Animal Science Papers and Reports vol. 22 (2004) no. 1, 115-118
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Presented at the Conference
„Gene polymorphisms affecting health and production traits in farm animals"
held at the „ANIMBIOGEN" Centre of Excellence in Genomics and Biotechnology Improving Functional Traits in Farm Animals and Quality of their Products,
2-3 October 2003, Jastrzębiec, Poland

Candidate genes for meat production and meat quality – the *MRF* genes

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Introduction

Muscle tissue becomes meat after slaughtering the animal. Meat quality is a complex trait affected by both environmental and genetic factors. The influence of genetic factors relate back to the prenatal formation of muscle tissue (myogenesis), *i.e.* determination of precursor cells (myoblasts), proliferation (cell division), and differentiation into multinucleated myofibers. This process is an exclusive prenatal event taking place twice, *i.e*. primary and secondary muscle fiber formation, together called myogenesis. Postnatal growth of muscle tissue is characterized by hypertrophic growth of myofibers without the formation of new myofibers. Myogenesis and postnatal muscle tissue growth are regulated by the myogenic regulatory factors (MRF) gene family. For a review of the genetic effects of the MRF gene family on livestock meat production see te Pas and Soumillion [2001].

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Genetic regulation of meat production – the role of the *MRF* genes

The *MRF* gene family consists of four transcription factors. The MyoD and myf-5 genes regulate proliferation of myoblasts and satellite cells (postnatal type myoblasts having the ability to fuse with existing myofibers, but lacking the ability to form new myofibers). Myogenin is expressed during fusion of cells to form multinucleated myofibers. The role of myf-6 has been mainly described as maintaining the muscle tissue. For our studies we focused on the role of myogenin in the first place, and than to the role of myf-5 and MyoD.

First we studied the role of genetic variation in myogenin on pork production. Myogenin was argued to be important for meat production because its expression limits the production of (new) precursor cells. Delayed expression could result in increased numbers of myoblasts, and subsequently myofibers. The porcine myogenin gene was sequenced and its genetic variation detected [Soumillion *et al.* 1997]. In a subsequent association study involving over 2000 pigs of four different commercial populations we showed differential effects of the genotype of the myogenin marker [te Pas *et al.* 1999]. Table 1 shows the differences in growth rate and carcass data of pigs of the two homozygous myogenin marker genotypes. Pigs homozygous for the optimum allele have a higher birth weight, increased growth rate, and increased lean per cent of carcass without increased backfat thickness.

Table 1. Differences between pigs with homozygous genotypes for the myogenin gene *locus* marker

Trait	Effect	P-value
Birth weight (g)	70	0.01
Carcass weight at slaughter age (kg)	2	0.05
Carcass lean weight (kg)	1	0.22
Backfat thickness (mm)	0.3	>0.5

Similar research was done for the myf-5 gene. Using the same animals we found no myf-5 genotype effect (data not shown) [te Pas *et al.*1999]. It is possible that genetic variation in the myf-5 gene *locus* does not affect pork production. However, since myf-5 and MyoD can substitute for each other, a negative allele in one gene may be compensated by the other gene fading out effects on livestock meat production [discussed by te Pas and Soumillion 2001].

Next we studied MRF mRNA expression in muscle tissue at slaughter, which may be an indication for lean growth potential at slaughter age. Samples from 14 different muscles were collected from boars of two different selection lines, a fast growing line (F-line), and a lean growing line (L-line), five boars each. The results showed that the mRNA expression level of the MyoD, myf-5, and myogenin genes in muscle tissue at slaughter of the F-line was higher than in muscle tissue of the L-line pigs. The opposite was found for the myf-6 gene [te Pas *et al.* 2000]. These results suggest that selec-

tion for increased growth rate is associated with increased MRF gene expression, regulation of satellite cell proliferation and differentiation, while selection for increased lean percentage is associated with increased maintenance of muscle tissue.

Discovering genes regulated by the *MRF* genes – ChIP technique

The *MRF* genes are transcription factors regulating myogenesis by influencing the expression of other genes which proteins affect the status of the cell for proliferation or fusion. However, the nature of many of these genes remains unknown. To start the study to discover these genes we adapted the chromatin-immunoprecipitation (ChIP) technique. In short: During the isolation of genomic DNA the protein complexes bound to the DNA at the moment of sampling the muscle tissue were cross-linked to the DNA with a cross-linking agent. After shearing the DNA the MRF-DNA fragments were isolated using immunoprecipitation with anti-MRF antibodies. De-cross-linking resulted in fragments to which the *MRF* genes were bound, thus putative promoters of genes regulated by the *MRF* genes during myogenesis. Since shearing resulted in random chromosome breaking part of exon 1 may be in the fragment. Thus, cloning and sequencing should uncover the nature of the genes regulated by the *MRF* genes during myogenesis. Present results indicate that we have isolated DNA fragments with promoter characteristics, preferentially of the E-box type, which is bound by the *MRF* genes.

Regulating the regulators – microarray studies

While the *MRF* genes are the central regulators of myogenesis, they act in a network of many other genes regulating the expression of the *MRF* genes. This ensures correct timing and positional expression of expression. Many of these genes remain unknown although an increasing number of genes affecting myogenesis is reported. However, the genetic effect of these genes on livestock meat production and meat quality remains unknown. We have selected about 300 of these genes from the literature, cloned the pig genes and placed them on a microarray together with well-characterized genes from a pig muscle cDNA library.

In an EU-funded project (PorDictor, EC31363) we investigated prenatal expression patterns related to post mortem meat quality differences between two pig breeds extreme for meat quality, *i.e*. Duroc and Pietrain. From both breeds embryos/fetuses were collected at 14, 21, 35, 49, 63, 77, and 91 days of age [Ducro-Steverink *et al.* unpublished]. Embryos/fetuses were mainly from a second litter. The first litter was born and slaughtered at slaughter age, and meat quality data were collected. Thus, the mean meat quality for the second litter was known by extrapolation from the first litter. Pools of RNA samples were labelled and hybridized to the microarrays. First analyses indicated that a number of genes in several pathways including myogenesis, energy metabolism, and muscle structural genes show different expression between

Duroc and Pietrain embryos/fetuses at several different prenatal ages. Furthermore, the expression levels of several genes fluctuate in time. The analysis of the microarray experiments is still ongoing.

Acknowledgement. *The author would like to thank his collaborators in the laboratory during the several projects on the MRF genes, especially Drs. Soumillion and Houba, and the laboratory technicians Harders, Erkens, Priem, and de Wit. The collaborators of the ongoing PorDictor project, Drs. Wimmers, Chang, Davoli, Merks, Harlizius, Henne, and Cagnazzo are acknowledged. Funding for the projects was received from the Institue for Pig Genetics (IPG), the Dutch Ministry for Economic Affairs, the Dutch Ministry for Agriculture, Food Quality and Nature Management, the Animal Sciences Group Strategic Research, and the European Union.*

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Please note that the references listed here are just a short list of some of the publications the group of the author has published on this subject. For a comprehensive list of publications on myogenesis in general and for livestock in particular see te Pas and Soumillion [2001].

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