Animal Science Papers and Reports vol. 28 (2010) no. 2, 115-122
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Pre- and postnatal differential gene expression with relevance for meat and carcass traits in pigs – a review

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(Received May 19, 2009; accepted April 21, 2010)

Pre- and postnatal processes determine the final outcome of breeding of pigs in terms of traits related to carcass and meat quality at slaughter. In particular, the number of myofibers and to a large extent their metabolic and contractile properties, which also influence their size, are determined prenatally during the process of myogenesis. By this, postnatal muscle growth and parameters of meat quality are modulated. The metabolic balance, biochemical and biophysical preslaughter properties of muscle prior to slaughter determine the process of maturation of muscle to meat. Thus, differential regulation of the abundance of transcripts of biological networks in prenatal and postnatal muscle affect biochemical processes of meat maturation. In general, because the traits of interest are typically not expressed at prenatal stages, no direct relationship between phenotype and gene expression pattern can be established. However, trait-related differential expression within any prenatal developmental stage can be assessed based on known estimated breeding values, known QTL-genotypes and/or based on breed differences. Expression profiles of muscle at slaughter can directly be linked to meat quality. A suitable experimental design of "matched samples" is the discordant sib pair design. Here it is exemplarily discussed that differentially expressed transcript profiles of M. *longissimus dorsi* at prenatal and postnatal stages offer an insight into the biological processes in the live muscle that affect the process of meat maturation and finally meat quality.

KEY WORDS: microarray / transcriptome / meat quality / muscle / myogenesis

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In animal science attempts to use holistic gene expression profiling techniques were made in order to elucidate tissue-specific differential gene expression in relation to developmental processes and/or phenotypes. The results significantly improve and specify lists of candidate genes for economic important traits including carcass composition and meat quality. These traits depend on prenatal events and postnatal nutrient utilization affecting growth and tissue deposition [Wu *et al.* 2008]. Consequently, prenatal and postnatal expression pattern were surveyed to obtain comprehensive inventories of genes and functional networks relevant to traits related to body composition and meat quality.

Microarrays were successfully used to obtain genes that are differentially expressed in muscle tissue due to developmental stage/age, breed, or phenotype in the pig. Application-specific cDNA macro- and microarrays enabled to monitor the expression of several hundred to thousand of genes along myogenesis [Seo and Beever, 2001; Ernst *et al.* 2002; Zhao *et al.* 2003; Bai *et al.* 2003; TePas *et al* 2005, Cagnazzo *et al.* 2006]. Genome wide microarrays covering up to more than 20,000 transcripts were used to elucidate mechanisms of control of carcass growth and meat quality traits [Davoli *et al.* 2007, Ponsuksili *et al.* 2008a,b]. As an alternative approach to microarray analysis the mRNA differential display technique (DD-RT-PCR) was applied. DD-RT-PCR is an open system potentially displaying all transcripts, whereas microarrays are restricted to the analysis of genes that are represented on the array. Genes differentially expressed in muscle of divergent breeds or in newborn piglets due to affection by the splay leg disorder compared to healthy littermates were identified by DD-RT-PCR [Zhao *et al.* 2001, Maak *et al.* 2001].

Here we summarize own attempts to derive functional networks and candidate genes for meat quality traits by analysing pre- and postnatal expression profiles of muscle.

Expression profiling of muscle at prenatal stages

In general, because the traits of interest are typically not expressed at prenatal stages, no direct relationship between phenotype and gene expression pattern can be established. However, trait-related differential expression within any prenatal developmental stages can be assessed based on known estimated breeding values, known QTL-genotypes and/or based on breed differences. In framework of an European initiative, PorDictor (FP5; QLK5_2000_01363) that aimed to derive functional candidate genes for meat quality from expression profiles of fetal muscle, transcripts of the trait divergent breeds Pietrain and Duroc were analysed at seven key developmental stages during myogenesis (14, 21, 35, 49, 63, 77, 91 days *post conceptionem* (dpc). Several techniques for expression profiling, *i.e.* cDNA-microarrays, DD-RT-PCR, construction of stage-specific muscle cDNA libraries and subtractive hybridization, were applied [Wimmers *et al.* 2002a, Te Pas *et al* 2005, Cagnazzo *et al*. 2006, Murani *et al.* 2007]. We generated DD-RT-PCRs of the

seven stages during porcine myogenesis using a total of 88 differential display primer combinations. Comparisons between breeds or between stages revealed 448 fragments varying either in their intensity or in their presence. Among these were 148 fragments showing differences between breeds and 310 fragments differentially displayed between stages. Eighty prominent cDNA fragments were sequenced, 43 showing stage-associated and 37 showing breed-associated differences in the expression, respectively. Out of the resulting 85 unique expressed sequence tags (EST), 52 could be assigned to known genes. The most frequent functional categories represented genes encoding myofibrillar proteins (8), genes involved in cell adhesion, cell-cell signaling and extracellular matrix synthesis/remodeling (8), genes regulating gene expression (8), and metabolism genes (8). Some of the EST that showed no identity to any known transcripts in the databases are located in introns of known genes and most likely represent novel exons (e.g. *HMGA2*). Expression of thirteen transcripts along with five reference genes was further analysed with real-time quantitative PCR. Nine of the target transcripts showed higher than twofold differences in the expression between the two breeds (*GATA3*, *HMGA2*, *NRAP*, *SMC6L1*, *SPP1*, *RAB6IP2, TJP1* and two EST).

Based on the analysis of breed differences the study revealed several genes differentially expressed during skeletal muscle development in domestic pig that were not yet associated with myogenesis and thus provide novel insights into molecular pathways employed in mammalian myogenesis (e.g. the autophagy pathway) and a foundation for future functional studies. Genes that exhibited differences between the divergent breeds represent candidate genes for muscle growth and structure [Murani *et al.* 2007]. Indeed, several of the identified genes map to known porcine QTL regions affecting muscle growth and/or structure, and their DNA variation is associated with variation in traits related to muscle deposition [Wimmers et al. 2006, Lui *et al.* 2007, 2008]. The expression of four candidate genes (*OPN, PDGFRA, ELKS* and *NME1*) at prenatal stages was linked to meat quality phenotypes by analysing foetuses with extreme breeding values. The qRT-PCR profiles obtained with individual samples essentially match those obtained using RNA pools in DD-RT-PCR analysis. Upregulation of the *OPN* gene on days 21, 49, and 77 and of *PDGFRA* on days 21 and 49 was associated ($P<0.05$) with low meat quality, however for Duroc embryos/foetuses only. Upregulation of *NME1* on either day 35 or 91 was associated ($P<0.1$) with low meat quality for Pietrain and Duroc foetuses. Expression levels of *ELKS* on day 21 were associated with meat quality in Pietrain ($P<0.05$) and Duroc ($P<0.001$) pigs. In addition, higher level of expression of the *ELKS* gene on day 49 in Duroc foetuses was associated with low meat quality.

Subsequently, ten genes shown to be regulated during myogenesis (*ANK1, bR10D1, CA3, EPOR, HMGA2, MYPN, NME1, PDGFRA, ERC1, TTN*) were subjected to analysis of 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 [Wimmers *et al.* 2007].

Comparative sequencing revealed polymorphic sites segregating across commercial breeds. 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 ≤ 0.05 threshold. In particular, *HMGA2*, *CA3*, *EPOR*, *NME1* and *TTN* were associated with meat colour, pH and conductivity of loin 24 h post-mortem; *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 with a particular trait across all populations.

Expression profiling revealed a number of genes whose candidacy for meat-quality and carcass traits arise from their differential expression among divergent breeds during myogenesis. Statistical–genetic evidence for association of the functional candidate genes with traits related to meat quality and muscle deposition was provided. However, the polymorphisms detected are not likely causal, but markers were identified being in linkage disequilibrium with causal genetic variation within particular populations. Focus on regulatory regions of the functional candidate genes is anticipated.

Expression profiling of muscle at peri-pubertal age

Functional genomics provide new opportunities for determining the molecular processes underlying phenotypic differences [Bernard *et al*. 2007, Ponsuksili *et al.* 2007]. The complexity of the relationship between physiological characteristics of the live muscle and meat quality is probably the reason why so few attempts have been made to improve meat quality by monitoring the physiological prerequisites of the muscle at the time of slaughter. Transcriptome profiles of *longissimus dorsi* muscle offer an insight into the biological processes in the muscle and maturing meat, and their effect on meat quality. Among the different meat quality traits drip loss has a major economic impact because it affects the weight of the carcass and thus the price the producers get paid for. In order to identify biological processes as well as molecular markers for drip loss, a parameter for water holding capacity (WHC) of meat, the *longissimus dorsi* muscle transcriptomes of six divergent sib pairs selected from 572 F2 crossbreds of Duroc and Pietrain (DUPI resource population) were analysed with Affymetrix Porcine Genome Array. The discordant sib pair design was applied in order to increase the probability of detecting differential expression due to differences in particular genes affecting the trait of interest rather, than due to the overall genetic background or bias arising from family effects [Ponsuksili *et al.* 2000, 2005, 2007; Wimmers *et al.* 2002b]. Earlier, QTLs for drip loss were identified on SSC5 and SSC18 in that population [Liu *et al.* 2007, 2008]. In order to further characterize the nature of the QTL/s/ for drip loss identified on SSC 5 and SSC 18 obtained in the DUPI population region-specific BAC arrays were constructed for expression profiling in these QTL regions. The region-specific BAC-arrays are not limited to annotated genes, which were covered by a commercially available genome-wide microarray that was applied in the complementary approach.

Comparative expression profiling by hybridization of the Affymetrix GeneChip Porcine Genome Arrays revealed 789 differential expressions of transcripts between high and low WHC group at $P<0.05$. Functional categories of differentially regulated transcripts were determined by single gene analysis and gene set analysis. The transcripts being upregulated at high drip loss belong to groups of genes functionally categorized as genes of membrane proteins, signal transduction, cell communication, response to stimuli and cytoskeleton. Among downregulated genes with high drip loss, functional groups of oxidoreductase activity, lipid metabolism and electron transport were identified. Differential regulation of the abundance of transcripts of these biological networks in live muscle affects post mortem biochemical processes of meat maturation [Ponsuksili *et al.* 2008a]. Knowledge of this functional link is indicative for the identification of candidate genes for improvement of meat quality. The analysis of the QTL region-specific BAC arrays supported and complemented the genome wide microarray analysis. Comparison of the BAC array analysis with the Affymetrix GeneChip analysis for SSC 5 showed that, out of 26 genes covered by the positive BACs according to the human-pig comparative map, 17 were not covered by the Affymetrix GeneChip. According to the Affymetrix GeneChip experiment the remaining 9 were not regulated. For SSC 18, the 2 positive BACs contained 3 genes: one was not represented on the Affymetrix GeneChip, one was not regulated according to the Affymetrix GeneChip experiment, and one (*GLI3*) that was significantly regulated according to the Affymetrix GeneChip experiment at $P\leq0.05$ and fold changes =1.5.

In a complementary approach to integrate the map-based QTL analysis and function-driven expression analysis and in order to identify genes and pathways with multiple evidence of their role in the biology of traits related to meat quality we aimed at combining (1) information on QTL for drip loss (=pheneQTL=pQTL) with analysis of (2) trait-correlated expression and with (3) mapping of expression QTL (eQTL) for the corresponding trait-dependent regulated genes [Ponsuksili *et al*. 2007b] Therefore, expression microarray analysis of *longissimus dorsi* muscle RNAs of 74 F2 animals of a resource population was conducted aiming at detecting genes regulated, depending on the phenotype. A total of 1,279 genes were significantly trait-associated at $P\leq0.001$ corresponding to $q \leq 0.004$, with 601 genes showing negative correlation and 678 showing positive correlation of their transcript abundance with drip loss. The correlations ranged between 0.37-0.67. Negatively correlated transcripts were enriched in functional categories and pathways like extracellular matrix receptor interaction and calcium signalling. Transcripts with positive correlation dominantly represented biochemical processes including oxidative phosphorylation, mitochondrial pathways, as well as transporter activity. In a second step a linkage analysis of abundance of trait-correlated transcripts was done and revealed 897 expression QTLs (eQTLs) with 104 eQTLs coinciding with QTL regions for WHC; 96 transcripts had *trans*-acting and 8 had *cis*-acting regulation. The cis-regulated positional functional candidate genes included *AHNAK, SLC3A2, MAP4K4, USP39,* hypothetical protein (*LOC162073*), *PRC, BBS2* and *COQ9*.

The complex relationships between biological processes taking place in live skeletal muscle and meat quality are driven on one hand by the energy reserves and their utilization in the muscle and by the muscle structure itself on the other. Holistic expression profiling was integrated with QTL analysis for the trait of interest and for gene expression levels for creation of a priority list of genes out of the orchestra of genes of biological networks relevant to the liability to develop elevated drip loss. By combining map-based and function-driven data functional positional candidate genes could be identified. Adding data derived from eQTL analysis and matching these to the gene map and pQTL map allowed addressing genes with *trans* and *cis* mode of transcriptional control. In particular functional positional candidate genes under *cis* acting regulation are of high priority for further analysis. The first porcine eQTL map of drip- correlated transcripts in pQTL regions will facilitate cloning causal genes.

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Pre- i postnatalne różnice w ekspresji genów w odniesieniu do cech mięsa i tuszy u trzody chlewnej – praca przeglądowa

Streszczenie

Procesy pre- i postnatalne determinują ostateczny wynik w produkcji mięsa wieprzowego, tak w odniesieniu do jakości tuszy, jak i samego mięsa. Szczególnie, liczba miofibryli, ale także w znacznym stopniu ich właściwości metaboliczne i zdolności skurczowe, determinowane są prenatalnie w czasie miogenezy. Procesy prenatalne oddziałują także na postnatalny przyrost mięśni i jakość mięsa. Równowaga metaboliczna, biochemiczne i biofizyczne właściwości mięsa przed ubojem warunkują dalszy proces dojrzewania mięsa. Stąd, zróżnicowana regulacja mnogości transkryptów w szlakach biologicznych w pre- i postnatalnej mięśniówce wpływa na biochemiczne procesy dojrzewania mięsa. Ogólnie, ponieważ cechy mięsa, którymi jesteśmy zainteresowani, nie są ujawniane w stadium prenatalnym, nie można wyznaczyć bezpośrednich zależności między fenotypem a modelem ekspresji genów. Jednakże prenatalne zróżnicowanie ekspresji genów związane z poszczególnymi cechami może być opisane w oparciu o znane wartości hodowlane, genotypy QTL i różnice rasowe. Profile ekspresji w mięśniach mogą być bezpośrednio związane z jakością mięsa. Odpowiednim układem doświadczalnym w tym przypadku jest analiza różnic między rodzeństwem (*discordant sib pair design*). W niniejszym opracowaniu dyskutowane są przykładowo zróżnicowane profile transkrypcyjne w *m. longissimus dorsi* w stadium pre- i postnatalnym, jako umożliwiające wgląd w procesy biologiczne w funkcjonujących mięśniach, które następnie wpływają na proces dojrzewania mięsa, a tym samym na jego jakość.