4	22.9 ± 5.2	21.4 ± 4.1
Carcass traits	*bR10D1*	DuPi	–	51	123	FOM	0.008		68.1 ± 4.1	68.9 ± 5.3
		DuPi	12	111	203	LEA	0.005	48.5 ± 6.1	50.8 ± 4.9	51.8 ± 5.6
	CA3	DuPi	–	70	254	**HAM**	**0.0008**		**16.0 ± 1.5**	**16.3 ± 1.2**
	EPOR	Pi	16	88	120	HAM	0.008	18.1 ± 2.1	18.3 ± 1.7	18.8 ± 1.7
		DuPi	62	91	20	FOM	0.009	68.9 ± 5.1	68.6 ± 5.1	69.1 ± 4.2
		DuPi	132	160	32	**HAM**	**0.0005**	**16.3 ± 1.2**	**16.4 ± 1.3**	**15.7 ± 1.2**
	MYPN	DuPi	–	68	108	**FOM**	**0.0001**		**67.4 ± 4.2**	**69.5 ± 5.2**
		DuPi	5	109	190	LD	0.04	69.1 ± 4.4	63.2 ± 4.1	63.8 ± 4.1
		DuPi	5	110	191	LEA	0.03	50.9 ± 5.5	50.2 ± 4.9	51.9 ± 5.6
		DuF1	86	298	76	LEA[1]	0.03	54.4 ± 5.5	56.2 ± 5.7	55.3 ± 5.6
	NME1	DuPi	–	136	186	LD	0.02		64.4 ± 4.1	63.4 ± 4.2
		DuPi	–	136	187	LOIN	0.01		6.33 ± 0.59	6.22 ± 0.51
	ERC1	PiF1	82	287	80	LEA	0.02	52.6 ± 7.2	52.1 ± 5.7	51.0 ± 5.8
		Pi	33	135	55	LOIN	0.03	7.16 ± 0.77	6.94 ± 0.79	7.08 ± 0.69
		DuPi	145	156	28	HAM	0.02	16.1 ± 1.3	16.3 ± 1.2	16.5 ± 1.2
	TTN	Pi	48	137	68	LC	0.05	63.2 ± 2.2	64.0 ± 2.2	63.3 ± 1.7
		Pi	48	137	68	LEA	0.03	57.0 ± 6.6	60.3 ± 7.3	58.9 ± 5.7

**P*-values of 0.05, 0.01, 0.001, 0.0001 correspond to false discovery rates of 0.467, 0.233, 0.077 and 0.046 respectively; *P*-values of <0.003 correspond to false discovery rates of $Q < 0.05$; associations reaching this level are marked in bold.

[1]HGP loin.

[2]Alleles designated as '1' are shown in Table 3.

A proportion of carriers of Duchenne muscle dystrophy have a higher *CA3* level than normal (Mokuno *et al.* 1985). *CA3* showed highly significant association with conductivity in Pi (Tables 4 and 5). Effects on thaw loss were found in DuPi. IMF in DuF1 and ham weight in DuPi was highly significantly associated with *CA3* ($P < 0.003$, $Q < 0.05$). The observed associations suggest that this may be an interesting gene for further research. Davoli *et al.* (2006) observed significantly different allele frequencies ($P < 0.05$) between the Duroc pigs divergent for visible intermuscular fat and supposed a likely association of *CA3* with IMF. An association between polymorphisms at this gene and IMF and percentage of ham was also suggested by Wang *et al.* (2006). Moreover, *CA3* mapped to the central region of SSC4 where QTL for carcass traits as well as meat-quality traits and muscle fibre size were detected in various resource populations including those based on Pietrain or Duroc (Geldermann *et al.* 2003; Wimmers *et al.* 2005, 2006b). Recently, a QTL for water-holding capacity was found close to *CA3* on SSC4 (Su *et al.* 2004).

Erythropoietin receptor (*EPOR*) is a member of the cytokine receptor family that is involved in regulating growth and proliferation. Interestingly, a number of QTL for meat colour and traits related to water-holding capacity were detected in the region of SSC2 (Malek *et al.* 2001) where *EPOR* was genetically mapped in this study. In the DuPi, significant QTL for lean traits were identified; and in the DUMI, significant QTL were identified for muscle fibre size, number and proportion of intermediate (fast twitch oxidative) fibres (Wimmers *et al.* 2006b; Liu *et al.* 2007). The study revealed association of *EPOR* on muscularity in Pi and DuPi and on water-binding properties (cook loss, pH) in PiF1 and DuPi (Tables 4 and 5). Association with $pH24_{loin}$ in DuPi was highly significant ($P < 0.003$, $Q < 0.05$). Effects on shear force and IMF were found in PiF1 and DuF1 respectively (Tables 4 and 5).

High mobility group AT-hook 2 (*HMGA2*) encodes a protein that belongs to the non-histone chromosomal high mobility group (HMG) protein family. HMG proteins function as architectural factors and are essential components of enhancers and act as a transcriptional regulating factor. Recently, experiments with embryonic stem cells revealed involvement of *HMGA2* in skeletal muscle differentiation *in vitro* (Caron *et al.* 2005). *HGMA2* is a positional candidate for QTL for meat colour, pH and conductivity identified on SSC5 (Malek *et al.* 2001; Geldermann *et al.* 2003). Only in DuF1 effects of *HMGA2* were found on meat colour, pH as well as shear force (Tables 4 and 5). This locus is fixed in the Pi examined but segregated in the other populations (Table 3). In DuF1 we observed association of *HMGA2* with meat colour at $P < 0.003$ ($Q < 0.05$). Other associations at $P < 0.05$ with pH and shear force were also observed in DuF1. Thus, it appears that the SNP we analysed is in LD with a DNA variable site in *HMGA2* that is segregating exclusively in the DuF1 population.

MYPN is a structural component of muscle. As a component of the sarcomere, it tethers nebulin in skeletal muscle and nebulette in cardiac muscle to alpha-actinin at the Z lines. Effects of *MYPN* on carcass traits were hypothesized by Davoli *et al.* (2003). Moreover, on SSC14, where *MYPN* is located, there are indications of the presence of QTL for several carcass and growth traits including loin weight and daily gain (Rohrer & Keele 1998). *MYPN* showed association with traits related to muscularity in DuF1 and DuPi: in particular association with FOM muscle in DuPi was significant at $P < 0.003$ ($Q < 0.05$) (Tables 4 and 5). It is noteworthy that for FOM muscle trait, there are no samples with genotype 11 in DuPi.

Non-metastatic cells 1 (*NME1*) was identified because of its reduced mRNA transcript levels in highly metastatic cells. *NME1* encodes the 'A' isoform of nucleoside diphosphate kinase (NDK) and is involved in the regulation of cell proliferation. *NME1* maps on the proximal region of SSC12 while QTL for chewiness score and meat colour were identified in the more distal region (Malek *et al.* 2001). For *NME1* effects on conductivity were found in PiF1 at $P < 0.003$ ($Q < 0.05$), while in DuPi effects on muscularity were shown (Tables 4 and 5).

ERC1 (previously known as *RAB6IP2*) was found to associated with meat colour and muscularity consistently in DuF1, Pi and DuPi on one hand, and Pi, PiF1 and DuPi on the other hand (Tables 4 and 5). The function of the *ERC1* gene is less well understood. Recently, Ducut Sigala *et al.* (2004) proposed that the ERC1 protein is a part of the IKK complex playing a role in the activation of NF-κB transcription factor. Activation of NF-κB is important in the induction of proteasome expression and protein degradation (Wyke *et al.* 2004). The NF-κB transcription factor also functions as a negative regulator of myogenesis by inhibiting MyoD (Guttridge 2004). We mapped *ERC1* physically and genetically on chromosome 5, in the middle of marker-interval *S0005-IGF1* at approximately 100 cM (*TNFR-SW152*) on the USDA linkage map. According to the PigQTL database downstream of this position is a QTL for ham weight, loin and ham percentage in carcass, pH and meat colour. Interestingly, a QTL for muscle structure was found in SSC5 in the proximity of *S0005* (Haley, US Patent App. 20040101842).

Titin (*TTN*) encodes a large abundant protein of striated muscle. The product of this gene is divided into two regions, an N-terminal I-band and a C-terminal A-band. A single titin molecule spans half the length of a sarcomere. Via binding sites for muscle-associated proteins titin serves as an adhesion template for the assembly of the contractile machinery in muscle cells. *TTN* is located in SSC15 (Bertani *et al.* 1999; Davoli *et al.* 2003) within a region exhibiting QTL for pH, flavour and tenderness (Malek *et al.* 2001). In agreement with Davoli *et al.* (2003) we found association of *TTN* and several lean content traits in Pi. Association of *TTN* was also found with meat colour in PiF1 and conductivity

in DuPi ($P < 0.003$, $Q < 0.05$; Tables 4 and 5). This is in agreement with previous reports of association with drip loss and colour (Pospiech *et al.* 2003; Toldrà 2003; Melody *et al.* 2004).

Platelet-derived growth factor receptor, α polypeptide (*PDGFRA*) encodes a cell surface tyrosine kinase receptor for members of the platelet-derived growth factor family. These growth factors are mitogens for cells of mesenchymal origin. *PDGFRA* was genetically mapped to SSC8 within a region where QTL for meat colour and type I fibre proportion have been shown (Ovilo *et al.* 2002; Geldermann *et al.* 2003; Malek *et al.* 2001). *PDGFRA* showed no effects reaching the $P \leq 0.05$ threshold.

The significant values presented are nominal *P*-values not adjusted for multiple testing which inflate the risk of finding false positives. Correction for multiple testing by Bonferroni correction is very conservative because it does not take into account correlation between traits and would probably lead to high type II error rate. With 544 tests made (genes × populations × trait records; Tables 1 and 3) some 29 results significant at the nominal 5% level can be expected by chance. However, the study revealed 50 associations, which is considerably more. Correspondingly, taking into account all 544 tests a nominal *P*-value of 0.05 equals a false discovery rate of $Q = 0.47$; *P*-values of <0.003 correspond to false discovery rates of <0.05.

The polymorphisms analysed are most likely non-functional mutations. The observed effects are not consistent across the analysed populations. This may be due to breed-specific effects that are related to the different extreme muscle phenotypes of the pig breeds (Jones 1998; Sellier 1998) that may also be related to well-known differences in meat quality of these pig breeds such as drip loss. This is not unexpected as the traits analysed are quantitative traits controlled by several loci. Also, the population size as well as the marker allele frequency varies and consequently some populations are less informative for a particular marker. The partly inconsistent gene effects between populations also indicate that the polymorphisms may not be in linkage disequilibrium to the same extent across different populations with the causative genetic variation. In general, association studies often suffer from the fact that the nature and extent of linkage disequilibrium will differ from population to population and could be extensive and long-range for some of the populations that involve crosses while linkage disequilibrium is expected to be least in purebreds. For some genes in some populations the distribution of genotypes was unequal, which may lead to reduced power of the analysis on one hand or positive results on the other hand that are due to extended linkage disequilibrium especially in crosses. In particular, for *ANK* in Pi, *TTN* in DuPi, *bR10D1* in PiF1 and *PDGFRA* in DiF1 one of the genotype classes was represented by <1% of the animals tested (Table 5). In the crosses we found deviation from Hardy–Weinberg equilibrium for most genes that is likely due to differences in allele frequencies of the parental lines; deviation from Hardy–Weinberg equilibrium observed in the purebreds is potentially because of selection (Table 3).

Expression analysis of the transcriptome of Duroc and Pietrain prenatal pig muscle revealed a number of genes that show stage- and/or breed-specific expression in prenatal muscle and represent as such, functional candidate genes for meat-quality and carcass traits (Murani *et al.* 2003; E. Murani, unpublished data; Te Pas *et al.* 2005; Cagnazzo *et al.* 2006). It is remarkable that by using functional expression data to select candidate genes for SNP detection and association we addressed genes with no obvious candidacy for meat quality, for example, *NME1* and *ERC1*, or even ESTs with no known function [EST *bR10D1* (*FLJ26539*)] which probably would not have been selected for further study based on mapping data. Here, we showed that for most of the genes, knowledge on their physiological role support their putative involvement in genetic regulation of these traits. Moreover, association studies provided statistical evidence for a link of DNA variation at these loci or close to them with traits of interest. The regional assignments to QTL regions also support the findings. These genes are thus functional positional candidate genes, for which linkage and association with the traits analysed could be demonstrated.

Summarizing this study revealed 10 candidate genes derived from prenatal muscle expression profiles that were associated with several pork production and quality traits. The results encourage performing further evaluation of these candidate genes, including analyses in independent populations, attempts to identify causal mutations and possible gene interactions, to promote their use for gene-assisted selection in breeding population.

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References

Bertani G.R., Larsen N.J., Marklund S., Hu Z.L. & Rothschild M.F. (1999) Rapid communication: mapping of the *titin* (*TTN*) gene to pig chromosome 15. *Journal of Animal Science* **77**, 2857–8.

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.

Caron L., Bost F., Prot M., Hofman P. & Binetruy B. (2005) A new role for the oncogenic high-mobility group A2 transcription factor in myogenesis of embryonic stem cells. *Oncogene* **24**, 6281–91.

Davoli R., Braglia S., Lama B., Fontanesi L., Buttazzoni L., Baiocco C. & Russo V. (2003) Mapping, identification of polymorphisms and analysis of allele frequencies in the porcine skeletal muscle *myopalladin* and *titin* genes. *Cytogenetic and Genome Research* **102,** 152–6.

Davoli R., Fontanesi L., Braglia S., Nisi I., Scotti E., Buttazzoni L. & Russo V. (2006) Investigation of SNPs in the *ATP1A2, CA3* and *DECR1* genes mapped to porcine chromosome 4: analysis in groups of pigs divergent for meat production and quality traits. *Italian Journal of Animal Science* **5,** 249–63.

Ducut Sigala J.L., Bottero V., Young D.B., Shevchenko A., Mercurio F. & Verma I.M. (2004) Activation of transcription factor NF-kappaB requires ELKS, an IkappaB kinase regulatory subunit. *Science* **304,** 1963–7.

Ernst C.W., Vaske D.A., Larson R.G., White M.E. & Rothschild M.F. (1994) Rapid communication: MspI restriction fragment length polymorphism at the swine *MYF6* locus. *Journal of Animal Science* **72,** 799.

Fahrenkrug S.C., Campbell E.M., Vallet J.L. & Rohrer G.A. (2000) Physical assignment of the porcine *erythropoietin receptor* gene to SSC2. *Animal Genetics* **31,** 69–70.

Fahrenkrug S.C., Freking B.A., Smith T.P., Rohrer G.A. & Keele J.W. (2002) Single nucleotide polymorphism (SNP) discovery in porcine expressed genes. *Animal Genetics* **33,** 186–95.

Fujishima-Kanaya N., Ito Y., Suzuki K., Sawazaki T., Hiraiwa H., Uenishi H. & Awata T. (2004) The porcine homologues of six genes located on human chromosome 8 (*RAB2, CA3, PTDSS1, MATN2, FZD6* and *SQLE*) assigned to porcine chromosome 4 by fluorescence *in situ* hybridization. *Animal Genetics* **35,** 501–2.

Geldermann H., Müller E., Moser G. *et al.* (2003) Genome-wide linkage and QTL mapping in porcine F_2 families generated from Pietrain, Meishan and Wild Boar crosses. *Journal of Animal Breeding and Genetics* **120,** 363–93.

Green P., Falls K. & Crooks S. (1990) *Documentation for CRI-MAP,* Version 2.4. http://www.animalgenome.org/~hu/CRIMAPwkshp/crimap-doc.html.

Guttridge D.C. (2004) Signaling pathways weigh in on decisions to make or break skeletal muscle. *Current Opinions in Clinical Nutrition and Metabolic Care* **7,** 443–50.

Honikel K.O. (1986) Wasserbindungsvermögen von Fleisch. *Mitteilungsblatt der BAFF* **94,** 7150–4.

Johansson M., Ellegren H., Marklund L., Gustavsson U., Ringmar-Cederberg E., Andersson K., Edfors-Lilja I. & Andersson L. (1992) The gene for dominant white color in the pig is closely linked to *ALB* and *PDGRFRA* on chromosome 8. *Genomics* **14,** 965–9.

Jones G.F. (1998) Genetic aspects of domestication. Common breeds and their origin. In: *The Genetics of the Pig* (Ed. by M.F. Rothschild & A. Ruvinsky), pp. 17–50. CAB Int., Wallingford, Oxon, UK.

Jorgensen C.B., Wintero A.K., Yerle M. & Fredholm M. (1997) Mapping of twenty-two expressed sequence tags isolated from a porcine small intestine cDNA library. *Mammalian Genome* **8,** 423–7.

Jungerius B.J., Rattink A.P., Crooijmans R.P., van der Poel J.J., van Oost BA., te Pas M.F. & Groenen M.A. (2003) Development of a single nucleotide polymorphism map of porcine chromosome 2. *Animal Genetics* **34,** 429–37.

Kauffman R.G., Eikeienboom G., van der Wal P.G., Merkus G. & Zaar M. (1986) The use of filter paper to estimate drip loss of porcine musculature. *Meat Science* **18,** 191–200.

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.

Lefaucheur L. (2006) Myofibre typing and its relationships to growth performance and meat quality. *Archives of Animal Breeding* **49** (Special Issue)**,** 4–17.

Lengerken von G., Maak S., Wicke M., Fiedler I. & Ender K. (1994) Suitability of structural and functional traits of skeletal muscle for the improvement of meat quality in pigs. *Archives of Animal Breeding* **37,** 133–43.

Liu G., Jennen D.G.J., 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.

Malek M., Dekkers J.C., Lee H.K., Baas T.J., Prusa K., Huff-Lonergan E. & Rothschild M.F. (2001) 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.

Melody J.L., Lonergan S.M., Rowe L.J., Huiatt T.W., Mayes M.S. & Huff-Lonergan E. (2004) Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. *Journal of Animal Science* **82,** 1195–205.

Mokuno K., Riku S., Matsuoka Y., Sobue I. & Kato K. (1985) Serum carbonic anhydrase III in progressive muscular dystrophy. *Journal of Neurological Science* **67,** 223–8.

Murani E., Muraniova M., Ponsuksili S., Schellander K. & Wimmers K. (2003) Expressed Sequence Tags Derived from Prenatal Development of Skeletal Muscle in Pigs: A Source of Functional Candidate Genes for Pork Quality. *Book of Abstracts of the 54th Annual Meeting of the European Association for Animal Production* (Ed. by Y. van der Honing), No. 9, 247. Wageningen Academic Publishers, Wageningen, the Netherlands.

Murani E., Ponsuksili S. & Wimmers K. (2005) Simultaneous detection of SNPs in four porcine genes using hybridisation probes and the Light Cycler 2.0 instrument. *Biochemica* **2,** 7–9.

Nakanishi H., Kanzaki A., Yawata A., Yamada O. & Yawata Y. (2001) Ankyrin gene mutations in Japanese patients with hereditary spherocytosis. *International Journal of Hematology* **73,** 54–63.

Ovilo C., Clop A., Noguera J.L. *et al.* (2002) Quantitative trait locus mapping for meat quality traits in an Iberian × Landrace F2 pig population. *Journal of Animal Science* **80,** 2801–8.

Ponsuksili S., Wimmers K., Yerle M. & Schellander K. (2001) Mapping of 93 porcine ESTs preferentially expressed in liver. *Mammalian Genome* **12,** 869–72.

Pospiech E., Grzes B., Lyczynski A., Borzuta K., Szalata M. & Mikolajczak B. (2003) Muscle proteins and their changes in the process of meat tenderization. *Animal Science Papers and Reports* **21** (Suppl. 1)**,** 133–51.

Rehfeldt C., Fiedler I. & Stickland N.C. (2004) Number and size of muscle fibres in relation to meat production. In: *Muscle Development of Livestock Animals Physiology, Genetics, and Meat Quality* (Ed. by M.F.W. Te Pas, M.E. Everts & H.P. Haagsman), pp. 1–37. CAB Int., Wallingford, Oxon, UK.

Rohrer G.A. & Keele J.W. (1998) Identification of quantitative trait loci affecting carcass composition in swine: II. Muscling and wholesale product yield traits. *Journal of Animal Science* **76,** 2255–62.

Sellier P. (1998) Genetics of meat and carcass traits. In: *The Genetics of the Pig* (Ed. by M.F. Rothschild & A. Ruvinsky), pp. 463–510. CAB Int., Wallingford, Oxon, UK.

Stratil A. & Cepica S. (1999) Three polymorphisms in the porcine *myogenic factor 5* (*MYF5*) gene detected by PCR-RFLP. *Animal Genetics* **30**, 79–80.

Su Y., Xiong Y., Jiang S., Zhang Q., Lei M., Zheng R. & Deng C. (2004) Mapping quantitative trait loci for meat quality traits in a Large White x Meishan cross. *Acta Genetica Sinica* **31**, 132–6.

Swatland H.J. (1973) Muscle growth in the fetal and neonatal pig. *Journal of Animal Science* **37**, 536–45.

Swatland H.J. (2005) On correlating penetrometry with photometry in beef roasts. *Archives of Animal Breeding* **48**, 318–23.

Te Pas M.F., Soumillion A., Harders F.L., Verburg F.J., den Bosch T.J., Galesloot P. & Meuwissen T.H. (1999a) Influences of myogenin genotypes on birth weight, growth rate, carcass weight, backfat thickness, and lean weight of pigs. *Journal of Animal Science* **77**, 2352–6.

Te Pas M.F., Harders F.L., Soumillion A., Born L., Buist W. & Meuwissen T.H. (1999b) Genetic variation at the porcine *MYF-5* gene locus. Lack of association with meat production traits. *Mammalian Genome* **10**, 123–7.

Te Pas M.F.W. & Soumillion A. (2001) Improvement of livestock breeding strategies using physiologic and functional genomic information of the muscle regulatory factors gene family for skeletal muscle development. *Current Genomics* **2**, 285–304.

Te Pas M.F., De Wit A.A., Priem J., Cagnazzo M., Davoli R., Russo V. & Pool M.H. (2005) Transcriptome expression profiles in prenatal pigs in relation to myogenesis. *Journal of Muscle Research and Cell Motility* **26**, 157–65.

Toldrà F. (2003) Muscle foods: water, structure and functionality. *Food Science and Technology International* **9**, 173–7.

Urbanski P. & Kuryl J. (2004) New SNPs in the coding and 5′ flanking regions of porcine *MYOD1* (*MYF3*) and *MYF5* genes. *Journal of Applied Genetics* **45**, 325–9.

Urbanski P., Flisikowski K., Starzynski R.R., Kuryl J. & Kamyczek M. (2006) A new SNP in the promoter region of the porcine *MYF5* gene has no effect on its transcript level in m. longissimus dorsi. *Journal of Applied Genetics* **47**, 59–61.

Wang H.L., Zhu Z.M., Wang H., Yang S.L., Zhao S.H. & Li K. (2006) Molecular characterization and association analysis of porcine *CA3*. *Cytogenetic and Genome Research* **115**, 129–33.

Wimmers K., Murani E., Schellander K. & Ponsuksili S. (2005) Combining QTL- and expression-analysis: identification of functional positional candidate genes for meat quality and carcass traits. *Archives of Animal Breeding* **48** (Special Issue), 23–31.

Wimmers K., Trong Ngu N., Murani E., Schellander K. & Ponsuksili S. (2006a) Linkage and expression analysis to elucidate the genetic background of muscle structure and meat quality in the pig. *Archives of Animal Breeding* **49** (Special Issue), 116–25.

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

Wyke S.M., Russell S.T. & Tisdale M.J. (2004) Induction of proteasome expression in skeletal muscle is attenuated by inhibitors of NF-kappaB activation. *British Journal of Cancer* **91**, 1742–50.

Wyszynska-Koko J. & Kuryl J. (2004) Porcine *MYF6* gene: sequence, homology analysis, and variation in the promoter region. *Animal Biotechnology* **15**, 159–73.

Wyszynska-Koko J., Pierzchala M., Flisikowski K., Kamyczek M., Rozycki M. & Kuryl J. (2006) Polymorphisms in coding and regulatory regions of the porcine *MYF6* and *MYOG* genes and expression of the *MYF6* gene in m. longissimus dorsi versus productive traits in pigs. *Journal of Applied Genetics* **47**, 131–8.

ZDS, Zentral Verband der Deutschen Schweineproduktion e. V. (2004) *Richtlinie fuer die Stationspruefung auf Mastleistung, Schlachtkoerperwert und Fleischbeschaffenheit beim Schwein*. Bonn, Germany 01.01.2004, http://www.zds-bonn.de/section_name_publikationen.html.