

A genetical genomics approach reveals new candidates and confirms known candidate genes for drip loss in a porcine resource population

Hanna Heidt · Mehmet Ulas Cinar · Muhammad Jasim Uddin · Christian Looft · Heinz Jüngst · Dawit Tesfaye · Astrid Becker · Andreas Zimmer · Siriluck Ponsuksili · Klaus Wimmers · Ernst Tholen · Karl Schellander · Christine Große-Brinkhaus

Received: 3 May 2013 / Accepted: 5 August 2013 / Published online: 12 September 2013
© Springer Science+Business Media New York 2013

Abstract In this study lean meat water-holding capacity (WHC) of a Duroc × Pietrain (DuPi) resource population with corresponding genotypes and transcriptomes was investigated using genetical genomics. WHC was characterized by drip loss measured in *M. longissimus dorsi*. The 60K Illumina SNP chips identified genotypes of 169 F₂ DuPi animals. Whole-genome transcriptomes of muscle samples were available for 132 F₂ animals using the Affymetrix 24K GeneChip® Porcine Genome Array. Performing genome-wide association studies of transcriptional profiles, which are correlated with phenotypes, allows

elucidation of *cis*- and *trans*-regulation. Expression levels of 1,228 genes were significantly correlated with drip loss and were further analyzed for enrichment of functional annotation groups as defined by Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathways. A hypergeometric gene set enrichment test was performed and revealed glycolysis/glyconeogenesis, pentose phosphate pathway, and pyruvate metabolism as the most promising pathways. For 267 selected transcripts, expression quantitative trait loci (eQTL) analysis was performed and revealed a total of 1,541 significant associations. Because of positional accordance of the gene underlying transcript and the eQTL location, it was possible to identify eight eQTL that can be assumed to be *cis*-regulated. Comparing the results of gene set enrichment and the eQTL detection tests, molecular networks and potential candidate genes, which seemed to play key roles in the expression of WHC, were detected. The *α-1-microglobulin/bikunin precursor (AMBP)* gene was assumed to be *cis*-regulated and was part of the glycolysis pathway. This approach supports the identification of trait-associated SNPs and the further biological understanding of complex traits.

Electronic supplementary material The online version of this article (doi:10.1007/s00335-013-9473-z) contains supplementary material, which is available to authorized users.

H. Heidt · M. U. Cinar · M. J. Uddin · C. Looft · H. Jüngst · D. Tesfaye · E. Tholen · K. Schellander · C. Große-Brinkhaus (✉)
Institute of Animal Science, University of Bonn,
Endenicher Allee 15, 53115 Bonn, Germany
e-mail: cgro@itw.uni-bonn.de

M. U. Cinar
Department of Animal Science, Faculty of Agriculture, Erciyes
University, 38039 Kayseri, Turkey

A. Becker · A. Zimmer
Institute of Molecular Psychiatry, University of Bonn,
Sigmund-Freud-Str. 25, 53127 Bonn, Germany

S. Ponsuksili
Research Group Functional Genomics, Research Institute for the
Biology of Farm Animals (FBN), Wilhelm-Stahl-Allee 2,
18196 Dummerstorf, Germany

K. Wimmers
Research Unit Molecular Biology, Research Institute for the
Biology of Farm Animals (FBN), Wilhelm-Stahl-Allee 2,
18196 Dummerstorf, Germany

Introduction

Water-holding capacity (WHC), i.e., the capacity of meat to retain its water during application of external forces (Hamm 1985), is an important quality criterion for the meat-processing industry and consumers. WHC can be predicted by measuring drip loss, which is a fluid consisting of water and protein expelled from the meat surface without any mechanical force other than gravity and is influenced by shrinkage of the myofibrils, pH value, and temperature post mortem (Offer and Knight 1988).

The structural components and the biological processes of WHC and its associated traits have been investigated extensively (Greaser 2001; Huff-Lonergan and Lonergan 2005, 2007; Huff-Lonergan et al. 2010; Offer and Cousins 1992; Rosenvold and Andersen 2003). However, the genetic mechanisms underlying WHC during the conversion of muscle to meat are not fully understood. The presented heritability (h^2) for drip loss in different breeds varied between 0.01 and 0.31, which may be dependent on the different measurement methods (e.g., Borchers et al. 2007; Ciobanu et al. 2011), and demonstrates the complexity of this trait.

A genome scan is the most general approach to identify genomic regions with quantitative trait loci (QTL), classically used for complex phenotypic characteristics. Such QTL regions are generally large and may contain thousands of putative genes, which are all candidate loci for the trait (Wayne and McIntyre 2002). Until now, 6,397 QTL covering 578 phenotypic traits have been identified and submitted for entry into the pig QTLdb (www.animalgenome.org, release April 12, 2013). In total, 953 QTL were identified for drip loss in loin in different pig populations (Hu et al. 2010). These QTL were identified mostly via a genome scan based on linkage analyses and microsatellite genotyping. Liu et al. (2007, 2008) found seven QTL for drip loss [*Sus scrofa* chromosomes (SSC) 2, 3, 4, 5, 6, and 18] in loin muscle of the Duroc × Pietrain (DuPi) F₂ resource population by applying different statistical methods.

Quantitative expression studies such as microarray technology can indicate regulatory variation in genes for complex traits (Wayne and McIntyre 2002). By combining QTL mapping and microarray analyses, it is possible to identify the regulatory networks underlying the quantitative trait of interest and localize genomic variation, the so-called genetical genomics approach (Jansen 2003; Jansen and Nap 2001). Furthermore, a QTL analysis of expression levels of genes identifies genomic regions that are likely to contain at least one causal gene with a regulatory effect on the expression level, termed expression QTL (eQTL). The use of eQTL analyses has been demonstrated as a promising tool for narrowing the gap between detected phenotype-related QTL regions and confirmed causative variations for the pig species (Rothschild et al. 2007; Steibel et al. 2011). Detected eQTL can be classified into a locus, which is located close to a gene (*cis*-regulation) or distant (*trans*-regulation) (Jansen and Nap 2001). Differentially expressed genes, where eQTL mapping indicates *cis*-regulation, are more likely to represent the “cause,” e.g., the genetic background of the trait of interest, whereas differentially expressed genes revealing *trans*-regulation represent the “effect,” e.g., pathways that are affected by

causal variations (Wimmers et al. 2010). The largest and most significant reported eQTL are often *cis*-regulated (Haley and de Koning 2006). However, some *trans*-regulated eQTL seem to control expression in several or many genes spread across the genome. These findings provide general information on the organization of the control of expression (Haley and de Koning 2006).

Previous results showed eight *cis*-regulated eQTL significantly associated with drip loss that were located on SSC 2, 3, 4, and 6. Additionally, eight candidate genes were identified from these results (Ponsuksili et al. 2008a). Lobjois et al. (2008) showed that of 63 genes associated with the Warner–Bratzler shear force, only 22 were mapped in the pig genome and 12 were located in the areas previously associated with tenderness (SSC 2, 6, and 13). In the study by Steibel et al. (2011), expression profiles from loin muscle tissue were combined with linkage analyses of 124 microsatellites showing 62 eQTL, including 22 *cis*-regulated eQTL. As shown in these studies, differences in gene expression can be used in reverse genetic studies to generate well-defined hypotheses regarding downstream effects on molecular, cellular, and functional networks, and finally at the phenotype level (Ciobanu et al. 2011). However, genetical genomic studies have rarely been used to investigate the complex genetic structure and gene regulation of drip loss in pigs. Therefore, the aim of this study was to identify candidate genes related to drip loss by (1) analyzing the correlation of drip loss with transcript abundance and (2) combining genome-wide gene expression profiling and genotyping.

Materials and methods

Animals and phenotypic traits

In this study, 214 animals of a reciprocal cross of the Duroc and Pietrain breeds were used. These animals consisted of 169 F₂, 39 F₁, and 9 parental (P) animals, which came from 59 full- and half-sib families. All F₂ DuPi animals were kept and performance tested at the Frankenforst experimental farm of the University of Bonn. The phenotypes were recorded in a commercial slaughterhouse according to the rules of German performance stations (ZDS 2003). Further information can be found in Liu et al. (2007).

Drip loss was measured using the bag method of Honikel (1986). The samples from *M. longissimus dorsi* were collected 24 h post mortem (p.m.), weighed, and wrapped in a plastic bag. After storage for 48 h at 4 °C, the samples were reweighed and drip loss were calculated as a percentage of weight loss based on the initial weight of a sample.

Sample preparation, genome-wide genotyping, and quality control

DNA was extracted from *M. longissimus dorsi* using a Genomic DNA Purification Kit (Fermentas Life Science, Thermo Fisher Scientific, Waltham, MA, USA). DNA concentration was measured using a NanoDrop 8000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and concentration was adjusted to 100 ng/μl by using double-distilled RNase and DNase free water. Illumina bead array technology (Porcine SNP 60 K Bead Chip) was used for genotyping the samples (Illumina, Inc., San Diego, CA, USA) in accordance with the protocol for SNP Infinium HD assay (<http://Illumina.com>). DNA (200 ng) was used for genome-wide amplification and fragmentation. A quality score for each genotype was generated. Because of missing phenotype information, the P and F₁ generations were excluded from further analyses. In the final step, 169 F₂ samples were used for the genome-wide association analyses. The quality of the data was measured using PLINK software (Purcell et al. 2007). For quality control, samples with a call rate of <95 % and markers with a low minor allele frequency (MAF) of <5 % were chosen, and SNPs with a missing rate of more than 2 % were removed. After checking the quality of the data, 153 animals and 46,964 SNPs remained in the data set.

Whole-genome expression profiling

Gene expression profiling of the *M. longissimus dorsi* was conducted with 100 of the 169 F₂ animals. In brief, total RNA of the loin was isolated using TRI Reagent (Sigma, St. Louis, MO, USA) according to the manufacturer's protocol. The RNA was cleaned up using an RNeasy Kit (Qiagen, Valencia, CA, USA). RNA concentration was measured using a NanoDrop 8000 spectrophotometer. The integrity and the absence of contamination were checked using the Agilent RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, CA, USA). According to Affymetrix protocols, 500 ng of total RNA was reversely transcribed into cDNA, transcribed into cRNA, and labeled using the Affymetrix One Cycle Synthesis and Labeling Kit (Affymetrix, Santa Clara, CA, USA). Muscle expression patterns were assessed using the GeneChip® Porcine Genome Array (Affymetrix), which contains 23,937 probe sets that interrogate 23,256 transcripts which represent 20,201 genes in pig. Hybridization and scanning using the GeneChip® Scanner 3000 (Affymetrix) were performed according to the manufacturer's protocols using 5 μg of total RNA. The quality of hybridization was assessed in all samples following the manufacturer's recommendations. Data were analyzed with Affymetrix's GCOS 1.1.1 software using global scaling to a target signal of 500.

Microarray data processing

To increase the depth of muscle transcriptome data, an additional array data set from a previous investigation was added (Ponsuksili et al. 2008a). To combine these two data sets, it was necessary to remove outliers and correct for the significant batch effect (Oldham et al. 2008). The whole microarray processing is described in Supplementary Methods. After microarray processing, 132 F₂ DuPi animals were left for further investigation.

Correlation between traits and expression levels

Individual phenotypes of drip loss and gene expression levels were adjusted for systematic effects using a generalized linear model (glm) of R software (www.r-project.org). The linear model contained as fixed effects "gender," "season," and "place of slaughter" and as covariables "age of slaughter" and "weight of slaughter." Then Pearson's correlation coefficients were calculated using the residuals of the expression intensities and the trait drip loss. Genes that showed a significant correlation ($P \leq 0.05$) were analyzed further.

Gene set enrichment and pathway analyses

The array contained 23,937 probe sets, which represent 12,307 UniGenes to measure the global transcripts. The Porcine Genome Array annotation available from NetAffx™ Analyses Centre (Porcine.na30.annot.csv; November 2012) was used. An additional updated annotation was obtained from the Ensembl database using the biomaRt package (ver. 2.14.0) in R (www.r-project.org). In total, 8,059 probes were assigned to the terms Biological Processes (BP), 8,179 probes to Cellular Components (CC), and 8,867 probes to Molecular Functions (MF) of the Gene Ontology (GO; <http://www.geneontology.org/>) database.

A hypergeometric gene set enrichment test (GOstats package ver. 2.24.0) was performed based on the clusters identified in correlation analyses. Overrepresentation of gene sets defined by the groups BP, CC, and MF in the GO database or the Kyoto Encyclopedia of Genes and Genomes database (KEGG; <http://www.genome.jp/kegg/>) was tested using Fisher's exact test. For this test, only the significant genes that were annotated with an Entrez gene ID were included. When a gene had a duplicate probe set on the array and only one Entrez gene ID, only this gene ID was used for gene set enrichment analysis (GSEA). A gene set was considered significant if $P \leq 0.05$.

Genome-wide association (GWA) analysis

For the genome-wide association study, the gene expression values were corrected for environmental effects by

using a glm of R software. The model contained the same effects used for the correlation analysis.

The GWA analysis was conducted with PLINK software, which is based on the family association tests for quantitative traits (QFAM) and performed using permutation techniques to account for the dependence between related animals. The within-sibship test of QFAM is robust for population stratification. Nominal scores were permuted to obtain an empirical P value while obtaining familial correlation between phenotype and genotype. The permutation procedure applied by QFAM corrects for relatedness within families and was performed 100,000 times. Genetic effects and the proportion of explained variance were also calculated using PLINK (Purcell et al. 2007). In addition, a false discovery rate (FDR) ≤ 0.1 was used to correct for multiple testing (Benjamini and Hochberg 1995).

Results

Expression profiling and eQTL analyses were performed on 132 animals of the DuPi population, including 100 animals in this study and 32 animals from previous studies (Ponsuksili et al. 2008a, 2010). The animals chosen for gene expression profiling were selected based on the high degree of relationship within the F_2 crosses and their extreme values of drip loss. Therefore, to avoid confounding of extreme phenotypes with families and litters, two discordant full sibs of each family were selected.

The animals were slaughtered at an average of 183.7 days and an average carcass weight of 86 kg. The mean drip loss was 2.0 %, with a minimum of 0.4 % and a maximum of 5.3 %. Using the Affymetrix Porcine Genome Array, 24,123 expression measurements were performed from each *M. longissimus dorsi* RNA sample from the 132 F_2 animals and corrected for the batch effect using “ComBat” (Johnson et al. 2007). The preselected gene expression data were further analyzed with the hybrid algorithm of PLIER (AffymetrixTechnicalNote 2005), resulting in 20,733 probe sets. Pearson’s correlation coefficient was calculated between each of the 20,733 probe set expression levels and the drip loss phenotypes. A total of 1,228 transcripts were significantly correlated with drip loss ($P \leq 0.05$), with 406 negative and 822 positive correlated genes.

Biological pathways associated with drip loss

We tested the list of significantly positive and negative correlated genes ($P \leq 0.05$) for gene set enrichment of functional annotation groups in the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto

2000) and annotation term Gene Ontology (GO) databases. This data set was represented by 1,227 probe sets, which represent 688 annotated genes. Eight significant KEGG pathways ($P \leq 0.05$), including “Glycolysis/glyconeogenesis,” “Pentose phosphate pathway,” and “Pyruvate metabolism,” for all genes significantly associated with drip loss were found (Supplementary Table 1). For the negatively correlated genes, 19 significant KEGG pathways, which were mostly disease pathways, were observed (Table 1). In addition, ten significant KEGG pathways were identified for positively correlated genes (Table 2).

For the GO Biological Process (BP) term, enrichment of 114 BP terms in the list of correlated genes was found. The GO terms “Hexose catabolic process,” “Primary metabolic process,” “Gluconeogenesis,” “Glycolysis,” “Monosaccharide biosynthetic process,” “Protein modification process,” “Cellular metabolic process,” “Cellular carbohydrate catabolic processes,” and “Carbohydrate catabolic process” annotated most of the genes with a significant association to drip loss (Supplementary Table 2).

For the genes that were negatively correlated with drip loss, enrichment of 235 BP terms were identified compared to the genes that were positively correlated with drip loss with 129 enriched BP terms. For the GO Cellular Component (CC) term, only 28 GO terms were observed for all significantly associated genes (Supplementary Table 3). The lists of positively and negatively correlated genes were enriched with 45 CC terms, consisting mainly of intracellular components, and with 21 CC terms, representing extracellular components, respectively.

Forty-six GO terms for MF were enriched in the list of all significantly associated genes. These included four MF terms: “Phosphorylase activity,” “Oxidoreductase activity, acting on the aldehyde or oxo group of donors,” “Fructose 1,6-bisphosphate 1-phosphatase activity,” and “Catalytic activity” (Supplementary Table 4). Within the list of genes positively correlated with drip loss, 77 MF terms were enriched compared to the gene list negatively correlated with drip loss enriched for 58 MF terms.

Whole-genome association analysis for transcripts correlated with drip loss (eQTL)

A total of 246 genes, which were highly correlated with drip loss ($P \leq 0.01$), were selected for eQTL analysis. In addition, 21 genes were selected from the gene set enrichment analysis because of their functional role in the development of drip loss and were added for eQTL analysis. In total, 46,946 SNPs were tested for association with the expression level of each of the 267 transcripts significantly associated with drip loss. The analysis revealed

Table 1 Significant KEGG identifiers detected based on negatively correlated transcripts with drip loss

KEGG ID	<i>P</i> value*	Odds ratio	ExpCount ^a	Count ^b (size ^c)	Term ^d
4650	0.004	5.219	1.089	5 (66)	Natural killer cell mediated cytotoxicity
4920	0.006	4.893	1.155	5 (70)	Adipocytokine signaling pathway
5160	0.006	4.099	1.651	6 (100)	Hepatitis C
4660	0.008	4.409	1.271	5 (77)	T cell receptor signaling pathway
5220	0.012	5.022	0.891	4 (54)	Chronic myeloid leukemia
4012	0.012	4.923	0.908	4 (55)	ErbB signaling pathway
4115	0.019	4.246	1.040	4 (63)	p53 signaling pathway
4380	0.023	3.357	1.634	5 (99)	Osteoclast differentiation
5213	0.026	5.164	0.644	3 (39)	Endometrial cancer
5214	0.027	5.023	0.660	3 (40)	Glioma
4662	0.029	4.890	0.677	3 (41)	B cell receptor signaling pathway
4370	0.029	4.890	0.677	3 (41)	VEGF signaling pathway
4610	0.034	3.517	1.238	4 (75)	Complement and coagulation cascades
5221	0.037	4.419	0.743	3 (45)	Acute myeloid leukemia
4940	0.039	4.315	0.759	3 (46)	Type I diabetes mellitus
5215	0.042	3.281	1.320	4 (80)	Prostate cancer
600	0.042	6.805	0.330	2 (20)	Sphingolipid metabolism
650	0.042	6.805	0.330	2 (20)	Butanoate metabolism
4010	0.045	2.495	2.608	6 (158)	MAPK signaling pathway

A hypergeometric gene set test was performed based on negatively correlated transcripts with drip loss. Over representation of gene sets defined by the KEGG database was tested using Fisher's exact test. The gene set was considered significant if $P < 0.05$

KEGG ID = KEGG pathway ID

^a ExpCount is the expected number of genes in the selected gene list to be found at each category term

^b Count is the number of genes identified in each category term

^c Size is the total number of genes found at each category term

^d Term is the definition of each category term

* *P* value calculated for hypergeometric test using Fisher's exact test

1,451 eQTL associated with the expression level of 30 total transcripts, with a nominal $P < 0.0001$ and a genome-wide FDR value of 0.1. The average number of eQTL for each transcript was 50, with a minimum of one eQTL and a maximum of 943 eQTL. Four transcripts had only one eQTL (Table 3). The calculated correlations with drip loss for all eQTL ranged between -0.25 and 0.30 . From these 1,451 eQTL, 466 eQTL were located on the same chromosome as the transcript. In this study, only eight eQTL were assumed to be potentially *cis*-regulatory because they were located close to the gene expressing the transcript whose level was the eQTL (Table 4). The explained variance of these eight eQTL ranged from 10 to 30 %. The gene *neuralized homolog (Drosophila) (NEURL)* showed two significantly associated SNPs, which were located within the gene. These two SNPs located within the *NEURL* gene were significantly associated with the *NEURL* expression level and may indicate a *cis*-eQTL at the *NEURL* gene position. An eQTL located on SSC 12 showing a significant association ($P < 2.58E^{-07}$) with expression of the gene *TPII* was found on SSC 5.

Discussion

Trait-dependent expression analyses

The genome-wide association between the quantitative trait drip loss and gene expression was analyzed using Pearson's correlation. This approach was similarly used in many studies such as those of Quackenbush (2001), Kraft et al. (2003), and Ponsuksili et al. (2008a). In the study by Kraft et al. (2003), a within-family correlation analysis was utilized to remove the effect of family stratification. In contrast, we used a general linear model to correct both drip loss phenotype and expression levels for environmental effects in the correlation analysis. In our additional analyses, we used the family approach of PLINK (Purcell et al. 2007) to account for family stratification. For the pathway and GO term analyses, we considered correlation significant at $P < 0.05$, as in the studies of Blalock et al. (2004) and Ponsuksili et al. (2008a).

To control for the problem of multiple testing, a genome-wide Bonferroni correction may be used but it will

Table 2 Significantly enriched KEGG identifiers detected based on positively correlated transcripts with drip loss

KEGG ID	<i>P</i> value*	Odds ratio	ExpCount ^a	Count ^b (size ^c)	Term ^d
4120	<0.001	3.829	3.661	12 (102)	Ubiquitin-mediated proteolysis
10	<0.001	6.551	1.328	7 (37)	Glycolysis/gluconeogenesis
620	0.006	4.941	1.184	5 (33)	Pyruvate metabolism
3040	0.007	2.735	4.056	10 (113)	Spliceosome
4130	0.010	5.504	0.861	4 (24)	SNARE interactions in vesicular transport
310	0.024	4.069	1.113	4 (31)	Lysine degradation
4115	0.024	2.911	2.261	6 (63)	p53 Signaling pathway
30	0.033	4.824	0.718	3 (20)	Pentose phosphate pathway
230	0.034	2.291	3.769	8 (105)	Purine metabolism
4720	0.039	3.429	1.292	4 (36)	Long-term potentiation

A hypergeometric gene set test was performed based on positively correlated transcripts with drip loss. Over representation of gene sets defined by the KEGG database was tested using Fisher's exact test. The gene set was considered significant if $P < 0.05$

KEGG ID = KEGG pathway ID

^a ExpCount is the expected number of genes in the selected gene list to be found at each category term

^b Count is the number of genes identified in each category term

^c Size is the total number of genes found at each category term

^d Term is the definition of each category term

* *P* value calculated for hypergeometric test using Fisher's exact test

result in high false negatives (Han et al. 2009). Thus, instead of a genome-wide Bonferroni correction, we set a more relaxed threshold by using a correction following Benjamini and Hochberg with a FDR <0.10 (Benjamini and Hochberg 1995). Storey and Tibshirani (2003) proposed an adaptation of the FDR for use with genome-wide experiments to provide a better balance between statistical stringency and power to detect true effects. However, for some genes even this method seems to be too stringent, e.g., for the gene *solute carrier family 37, member 4* (*SLC37A4*), which does not reach the FDR level of 0.1. This gene is located in a region on SSC 9 and showed eQTL on SSC 18, where several promising candidate genes influencing drip loss are located (Jennen et al. 2007).

Gene set enrichment of significant correlated genes with drip loss

The genetic background of WHC during the conversion of muscle to meat is not yet completely understood because of the highly complex nature of this conversion, which may be due to the complex structure of the muscle itself (Huff-Lonergan and Lonergan 2005). In this study, individual transcript levels in muscle at slaughter were correlated with drip loss after slaughter to find biological processes that are relevant in the development of drip loss. Functional annotation analyses are based essentially on the extrapolation of pathway information and gene ontology data from other species to the pig (Ponsuksili et al. 2008a). In our study, different correlations between specific cellular

pathways and quantities of drip loss among individuals were observed. The genes that were positively correlated with drip loss were those of the group belonging to energy metabolism (Table 2); the same results were observed in the GO term analyses. For example, glycolytic metabolism is a process characteristic of skeletal muscle (Hamill et al. 2012). Each muscle consists of three main fiber types: slow-twitch oxidative, fast-twitch oxidoglycolytic, and fast-twitch glycolytic (Peter et al. 1972). A high proportion of fast-twitch glycolytic fibers are associated with drip loss, low vascularization, reduced mitochondrial activity, and higher glycogen content (Wimmers et al. 2006).

In the recent study, the negatively correlated transcripts were enriched mostly in disease pathways; the same results were found in GO analyses.

In summary, analyses of the positively trait-correlated expression revealed that the complex relationships between biological processes taking place in skeletal muscle and meat qualities like drip loss are driven by the energy reserves in the muscle and their metabolism (Ponsuksili et al. 2008a).

Candidate genes for drip loss and their regulation patterns

Drip loss is a complex trait that is genetically controlled by many different genes. With the growing knowledge of genome sequences and gene annotation, eQTL analyses give insight into the architecture of regulatory networks (Ponsuksili et al. 2010). Eight eQTL that control expression of seven transcripts were predicted to be putative *cis*-

Table 3 Transcripts with their number of potential *cis*- and *trans*-regulated eQTL and their correlation coefficients to drip loss level

Probe set id	Gene name	Chromosome ^a	Estimated correlation ^b	No. of eQTL
Ssc.11793.1.A1_at	<i>ZNF79</i>	1	0.255**	1
Ssc.1801.2.S1_at	<i>FNBP1</i>	1	-0.251**	1
Ssc.18261.1.S1_at	<i>PSTPIP2</i>	1	0.275***	45
Ssc.1894.1.S1_at	<i>AMBP</i>	1	-0.178*	4
Ssc.19564.1.S1_at	<i>PCDH9</i>	1	0.242**	943
Ssc.3339.1.S1_at	<i>BCKDHB</i>	1	0.252**	15
Ssc.16250.1.S2_at	<i>IL1RN</i>	2	-0.228**	5
Ssc.20525.1.S1_at	<i>IGF2</i>	2	-0.194*	14
Ssc.30674.1.S1_at	<i>PTBP1</i>	2	0.242**	12
Ssc.11624.1.A1_at	<i>ATL2</i>	3	0.239**	10
Ssc.14375.1.A1_at	<i>RRM2B</i>	4	0.223**	11
Ssc.1589.1.A1_at	<i>JTB</i>	4	0.235**	37
Ssc.1297.1.S1_at	<i>TPI1</i>	5	0.267**	17
Ssc.15905.1.A1_at	<i>GDF11</i>	5	0.240**	11
Ssc.16770.1.S1_at	<i>EMG1</i>	5	0.237**	59
Ssc.14396.1.S1_at	<i>TAF12</i>	6	0.226**	22
Ssc.8727.1.A1_at	<i>RPRD1A</i>	6	0.255**	21
Ssc.30641.1.S1_at	<i>ATP5L</i>	9	0.249**	10
Ssc.10209.1.A1_at	<i>CHURC1</i>	10	0.232**	12
Ssc.21622.1.A1_at	<i>FBP2</i>	10	0.172*	1
Ssc.3766.1.S1_at	<i>RAB18</i>	10	0.222**	4
Ssc.6339.1.A1_at	<i>DHTKD1</i>	10	0.230**	1
Ssc.7225.1.A1_at	<i>BAG1</i>	10	0.245**	7
Ssc.7523.1.A1_at	<i>PHB</i>	12	0.307***	5
Ssc.11661.1.A1_at	<i>PPP1CC</i>	14	0.210*	46
Ssc.17499.1.S1_at	<i>NEURL</i>	14	0.244**	86
Ssc.30435.1.A1_at	<i>BAG3</i>	14	0.273***	38
Ssc.1473.1.S1_at	<i>SMARCD3</i>	18	0.227**	3
Ssc.7292.1.S1_at	Unknown	18	0.234**	10

^a Number of *Sus scrofa* chromosomes where the gene is located

^b The potential candidate genes with their number of significant transcripts and their calculated Pearson's correlation coefficient with the phenotypic trait drip loss

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 4 Potential *cis*-regulated eQTL and their explained variance

Chr ^a	Probe set id	Gene name	Position of the transcript	SNP	Position of SNP	P value	Variance ^b
1	Ssc.11793.1.A1_at	<i>ZNF79</i>	301852485–301870268	ALGA0010677	303486951	5.10E-07**	0.175
1	Ssc.1894.1.S1_at	<i>AMBP</i>	285708391–285725500	ASGA0007079	287975124	7.85E-06*	0.141
1	Ssc.19564.1.S1_at	<i>PCDH9</i>	86706491–86706515	ALGA0004442	86848196	0.0001701**	0.102
4	Ssc.1589.1.A1_at	<i>JTB</i>	104615009–104617469	DIAS0000629	109076487	4.78E-05*	0.118
5	Ssc.16770.1.S1_at	<i>EMG1</i>	66181482–66187680	ASGA0025815	66243536	1.18E-05*	0.136
10	Ssc.3766.1.S1_at	<i>RAB18</i>	44073199–44073223	ALGA0058578	44475243	1.56E-06*	0.161
14	Ssc.17499.1.S1_at	<i>NEURL</i>	124318583–124396585	ASGA0066211	124326965	5.73E-12***	0.303
14	Ssc.17499.1.S1_at	<i>NEURL</i>	124318583–124396585	H3GA0042143	124382934	3.71E-05*	0.121

^a *Sus scrofa* chromosome

^b Gene expression variation explained by the associated SNP

* $FDR < 0.1$; ** $FDR < 0.05$; *** $FDR < 0.01$

regulatory following the criteria used in the study by Ponsuksili et al. (2011). In our study, 24 % of the significantly associated genes were *cis*-regulated and 76 % were *trans*-regulated. This proportion of *cis*-regulated genes is

higher than that of (Ponsuksili et al. 2008a, 2010, 2011), where 10 % of all genes were *cis*-regulated.

The *cis*-regulated genes are of interest because the underlying genes are expected to harbor genetic variants that

influence their own expression level, which thus may influence the physiological trait of interest if transcript abundance is correlated with the target phenotype such as drip loss (Göring et al. 2007). For example, the polypeptide α -1-microglobulin/bikunin precursor (*AMBP*), which is located on SSC 1, was already known to be involved in the formation of drip loss (Cinar et al. 2012; Ponsuksili et al. 2008b), and we found its expression to be significantly associated with drip loss (Fig. 1). *AMBP* is the precursor of bikunin, which plays an important role in the stabilization of the extracellular matrix (Tyagi et al. 2002). In addition, *AMBP* is important for cell growth, development, metabolism, immune response, and the level of intracellular calcium (Grewal et al. 2005). The extracellular matrix is reported to influence meat quality (Velleman 2000) because signal transduction from the extracellular matrix to the myoblast plays a significant role in muscle formation and growth (Velleman 2002). Furthermore, the extracellular matrix consists of proteins, including collagens and proteoglycans that contribute to the increase in WHC of tissue (Velleman 2002). In the studies by Ponsuksili et al. (2008a, b), *AMBP* was differentially expressed in pig muscles with high vs. low drip loss and low vs. high pH. These results were confirmed in the study by Cinar et al. (2012). *AMBP* was mapped to SSC 1, where QTL for meat quality traits are reported in different pig breeds and crosses (Hu et al. 2010). In contrast to our results, Cinar et al. (2012) identified *AMBP* as *trans*-regulated. One reason for this might be that we used a higher number of genetic markers. However, this high number of genetic markers may not track perfectly with the causative mutations but they are potentially useful tools for identifying chromosomal regions associated with the trait (Cole et al. 2009). The functions of the other potential *cis*-regulated genes (Table 4) have not yet been correlated with porcine skeletal muscle biology.

Many loci are *trans*-acting modulators of gene expression (Jansen and Nap 2004) and are also of great interest because these genes are likely to play a role in explaining trait variation (Wang and Nettleton 2006). For example, the gene *insulin-like growth factor 2* (*IGF2*), located on SSC 2

(Fig. 2) and also found to be differentially expressed in pigs with high and low drip loss (Ponsuksili et al. 2008b), and the gene *triosephosphate isomerase 1* (*TPI1*), located on SSC 5 (Fig. 3), were *trans*-regulated. *IGF2* promotes growth and plays a role in regulating proliferation, differentiation, and apoptosis of cells in many different tissues, such as skeletal muscle (Pavelic et al. 2002; van Wyk and Smith 1999). The *IGF2* gene is imprinted in most mammalian tissues and is expressed exclusively from the paternal allele (Jeon et al. 1999; Nezer et al. 1999). In addition, *IGF2* is involved in myogenesis and controls primarily muscle mass and fat deposition (Jeon et al. 1999; Nezer et al. 1999). It is responsible for 15–30 % of the phenotypic variation in muscle mass (Van Laere et al. 2003). The *TPI1* gene encodes a glycolytic enzyme, which plays an important role in energy generation for muscle cells (Solem et al. 2008). In the study by Kwasiborski et al. (2008), protein analyses were performed and *TPI1* was positively correlated with drip loss in a Large White population. In our study, a positive correlation ($r = 0.27$ with $P < 0.001$) between the expression level of this gene and drip loss was observed in the DuPi population.

In this study we identified an eQTL for *SLC37A4* on SSC 18 with a FDR of 0.53. Although this eQTL failed the FDR level of 0.1 test, the *trans*-regulated *SLC37A4*, which is located on SSC 9, is a promising candidate gene for drip loss (Fig. 4). *SLC37A4*, also known as *glucose-6-phosphate transporter* (*G6PT*), is expressed in several tissues such as heart, brain, and skeletal muscle (Lin et al. 2000). *G6PT* translocates glucose-6-phosphate (G6P), the product of gluconeogenesis and glycogenolysis, from the cytoplasm to the lumen of the endoplasmic reticulum (ER) (Arion et al. 1980). Inside the ER, *G6Pase* catalyzes the conversion of G6P to glucose. Therefore, *G6PT* and *G6Pase* both work in the glucose homeostasis pathway (Lin et al. 2000). *G6PT* plays a role in antioxidant protection because defective G6P transport leads to an increase in reactive oxygen species (ROS) (Leuzzi et al. 2003), which act as important signaling molecules in muscle contraction and adaptation (Musaro et al. 2010). An increase in ROS

Fig. 1 Most significant autosomal eQTL controlling expression of polypeptide α -1-microglobulin/bikunin precursor (*AMBP*), a gene associated with drip loss, mapped to the *AMBP* location on SSC 1

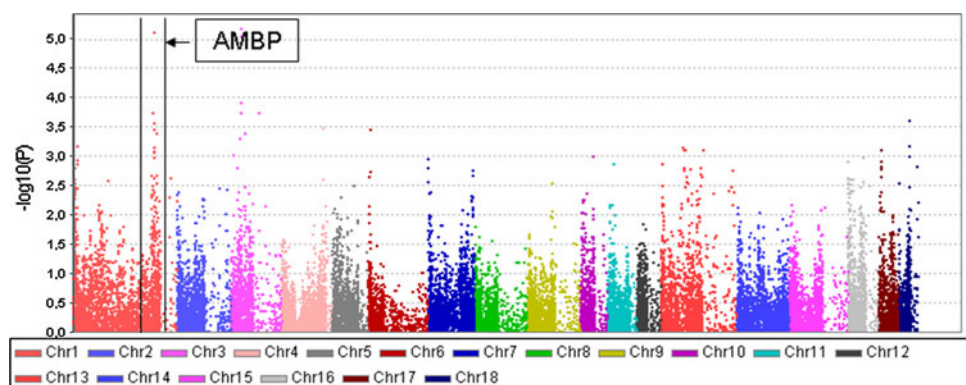


Fig. 2 Most significant autosomal eQTL controlling expression of gene *Insulin-like growth factor 2 (IGF2)*, a gene associated with drip loss and located on SSC 2, mapped to SSC 10 and 12

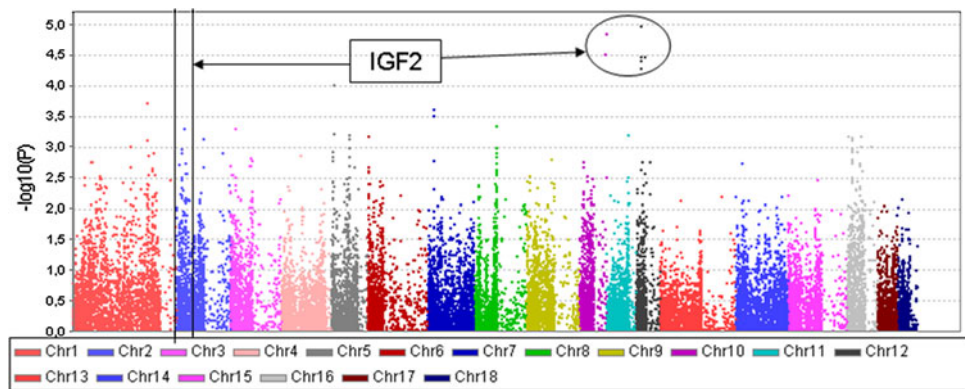


Fig. 3 Most significant autosomal eQTL controlling expression of *triosephosphate isomerase 1 (TPI1)*, a gene associated with drip loss and located on SSC 5, mapped to SSC 12

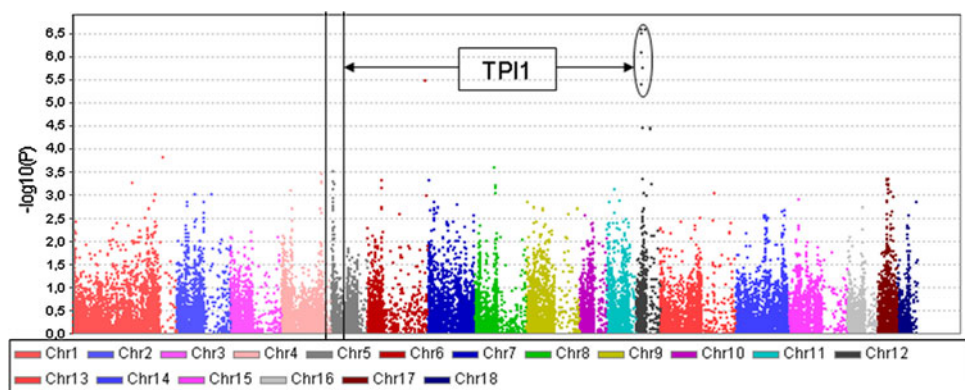
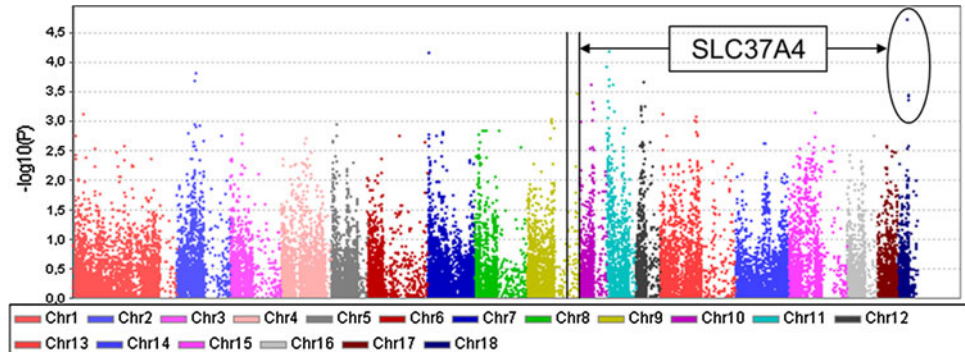


Fig. 4 Most significant autosomal eQTL controlling expression of *solute carrier family 37, member 4 (SLC37A4)*, a gene associated with drip loss and located on SSC 9, mapped to SSC 18



production in osmotic cell swelling, muscle contraction, anoxia, and sepsis has been demonstrated. It may cause a decline in pH and drip loss formation in skeletal muscle (Ortenblad et al. 2003).

From previous eQTL studies (Ponsuksili et al. 2008a, b, 2010), only the genes *AMBP* on SSC 1 and *IGF2* on SSC 2 could be identified in this study. This might be due to a greater number of animals and a higher marker density for calculating the eQTL used in this study.

In comparison to the QTL studies performed in the DuPi population (Edwards et al. 2008; Große-Brinkhaus et al. 2010; Liu et al. 2007, 2008), it can be shown that the *cis* eQTL found in this study were located close to previously identified meat quality QTL. In particular, the genes

jumping translocation breakpoint (JTB) on SSC 4 and *EMG1 nucleolar protein homolog (EMG1)* on SSC 5 were located near the QTL for drip loss identified by Liu et al. (2007, 2008). However, the *cis*-regulated candidate gene *RAB18, member RAS oncogene family (RAB18)*, located on SSC 10, was found only near the QTL region associated with connective tissue in a different DuPi population (Edwards et al. 2008). In our study, it was possible to identify candidate genes on SSC 1 and 14 close to epistatic QTL, independent from a second QTL, because of the high number of genetic markers provided by the SNP chips. For the *trans*-regulated candidate genes, *IGF2* on SSC 2 was located close to the QTL associated with drip loss identified by Liu et al. (2007).

Conclusion

Mapping quantitative traits and unraveling their transcriptional control is challenging, when applied to one phenotype at a time. In studies of typical quantitative porcine traits like WHC, strong effects are rarely found. In this study we coupled genomic technologies for expression profiling with genome-wide genetic mapping using SNP markers and showed that specific chromosomal regions on SSC 1, 2, 4, 5, 10, and 14 contain functional candidate genes. These approaches and results allowed us to investigate and dissect the genetic contribution to natural variation in porcine muscle gene expression. Besides confirming that the genes *AMBP* and *IGF2* influence drip loss, additional promising candidate genes such as *TPII* and *SLC37A4*, located in interesting regions, were identified. These promising candidate genes need further validation in other pig populations and the gene regulation networks have to be more closely investigated.

Acknowledgments Authors are grateful to Prof. Dr. med. Markus M. Nöthen from the Institute of Human Genetics, University of Bonn, for genotyping the samples with an Illumina 60K Porcine SNP Chip. This work was part of the DRIP phase II (Genetic-functional background of the water-holding capacity in pork) project and supported by the German Research Foundation (DFG), Germany (Grant No. SCHE562/13-1).

References

- Affymetrix Technical Note (2005) Guide to probe logarithmic intensity error (PLIER) estimation. Affymetrix, Santa Clara
- Arion WJ, Lange AJ, Walls HE, Ballas LM (1980) Evidence for the participation of independent translocation for phosphate and glucose 6-phosphate in the microsomal glucose-6-phosphatase system. Interactions of the system with orthophosphate, inorganic pyrophosphate, and carbamyl phosphate. *J Biol Chem* 255:396–406
- Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate—a practical and powerful approach to multiple testing. *J R Stat Soc Ser B* 57:289–300
- Blalock EM, Geddes JW, Chen KC, Porter NM, Markesbery WR, Landfield PW (2004) Incipient Alzheimer's disease: microarray correlation analyses reveal major transcriptional and tumor suppressor responses. *Proc Natl Acad Sci USA* 101:2173–2178
- Borchers N, Otto G, Kalm E (2007) Genetic relationship of drip loss to further meat quality traits in purebred Pietrains. *Arch Tierz- Arch Anim Breed* 50:84–91
- Cinar MU, Kayan A, Uddin MJ, Jonas E, Tesfaye D, Phatsara C, Ponsuksili S, Wimmers K, Tholen E, Looft C, Jungst H, Schellander K (2012) Association and expression quantitative trait loci (eQTL) analysis of porcine *AMBP*, *GC* and *PPP1R3B* genes with meat quality traits. *Mol Biol Rep* 39:4809–4821
- Ciobanu DC, Lonergan SM, Huff-Lonergan EJ (2011) Genetics of meat quality and carcass traits. In: Ruvinsky A (ed) *The Genetics of the Pig*. CAB International, New York
- Cole JB, VanRaden PM, O'Connell JR, Van Tassell CP, Sonstegard TS, Schnabel RD, Taylor JF, Wiggans GR (2009) Distribution and location of genetic effects for dairy traits. *J Dairy Sci* 92:2931–2946
- Edwards DB, Ernst CW, Raney NE, Doumit ME, Hoge MD, Bates RO (2008) Quantitative trait locus mapping in an F-2 duroc × pietrain resource population: II. Carcass and meat quality traits. *J Anim Sci* 86:254–266
- Göring HH, Curran JE, Johnson MP, Dyer TD, Charlesworth J, Cole SA, Jowett JBM, Abraham LJ, Rainwater DL, Comuzzie AG, Mahaney MC, Almasy L, MacCluer JW, Kissebah AH, Collier GR, Moses EK, Blangero J (2007) Discovery of expression QTLs using large-scale transcriptional profiling in human lymphocytes. *Nat Genet* 39:1208–1216
- Greaser ML (2001) Postmortem muscle chemistry. In: Hui YH, Nip WK, Roger RW, Young OA (eds) *Meat science and applications*. Marcel Dekker, New York
- Grewal JS, Tsai JY, Khan SR (2005) Oxalate-inducible *AMBP* gene and its regulatory mechanism in renal tubular epithelial cells. *Biochem J* 387:609–616
- Große-Brinkhaus C, Jonas E, Buschbell H, Phatsara C, Tesfaye D, Jungst H, Looft C, Schellander K, Tholen E (2010) Epistatic QTL pairs associated with meat quality and carcass composition traits in a porcine duroc × pietrain population. *Genet Sel Evol* 42:39
- Haley C, de Koning DJ (2006) Genetical genomics in livestock: potentials and pitfalls. *Anim Genet* 37(Suppl 1):10–12
- Hamill RM, McBryan J, Mcgee C, Mullen AM, Sweeney T, Talbot A, Cairns MT, Davey GC (2012) Functional analysis of muscle gene expression profiles associated with tenderness and intramuscular fat content in pork. *Meat Sci* 92:440–450
- Hamm R (1985) Wasserbindungsvermögen des Fleisches-Aspekte eines wichtigen Qualitätsmerkmals. *Mitteilungsblatt BAF* 88:6383–6387
- Han B, Kang HM, Eskin E (2009) Rapid and accurate multiple testing correction and power estimation for millions of correlated markers. *PLoS Genet* 5:e10000456
- Honikel KO, Kim CJ, Hamm R, Roncales P (1986) Sarcomere shortening of pre-rigor muscles and its influence on drip loss. *Meat Sci* 16:267–282
- Hu Z, Park CA, Fritz ER, Reecy JM (2010) QTLdb: a comprehensive database tool building bridges between genotypes and phenotypes. In: *The 9th World Congress on Genetics Applied to Livestock Production*, Leipzig, Germany, August 1–6, 2010
- Huff Lonergan E, Zhang W, Lonergan SM (2010) Biochemistry of postmortem muscle—lessons on mechanisms of meat tenderization. *Meat Sci* 86:184–195
- Huff-Lonergan E, Lonergan SM (2005) Mechanisms of water-holding capacity of meat: the role of postmortem biochemical and structural changes. *Meat Sci* 71:194–204
- Huff-Lonergan E, Lonergan SM (2007) New frontiers in understanding drip loss in pork: recent insights on the role of postmortem muscle biochemistry. *J Anim Breed Genet* 124:19–26
- Jansen RC (2003) Studying complex biological systems using multifactorial perturbation. *Nat Rev Genet* 4:145–151
- Jansen RC, Nap JP (2001) Genetical genomics: the added value from segregation. *Trends Genet* 17:388–391
- Jansen RC, Nap JP (2004) Regulating gene expression: surprises still in store. *Trends Genet* 20:223–225
- Jennen DG, Brings AD, Liu G, Jungst H, Tholen E, Jonas E, Tesfaye D, Schellander K, Phatsara C (2007) Genetic aspects concerning drip loss and water-holding capacity of porcine meat. *J Anim Breed Genet* 124:2–11
- Jeon JT, Carlborg O, Tornsten A, Giuffra E, Amarger V, Chardon P, Andersson-Eklund L, Andersson K, Hansson I, Lundstrom K, Andersson L (1999) A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the *IGF2* locus. *Nat Genet* 21:157–158
- Johnson WE, Li C, Rabinovic A (2007) Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics* 8:118–127

- Kanehisa M, Goto S (2000) KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 28:27–30
- Kraft P, Schadt E, Aten J, Horvath S (2003) A family-based test for correlation between gene expression and trait values. *Am J Hum Genet* 72:1323–1330
- Kwasiborski A, Sayd T, Chambon C, Sante-Lhoutellier V, Rocha D, Terlouw C (2008) Pig *Longissimus lumborum* proteome: part II: relationships between protein content and meat quality. *Meat Sci* 80:982–996
- Leuzzi R, Banhegyi G, Kardon T, Marcolongo P, Capecchi PL, Burger HJ, Benedetti A, Fulceri R (2003) Inhibition of microsomal glucose-6-phosphate transport in human neutrophils results in apoptosis: a potential explanation for neutrophil dysfunction in glycogen storage disease type 1b. *Blood* 101:2381–2387
- Lin BC, Pan CJ, Chou JY (2000) Human variant glucose-6-phosphate transporter is active in microsomal transport. *Hum Genet* 107:526–529
- Liu G, Jennen DGJ, Tholen E, Juengst H, Kleinwachter T, Holker M, Tesfaye D, Un G, Schreinemachers HJ, Murani E, Ponsuksili S, Kim JJ, Schellander K, Wimmers K (2007) A genome scan reveals QTL for growth, fatness, leanness and meat quality in a Duroc-Pietrain resource population. *Anim Genet* 38:241–252
- Liu GS, Kim JJ, Jonas E, Wimmers K, Ponsuksili S, Murani E, Phatsara C, Tholen E, Juengst H, Tesfaye D, Chen JL, Schellander K (2008) Combined line-cross and half-sib QTL analysis in Duroc-Pietrain population. *Mamm Genome* 19:429–438
- Lobjois V, Liaubet L, SanCristobal M, Glenisson J, Feve K, Rallieres J, Le Roy P, Milan D, Cherel P, Hately F (2008) A muscle transcriptome analysis identifies positional candidate genes for a complex trait in pig. *Anim Genet* 39:147–162
- Musarò A, Fulle S, Fanò G (2010) Oxidative stress and muscle homeostasis. *Curr Opin Clin Nutr Metab Care* 13(3):236–242
- Nezer C, Moreau L, Brouwers B, Coppieters W, Detilleux J, Hanset R, Karim L, Kvasz A, Leroy P, Georges M (1999) An imprinted QTL with major effect on muscle mass and fat deposition maps to the *IGF2* locus in pigs. *Nat Genet* 21:155–156
- Offer G, Cousins T (1992) The mechanism of drip production—formation of two compartments of extracellular space in muscle postmortem. *J Sci Food Agric* 58:107–116
- Offer G, Knight P (eds) (1988) *The structural basis of water-holding capacity in meat part 1: general principles and water uptake in meat processing*. Elsevier Applied Science, New York
- Oldham MC, Konopka G, Iwamoto K, Langfelder P, Kato T, Horvath S, Geschwind DH (2008) Functional organization of the transcriptome in human brain. *Nat Neurosci* 11:1271–1282
- Ortenblad N, Young JF, Oksbjerg N, Nielsen JH, Lambert IH (2003) Reactive oxygen species are important mediators of taurine release from skeletal muscle cells. *Am J Physiol Cell Physiol* 284:C1362–C1373
- Pavelic K, Bukovic D, Pavelic J (2002) The role of insulin-like growth factor 2 and its receptors in human tumors. *Mol Med* 8:771–780
- Peter JB, Barnard RJ, Edgerton VR, Gillespie CA, Stempel KE (1972) Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. *Biochemistry* 11:2627–2633
- Ponsuksili S, Jonas E, Murani E, Phatsara C, Srikanchai T, Walz C, Schwerin M, Schellander K, Wimmers K (2008a) Trait correlated expression combined with expression QTL analysis reveals biological pathways and candidate genes affecting water holding capacity of muscle. *BMC Genomics* 9:367
- Ponsuksili S, Murani E, Phatsara C, Jonas E, Walz C, Schwerin M, Schellander K, Wimmers K (2008b) Expression profiling of muscle reveals transcripts differentially expressed in muscle that affect water-holding capacity of pork. *J Agric Food Chem* 56:10311–10317
- Ponsuksili S, Murani E, Schwerin M, Schellander K, Wimmers K (2010) Identification of expression QTL (eQTL) of genes expressed in porcine *M. longissimus dorsi* and associated with meat quality traits. *BMC Genomics* 11:572
- Ponsuksili S, Murani E, Brand B, Schwerin M, Wimmers K (2011) Integrating expression profiling and whole-genome association for dissection of fat traits in a porcine model. *J Lipid Res* 52:668–678
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, de Bakker PIW, Daly MJ, Sham PC (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81:559–575
- Quackenbush J (2001) Computational analysis of microarray data. *Nat Rev Genet* 2:418–427
- Rosenvold K, Andersen HJ (2003) Factors of significance, for pork quality—a review. *Meat Sci* 64:219–237
- Rothschild MF, Hu ZL, Jiang Z (2007) Advances in QTL mapping in pigs. *Int J Biol Sci* 3:192–197
- Solem C, Koebmann B, Jensen PR (2008) Control analysis of the role of triosephosphate isomerase in glucose metabolism in *Lactococcus lactis*. *IET Syst Biol* 2:64–72
- Steibel JP, Bates RO, Rosa GJ, Tempelman RJ, Rillington VD, Ragavendran A, Raney NE, Ramos AM, Cardoso FF, Edwards DB, Ernst CW (2011) Genome-wide linkage analysis of global gene expression in loin muscle tissue identifies candidate genes in pigs. *PLoS ONE* 6:e16766
- Storey JD, Tibshirani R (2003) Statistical significance for genome-wide studies. *Proc Natl Acad Sci USA* 100:9440–9445
- Tyagi S, Salier JP, Lal SK (2002) The liver-specific human alpha(1)-microglobulin/bikunin precursor (AMBIP) is capable of self-association. *Arch Biochem Biophys* 399:66–72
- Van Laere AS, Nguyen M, Braunschweig M, Nezer C, Collette C, Moreau L, Archibald AL, Haley CS, Buys N, Tally M, Andersson G, Georges M, Andersson L (2003) A regulatory mutation in *IGF2* causes a major QTL effect on muscle growth in the pig. *Nature* 425:832–836
- van Wyk JJ, Smith EP (1999) Commentary—insulin-like growth factors and skeletal growth: possibilities for therapeutic interventions. *J Clin Endocrinol Metab* 84:4349–4354
- Velleman SG (2000) The role of the extracellular matrix in skeletal development. *Poult Sci* 79:985–989
- Velleman SG (2002) Role of extracellular matrix (ECM) in growth and development: role of the extracellular matrix in muscle growth and development. *J Anim Sci* 80:E8–E13
- Wang D, Nettleton D (2006) Identifying genes associated with a quantitative trait or quantitative trait locus via selective transcriptional profiling. *Biometrics* 62:504–514
- Wayne ML, McIntyre LM (2002) Combining mapping and arraying: an approach to candidate gene identification. *Proc Natl Acad Sci USA* 99:14903–14906
- 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 Genet* 7:15
- Wimmers K, Murani E, Ponsuksili S (2010) Functional genomics and genetical genomics approaches towards elucidating networks of genes affecting meat performance in pigs. *Brief Funct Genomics* 9:251–258
- Zentralverband der Deutschen Schweineproduktion (ZDS) (2003) Richtlinie für die Stationsprüfung auf Mastleistung, Schlachtkörperwert und Fleischbeschaffenheit beim Schwein, 10.12.2003, Bonn, Germany