56th Annual Meeting of the European Association for Animal Production, June 5-8, 2005, Uppsala, Schweden
Commission G6,Session 32, Poster 542

ANALYSIS OF EFFECTS OF GENES DIFFERENTIALLY EXPRESSED DURING MYOGENESIS ON PORK QUALITY

E. Murani[1,8], M.F.W. te Pas[2], K.C. Chang[3], R. Davoli[4], J.W.M. Merks[5], H. Henne[6], R. Wörner[6], H. Eping[7], S. Ponsuksili[1,8], K. Schellander[1], N. da Costa[3], D. Prins[5], B. Harlizius[5], Egbert Knol[5], M. Cagnazzo[4], S. Braglia[4] and K. Wimmers[1,8,], [1] University of Bonn, 53115 Bonn, Germany, [2] Wageningen University and Research Centre, Animal Sciences Group, 8200 AB Lelystad, The Netherlands, [3] University of Glasgow, Glasgow G611QH, UK, [4] DIPROVAL University of Bologna, 42100 Reggio Emilia, Italy, [5] IPG, 6641 SZ Beuningen , The Netherlands, [6] BHZP Lueneburg, 21335 Lueneburg, Germany, [7] LRS, 53115 Bonn, Germany, [8] Research Institute for the Biology of Farm Animals, 18196 Dummerstorf, Germany*

Abstract: Genes regulated during myogenesis may be involved in the development and control of muscle-(structure) and consequently may have an effect on meat quality. Transcription profiles of embryonic (presumptive) and foetal M. longissimus dorsi were compared between Pietrain and Duroc breeds at 7 key stages of myogenesis employing microarrays, SSH and DD-RT-PCR. Fifty three differentially expressed genes were selected for further study. For 35 genes DNA polymorphisms were detected. The association between DNA variation of 23 candidates and meat quality and content was analysed in four Duroc and Pietrain based commercial lines and one Duroc × Pietrain experimental cross. The most interesting effects were found for genes on chromosomes 2, 4, 5 and 14 in regions harboring QTL for muscle structure and meat quality traits. This work is part of an EU-funded project (PorDictor – QLK5-2000-01363).

Introduction: Skeletal muscle is composed of muscle fibres and non-myofibre elements, like adipose and connective tissue. It becomes meat at slaughter. The number and type of muscle fibres are generally regarded to be determined prenatally by genetic factors. Muscle fibre number and composition are not only important physiological parameters in the live animal but also are key determinants of meat quality at post-mortem. Meat quality parameters, like shear force, colour, pH, conductivity, and quantity of meat are directly related to the number and proportion of different types of muscle fibres. The earliest embryonic development that is important for meat quality is the development of muscle fibres. The genotype may determine the timing and level of expression of certain genes during prenatal muscle development. The resulting phenotype in number of muscle fibres is fixed at birth, and will influence the final phenotype of muscle at slaughter related to meat quality. A huge number of genes are expressed in muscle tissue at different times of development all of which more or less contribute to the muscle phenotype. These are candidate genes for carcass and meat quality traits. We aimed to short list genes with strong functional and positional evidence for effects on meat production and quality traits (functional positional candidate genes) (Wimmers et al., 2005). Here we report on the association of functional candidate genes for meat production and quality traits derived from expression profiling during prenatal muscle development.

Material and Methods: Expression profiles of embryonic (presumptive) and foetal M. longissimus dorsi were compared between Pietrain and Duroc breeds at 7 key stages of myogenesis (d14, 21, 35, 49, 63, 77, 91) employing microarrays, SSH and DD-RT-PCR. The various techniques of expression profiling revealed in total 584 genes that were either temporal regulated during myogenesis or differentially expressed between the two breeds. Selection of loci for further analysis was based on (1) the consistency of the expression pattern and its reproducibility (2) knowledge on the function of the particular gene (categorized as structural gene, metabolic ~, translational ~, transcriptional ~, receptor/endocrine factors, differentiation ~, proliferation ~ unknown), (3) the map position allowing to give preference to those genes located in QTL regions for meat quality traits. Loci of the short list of functional candidate genes were screened for polymorphism by comparative sequencing of a set of DNAs of animals of the breeds Duroc, Pietrain, and German Landrace. Subsequently PCR-RFLPs, PCR-SSCPs, single base extension assays, TaqMan assays, as well as melting curve analysis protocols were established for high throughput genotyping of the polymorphisms (Murani et al., 2005).

For a number of loci mapping information was available from published porcine genome maps as well as comparative maps. For others regional assignment was done using the IMpRH-panel (INRA-University of Minnesota pig Radiation Hybrid) (Yerle et al., 1998). In addition those loci that were genotyped in animals of the experimental F2-Population of Duroc and Pietrain, DUPI, were genetically mapped using the CRI-MAP package (Version 2.4).
A total of 22 candidate loci were evaluated for association to meat quality traits by genotyping offspring of boars with extreme meat quality breeding values of the commercial herds first (selective genotyping). Taking into account any indication of association of the selective genotyping procedure, positional and functional information on the loci, a subset of 10 loci was selected for the association analysis for various carcass and meat quality traits using about 2000 performance tested pigs of Duroc and Pietrain based commercial purebred and crossbred lines and a Duroc × Pietrain experimental cross.
Analyses were done separately for purebred Pietrain, Pietrain X F1, Duroc X F1 and Duroc X Pietrain (total dataset) using the model:

$$Y_{ijklm} = \mu + sex_i + sladate_j + gene_k + litter_l + animal_{ijklm} + e_{ijklm}$$

where Y = meat quality and carcass traits, sex = fixed effect of gender, sladate = fixed effect of day of slaughter, gene = fixed effect of genotype at a candidate gene, animals = random effect of animals, litter = fixed effect of litter. Analyses were performed using ASReml and in all analyses pedigree was included.

Results and Discussion: Results of the association study are summarized in table 1. For 9 of the 10 selected genes significant (P<0.05) associations with one or more pork quality traits are reported. The most interesting effects were found for genes on chromosomes 2, 4, 5 and 14 in regions harboring QTL for muscle structure and meat quality traits.
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.
Carbonic anhydrase III, CA3, is a member of a multigene family (at least six separate genes are known) that encode carbonic anhydrase isozymes. These carbonic anhydrases are a class of metalloenzymes that catalyze the reversible hydration of carbon dioxide and are differentially expressed in a number of cell types. The expression of the CA3 gene is strictly tissue specific and present at high levels in skeletal muscle. A proportion of carriers of Duchenne muscle dystrophy have a higher CA3 level than normal. CA3 maps to the central region of SSC4 where QTL for carcass traits as well as meat quality traits were detected (Andersson et al., 1994; Geldermann et al., 2003). Recently a QTL for water holding capacity was found close to CA3 on SSC4 (Su Yu-Hong, et al. 2004)
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. HGMA2 is a positional candidate for QTL for meat colour, pH and conductivity identified on SSC5 (Malek et al., 2001; Geldermann et al., 2003).
The function of the ELKS gene is less well understood. Recently Ducut Sigala et al. (2004) proposed ELKS as a part of IKK complex playing a role in the activation of NF-kappaB transcription factor. The NF-kappaB transcription factor functions as a negative regulator of myogenesis by inhibiting MyoD (Guttridge, 2004). We mapped ELKS physically and genetically on chromosome 5. According to the PigQTL database close to ELKS QTL are located for ham weight, loin and ham percentage in carcass, pH and meat colour.
ANK1, ankyrin1, belongs to a family of proteins that link the integral membrane proteins to the underlying spectrin-actin cytoskeleton and play 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, 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 in approximately half of all patients with hereditary spherocytosis. According to the current comparative map the porcine ANK1 maps to SSC14p11-16. To the proximal region of SSC14 QTL for driploss, cookloss as well as loin eye area have been previously assigned (de Koning et al., 2001; Malek et al., 2001; Rohrer et al., 1998). Thus there is functional and positional evidence for effects of ANK1 on meat quality and carcass traits.

Table 1: Summary of results of the association analysis (P<0.1, **bold:** P<0.05)

	Duroc X F1	Pietrain	Pietrain X F1	Duroc X Pietrain
ANK1	**Driploss** **pH loin** **Cookloss** **IMF**/marbling	Minolta a loin Loin eye area	**Thawloss** **Shearforce** Loin eye area	
bR10D1	Driploss Thawloss FOP loin **Shearforce**	**Opto colour** **Thawloss** Minolta L loin Minolta b loin	**Shearforce**	**Loin eye area** **FOM muscle**
PDGFRA	Shearforce HGP loin	Loin eye area		
HMGA2	**pH loin** FOP loin pH ham **shearforce** **Minolta L loin** **Minolta b loin**			
ELKS	Jap colour score Deboned loin	**Minolta L loin** **Opto colour** Loin weight	Loin eye area	**Opto colour** **Ham weight**
NME1			Cookloss **Conductivity**	Shearforce **Loin depth** **Loin Weight** Ham weight
EPOR	Shearforce Minolta a loin IMF	Driploss **Ham weight**	**Cookloss** **Shearforce**	**Cookloss** **pH loin** Opto colour pH ham **FOM muscle** **Ham weight**
CA3	**IMF**	Thawloss **Conductivity**	Cookloss	**Thawloss** **Ham weight**
TTN		Shearforce Lean content Loin weight **Loin eye area**	Shearforce **Opto colour**	Driploss **Conductivity**
MYOP	Minolta L loin **HGP loin** Deboned loin			pH loin pH ham **Loin depth** **FOM muscle** **Loin eye area**

Myopalladin, MYOP, is another structural component of muscle. As a component of the sarcomere it tethers nebulin in skeletal muscle and nebulette in cardiac muscle to α-actinin at the Z lines. Effects of MYOP on carcass traits have already been shown (Davoli et al., 2003). QTL for pH exists close to MYOP on SSC14 (Malek et al., 2001).

The EST bR10D1 (FLJ26539) maps to SSC14, in accordance with the human-porcine comparative map. The position of bR10D1 falls in the confidence interval of pH and meat colour QTL reported by de Koning et al. (2001). The function of FLJ26539 is unknown, however it is highly conserved between human, mouse and chicken (http:ecrbrowser.dcode.org).

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 map to SSC8 within a region where QTL for meat colour and fiber type I proportion have been shown (Geldermann et al., 2003; Ovilo et al., 2002; Malek et al., 2001).

Non-metastatic cells 1, protein 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).
Titin, TTN, encodes a large abundant protein of striated muscle. The product of this gene is divided into two regions, a N-terminal I-band and a C-terminal A-band. A N-terminal Z-disc region and a C-terminal M-line region bind to the Z-line and M-line of the sarcomere respectively so that a single titin molecule spans half the length of a sarcomere. Titin also contains binding sites for muscle associated proteins so it serves as an adhesion template for the assembly of contractile machinery in muscle cells. TTN is located on SSC15 (Davoli et al., 2003) within a region exhibiting QTL for pH, flavor and tenderness (Malek et al., 2001).

The study 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. For most of the genes knowledge on their physiological role support their putative involvement in genetic regulation of these traits. Moreover, association study provided statistical evidence for effect of DNA variation at these loci on the traits of interest. Also the regional assignments to QTL regions support the findings. These genes are thus functional positional candidate genes, for which linkage and association to the traits analyzed could be demonstrated. The polymorphisms analyzed are most likely non-functional mutations. However, the observed effects are not consistent across populations. This is not unexpected as the traits analyzed are quantitative traits controlled by several loci, i.e. the genetic background has an important impact on the effects observed for a single locus. The partly inconsistent gene effects within and between populations indicate that the polymorphisms may not be in linkage disequilibrium with the causative genetic variation.
This study revealed 10 genetic markers that are significantly associated with several pork production and quality traits and that were derived from prenatal muscle expression profiles.

References

Andersson, L.; Haley, C.S.; Ellegren, H.; Knott Sa.; Johansson M.; Andersson K.; Andersson-Eklund L.; Edfors-Lilja I.; Fredholm M.; Hansson I.; Hakansson J.; Lundström K. (1994) Genetic mapping of quantitative trait loci for growth and fatness in pigs. Science 263, 1771-74

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. Cytogenet Genome Res 102, 152-6

de Koning DJ, Harlizius B, Rattink AP, Groenen MA, Brascamp EW, van Arendonk JA. (2001) Detection and characterization of quantitative trait loci for meat quality traits in pigs. J Anim Sci 79, 2812-19

Ducut Sigala JL, Bottero V, Young DB, Shevchenko A, Mercurio F, Verma IM. (2004) Activation of transcription factor NF-kappaB requires ELKS, an IkappaB kinase regulatory subunit. Science 304, 1963-7

Geldermann H, Müller E, Moser G, Reiner G, Bartenschlager H, Cepica S, Stratil A, Kuryl J, Moran C, Davoli R, Brunsch C. (2003) Genome-wide linkage and QTL mapping in porcine F_2 families generated from Pietrain, Meishan and Wild Boar crosses. J Anim Breed Genet 120, 363-93

Guttridge DC. (2004) Signaling pathways weigh in on decisions to make or break skeletal muscle. Curr Opin Clin Nutr Metab Care 7, 443-50

Malek M, Dekkers JC, Lee HK, Baas TJ, Prusa K, Huff-Lonergan E, Rothschild MF. (2001) A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. II. Meat and muscle composition. Mamm Genome 12, 637-45

Murani E, Ponsuksili S, Wimmers K. (2005): Simultaneous detection of SNPs in four porcine genes using hybridization probes and the LightCycler 2.0 Instrument. Biochemica 2, 7-9

Ovilo, C, Clop A, Noguera JL, Oliver MA, Barragan C, Rodriguez C, Silio L, Toro MA, Coll A, Folch JM, Sanchez A, Babot D, Varona L, Perez-Enciso M. (2002) Quantitative trait locus mapping for meat quality traits in an Iberian x Landrace F2 pig population. J Anim Sci 80, 2801-8

Rohrer GA, Keele JW (1998) Identification of quantitative trait loci affecting carcass composition in swine: II. Muscling and wholesale product yield traits. J Anim Sci 76, 2255 - 62

Su Yu-Hong, Xiong Yuan-Zhu, Jiang Si-Wen, Zhang Qin, Lei Ming-Gang, Zheng Rong, Deng Chang-Yan (2004): Mapping quantitative trait loci for meat quality traits in a Large White x Meishan cross. Acta Genetica Sinica 3,132-6

Wimmers K, Murani E, Schellander K, Ponsuksili S. (2005) Combioning QTL- and expression-analysis: identifcation of functional positional candidate genes for meat quality and carcass traits. Arch Tierz 48, 23-31

Yerle M, Pinton P, Robic A, Alfonso A, Palvadeau Y, Delcros C, Hawken R, Alexander L, Beattie C, Schook L, Milan D, Gellin J. (1998) Construction of a whole-genome radiation hybrid panel for high-resolution gene mapping in pigs. Cytogenet Cell Genet 82,182-188