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Identification of expression quantitative trait loci (eQTL) and candidate genes associated with water holding capacity in porcine meat

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Mehmet Ulas Cinar

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Referent :	Prof. Dr. Karl Schellander
Korreferent:	Prof. Dr. Brigitte Petersen
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This dissertation is dedicated to my parents and my dear sister Fatma Zeynep Cinar for their love, endless support and encouragement

Identifizierung von expressions quantitativ trait Loci (eQTL) und Kandidatengenen für das Wasserhaltevermögen im Schweinefleisch

Die Beurteilung von Fleischqualität durch den Konsumenten ist definiert durch sensorische Merkmale, wie Saftigkeit, Muskel pH, Wasserhaltevermögen und Farbe. Das Wasserhaltevermögen in Bezug auf den Tropfsaftverlust hat sowohl eine genetische als auch eine ökologische Komponente mit geringer bis mittlerer Heritabilität. Die Anzahl der Gene die an dem Merkmal Tropfsaftverlust beteiligt sind, sind bislang noch unbekannt. Das Ziel dieser Studie war es, geeignete Kandidatengene, die für den Tropfsaftverlust im Schweinefleisch verantwortlich sind, zu identifizieren.

Für die Kandidatengenanalyse und ihre eQTL Positionen wurden zwanzig Gene selektiert. Zur Erstellung der Expressionsprofile von 300 DUPI Tieren wurde einerseits eine quantitative Real-Time PCR verwendet, sowie das GenomeLab GeXP Multiplex Verfahren. Zur Normalisierung der Expression wurden mehrere Referenz Gene eingesetzt. Die Analyse zeigte das die Gene Peroxisome proliferator activated receptor gamma, coactivator 1 alpha (*PPARGC1*) und Alpha 1 microglobulin/bikunin (*AMBP*) in Vergleich zwischen hohem und niedrigem Tropfsaftverlust unterschiedlich exprimierten. Darüber, hinaus zeigte die Expression weiterer Gene signifikante Assoziationen mit verschiedenen Parametern der Fleischqualität. Die Analyse der eQTL erbrachte das diese Gene in der DUPI Population *trans*-reguliert waren. Durch die Verwendung verschiedener QTL Modelle wurde auf SSC2 in der Nähe des Markers S0141 eine vielversprechende chromosomale Region für Tropfsaftverlust entdeckt, dies konnte durch Fachliteratur bestätigt werden. Durch die Übereinstimmung entdeckter eQTL mit QTL in der DUPI Population und in anderen Schweine Populationen konnten weitere vielversprechende chromosomale Regionen für zukünftige Feinkartierungen und Assoziationsstudien a werden.

Identification of expression quantitative trait loci (eQTL) and candidate genes associated with water holding capacity in porcine meat

Consumer assessment of meat quality is defined by the characteristics of sensory experience such as juiciness, muscle pH, water-holding capacity and colour. Water-holding capacity in terms of drip loss has a genetic as well as environmental component with low to medium heritability. The number of genes involved in the development of drip loss is unknown. The aim of this study was to identify the candidate genes and their transcriptional regulation responsible for the drip loss in pig meat.

Twenty genes were selected for the candidate gene analysis and for their eQTL study. For the expression of genes quantitative real-time PCR and GenomeLab GeXP multiplex were used in 300 DUPI animals. Multiple housekeeping genes were used for the accurate gene expression normalization. Analysis revealed expression of peroxisome proliferator activated receptor gamma, coactivator 1 alpha (*PPARGC1*) and alpha 1 microglobulin/bikunin (*AMBP*) genes were differentially regulated in animals with higher drip loss compared to lower drip loss. Moreover, expression of other genes showed significant association with different meat quality parameters. eQTL analysis showed that these genes are *trans*-regulated in DUPI population. By using different QTL models, on SSC2 vicinity of marker S0141 was detected as the most promising chromosomal region for drip loss supported by the literature as well. Ovelapping of detected eQTL with QTL in DUPI population and other pig populations showed promising chromosomal regions for further fine mapping and association studies.

Contents

List of abbreviations

A2M	:alpha-2-macroglobulin
ACTB	:actin, beta
ALB	:albumin
AMBP	:alpha 1 microglobulin/bikunin
ANGPTL4	:angiopoietin-like 4
APOA1	:apolipoprotein A-I
APOC3	:apolipoprotein C-III
ATF4	:activating transcription factor 4
BMP	: Bone morphogenetic potein
bp	: Base pairs
C_{1L}	: Conductivity, longissimus dorsi, 45 min post-mortem (mS/cm)
C_{24L}	: Conductivity, Longissimus dorsi, 24 h post mortem (mS/cm)
C_{24S}	: Conductivity, Semimembranosus 24 h post mortem (mS/cm)
cM	: Centimorgan
CAPNS1	: Calpain, small subunit 1
CYP2C33	: Cytochrome P450 2C33
cDNA	: complementary DNA
d	: day
ddH_2O	: Distilled & deionized water
dH_2O	: Deionized or distilled water
ddNTP	: Deoxyribonucleoside triphosphate (usually one of dATP, dTTP, dCTP and dGTP)
drip	: Drip loss
DNA	: Deoxynucleic acid
DUPI	: Duroc × Pietrain cross
E.coli	: Escherichia coli
EST	: Expressed sequence tag
eQTL	: Expression quantitative trait loci

Gb	: Giga base
GSTA2	: Glutathione S-transferase A2
EDTA	: Ethylenediaminetetraacetic acid (powder is a disodium salt)
EtBr	: Ethidium bromide
EtOH	: Ethanol
Fig	: Figure
g	: Gram
GC	: Group-specific component (vitamin D binding protein)
HAL (RYR1)	: Halothane (ryanodine receptor)
h	: Hour
HMBS	: Hydroxymethylbilane synthase
IGF2	: Insulin-like growth factor
IPTG	: Isopropylthio-ß-D-galactoside
kb	: Kilobases
l	: Litre
LD	: Longissimus dorsi
mg	: Milligrams
$MgCl_2$	: Magnesium chloride
ml	: Milliliters
mg	: Milligrams
$MgCl_2$	: Magnesium chloride
min	: Minute
ml	: Milliliters
Myf5	: Myogenic factor 5
MRF4	: Myogenic regulatory factor 4
MyoD	: Myogenic differentiation protein
MyHC	: Myosin heavy changes
mmole	: Milimole
MRF	: Myogenic regulatory factors
mRNA	: Messenger RNA
NaCl	: Sodium chloride
N	: Newton

ng	: Nanograms
nm	: Nanometers
OD260	: Optical density at 260 nm wavelength (UVlight); = A260
PAX3	: Paired box 3
PAX7	: Paired box 7
pm	: Post mortem
PI	: Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
PPARGC1	: Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha
PPP1R3B	: Protein phosphatase 1, regulatory (inhibitor) subunit 3B
PSE	: Pale, soft, exudative
pH1	: pH value, 45 min post mortem
pH_{1L}	: pH value, Longissimus dorsi, 45 min post mortem
pH_{24L}	: pH value, Longissimus dorsi, 24 hours post mortem
pH_{24H}	: pH value, Semimembranosus 24 hours post mortem
pH_u	: pH ultimate
pmol	: Picomolar
RBP4	: Retinol binding protein 4
SERPINA3-2	: α-1-antichymotrypsin 2
SNP	: Single nucleotide polymorphism
SR	: Sarcoplasmic reticulum
TAE	: Tris-acetate buffer
TBE	: Tris- borate buffer
TBP	: TATA-box binding protein
TF	: Transferrin
TNC	: Tenascin C
TTR	: Transthyretin
TYROBP	: TYRO protein tyrosine kinase binding protein
WBSF	: Warner-Bratzler Shear Force
WHC	: Water-holding capacity

WHC	: Water-holding capacity
vs.	: versus
v/v	: Volume per volume
X-gal	: 5-Bromo-4-chloro-3-indolyl-beta-D-galactoside
µ	: Micro

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1 Introduction

Amount and distribution of water within the meat is a key factor which influences many technological and sensory properties of pork. Water-holding capacity (WHC), i.e. the capacity of meat to retain its water during application of external forces in particular is of special interest (Hamm 1985). During the post-mortem conversion of muscle to meat changes to WHC occur and as a result, a fluid consisting of water and dissolved proteins is released without application of external forces (except gravity), the so-called drip loss (drip) (Hamm 1985; Jennen et al. 2007). Several factors can influence the amount of drip loss. Besides the genetics, other aspects, including animal and carcass handling, post-mortem temperature management, nutrition, processing, also play an important role. Drip loss can cause product weight losses due to purge can average as much as 1–3% in fresh retail cuts (Offer and Knight 1988) and can be as high at 10% in PSE products (Melody et al. 2004). In addition to the loss of salable weight, purge loss also entails the loss of a significant amount of protein (Offer and Knight 1988; Offer et al. 1989).

The majority of water in muscle is held either within the myofibrils, between the myofibrils and between the myofibrils and the cell membrane (sarcolemma), between muscle cells and between muscles bundles (groups of muscle cells). Once muscle is harvested the amount of water and location of that water in meat can change depending on numerous factors related to the tissue itself and how the product is handled (Honikel, 2004). The main constituent of meat is water, comprising 75% of its weight. About 90% of the water is bound in the muscle cell, and the rest is present in the interstitium. As 80% of the volume of the muscle is occupied by myofibrils, it is generally accepted that 90-95% of the water in the muscle cell is present between the myofibrils and 5% is chemically bound to the charged groups of the intracellular muscle proteins (Lambert et al. 2001).

The biochemical, physiological, and structural events that are initiated by slaughtering and which lead to drip loss are complex and poorly understood. However, loss of osmolytes and cell water, following osmotic perturbation, hormonal stimulation, and limitation in oxygen supply (anoxia), has been described in a variety of mammalian cells. This knowledge provides an obvious starting point in the understanding of the initial processes leading to water reorganization in muscle during its conversion to meat.

Several economic traits have been explored in pig industry through QTL studies including fattening traits (daily gain, feed efficiency), body composition traits (meat content, fat content, loin eye area) and meat quality traits (color, water-holding capacity, muscle pH, tenderness, intramuscular fat). These traits are caused by a number of genotypic and environmental factors. From the view of molecular genetics, genotypic effects rely on the variation of the nucleotide sequence in the genome that either influences the expression level of a given gene or the functional properties of the encoded proteins. Contributing greatly to this variation are the effects of extreme breeds and the structural and functional properties of muscle itself.

The finding of genes responsible for genetic variation in the traits of interest in animal species is of importance in genomic analysis (Rothschild and Soller 1997). Currently, there are two different approaches known as QTL mapping and candidate gene analysis. While the first technique is to discover genomic regions related to quantitative traits, the other one focuses on detection of mutations in candidate genes and their possible association with economical production traits (Ovilo et al. 2002) and thereby exploits information from previous cellular, biochemical or physiological functional studies to target a gene of interest. Candidate genes can be derived based on knowledge of the function of the gene product (direct biological candidate) or its specific expression pattern (functional candidate).

The present study was undertaken with the following objectives:

1. To quantify transcript abundance of drip loss related genes and further figure out the expression profile of these genes in low and high performing pigs in Duroc / Pietrain (DUPI) F_2 resource populations.

2. To understand the association of gene expression with meat quality traits.

3. To identify expression quantitative loci (eQTL) of drip loss related genes.

2 Literature review

2.1 The skeletal muscle development

In farm animals, the structural and functional diversity of skeletal muscle is represented by a variety of myosin isoforms. Understanding muscle fiber characteristics will help to optimize the efficiency of the muscle growth and meat quality, the two important concerns in animal production. To this point, fiber classification, myosin heavy chain (MyHC) expression, their role in muscle development and meat quality traits and possible factors influencing the fiber proportions are briefly described in the next section.

2.1.1 Prenatal development of skeletal muscle

Skeletal muscle represents nearly half of the total body mass and thus is the most abundant tissue of the human body. A skeletal muscle is composed of many bundles of myofibers, which are the functional units. A single myofiber is derived from the fusion of numerous myoblasts and therefore contains many nuclei. Each myofiber contains many myofibrils, which are composed of repeating sarcomeres. A sarcomere is an arrangement of the contractile proteins myosin and actin, which form the thick and thin filaments, respectively (Figure 2.1) (Grefte et al. 2007).

In early stages of embryonic development, the major function of gastrulation is to create a mesodermal layer between ectoderm and the endoderm. The mesoderm forms the blood, blood vessels, bones, cartilage, connective tissue, and the muscle of the body trunk (Grefte et al. 2007). The somites are generally regarded as the site of myogenesis. The somites are derived from the mesoderm which, in the body (excluding the head), is subdivided into four compartments: the axial, paraxial, intermediate and lateral plate mesoderm. Somites develop from the paraxial mesoderm and constitute the segmental pattern of the body. Each somite is surrounded by extracellular matrix material connecting the somite with adjacent structures. The competence to form skeletal muscle is a unique property of the somites and becomes realized during compartmentalization, under control of signals emanating from surrounding tissues (Christ and Ordahl 1995). Compartmentalization is accompanied by altered patterns of expression of regulatory factors *PAX-3* and *PAX-7* genes within the somite (Mozdziak 2006). A crucial step in the formation of skeletal muscle is the appearance of myotome (Figure 2.2).

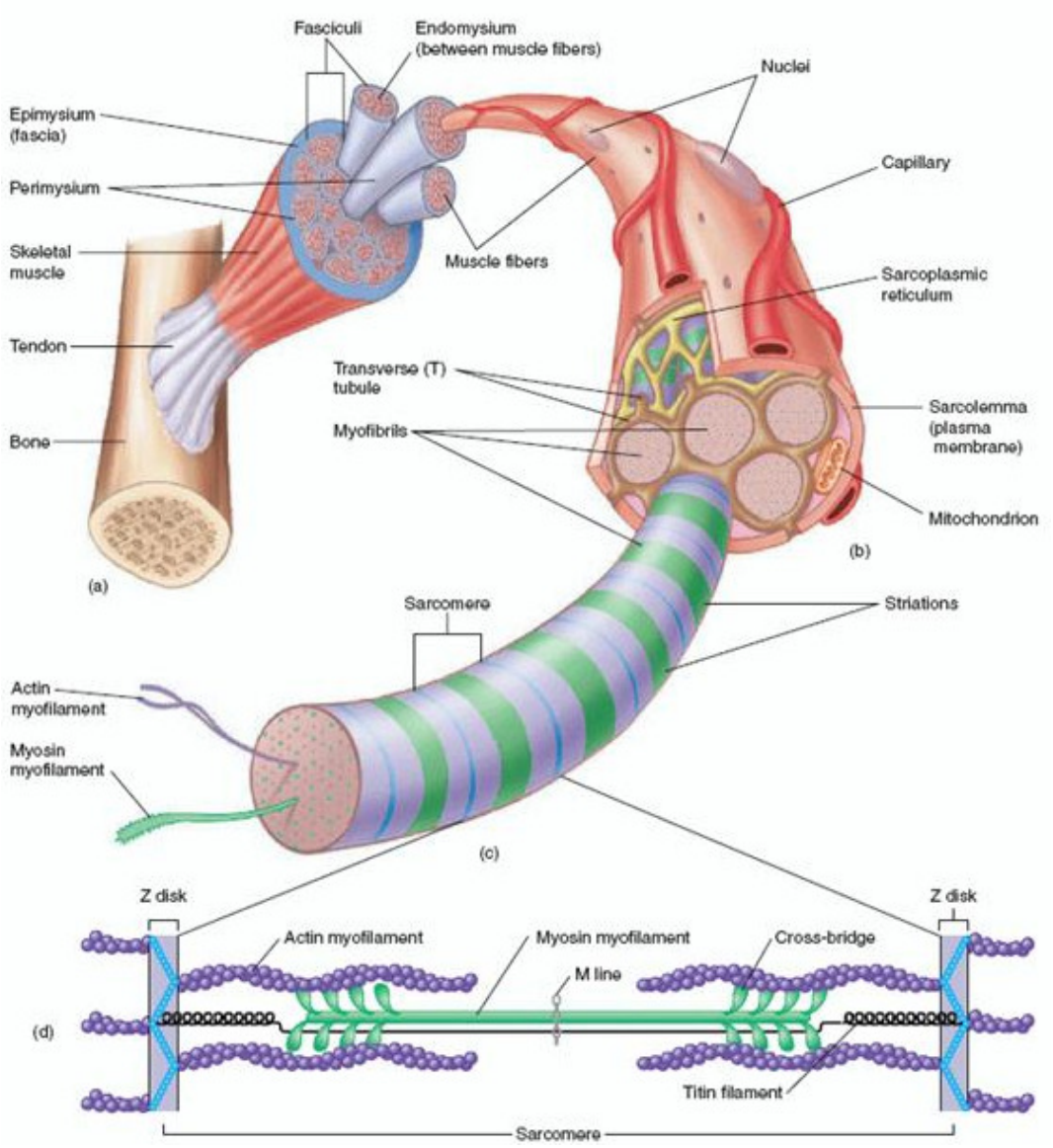

Figure 2.1: Shows the structure of skeletal muscle. Skeletal muscle made up of clusters of myofibers. The bundles of fibres, known as Fasciculi, which are surrounded by another connective tissue, called the Perimysium. Each Fasciculi contains anywhere between 10 and 100 muscle fibres. Muscle fibres are covered in a fibrous connective tissue, known as Endomysium which insulates each muscle fibre. Muscle fibres can range from 10 to 80 micrometers in diameter and may be up to 35cm long. Under the Endomysium and surrounding the muscle fibre is the Sarcolemma which is the fibres cell membrane and under this is the Sarcoplasm, which is the cells cytoplasm, a gelatinous fluid which fills most cells. This contains glycogen and fats for energy and also mitochondria which are the cells powerhouses, inside which the cells energy is produced

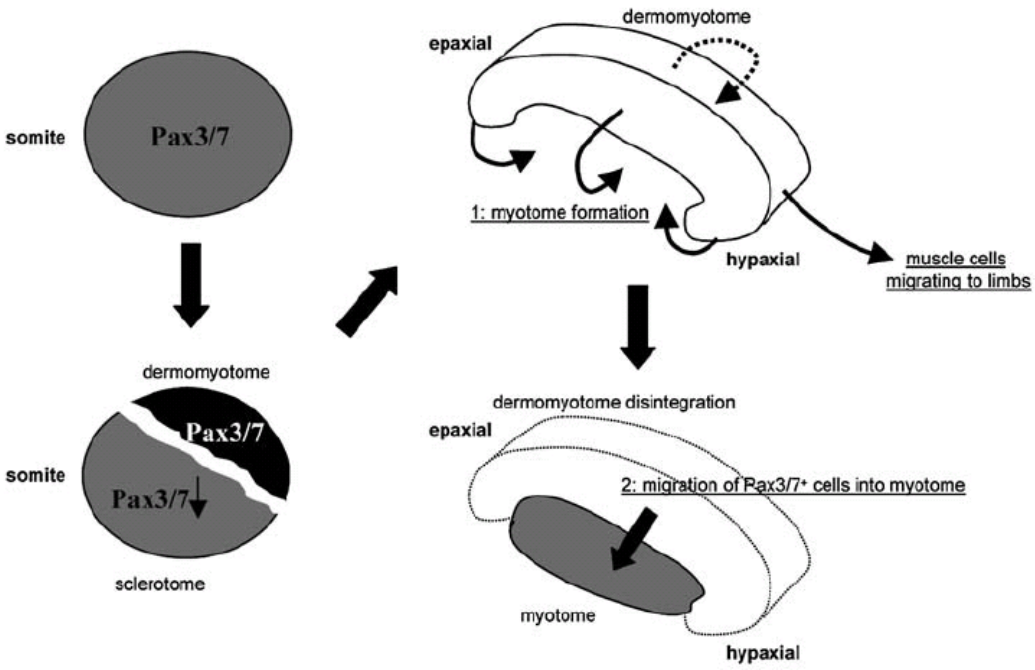

Figure 2.2: Formation of the somites and myotomes. Adapted from Grefte et al. (2007).

Muscle progenitors cells delaminate from the four edges of dermomyotome and muscle progenitor cells migrate into limb buds. In later embroyogenesis, muscle masses separate into epaxial (deepback) muscles and hypaxial (abdominal and apendicular) muscles (Gross et al. 2004). Schematic reprasantation of muscle formation during embryo development is given in figure 2.3.

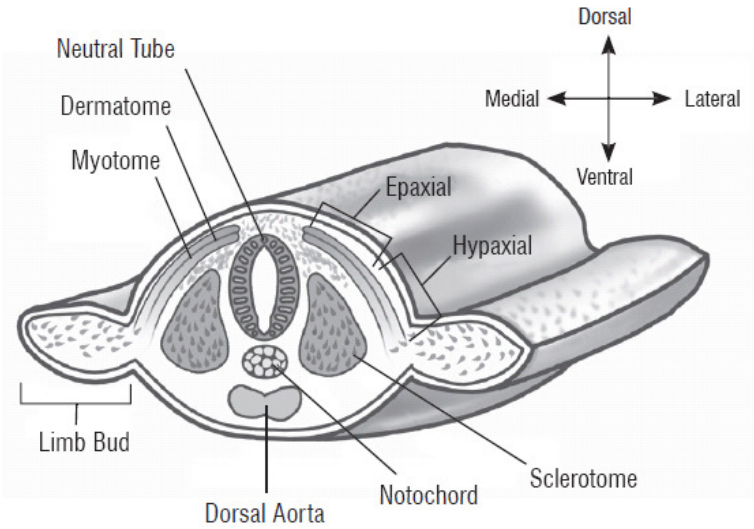

Figure 2.3: Schematic representation of muscle formation. Adapted from Mozdziak et al. (2006)

Tyrosine kinase receptor (C-MET) that binds hepatocyte growth factor and *PAX3* are major contributors to this delamination and migration. Because embryos lacking functional *C-MET* and *PAX3* do not form skeletal muscle in limbs (Relaix et al. 2003; Epstein et al. 1996). Myogenesis regulated by basic-helix-loop-helix transcription factors through the action of myogenic regulatory factors (*MRF*s). *MRF*s are essential to the specification and determination of muscle cells lineage (Cole et al. 2004). Delaminating progenitor cells down-regulate *PAX3* and become myoblasts by the action of *MRF*s. These myoblasts increase their expression of *MYF5*, *MRF4*, and *MYOD* (Cole et al. 2004; Rudnicki et al. 1993), and differentiate into myocytes through the cation of myogenin, *MRF4* and *MYOD* (Grefte et al. 2007). These myocytes fuse and mature into multinucleated musclefibers forming continues muscle layer, the myotome (Kalcheim et al. 1999). Myogenesis processes stimulated by signals which are released from neural tube, notochord, and surface ectoderm (Figure 2.3). Sonic hedgehoh (Shh) and Wnt proteins are the secreted signaling molecules, are involved in muscle development (Münstreberg et al. 1995). Bone morphogenetic proteins (BMPs), another family of secreted signaling proteins, are released from the neural tube and the lateral plate mesoderm and inhibits myogenesis (Grefte et al. 2007; Duprez et al. 1996). During embryonic development, two distinct types of skeletal muscle fibers appear. The first muscle fibers that emerge are called primary or ambryonic fibers; the secondry or fetal fibers arise later. The primary and secondry fibers have distinct morphological and and biochemical properties and can be classified into slow-twitch and fast-twitch fibers. Toward the end of embryogenesis, the satellite cells appear. They are the major players in postnatal muscle growth and regeneration (Grefte et al. 2007).

2.1.2 Postnatal muscle growth

During postnatal growth, the increase in skeletal muscle mass is mainly due to an increase in muscle fibre size (hyperthrophy). This process is accompanied by the proliferative activity of satellite cells, which are the source of new nuclei incorporated into muscle fibres (Rehfeldt et al. 2004). At birth, satellite cells, which have yet to exit the cell cycle, account for ~30% of the nuclei in rodent limb muscle (Knapp et al. 2006; Cardasis and Cooper, 1975). During the first few weeks of life, satellite cells fuse to growing fibers so that the cells eventually account for at least 50% of the nuclei inside

the fiber. As adulthood is reached, muscle growth declines and the residual satellite cell population accounts for only 6% of the nuclei in limb muscles (Knapp et al. 2006; Cardasis and Cooper, 1975). The residual satellite cells become quiescent until receptor-mediated signaling triggered by exercise or wounding causes them to re-enter the cell cycle, proliferate and differentiate into muscle (Knapp et al. 2006; Charge and Rudnicki, 2004; Seale et al. 2001).

In general, adult skin wound healing occurs in three overlapping phase: inflammation, tissue formation and tissue remodelling (Grefte et al. 2007). At the site of injury, many growth factors are expressed and several of these are able to activate satellite cells. The function of satellite cells during muscle regeneration is regulated by many growth factors and cytokines such as fibroblast growth factor (*FGF*) and transforming growth factor-ß (*TGF-ß*) families, insulin-like growth factors-1 and -2 (*IGF-1*, *IGF-2*), hepatocyte growth factor (*HGF*), and interleukin-6 (*IL-6*) (Grefte et al. 2007).

After birth, total muscle fiber number has been reported to remain unchanged in mammals and birds (Figure 2.4) (Rehfeldt et al. 2004). However, some reports have indicated increases in muscle fiber number after birth in rodents, chickens and pigs. A definite increase in the number of muscle fibers was reported in rats during the first three weeks after birth which is the result of the differentiation into myofibers of myoblasts present in muscle fiber bundles at birth however in fish the number of muscle fibers increases throughout life (Rehfeldt et al. 2004; Stickland 1983).

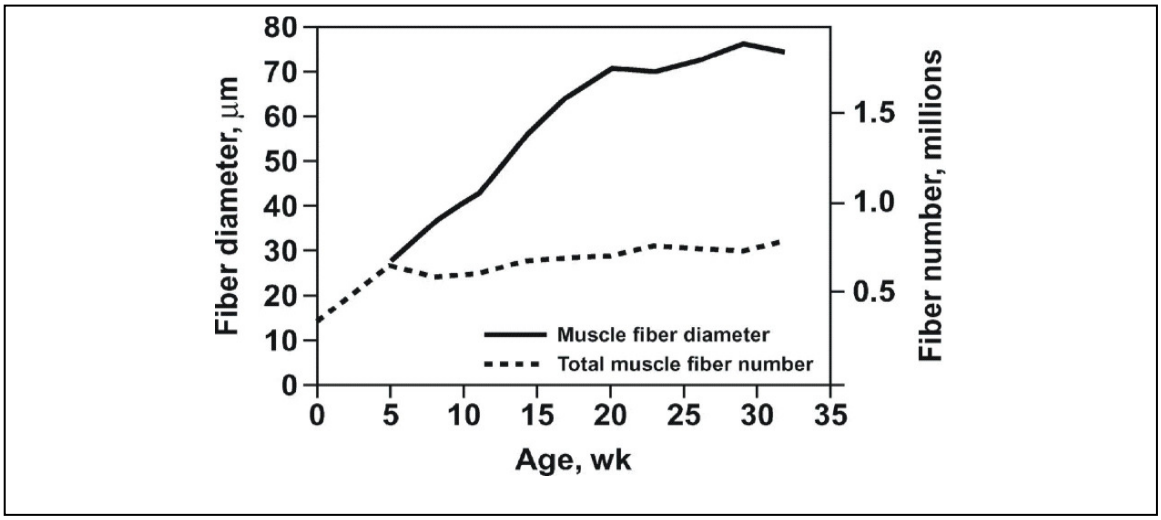

Figure 2.4: Postnatal development of fiber diameter and total fiber number per cross section in the semitendinosus muscle of German Landrace pigs, adapted from Rehfeldt and Kuhn (2006)

2.1.3 Skeletal muscle to meat conversion

The conversion of muscle into meat is a complex process in which all mechanisms responsible for the development of meat qualities are very likely independent. Colour and flavour are thus both dependent on oxidative mechanism. Oxidation and proteolysis are probably two processes involved in the development of meat tenderness (Ouali et al. 2006). After slaughter, meat is dressed, deboned and stored at refrigerated temperature for duration of one week or more depending on practice which applied in national level. Storage of muscles for a reasonable length of a time is prerequisite for the development of organoleptic qualities of the final product namely meat (Ouali et al. 2006). After bleeding, muscle cells have no other alternative to only enter the programmed cell death procedure or apoptosis. Consequently, all cells and tissues will be irreversibly deprived of nutrients and oxygen. Under these very harmful environmental conditions, muscular cells will have no alternative but engage towards “suicide”, with all the consequences described above.

Caspases (structure and functions) play an important role and regulate apoptotic processes in meat animal species (Fuentes-Prior and Salvesen 2004). Apoptosis in human muscle was shown in many publications (Tews 2002, 2005; Tews and Goebel 1997). Cell death changes the inversion of the membrane polarity. In vivo, cellular membranes have a well defined polarity dependent on the distribution of phospholipids. This process has also consequences on some feature of the muscle such as pH fall, muscle thrombin activation (neuromuscular junction), calcium and meat ageing, variation of intra- and extracellular spaces in postmortem muscle, deterioration of mitochondria and cellular oxidation.

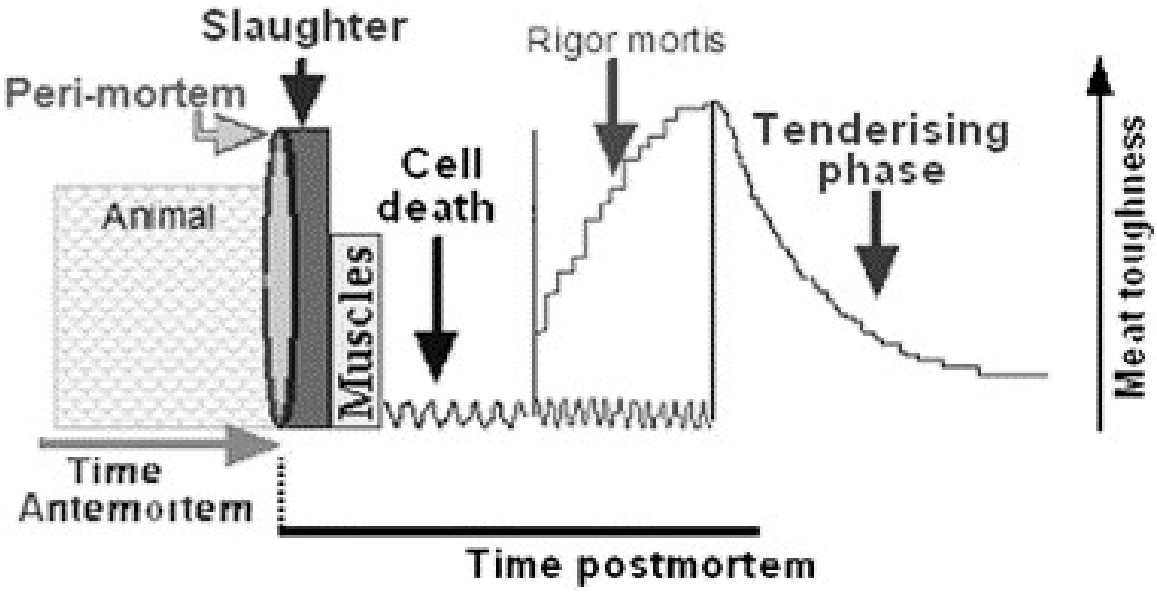

Figure 2.5: Different phases of the conversion of muscle into meat. Adapted from Ouali et al. (2006)

2.2 Meat quality in pigs

Pork quality can be considered from technological, nutritional, hygienical and sensory point of view, which may be influenced by multiple interacting factors acting before and after slaughter. Technological quality refers to the utility characteristics of meat in the current production processes, which constitute a set of technological and physicochemical properties such as: water holding capacity, pH, intensity and homogeneity of colour, firmness and processing yield. Nutritional quality concerns fatty acids profile and content of cholesterol, fat, conjugated linoleic acid (CLA), vitamins and minerals in meat. Sensory quality of pork is measured instrumentally as well as in sensory panel evaluation, and involves such elements as colour, marbling, tenderness, juiciness and flavour. The main factor influencing the meat quality, especially in technological, but also in sensory context, is muscle pH.

2.2.1 Muscle pH

Many qualities of meat depend on its pH. Meat pH influences such characteristics like colour, water-holding capacity, cooking losses, processing yield, etc. The pH of living skeletal muscle is usually above pH 7. It may decrease after slaughter to pH 5.4 to 5.6 in normal meat. If initial glycogen is limited, the pH stays high and the meat remains DFD (dark, firm, dry). If the pH decline is rapid (affecting muscle proteins while still warm) or extensive (giving a low ultimate pH), the meat becomes PSE (pale, soft exudative). Muscle pH is measured 45 minutes and 24 hours after slaughter and shows dynamics of biochemical changes in muscle post-mortem. These may be termed pH_1 and pH_{24} or pH_{45} and pH_{24} respectively. When the muscle becomes anaerobic after slaughter, glycogen is converted to lactate by way of the glycolytic pathway. Hydrogen ions are produced at the same time, causing the pH to fall (Young et al. 2004a). A quick acidification of muscle causes protein denaturation, and improper energetic processes which underlay the lactate production, cause the myofibrils damage. Denaturated proteins and damaged sarcolemma are not able to hold water and are the reason of a drip loss. These changes are accompanied by a paler colour of meat. Described features characterize a meat defect called PSE (Swatland, 2001).

2.2.1.1 Muscle pH 45 minutes post-mortem (pH_{45})

The muscle condition known as PSE pale, soft, exudative, which mainly affects the longissimus dorsi and the semi-membranosus, results in less attractive, less tender and less juicy meat. Although the cause is not fully understood it is associated with an unusually rapid fall in the muscle pH after slaughter and is linked to short-term stress. Much effort has been put into detecting PSE meat in intact carcasses. This would allow abattoirs to identify a problem at an early stage and take remedial action, by ensuring considerate preslaughter handling for example. It would also enable the carcass to be diverted away from fresh meat sale into other uses. Readings of pH at 45 min vary with the rate of pH fall (i.e. rapid fall in pH gives a lower value at 45 min). Therefore low pH values at 45 min post-slaughter are seen as indicative of PSE muscle (Homer and Matthews 1998). A pH 45 min pm < 6.0 is typically taken as the critical point below which commercially important PSE develops in pork (Bendall and Swatland 1988).

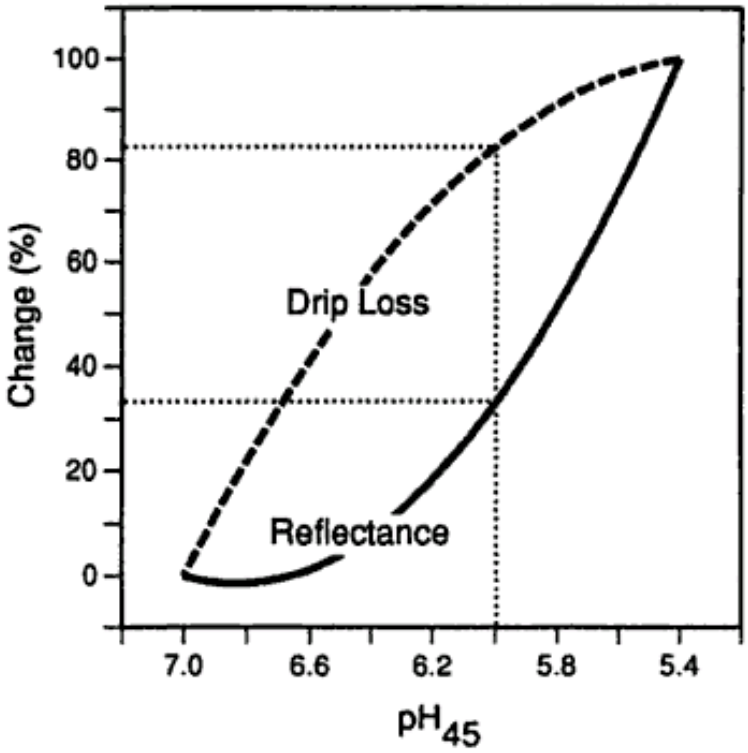

Figure 2.6: Effect of pH_{45} on reflectance and and drip loss. Adapted from Murray (1995)

2.2.1.2 Muscle pH 24 hours post-mortem (pH_{24} or pH_u)

Ultimate pH has been implicated as a major factor affecting pork quality (Offer 1991). Many qualities of meat depend on its pH. Generally, meat in the pH range 5.4 to 5.6 has the most desirable properties for table cuts. Higher values, which can reach pH 6.9, result in several defects, the most obvious being its colour, which becomes progressively darker as pH increases. As a result, high pH meat is sometimes called 'dark-cutting' or 'DFD', dark, firm, and dry, referring to the meat's physical properties. Moreover, the microbiological stability of high pH meat is poor (Homer and Matthews 1998), tenderness is more variable, and cooked flavour is inferior (van Laack et al. 2001). In the 24 h following slaughter, a decrease in glycogen, as determined by glucose, occurred in parallel with the decline in pH. At the same time, lactate progressively accumulated as expected (Young et al. 2004b). The variation in ultimate pH influences factors such as colour and the ability of the meat to retain water. A low ultimate pH results in meat proteins having decreased water-holding capacity and a

lighter colour. Conversely, a higher ultimate pH will give a darker colour and less drip loss (Huff-Lonergan and Lonergan 2005).

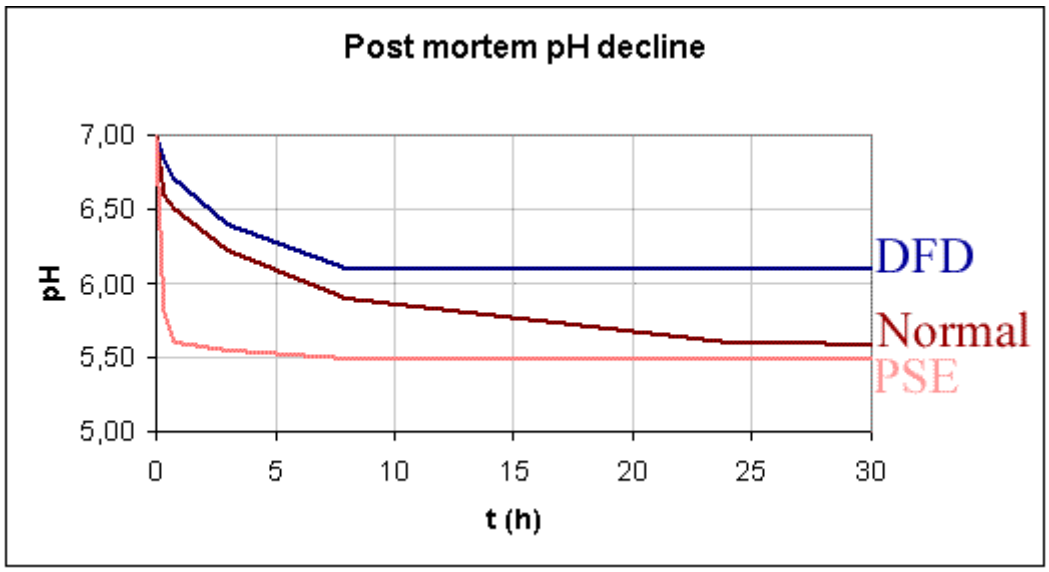

Figure 2.7: A schematic representation of the relationship between post-mortem change of pig muscle pH (longissimus dorsi) and meat quality

2.2.2 Drip loss

Water is the major constituent of meat accounting for approximately 75% of its weight (Borisova and Oreshkin 1992; Offer and Knight 1988). The amount and distribution of water inside the meat has a considerable influence on its properties. High losses of fluid in the form of drip may affect financial output, nutritional value, consumer appeal and/or technological properties of porcine meat (Jennen et al. 2007). Water-holding capacity (WHC), i.e. the capacity of meat to retain its water during application of external forces (Hamm 1985) in particular is of special interest. During the post-mortem conversion of muscle to meat changes to WHC occur and as a result, a fluid consisting of water and dissolved proteins is released without application of external forces (except gravity), the so-called drip loss (DRIP) (Offer and Knight 1988). In the past the assessment of drip loss was done by several methods (Borchers et al. 2007; Otto et al.

2004). Among them, bag method of Honikel (1987) is widespread and accepted as internationally method. A variety of different methods can be found in table 2.1.

Table 2.1: Different methods of measurement drip loss and water-holding capacity of meat. Adapted from Borchers et al. (2007)

Filterpaper-press method	Grau and Hamm, 1953
Loose bound water	Beutling, 1969
Capillary volumeter	Hofmann, 1975
Tray method	Lundström and Malmfors, 1985
Filterpaper method	Kauffman et al., 1986
Bag method	Honikel, 1987
Centrifugation methods	Honikel and Hamm, 1994
EZ-DripLoss method	Rasmussen and Andersson, 1996
Absorptive material	Walukonis et al., 2002

2.2.3 Water-holding capacity

The definition of water holding capacity (WHC) is the ability of meat or meat systems to retain all water or part of its own and/or added water (Honikel 2004). Muscle contains approximately 75% water and other components such as protein (20%), lipids (5%), carbohydrate (1%) and vitamins and minerals (1%) (Huff-Lonergan and Lonergan 2005).

The water-holding capacity of meat products is a very important quality attribute which has an influence on product yield, which in turn has economic implications, but is also important in terms of eating quality. A number of pre- and post-mortem factors influence the water holding capacity (WHC) of meat. During the growth and development of meat animals, genotype and animal diet are important due to their direct influence on muscle characteristics. In the immediate pre-slaughter period, stresses on the animal such as fasting, and different stunning methods are likely to influence meat WHC. In the post-slaughter period chilling, ageing, injecting non-meat ingredients, as well as tumbling have important influences on WHC. Furthermore, cooking and cooling procedures for the final meat products can also affect the WHC of the product, in particular the cooking and the cooling methods, the heating and the cooling rate, the cooking temperature, and the endpoint temperature (Cheng and Sun 2008).

Two major genes have been identified to cause watery meat (PSE meat) within pig populations Halothane and *RN−* gene, the former being associated with an abnormally fast rate of pH decline. The Halothane gene offers producers a rapid way of producing lean, heavily muscled market hogs that will receive higher packer premiums. However, Halothane positive and carrier animals have poorer meat quality and processing characteristics, when compared to Halothane negative pigs. The meat quality problems associated with the Halothane gene result from a high incidence of the pale, soft, exudative (PSE) condition. Animals that carry the dominant allele of the Rendement Napole gene (*RN-*) have been found to produce paler meat with reduced water holding capacity and processing yields. However, *RN-* carriers have also been found to have higher carcass lean meat percentages and lower shear force values, indicating more tender meat. Thus, both the Halothane and *RN* genes independently have both negative and positive effects on carcass and meat quality and because of their different modes of action may have a greater combined effect on meat quality.

Recently, mutation of the *PRKAG3* gene encoding the γ_3 subunit of the AMP dependent kinase (*AMPK*) was associated with excess glycogen content in pig skeletal muscle; one can thought that mutations of the same gene could be responsible for the biological variability in drip loss. The *AMPK*, comprising three subunits (α, β,γ), has been pointed out as one of the main actors in the regulation of intracellular energy metabolism (Carling 2004). The consequences of these mutations on muscle WHC, meat juiciness and drip loss are however still unknown. Investigation on the *AMPK* gene function and its polymorphic allelic expression would be an alternative way to provide answers to carcasses, muscles and meat exudation. In a longer term, these studies would probably be also very helpful for the genetic selection of animals expressing the most suitable isoform of the corresponding *AMPK* subunit. In addition, this gene is very likely common to all meat animal species and findings obtained can be therefore extended to any species (Cheng and Sun 2008).

2.3 Molecular genetic methods for dissecting meat quality in pigs

The genome of the pig (*Sus scrofa*) comprises 18 autosomes, with X and Y sex chromosomes. The genome size is similar to that of human and is estimated at 2.7 Gb. There is extensive conserved homology with the human genome. The pig is a member

of the artiodactyls, (cloven-hoofed mammal), which are an evolutionary clade distinct from the primates and rodents. It is an important model for human health particularly for understanding complex traits such as obesity and cardiovascular disease (http://www.sanger.ac.uk/Projects/S_scrofa/). Currently, the first release of the high-coverage Sscrofa9 (April 2009) assembly for chromosomes 1 to 18 and X of the pig genome is available in Ensembl database. The Pig Sscrofa9 was annotated using a standard Ensembl mammalian pipeline. Predictions from vertebrates as well as pig proteins have been used to create the gene set, along with human 1:1 orthologs aligned to the pig genome using Exonerate. Pig cDNAs have been used to add UTRs to pig protein based predictions, and pig ESTs were used to add UTR to predictions made from non-pig proteins (Ensembl). According to Ensembl release 58.9b (May 2010), there has been already 660 known protein coding genes are available, however, 11,873 protein coding genes were projected for the genome assembly. Up to now, 159,872 gene exons and 22,013 transcripts have already been identified by the pig genome sequencing consortium.

2.3.1 Muscle transcriptome analysis for identifiying the positional candidate genes for pig meat quality

The high-throughput, recently developed ‘omics’ techniques are capable of uncovering associations between previously unknown molecules (DNA, RNA, proteins and metabolites) or previously uncharacterized DNA/protein sequences and physiological traits of interest (Davoli and Braglia 2007). Earlier attempts for analyzing the expression of thousands of genes simultaneously in diverse biological systems started with the cDNA libraries. For application of microarray technologies to studies of skeletal muscle growth and development in swine, Yao et al. (2002) constructed a normalized cDNA library from porcine skeletal muscle. Among 742 EST sequences against the public database (dbEST), they found 139 novel porcine ESTs, suggesting the possibility of their specific expression in porcine skeletal muscle. Microarray technology facilitates quantitative assessment of gene expression levels for several thousand genes simultaneously. A porcine cDNA microarray comprising 5500 clones has been used to analyze differential transcript expression in phenotypically distinct muscle with the aim of identifying the genes involved in muscle phenotype

determination (Bai et al. 2003). The "Quality Pork GENES" project (www.qualityporkgenes.com) was initiated in order to create a unique phenotypic resource that could be exploited through the application of new functional genomics tools that determine differences in the transcriptome and proteome of muscle and relate this to the different aspects of meat quality. Cagnazzo et al. (2006) investigated the difference in the prenatal muscle-specific transcriptome profiles of Duroc and Pietrain pigs using microarray technology that contained more than 500 genes affecting myogenesis, energy metabolism, muscle structural genes, and other genes from a porcine muscle cDNA library. They conclude that the expression of the myogenesis-related genes was greater in early Duroc embryos than in early Pietrain embryos (14 to 49 d of gestation), whereas the opposite was found in late embryos (63 to 91 d of gestation). Their findings suggested the myogenesis process is more intense in early Duroc embryos than in Pietrain embryos but that myogenesis is more intense in late Pietrain fetuses than in Duroc fetuses. Murani et al. (2007) profiled the transcriptome changes during the myogenesis in vivo. In order to address this, they performed transcriptome profiling of prenatal skeletal muscle using differential display RT-PCR as on open system with the potential to detect novel transcripts. Seven key stages of myogenesis (days 14, 21, 35, 49, 63, 77 and 91 post conceptions) were studied in two breeds, Pietrain and Duroc, differing markedly in muscularity and muscle structure. 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, genes involved in cell adhesion, cell-cell signaling and extracellular matrix synthesis/remodeling, genes regulating gene expression, and metabolism genes. 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. high mobility group AT-hook 2; *HMGA2*). Expression of thirteen transcripts along with five reference genes was further analyzed by means of real-time quantitative PCR. Nine of the target transcripts showed higher than two-fold differences in the expression between the two breeds (*GATA3*, *HMGA2*, *NRAP*, *SMC6L1*, *SPP1*, *RAB6IP2*, *TJP1* and two EST). Their study revealed several genes and novel transcripts not previously associated with myogenesis. Moreover, they also gained knowledge of genetic factors operating during

myogenesis. Genes that exhibited differences between the divergent breeds represent candidate genes for muscle growth and structure.

Te Pas et al. (2005) studied expression profiles of genes known to affect myogenesis, muscle structural proteins, and energy metabolism in prenatal pigs from 14 to 91 days of gestation by using microarray technology. Gene activation and repression profiles were studied counting the number of spots with detectable signal. The number of spots for muscle tissue structural protein genes showing upregulated expression increased constantly from day 14 until day 91 of gestation indicating continued activation of genes during this period. The mRNA expression level of the genes showed a peak around day 35 of gestation. The expression levels of genes affecting myogenic differentiation (stimulating and inhibiting) showed a peak at day 35 of gestation. The number of spots for differentiation-stimulating genes showing differential expression reaches a first peak around day 35 of gestation and a nadir at day 49 of gestation while the number of spots for differentiation-inhibiting genes reaches a nadir at day 35 of gestation. Myogenic differentiation seems less a matter of the expression level of genes affecting differentiation, but depends on the balance between the number of significantly activated genes for stimulating and inhibiting differentiation. Genes stimulating myoblast proliferation showed a small peak expression prior to day 35 of gestation indicating myoblast proliferation before differentiation. The number of spots and the expression levels of genes for glycolysis and ATP-metabolism are at a nadir around days 35 and 49-63 of gestation suggesting that the energy metabolism is low during fusion of myoblasts into multinucleated muscle fibers.

Schulz et al. (2006) developed microarrays to profile the level of proteins associated with calcium regulation in sarcoplasmic reticulum (SR) isolated from porcine longissimus dorsi muscle. The microarrays consisted of SR preparations printed onto to glass slides and probed with monoclonal antibodies to 7 target proteins. Proteins investigated included: ryanodine receptor, (RyR), dihydropyridine receptor, (DHPR), triadin (TRI), calsequestrin (CSQ), 90 kDa junctional protein (JSR90), and fast-twitch and slow-twitch SR calcium ATPases (SERCA1 and SERCA2). The microarray developed was also employed to profile Longissimus muscle SR proteins from halothane genotyped animals. Significant ($p<0.05$) reductions in levels of several proteins were found including: RyR, CSQ, TRI, DHPR and SERCA2 in SR samples from halothane positive animals. Their results illustrated the potential of microarrays as

a tool for profiling SR proteins and aiding investigations of calcium regulation. Lin and Hsu (2005) compared gene transcription profiles of longissimus dorsi (LD) muscle between two pig breeds, Duroc and Taoyuan, which display dramatically different postnatal muscle growth. They isolated LD from neonatal pigs, and the Duroc muscle length and mass were greater ($p<0.01$) than for Taoyuan pigs; however, insignificant differences in the muscle fiber area and the percentage of fiber types were found. A human high-density complementary DNA (cDNA) microarray consisting of 9,182 probes was used to compare gene transcription profiles of LM between the two breeds. The results showed that the transcription level of 73 genes and 44 genes in Duroc LD were up-regulated and down-regulated by at least 1.75-fold ($p<0.05$) compared with Taoyuan, respectively. The strongly up-regulated genes in Duroc pigs included those encoding the complex of myofibrillar proteins (e.g., myosin light and heavy chains, and troponin), ribosomal proteins, transcription regulatory proteins (e.g., skeletal muscle LIM protein 1; SLIM1 and high-mobility group proteins), and energy metabolic enzymes (e.g., electron-transferring flavo-protein dehydrogenase, NADH dehydrogenase, malate dehydrogenase, and ATP synthases). The highly transcribed genes that encode energy metabolic enzymes indicate a more glycolytic metabolism in Duroc LD, thereby favoring carbohydrates rather than lipids for use as energy substrates in this tissue. The over-transcribed genes that encode skeletal muscle predominant proteins or transcription regulators that control myogenesis and/or muscle growth suggest a general mechanism for the observed higher rate of postnatal muscle growth in Duroc pigs. The transcription of one such gene, *SLIM1*, was more highly transcribed ($p<0.01$) in Duroc LM at birth and at postnatal d 7 than in Taoyuan. The transcription of *SLIM1* increased ($p<0.05$) in Duroc LD from neonate through 7 d of age, whereas its transcription remained essentially constant in Taoyuan during this period. They suggested that *SLIM1* may be useful for the development of markers associated with the postnatal muscle growth of pigs. Muscle tenderness is an important complex trait for meat quality and thus for genetic improvement through animal breeding. However, the physiological or genetic control of tenderness development in muscle is still poorly understood. Lobjois et al. (2008) by using transcriptome analysis, found a relationship between gene expression variability and tenderness. Muscle (longissimus dorsi) samples from 30 F_2 pigs were characterized by Warner-Bratzler Shear Force (WBSF) on cooked meat as a measurement of muscle tenderness. Gene expression levels were measured

using microarrays for 17 muscle samples selected to represent a range of WBSF values. Using a linear regression model, they determined that samples with WBSF values above 30 N could be effectively analysed for genes exhibiting a significant association of their expression level on shear force (FDR<0.05). These genes were shown to be involved in three functional networks: cell cycle, energy metabolism and muscle development. Twenty two genes were mapped on the pig genome and 12 were found to be located in regions previously reported to contain quantitative trait loci (QTL) affecting pig meat tenderness (chromosomes 2, 6 and 13). Some genes appear therefore as positional candidate genes for relevant QTL. There is considerable variation in the water-holding capacity of meat affecting economy of meat production. Water holding-capacity depends on numerous genetic and environmental factors relevant to structural and biochemical muscle fibre properties a well as ante and post slaughter metabolic processes. Microarray analysis of M. longissimus dorsi RNAs of 74 F_2 animals of a resource population showed 1,279 transcripts with trait correlated expression to water-holding capacity (Ponsuksili et al. 2008a). 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. A linkage analysis of abundance of trait correlated transcripts revealed 897 expression QTL (eQTL) with 104 eQTL coinciding with QTL regions for water holding capacity; 96 transcripts had *trans*-acting and 8 had cis-acting regulation. Moreover, they concluded, 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 (Ponsuksili et al. 2008a; Ponsuksili et al. 2008b).

2.3.2 Expression QTL (eQTL) and applications in farm animals

With the emergence of genome-wide gene expression arrays in the late 1990s it has become possible to consider genome-wide studies aimed at dissecting the genetic regulation of gene expression. Jansen and Nap (2001) published the formal description of this new research area and coined it genetical genomics. The study of expression

quantitative loci (eQTL) associates genetic variation in populations with variation of gene expression in order to pinpoint polymorphic genetic regions affecting gene expression. Conceptually eQTL are very simple: if the genotype at a certain locus is correlated with the phenotype (i.e. gene expression) of a certain gene, this region potentially contains a specific regulator of the target gene's expression (Michaelson et al. 2009; Rockman and Kruglyak 2006). These polymorphic regulators maybe protein coding regions, microRNAs, *cis*-regulator motifs, or other functional nucleotide sequences (Michaelson et al. 2009). In order to detect such genomic regions one has to genotype genetically diverse individuals and measures their expression pattern using, for example microarrays, quantitative real-time PCR or deep sequencing technologies. High correlations between a marker and the expression of specific gene constitute an eQTL. One of the most powerful features of this approach is the ability to discriminate between *cis*- and *trans*-acting influences on gene expression and potentially to dissect complex regulatory networks (Li et al. 2006; Li et al. 2005). A cis-acting eQTL maps to the physical location of the gene itself, whereas a *trans*-acting eQTL maps to a genomic region that is distant from the physical location of the gene being transcribed. By combining the genomic position of the gene encoding each transcript and the position of its eQTL, it is possible to discriminate between *cis*- and *trans*-regulatory control elements of gene expression for thousands of genes across the genome (Li et al. 2006; Li et al. 2005).

A number of genetical genomics studies show that sequence variation in cis-acting genes plays a considerable role in determining detectable variability in gene expression, and, accordingly, cis effects are usually mapped with high statistical significance (Hubner et al. 2005; Schadt et al. 2003). *Cis*-acting genes are generally easier to detect by linkage, since they explain a large fraction of the variance of gene expression, and are of great interest as positional candidates for physiological quantitative trait loci (QTLs) (Doss et al. 2005). Although *trans*-eQTLs may be associated with lesser statistical significance, they are often detected as clusters, reflecting coordinated regulation of many genes by a single "master regulator" (Yvert et al. 2003). Variation in gene expression is heritable and Petretto et al. (2006) report the influence of heritability and allelic effect of the quantitative trait locus on detection of *cis*- and *trans*-acting eQTLs and discuss how these factors operate in four different tissue-specific context.

They concluded, the median h^2 ranges from a minimum of 0.14 for the *trans*-eQTLs to a maximum of 0.37 for the *cis*-eQTLs.

Selecting a population for an eQTL study is an important factor affecting the outcome of experiment. The main difference among population is the degree of homozygosity, and the relatedness of individuals (Michaelson et al. 2009). Many different designs in different studies such as recombinant inbred strains (RILs), heteregenous stocks or other outbred populations can be used for the experiment. RILs are derived from two parental strains and have the advantage that individuals are homozygous at every marker and one can generate many identical individuals when it is needed. This mating design is mainly used for mice and rat studies (Tesson and Jansen 2009). This set up could be changed by inbred lines that were not derived from same parental strains. This creates more genetically diverse population. McClurg et al. (2007) used mouse diversity panel in their eQTL study and stated the advantages of this panel compared to traditional F_2 mapping populations. The third one, outbred population approach provides the largest genetic diversity. This design has been carried out in the human and livestock eQTL studies (Ponsuksili et al. 2008a).

Different strategies for eQTL mapping were published. Jia and Xu (2007) reviewed two simple approaches. Individual transcript analysis in which a single expression trait is mapped at a time and the entire eQTL mapping involves separate analysis of thousands of traits and secondly individual marker analysis where differentially expressed transcripts are detected on the basis of their association and with the segregation pattern of an individual marker and the entire analysis requires scanning markers of the entire genome. The first approach requires single-trait QTL mapping (interval mapping), composite interval mapping or multiple QTL mapping. On the other hand, the second approach requires only a method for differential expression analysis, hierarchical mixture model or model based cluster analysis. They suggested a Bayesian clustering method that analyzes all expressed transcripts and markers jointly in a single model. This approach covers: a transcript may be simultaneously associated with multiple markers and additionally, a marker simultaneously alters the expression of multiple transcripts. Univariate mapping method which correlates one marker at a time with expression of the target gene. Linear model concept is the established univariate method for eQTL mapping. This depends on observed variation broken into component attributed to the eQTL under consideration, and another component of residual or

unexplained variation. Residual variation can contain environmental effect, effects from other genetic loci, or random noise. Two main algorithm could be considered under univariate methods: (1) Lander and Botstein (1989) suggested the term interval mapping which considers regression between ungenotyped intervals to markers flanking the intervals. Markers flanking the interval can be used to compute a probability distribution for the interval genotypes. (2) Haley and Knott (1992) improved the strategy of interval mapping which is proposed by Lander and Botstein (1989). They suggested a different parameterization of the linear model which incorporates additive and dominant effects.

Removing the various factors other than genetic effect on gene expression is important. Recent studies have demonstrated the several confounding factors affect gene expressions and eQTL analysis: (1) sex specificity. It is well established that many genes are expressed in sex specific manner (Michaelson et al. 2009; Wang et al. 2006; Yang et al. 2006). By using equal number of sexes, only one sex or different statistical modelling which consider sex as covariable, this problem can be adressed. (2) It has been reported that sequence polymorphisms can affect the mRNA levels, especially cis-acting factors. Microarray platforms which are using shorter oligonucleotides to probe the samples should be more affected compare to the platforms which are using longer probe sets. The relative effect of SNPs in probe regions is higher in shorter oligonucleotide platforms. Although, one can exclude probes with SNPs from the experiment, this could not be practical since not all SNPs are known in all populations. Another method was proposed by Alberts et al. (2007), they integrate hybridization errors due to SNPs in a multi-probe model. Nevertheless, this method is applicable in backcross, intercross and other experimental design with only Affymetrix GeneChips™. (Alberts et al. 2005; Alberts et al. 2007; Ghazalpour et al. 2008; Walter et al. 2007). Batch affects cover the non-biological variations due to microarray design or experimental artefacts such as sample preparation or expression measurements (Akey et al. 2007; Branham et al. 2007; Vartanian et al. 2009). Recently, Ponsuksili et al. (2010) reported eQTL differences due to population size and expression measurement methods (microarray vs. qReal-Time PCR). They conclude, a global microarray eQTL analysis of a limited number of samples can be used for exploring functional and regulatory gene networks and scanning for cis eQTL, whereas the subsequent analysis of a subset of

likely cis-regulated genes by real-time RT-PCR in a larger number of samples is relevant to narrow down a QTL region by targeting these positional candidate genes.

Association of eQTL data with complex traits were revealed in several publications. (Chen et al. 2008; Cookson et al. 2009; Schadt et al. 2005; Schadt et al. 2003; Wimmers et al. 2010). Most studies link trait associated loci to eQTL data by identifiying target genes whose expression is linked to the same or nearby locus (Michaelson et al. 2009). Although several eQTL studies have been performed in human (Franke and Jansen 2009), model species (Tesson and Jansen 2009) and plants (Druka et al. 2009), a few studies has been conducted in farm animals. Potential and pitfalls of eQTL studies in livestock were reviewed by Haley and de Köning (2006). They concluded, most of the studies are too limited in size and design to unlock the full potential of the approach. Limited statistical power of studies exacerbates the problem of detection of false-positive eQTL and some reported results should be interpreted with caution. However, combining expression studies with fine mapping has been proposed to facilitate the implementation of genetical genomis studies in farm animals (Haley and de Koning 2006). With the absence of recombinant inbred lines for livestock species, other options to increase the power of an eQTL design should be explored (de Koning et al. 2005). One possibility is to include knowledge about identified QTL into the design of the study. By selecting only individuals that are homozygous for the previously detected QTL (either ++ or −−) for disease challenge, expressionstudies and genome-wide molecular typing, the power to detect eQTL in the selected regions then becomes equal to that of an RI line (i.e. the same power as an F_2 of twice the actual size) (de Koning et al. 2005). For immune traits, the combination of traditional QTL mapping with eQTL mapping will provide crucial information about the nature of the disease QTL. It will elucidate how a disease QTL affects gene expression following infection. Identification of genes that are affected by the eQTL provides a first step to unravelling the genetic pathway underlying the response to the disease challenge. The eQTL analysis may identify further regions related to the disease response that remained unnoticed or nonsignificant in the standard QTL analysis (de Koning et al. 2005). The same approach was successfully applied for meat quality in pigs (Ponsuksili et al. 2008a; Wimmers et al. 2010). Overlapping pQTL and eQTL regarding meat pH and drip loss elucidated 104 genes, of those, 8 *cis*-acting and 96 trans-acting across the porcine genome. Kadarmideen et al. (2006) listed the benefits of eQTL in animal breeding. According to

them, the main uses of this systems genetics approach in quantitative genomics were shown to be in refinement of the identified QTL, candidate gene and SNP discovery, understanding gene-environment and gene-gene interactions, detection of candidate regulator genes/eQTL, discriminating multiple QTL/eQTL, and detection of pleiotropic QTL/eQTL, in addition to its use in reconstructing regulatory networks. The potential uses in animal breeding are direct selection on heritable gene expression measures, termed ‘‘expression assisted selection,’’ and genetical genomic selection of both QTL and eQTL based on breeding values of the respective genes, termed ‘‘expression-assisted evaluation.’’ Moreover, in chickens, using the breast tissue samples, cis and *trans*-regulation of corresponding genes were identified on chicken chromosome 4 for the breast weight (de Koning et al. 2007).

Although variety of eQTL mapping strategies in use and was published, there are two main approach; first correlation between the genotyping pattern at every individual marker and expression values and the second is deducing set of markers those explain the variation in expression values. When different confounding effects corrected, more accurate eQTL results would be handled to use in terms of expression assisted selection or genetical genomics selection. In farm animals, eQTL or SNPs within the eQTL regions can help us to predict traits that are difficult to measure on the farm and sex-limited traits (e.g., disease susceptibility and health traits, reproductive traits, carcass quality, feed intake, milk production, reproduction). Such fast-track and reliable decisions have clear commercial benefits to animal breeders and producers.

2.3.3 QTL analysis and important genes related to growth performance and meat quality traits

Initially many QTL experiments were undertaken by using linkage maps to help determine regions underlying traits of importance to the pig industry. These early QTL scans used families developed by generally crossing European Wild Boar with a commercial breed or crossing the exotic Chinese Meishan breed with a commercial breed (Rothschild et al. 2007). The current release of the Pig QTL database contains 5732 QTL representing 558 traits (www.animalgenome .org/QTLdb/pi.html) mostly for economically important traits like growth, carcass and meat quality. In QTL scan generally 300 to 700 pigs from F_2 design were used, more recently, researchers have

used two commercial breeds for F_2 families or larger synthetic lines or breed for candidate gene studies and large scale SNP association studies. Although, a lot of work has been carried out in finding potential genes or chromosome regions responsible for (or associated with) meat quality and processed meat products, the knowledge of genes and their interactions that are involved in meat properties are very limited. As a consequence, the understanding of meat quality on a genetic basis is scanty. Using the linkage analysis several QTL for the meat and carcass quality traits almost in all autosomes have been detected by using different pig crosses (Jennen et al. 2007; Liu et al. 2007; Liu et al. 2008; Ponsuksili et al. 2005; Wimmers et al. 2006; Wimmers et al. 2007).

3 Materials and methods

3.1 Materials

3.1.1 Animals

The animal population used for the meat quality and the genome scan was based on a Duroc / Pietrain F_2 cross. A detailed description of the population structure was reported earlier (Liu et al. 2007; Liu et al. 2008). In this study, genetic information of three generations P, F_1 and F_2 and phenotypes from 300 F_2 pigs were used. Crosses between these two breeds offer the possibility to identify the allelic variants responsible for their differences. This opportunity is particularly relevant since the corresponding variation is being exploited in the present breeding programs. The F_1 generation was produced by mating of four Duroc boars to eight Pietrain sows and two Pietrain boars mating to five Duroc sows separately. The F_1 animals were reciprocally assigned to produce the F_2 animals, therefore, 13 'DuPi' F_1 females were assigned to two 'PiDu' F_1 boars and 14 'PiDu' F_1 females were assigned to three 'DuPi' F_1 boars.

All pigs were kept at the experimental research farm 'Frankenforst' of the University of Bonn and exposed to uniform environmental conditions. Piglets were weaned at 28 days of age and placed in collective pens in the post-weaning unit until 10 weeks of age. Male piglets were castrated. All animals were individually weighed at birth, at weaning, at the beginning and at the end of the testing, respectively. The F_2 pigs were given an ad libitum diet containing 16 % crude protein, 1 % lysine, 0.6 % (methionine + cystine), 0.6 % threonine and 13 MJ metabolize energy during the whole tetsting period from 10 to 22 weeks of age, slaughtered approximately at 85 kg of slaughter weight, the average age at slaughter was 177 ± 15.6 days. A total of 1085 F_2 pigs from 38 full-sib families were generated between May 2000 and October 2003. The 19 founder animals were tested and were found to be free of the mutation at the ryanodine receptor locus which is responsible for halothane susceptibility. Among these 1085 F_2 animals, 300 animals were used for eQTL study. Skeletal muscle (longissimus dorsi) samples were collected for RNA isolation between 13^{th} and 14^{th} rib of each animal. All samples were taken immediately after slaughter in slaughterhouse. Muscle samples were snap frozen in liquid nitrogen after collection then stored at -80°C for further analysis. Figure 3.1

shows the picture of P, F1 and F2 animals and figure 3.2 show the the pedigree structure of DUPI population.

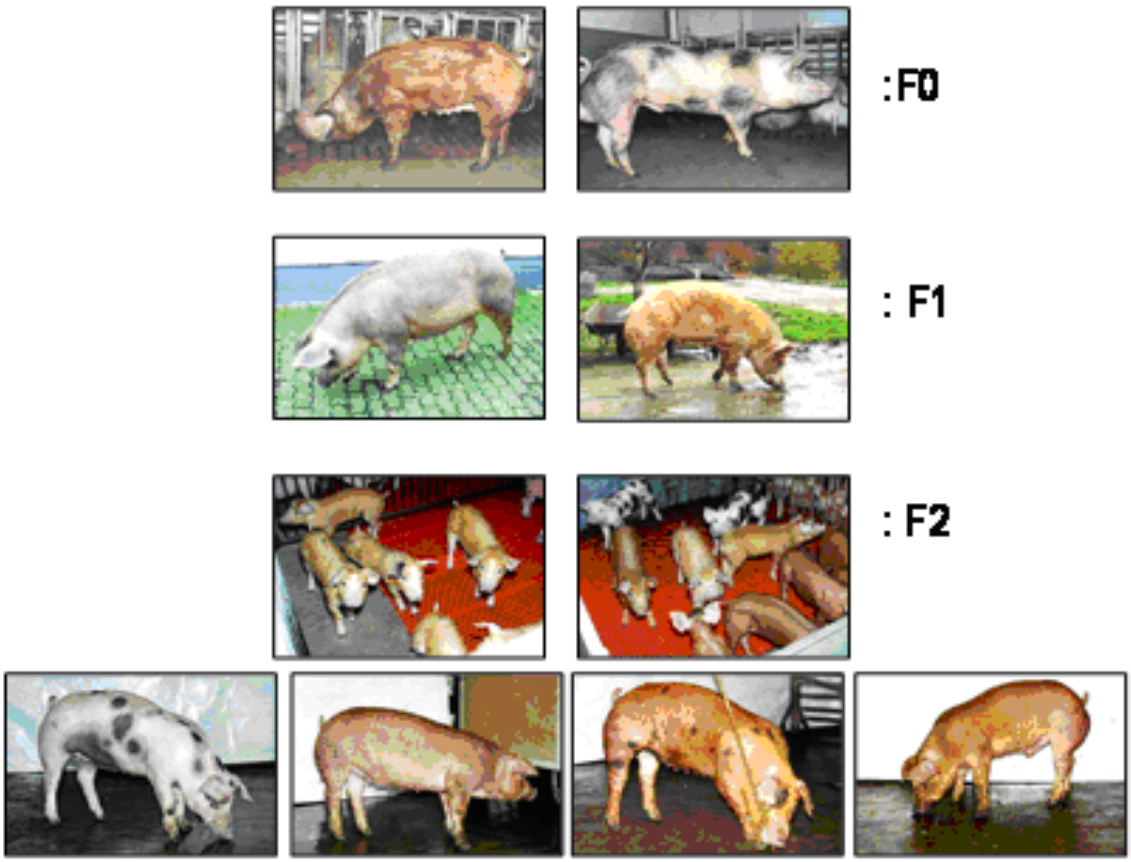

Figure 3.1: Structure of the F_2 Duroc / Pietrain (DUPI) resource population

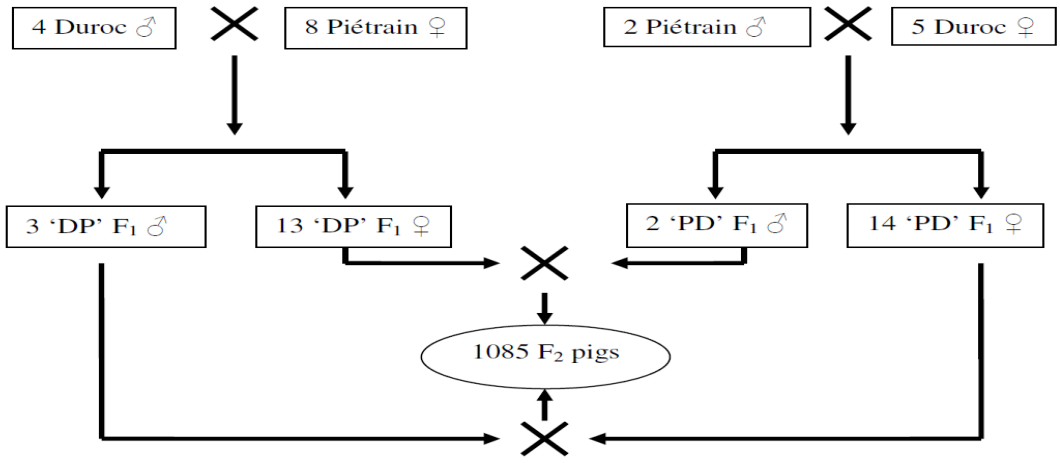

Figure 3.2: Pedigree structure of the Duroc / Pietrain (DUPI) F_2 resource population. Adapted from Liu (2005)

3.1.2 Materials for laboratory analysis

3.1.2.1 Chemicals, kits, biological and other materials

Applied Biosystems (Foster City): SYBR® Green Universal PCR Master Mix

Beckman Coulter (Krefeld): CEQ™ 8000 Genetic Analysis System, Dye Terminator Cycle Sequencing (DTCS), Glycogen

Beckman Coulter (Krefeld): CEQ™ 8000 GenomeLab GeXP, Start Kit

Beckman Coulter (Krefeld): CEQ™ 8000 GenomeLab GeXP, Thermo-Start® DNA polymerase

Bio-Rad (Hercules) iTaq™ SYBR® Green Supermix with ROX

Invitrogen Life Technologies (Karlsruhe): DTT, SuperScript™ II RNase H- Reverse Transcriptase, 5 X first strand buffer, Random Primers

MBI Fermentas (St. Leon-Rot): Glycogen

Promega (Mannheim): BSA, pGEM®-T vector, RQ1 RNase-free DNase. RNasin Ribonuclease inhibitor, 2X rapid ligation buffer, T4 DNA ligase, Pronto™ Plus systems

Qiagen (Hilden): RNeasy® Mini kit, QIAquick PCR Purification Kit, Mini Elute™ Reaction Cleanup Kit

Roth (Karlsruhe): Acetic acid, Agar-Agar, Ampicillin, Bromophenol blue, Dimethyl sulfoxide (DMSO), Ethylenediaminetetraacetic acid (EDTA), Ethanol, Ethidium bromide, Hydrochloric acid, Isopropyl -D-thiogalactoside (IPTG), Kohrso1in FF, Nitric acid, Peptone, Potassium dihydrogen phosphate, 2-Propanol, Silver nitrate, Sodium acetate, Sodium carbonate, Sodium chioride, Sodium hydroxide, Trichloromethane/chiorophorm, Tris, X-Gal (5 -bromo-4-chloro-3-indolylbeta-D-galactopyranoside), Yeast extract

Sigma-Aldrich Chemie GmbH (Munich): Agarose, Ammonium acetate, Calcium chloride, Formaldehyde, GenElute™ Plasmid Miniprep Kit, Glutamine, Hepes, Isopropanol, Magnesium chloride, 2-Mercaptoethanol, Ohigonucleotide primers, Penicillin, Phenol red solution, 10 X PCR reaction buffer, Potassium chloride, Sodium dodecyl sulfate (SDS), Taq DNA polymerase, yeast tRNA

Stratagene (Amsterdam): 5 c DH Escherichia coli competent cells

USB (Ohio): ExoSAP-IT

3.1.2.2 Buffers, reagents and media

All solutions used in these investigations were prepared with deionized Millipore water (ddH_2O) and pH was adjusted with sodium hydroxide (NaOH) or hydrochloric acid (HCl).

LB-agar plate	Sodium chloride	8.0 g
	Peptone	8.0 g
	Yeast extract	4.0 g
	Agar-Agar	12.0 g
	Sodium hydroxide (40 mg/ml)	480.0 µl
	ddH_2O added to	800.0 ml
LB-broth	Sodium chloride	8.0 g
	Peptone	8.0 g
	Yeast extract	4.0 g
	Sodium hydroxide (40 mg/ml)	480.0 µl
	ddH_2O added to	800.0 ml
TBE (10x) buffer	Tris	108.0 g
	Boric acid	55.0 g
	EDTA (0.5 M)	40.0 ml
	ddH_2O added to	1000.0 ml
TAE (50x) buffer, pH 8.0	Tris	242.0 mg
	Acetic acid	57.1 ml
	EDTA (0.5 M)	100.0 ml
	ddH_2O added to	1000.0 ml
TE (1x) buffer	Tris (1 M)	10.0 ml
	EDTA (0.5 M)	2.0 ml
	ddH_2O added to	1000.0 ml

X-gal	X-gal	50.0 mg
	N, N'-dimethylformamide	1.0 ml
10x FA buffer, pH 7.0	MOPS	41.8 g
	Sodium acetate	4.1 g
	EDTA (0.5M)	20.0 ml
	ddH_2O added to	1000.0 ml
1.2% FA gel	Agarose	1.2 g
	10 x FA buffer	10.0 ml
	RNase free H_2O	90.0 ml
	Ethidium bromide	2.0 µl
	Formaldehyde (37%)	1.8 ml
Agarose loading buffer	Bromophenol blue	0.0625 g
	Xylencyanol	0.0625 g
	Glycerol	7.5 ml
	ddH_2O added to	25 ml
Digestion buffer	NaCl	100 mM
	Tris-HCl	50 mM
	EDTA pH 8.0	1mM
SDS solution	Sodium dodecylsulfat in ddH_2O	10% (w/v)
Proteinase K solution	Protein K in 1 x TE bufer	2% (w/v)
dNTP solution	dATP (100 mM)	10.0 µl
	dCTP (100 mM)	10.0 µl
	dGTP (100 mM)	10.0 µl
	dTTP (100 mM)	10.0 µl
	ddH_2O added to	400.0 µl

IPTG solution	IPTG	1.2 g
	ddH_2O added to	10.0 µl
3M Sodium Acetate, pH 5.2	Sodium Acetate	123.1 g
	ddH_2O added to	500 ml
1M EDTA, pH 8.0	EDTA	37.3 g
	ddH_2O added to	1000 ml
Phenol Chloroform	Phenol : Chloroform	1 : 1 (v/v)

3.1.2.3 Used softwares

BLAST	http://www.ncbi.nlm.nih.gov/BLAST/
Multi sequence alignment	http://prodes.toulouse.inra.fr/multalin/multalin.html
Primer3	http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi
CRI-MAP (version 2.4)	http://compgen.rutgers.edu/multimap/crimap/
SAS (version 9.2)	SAS Institute Inc., NC, USA
Primer Express® Software	Applied Biosystems, Foster city, CA, USA
Express Designer	Beckman Coulter GmbH, Krefeld, Germany
Express Map	Beckman Coulter GmbH, Krefeld, Germany

3.1.2.4 Equipment

ABI PRISM® 7000 SDS	Applied Biosystems, Foster city, USA
Automated sequencer (LI-COR 4200)	MWG Biotech, Ebersberg
Centrifuge	Hermle, Wehingen
CEQ™ 8000 Genetic Analysis System	Beckman Coulter GmbH, Krefeld
CEQ™ 8000 GenomeLab GeXP	Beckman Coulter GmbH, Krefeld
Electrophoresis (for agarose gels)	BioRad, Munich

Electrophoresis (vertical apparatus)	Consort, Turnhout
HERA safe Bioflow safety hood	Heraeus Instruments, Meckenheim
Incubator	Heraeus, Hanau
Millipore apparatus	Millipore corporation, USA
PCR thermocycler (PTC100)	MJ Research, USA & BioRad, Germany
pH meter	Kohermann
Savant SpeedVac®	TeleChem International, Sunnyvale
Power supply PAC 3000	Biorad, Munich
Spectrophotometer, Ultrospec™ 2100 pro UV/Visible	Amersham Bioscience, Munich
Thermalshake Gerhardt	John Morris scientific, Melbourne
Tuttnauer autoclave	Connections unlimited, Wettenberg
Ultra low freezer (-80^{o}C)	Labotect GmbH, Gottingen Sanyo, Japan
UV Transilluminator (Uvi-tec)	Uni Equip, Martinsried, Germany
UV Transilluminator (Bio-Rad)	Hercules, CA, USA
Spectrophotometer (DU-62)	Beckman, Unterschleissheim-Lohhof
NanoDrop (ND-8000)	Thermo Scientific, Wilmington, DE, USA

3.2 Methods

In this section, basic phenotyping and molecular genetics methods used in this study were described. After whole genome transcription profiling and data analysis, gene expression quantification methodologies and eQTL analysis were given. Figure 3.1 explains the work flow for the given study.

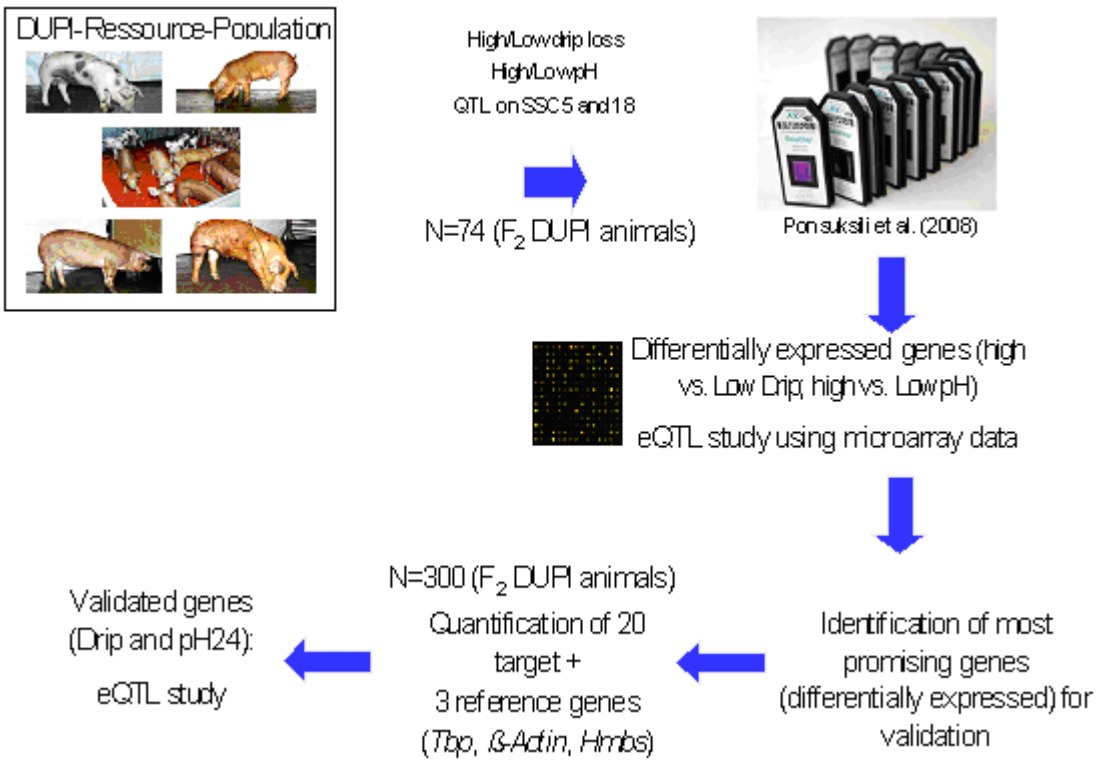

Figure 3.1 Overview of the experimental design

3.2.1 Phenotypes

The phenotypic data of F_2 animals were collected following the guidelines of the German performance testing procedures (ZDS 2003). The description of traits, numbers of records, means and standard deviation are summarised in result part. Meat pH value, and conductivity were measured using Star-series equipment (Rudolf Matthaeus Company, Germany). Muscle pH and conductivity measures were taken at 45 min post-mortem and 24 h post-mortem in loin (m. longissimus dorsi) between the 13th and 14th ribs, symbols of pH and conductivity are pH_{1L}, pH_{24L}, LF_{1L}, LF_{24L} respectively and 24 h post-mortem in the ham (musculus semimembranosus) (symbol: pH_{24si}, LF_{24si}), respectively. Muscle colour was measured at 24 h post-mortem by Opto-Star. Drip loss was scored using the bag method by a size-standardised sample from the longissimus dorsi that was collected at post-mortem. The sample was weighed, suspended in a plastic bag, held at 4°C for 48 h and re-weighed at the end of the holding time (Honikel 1987). Drip loss was calculated as a percentage of weight loss based on the start weight

of a sample. To obtain cooking loss, a loin cube was taken from the longissimus dorsi, weighed, placed in a polyethylene bag and incubated in water at 75°C for 50 min. The bag was then immersed in flowing water at room temperature for 30 min and the solid portion was reweighed. Cooking loss was obtained as the difference of the sample weights before and after the treatment. Thawing loss was determined similarly after at least 24 h freezing at -20°C. Shear force was measured by the Instron-4310 equipment and replicated four times.

3.2.2 DNA extraction

A hundred mg of sample tissue was cut into small pieces and placed in a 1.5 ml tube, after that 700 µl of digestion buffer, 70 µl of 10% SDS and 18 µl of proteinase K for protein digestion was added. The mixture was incubated overnight at 37^{o}C in a shaker at 90 rpm. Completely digested tissue resulted in a viscous homogeneous solution. To extract DNA, 700 µl of phenol-chloroform was added into each tube and gently mix by several inversions until an emulsion formed. The mixture was separated into 3 phases after centrifugation at 10,000 rpm for 10 min, a lower phenol-chloroform phase, an interphase of precipitated protein and an upper phase containing DNA. The aqueous phase was transferred to another 2 ml tube followed by an addition of 700 µl chloroform and centrifugation at 10,000 rpm for 10 min. For DNA precipitation, the DNA phase was transferred to a fresh tube and mixed with 700 µl of isopropanol and 70 µl of sodium acetate. After centrifuging at 10,000 rpm for 5 min, a DNA pellet was collected and the supernatant was removed. The pellet was washed with 200 µl of 70% ethanol for the removal of excess salt and left air dry after centrifugation. In the final step, the pellet was dissolved in 200-500 µl of 1x TA buffer. The DNA concentration and integrity was evaluated by a spectrophotometer. The working solution of DNA was prepared by diluting stock DNA in 1x TA buffer to the concentration of 50 ng/µl. Stock DNA solution was stored at -20^{o}C and the working solution was kept at 4^{o}C.

3.2.3 Marker analysis

A linkage map with the total length of 2159.3 cM and an average marker interval of 17.7 cM was constructed. P, F_1 and F_2 animals of the DUPI population were genotyped

at 122 markers loci covering all porcine autosomes. Marker positions and details of genotyping procedures were given in Liu et al. (2007) and for SSC1 in Große-Brinkhaus et al. (2009). Most of the markers were selected from the USDA/MARC map (http://www.marc.usda.gov). They are also available in porcine genome drafts Sscrofa5 (NCBI) and Sscrofa9 (Ensembl). Genotyping, electrophoresis, and allele determination were done using a LI-COR 4200 Automated Sequencer including the software OneDScan (Scanalytics). The CEQ8000 sequencer (Beckman Coulter) was used for genotyping of SSC1 and SSC18. Allele and inheritance genotyping errors were checked using Pedcheck software (version 1.1) (O'Connell and Weeks 1998). The relative positions of the markers were assigned using the build, twopoint and fixed options of CRI-Map software (version 2.4) (Green et al. 1990). Recombination units were converted to map distances using the Kosambi mapping function. Marker information content and segregation distortion were tested by following Knott et al. (1998).

3.2.4 RNA isolation of muscle samples

Total RNA was isolated from individual skeletal muscle (longissimus dorsi) by using Tri-Reagent (Sigma) according to the manufacturer's protocol. In brief, muscle sample was first grinded in a mortar, then mixed and homogenized with 1 ml TriReagent using syringes and needles. To ensure complete dissociation of nucleoprotein complexes, the sample was allowed to stand for 5 min before adding 0.2 ml of chloroform. The mixture was shaken and left at room temperature for 10 min and centrifuged at 12,000 x g for 15 min at 4^{o}C. The upper aqueous phase was transferred to another fresh centrifuge tube and RNA was precipitated with 0.5 ml of isopropanol. After being incubated at room temperature for 10 min, the sample was centrifuged at 12,000 x g for 10 min at 4^{o}C to get the RNA pellet, which was subsequently washed by 75% (v/v) ethanol. Centrifugation was then performed and the RNA pellet was air-dried and resuspended in 40 µl of DEPC treated water.

In order to remove possible contamination of genomic DNA, the extracted RNA was treated with 5 µl RQ1 DNase buffer, 5 units DNase and 40 units of RNase inhibitor in a 40 µl reaction volume. The mixture was incubated at 37^{o}C for 1h followed by purification with the RNeasy Mini Kit. Concentration of clean-up RNA was determined spectrophotometrically by using the NanoDrop (ND-8000) instrument; the purity of

RNA was estimated by the ratio A260/A280 with respect to contaminants that absorb in the UV. Additional examination of integrity was done by denaturing agarose gel electrophoresis and ethidium bromide staining. Finally, the purified RNA was stored at -80^{o}C for further analysis.

3.2.5 Microarray analysis and selection of genes

The RNA samples from skeletal muscle were sent to the Leibniz Institute for Farm Animal Biology in Dummerstorf. Immediately post mortem tissue samples were collected and snap frozen that were taken between the 13th and 14th rib from the center of Musculus longissimus dorsi of 74 animals. Animals were selected according to their drip loss, pH_{24ko} and drip loss QTL genotypes based on SSC5 and 18. Total RNA was isolated as explained previous chapter. After DNaseI treatment the RNA was cleaned up using the RNeasy Kit (Qiagen). The quantity of RNA was established using the NanoDrop ND-1000 spectrophotometer (Peqlab) and the integrity was checked by running 1 µg of RNA on 1% agarose gel. In addition absence of DNA contamination was checked using the RNA as a template in standard PCR amplifying fragments of RPL32 and HPRT housekeeping genes. Muscle expression pattern were assessed using 74 Porcine Genome Array which contains 23,937 probe sets that interrogate approximately 23,256 transcripts from 20,201 Sus scrofa genes. Preparation of target products, hybridization and scanning using the GeneChip scanner 3000 were performed according to Affymetrix protocols using 5 µg of total RNA to prepare antisense biotinylated RNA. The quality of hybridization was assessed in all samples following the manufacturer's recommendations. Data were analysed with Affymetrix GCOS 1.1.1 software using global scaling to a target signal of 500. Data were then imported into Arrays Assist software (Stratagene Europe) for subsequent analysis. The data were processed with MAS5.0 to generate cell intensity files (present or absent). Quantitative expression levels of the present transcripts were estimated using PLIER (Probe Logarithmic Intensity Error) for normalization. The microarray data related to all samples have been deposited in the Gene Expression Omnibus (GEO) public repository (GEO accession number: GSE10204).

In total 1279 genes with significant correlation of transcript abundance to drip loss were selected for eQTL linkage mapping. Among those, the eQTL analysis revealed 897

chromosome-wide significant eQTL detected in porcine autosomes. Combining QTL data for drip loss (pQTL) and eQTL data from the experiment, 104 significant eQTL were detected in the pQTL target regions for drip loss on SSC2, 3, 4, 5, 6, and 18. cis- and trans- acting regulation out of 104 eQTL that coincided with pQTL for drip, 96 belong to genes that had trans-acting regulation of transcription, 8 genes had cis-acting transcriptional regulation. Details regarding microarray and eQTL experiment could be found in Ponsuksili et al. (2008a) and Ponsuksili et al. (2008b). From the 104 promising genes, twenty up- or down-regulated genes (Table 3.1) were selected according to their statistical power and functionality in skeletal muscle for further gene expression and eQTL analysis in the 300 DUPI animals.

Table 3.1: Descriptive information of genes derived from microarray study

Gene	Affy. probe set ID	Gene Acc. nr.	Regulation	Biological process
A2M	Ssc.16603.1.A1_at	AY509877	↑	Negative regulation of complement activation, lectin pathway
ALB	Ssc.10439.1.S1_at	NM_001005208	↓	Cellular response to starvation; hemolysis by symbiont of host erythrocytes; maintenance of mitochondrion location; negative regulation of apoptosis; transport
AMBP	Ssc.1894.1.S1_at	X52087	↓	Cell adhesion; heme catabolic process; transport; negative regulation of immune response
ANGPTL4	Ssc.17345.1.S1_at	NM_001038644	↑	Angiogenesis; differentiation
APOA1	Ssc.807.1.S1_at	NM_214398		Cholesterol metabolism; lipid metabolism; lipid transport
APOC3	Ssc.1039.1.S1_at	NM_001002801	↓	Lipid degradation; lipid transport; transport
ATF4	Ssc.11072.2.A1_at	NM_001123078	↑	Transcription; transcription regulation
CAPNS1	Ssc.7158.1.A1_a_at	NM_214318	↓	Positive regulation of cell proliferation, calcium metabolism
CYP2C33	Ssc.955.1.S1_at	NM_214414	↓	Oxidation reduction; drug metabolic process
GC	Ssc.2992.1.A1_at	AY710291	↓	Transport of vitamin D sterols
GSTA2	Ssc.8516.1.A1_at	NM_214389	↓	Metabolic process
PI	Ssc.7090.1.A1_at	NM_214395	↓	Acute-phase response; blood coagulation
PPARGC1	Ssc.16864.1.S1_at	NM_213963	↓	Transcription; transcription regulation
PPP1R3B	Ssc.6382.1.A1_at	BW971859	↓	Carbonhydrate metabolism; glycogen metabolism
RBP4	Ssc.15695.1.S1_at	NM_214057	↓	Sensory transduction; transport; vision
SERPINA3-2	Ssc.15773.1.S1_at	NM_213787	↓	Acute-phase response; regulation of lipid metabolic process
TF	Ssc.4222.1.S1_at	X12386	↓	Ion transport; iron transport, transport

TNC	Ssc.16209.1.S1_at	NM_214230	↓	Cell adhesion; response to wounding; signal transduction
TTR	Ssc.640.1.S1_at	NM_214212	↓	Thyroid hormone generation; transport
TYROBP	Ssc.507.1.A1_at	NM_214202	↑	Cellular defence response; intracellular signalling cascade

↑ up-regulation

↓ down-regulation

3.2.6 Reverse transcription and cDNA synthesis

First-strand cDNA was synthesized from individual RNA using SuperScript II enzyme. The reaction started by adding 1 µl of Oligo $(dT)_{15}$ primer (100 µM) and 1 µl random primer to 1 µg of total RNA for annealing by incubation at 68°C for 5 min followed by cooling on ice for 2 min. A transcription mixture including 4 µl first strand 5X buffer, 1 µl 0.1 DTT, 1 µl dNTP mix (10 mM each), 1 µl (200 units) SuperScript II reverse transcriptase, 1 µl (40 units) of RNasin Ribonuclease inhibitor and RNase-free water was prepared to make a final volume of 20 µl. The reaction was incubated at 25°C for 5 min followed by 42°C for 1h and stopped by heating at 70°C for 15 min. The resulting cDNA was tested with housekeeping gene 18S primers and kept at -20°C until use.

3.2.7 PCR product purification

The PCR product (20 µl) was separated by running in a 1% (w/v) agarose gel containing ethidium bromide (5 µl) in 1x TAE buffer. DNA fragment, which was visualized under an ultraviolet transilluminator, was cut and placed in a fresh tube at -20°C for 30 min before adding 600 µl of 1 x TE buffer to be homogenized. An equal volume (600 µl) of phenol: chloroform (1:1) was added, mixed and vortexed well. After centrifugation at 14,000 g for 15 min at 4°C, the aqueous phase was carefully transferred to a new microcentrifuge tube. Subsequently, to remove the traced phenol, another 600 µl of chloroform was added, shaken vigorously and centrifuged for 10 min. The upper phase was transferred to a new tube and DNA was precipitated by adding double volume of cool absolute ethanol and 1:10 volume of 3 M sodium acetate (pH 5.2) acting to neutralize the highly charged phosphate backbone and promoting hydrophobic interactions. The solution was mixed gently and stored at -20°C overnight or alternatively at -80° for 2 hours. The DNA was later recovered by centrifugation at

14,000 g for 30 min at 4°C. To remove residual salt in the sample, the DNA pellet was washed by 75% (v/v) ethanol followed by drying at room temperature and resuspending in 7 µl of water. The purified cDNAs were kept at -20°C for ligation.
As an alternative way, Qiagen QIAquick PCR purification kit (Cat nr: 28104) was used as well. QIAquick Kits contain a silica membrane assembly for binding of DNA in high-salt buffer and elution with low-salt buffer or water. The purification procedure removes primers, nucleotides, enzymes, mineral oil, salts, agarose, ethidium bromide, and other impurities from DNA samples. The binding buffer contains a pH indicator, allowing easy determination of the optimal pH for DNA binding. Nucleic acids adsorb to the silica-gel membrane in the high-salt conditions provided by the buffer. Impurities are washed away and pure DNA is eluted with a small volume of low-salt buffer provided or water, ready to use in all subsequent applications.

3.2.8 Ligation

Ligation of a PCR fragment into plasmid pGEM®-T vector was done in a 5 µl reaction mix containing 2.5 µl of 2x ligation buffer, 0.5 µl of pGEM®-T (50 µg/µl), 0.5 µl of T4 DNA ligase (3 units/µl) and 1.5 µl target template. The reaction was incubated at room temperature for 1h or, to get a maximum of transformants, at 4°C overnight.

3.2.9 Transformation

For DNA transformation, the entire ligation reaction was added to an aliquot of 60 µl competent JM109 *Escherichia coli* (*E. coli*) cells and incubated on ice for 20 min. This mixture was then heat-shocked briefly in a 42°C water bath for 90 sec and immediately returned to ice for 2 min. Next, an addition of 600 µl nutrient medium (LB-broth) was performed and all were incubated at 37°C for 90 min in a shaker. At the same time, ampicillin treated LB-agar (50 mg/L LB-agar) plates including 20 µl of X-Gal (50 mg/ml in N, N'-dimethyl-formamide) and 20 µl of IPTG were prepared. At the end of incubation period, the transformation culture was plated on two previously prepared LB-agar plates and incubated at 37°C overnight.

3.2.10 Colony screening and plasmid DNA isolation

Colonies were screened based on the activity of β-galactosidase as white and blue for the presence and absence of insert DNA fragments. Three white colony representatives from each plate were picked up and suspended in 30 µl 1x buffer for M13 PCR for further confirmation of transformation. The same colonies were cultured in 600 µl ampicillin/LB-broth (5 mg / 100 ml) in a shaking incubator at 37^{o}C for further plasmid isolation. In addition, to be used as a control for later comparison of the length of amplified DNA fragments, a blue colony was also selected from any plate.

Plasmid DNA was isolated by using GenElute^{TM} Plasmid Miniprep Kit followed the manufacturer's instructions. Briefly, 5 ml of bacterial culture were centrifuged at 14,000 g for 1 min for harvesting cells, the supernatant was discarded. These cells were resuspended and vortexed in 200 µl of resuspension solution before adding 200 µl of lysis solution to lyse the solution. The mixture was subsequently mixed by inversion of tubes until it became clear and viscous. After incubating at room temperature for 4 min, cell precipitation was done by adding 350 µl of neutralization/binding buffer, mixed gently and centrifuged at 14000 g for 10 min. At the same time, the GeneElute Miniprep column was prepared by adding 500 µl of preparation solution, centrifuging shortly and discarding the flow-through. After that, the clear supernatant was transferred to this binding column and centrifuged at 14000 g for 1 min. The flow-through was discarded and the column was washed by adding 750 µl of wash solution followed by centrifugation at 14000 g for 1 min. To elute DNA, the column was transferred to a fresh collection tube; 50 µl of ddH_2O was added and centrifuged at 14000 g for 1 min. The column was discarded and the DNA plasmid was then collected.

For determination of plasmid size and quality, 5 µl of plasmid together with 2 µl loading buffer was checked by agarose gel electrophoresis. In addition, the quantity of plasmid was also measured by reading the absorbance at 260 nm in a spectrophotometer UV/visible light (Beckman Du® 62). An aliquot of DNA plasmid was subjected to sequence check; the rest was stored at -20^{o}C to be used as template for setting up the standard curve in real-time PCR.

3.2.11 M13 PCR and sequencing for product confirmation

M13 PCR was done to confirm the insertion of the fragment into the plasmid. Bacterial suspensions were boiled at 95°C for 15 min and these lysed bacterial solutions were used as templates. The M13 PCR was carried out in a 20 µl reaction including 1 µl 10x PCR buffer, 10 µl lysed bacterial solution, 0.5 µl dNTP (10 mM), 0.5 µl (10 µM) of each M13 primer (forward: 5'-TTGTAAAACGACGGCCAGT-3'; reverse: 5'-CAGGAAACAGCTATGACC-3') and 0.1 Unit of Taq polymerase. The PCR reaction was performed with a thermal cycling program of 95°C for 5 min followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec, 70°C for 1 min and an additional extension step for 5 min at 70°C. An aliquot of 5 µl PCR product was then electrophoresed in 2% (w/v) agarose gel with 0.8 µg/ml ethidium bromide in 1 x TAE buffer. Under UV-transilluminator, length differentiation of PCR fragments was identified. The M13 PCR products from white colonies were selected for subsequent sequencing while bacterial cultures of these colonies were expanded in a volume of 5 ml and incubated at 37°C overnight in a shaking incubator for plasmid isolation.

The M13 products were used as templates for sequencing according to the dideoxy chain-termination method using SequiTherm Excel™ II DNA Sequencing Kit (Epicentre Technologies, Biozym). For each PCR fragment, four sequencing reactions were prepared with different termination mix solutions namely deaza-dATP, ddCTP, ddTTP, ddGTP in a total volume of 3 µl containing 1 µl of termination mix and 2 µl of premix solution, which included of 3.5 µl of 3.5X sequencing buffer, 0.25 µl of 700-IRD labeled primer SP6 (10 µM): 5'-TAAATCCACTGTGATATCTTATG-3', 0.25 µl of 800-IRD labeled primer T7 (10 µM): 5'-ATTATGCTGAGTGATATCCCGCT-3', 5 units of SequiTherm Excel II DNA polymerase and 3.9 µl PCR products. These sequencing reactions were carried out at 95°C for 3 min followed by 29 cycles of 95°C for 15 sec, 59 °C for 15 sec, and 70°C for 1 min and the reactions were ended by adding 1.5 µl of stopping buffer. The sequence reaction products were then denatured at 95°C for 5 min and immediately kept on ice and loaded on a 6% Sequagel XR sequencing gel, 41 cm in length (National Diagnostics, Biozym). Electrophoresis was performed overnight (or at least 6h) in 1 x TBE buffer at 50°C, 50 W and 1200 V in the LI-COR

4200 automated DNA sequencer. The gel image was later analyzed by Image Analysis Program version 4.10 (LI-COR Biotechnology).

The results from sequence analysis were compared with published sequences using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). Plasmids from those clones with identity percentage higher than 90% were considered significant similarity and were isolated for downstream application.

3.2.12 Quantitative Real-Time PCR (qPCR)

qRT-PCR was carried out in an ABI Prism® 7000 SDS instrument based on the changes in fluorescence proportional to the increase of product. SYBR® Green, which emits a fluorescent signal upon binding to double stranded DNA, was used as a detector. Fluorescence values were recorded during every cycle representing the amount of product amplified to a point known as threshold cycle (Ct). The higher the initial transcript amount, the sooner accumulated product was detected in the PCR process.

The plasmid serial dilution was prepared by converting concentration of plasmid (ng/µl) into numbers of molecules using the website http://molbiol.ru/eng/scripts/01_07.html. The plasmid concentration was diluted several folds from 10^1 to 10^9 so that the concentration would cover the range of target concentration in the muscle samples. A further step was to start the PCR assay to test whether a suitable standard curve could be achieved for high PCR efficiency.

Prior to quantification, the optimum primer concentration was obtained by trying different combinations from 200 nM to 600 nM. Results from these primer combinations were compared and the one with lowest threshold cycle and minimizing non-specific amplification was selected for subsequent reaction. After selection of primer concentration, a final assay consisted of 1 µl cDNA as template, up and down stream primers and SYBR Green Universal PCR Master Mix containing SYBR Green I Dye, AmpliTaq Gold DNA polymerase, dNTPs with dUTP, passive reference and optimized buffer components were performed in a total volume of 20 µl reaction. Thermal parameters used to amplify the template started with an initial denaturation at 95°C for 10 min followed by 40 cycles of 95°C for 15s denaturation and 60°C for 1 min annealing and extension. A dissociation curve was generated at the end of the last cycle by collecting the fluorescence data at 60°C and taking measurements every 7s until the

temperature reached 95^{o}C. Final quantification analysis was done by amplifying serial dilutions of target plasmid DNA. The concentration of unknown cDNA was calculated according to the standard curve, and expression level of transcripts was normalized relatively to the transcript of TBP, GAPDH and RPL32 genes. Table 3.2 presents the list of primers used in qPCR quantification.

Table 3.2: List of primers used to quantify genes for eQTL analysis (qPCR)

Gene	Primer sequence 5'→3'	Pro. size (bp)	Ta (^{o}C)	Acc. nr.
AMBP	Fw: AAGGATCAGGAGCTGGACAA Rv: CAGCCACCATAGTGGAAGGT	153	54	NM_001164006
GC	Fw: CCCACAAACAAAGATGCGTGT Rv: TCAGAGTGGCAGCATTCATCA	151	54	AY710291
PPP1R3B	Fw: CGAAATCCAGCACGAAGGTC Rv: AGAAGCGGGTGTCCTTTGC	164	54	AK236976
TNC	Fw: ACAATGAGATGCGGGTCACAG Rv: CGCTGACAGGAATGCTCTTCTT	185	59	NM_214230
GAPDH	Fw: ACCCAGAAGACTGTGGATGG Rv: ACGCCTGCTTCACCACCTTC	247	60	DQ845173
RPL32	Fw: AGCCCAAGATCGTCAAAAAG Rv: TGTTGCTCCCATAACCAATG	164	54	NM_0010016361
TBP	Fw: GATGGACGTTCGGTTTAGG Rv: AGCAGCACAGTACGAGCAA	124	60	DQ845178

3.2.13 mRNA expression with GenomeLab (GeXP)

Concentration of skeletal muscle RNAs were measured by using the NanoDrop ND-8000 spectrophotometer and diluted to 10ng/µl. By using 5 µl of template our standard protocol entails the use of 50 ng RNA as starting material for reverse transcription (RT) with a pool of all reverse primers (Table 3.3) prepared at 50 nmol final concentrations in a 20 µL reaction volume. Each of these primers is chimeric, having a gene-specific and universal sequence. Gene specific primers are given in table 3.3. Primers are designed with the 5′ end containing a quasi-T7 universal sequence which serves as a template for universal primers for use in subsequent amplification steps. Kanamycin RNA internal positive control is also included and produces a peak at 326 bp when samples are separated via electrophoresis. The RT reaction was performed using Beckman Coulter (Fullerton, CA, USA) GenomeLab GeXP Start Kit under the conditions: 1 min at 48 °C, 60 min at 42 °C, 5 min at 95 °C, hold at 4 °C, in a thermal cycler. All experiments

include “no template” (i.e. without RNA) and “no enzyme” (i.e. no reverse transcriptase) negative controls to confirm the absence of peaks at the expected target sizes. The “no template” sample produces a single peak at 326 bp, corresponding to the externally spiked-in kanamycin RNA. This control confirms that all reagents are satisfactory and that the experiment is technically sound. The template used for PCR was 9.3 µl of the 20-µl RT reaction with 20 nmol (final concentration) of each forward primer. Beckman Coulter Thermo-Start® DNA Polymerase (Beckman Coulter, Fullerton, CA, USA) was used for amplification conditions. Each of the forward primers contains an SP6 universal sequence at the 5′ end and a gene-specific sequence at the 3′ end (Table 3.2). The PCR reaction was performed in a thermal cycler under the conditions: 10 min at 95 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 1 min at 70 °C; hold at 4 °C. The procedure for sample preparation and subsequent analysis follows manufacturer’s instructions, and is described here briefly. Amplicons are resolved based on size, with fragments measuring between 137 and 348 bp. Sample loading solution was prepared by adding 28.5 µl SLS buffer (Beckman Coulter, Fullerton, CA, USA) and 0.50 µl size standard-400 (Beckman Coulter, Fullerton, CA, USA) to each reaction. PCR products were separated by the fragment analysis method (Frag-3) on the GeXP by diluting 1 µl PCR reaction with 29.0 µl sample loading solution. The GeXP software matches each fragment peak with the appropriate gene, and reports peak height and area-under-the-curve (AUC) for all peaks in the electropherogram. This data were exported from the Express Analysis module of the GeXP software as a tab-delimited file for subsequent analyses. Electrophoretic separation was done by eight capillary GenomeLab™ GeXP Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA). The GeXP software matches each fragment peak with the appropriate gene, and reports peak height and AUC for all peaks in the electropherogram. This data can be exported from the Express Analysis module of the GeXP software as a tab-delimited file for subsequent analyses. The data set was exported from the GeXP software after normalization to kanamycin, with area-under-the-curve (AUC) set to 1. This step minimizes inter-capillary variation. All multiplexes were combined into one Excel data file for further statistical analysis, data were stored and checked using a SQL based database system.

Table 3.3 Multi-plex primer sequences and descriptive information regarding genes used for the experiment

Gene	Affy. probe set ID	Accession nr.	SSC	BP	Multi-plex primer sequence 5'→3'
A2M	Ssc.16603.1.A1	AY509877	5	263	Fw: AGCAGGAATCAGGTGAAACG Rv: GACTCTCCACCAAAGCAGGT
ACTB		DQ845171		312	Fw: GACATCCGCAAGGACCTCTA Rv: CAGTCCGCCTAGAAGCATTT
ALB	Ssc.10439.1.S1	NM_001005208	8q12	256	Fw: AGTCCTTGGTGAACAGACGG Rv: CAAAGTTGCCCAGGACAGTT
AMBP	Ssc.1894.1.S1	X52087	1q	270	Fw: CGTCTCGGAGAAGGAGTGTC Rv: GCAGCAGCTCTTCATCCTCT
ANGPTL4	Ssc.17345.1.S1	NM_001038644	2q21-q24	235	Fw: CTGGAGAGGCCTTTGATGAG Rv: TGACTGAATCTGCCATCGAG
APOA1	Ssc.807.1.S1	NM_214398	9p12-p11	284	Fw: TTTGGGAAAACACCTCAACC Rv: CATCTTCTGGCGGTACGTCT
APOC3	Ssc.1039.1.S1	NM_001002801	9p13	277	Fw: CTTCTTGTCGCTGGTCTCCT Rv: CCAGAAGTCGGTGAACTTGC
ATF4	Ssc.11072.2.A1	NM_001123078	5	193	Fw: AAACCATGCCAGATGAGCTT Rv: TTTGCAAGAACGTAAAGGGG
CAPNS1	Ssc.7158.1.A1_a	NM_214318	6q1.1-q1.2.	144	Fw: CATCTCATACCCGCTCCATT Rv: GGACTGATCCTCCCAGAACA
CYP2C33	Ssc.955.1.S1	NM_214414	14	332	Fw: TCATTGGGAATCTGATGCAA Rv: ATCCCAAAGTTCCTCAGGGT
GC	Ssc.2992.1.A1	AY710291	8	214	Fw: GAGCTGCCCGAATACACAGT Rv: ACACGCATCTTTGTTTGTGG
GSTA2	Ssc.8516.1.A1	NM_214389	7p	223	Fw: GTGGCCTCGATCAAAGAAAA Rv: CTTCAGCAGAGGGAAGTTGG
PI	Ssc.7090.1.A1	NM_214395	7q2.4-q2.6	298	Fw: AAATCTCTCCTGGGCAACCT Rv: TCCCATGAAGAGGACAGCTT
PPARGC1	Ssc.16864.1.S1	NM_213963	8p21	165	Fw: TTGTCAACAGCAAAAGCCAC Rv: TGGAGGTGCACTTGTCTCTG
PPP1R3B	Ssc.6382.1.A1	BW971859	14	186	Fw: ACCATGTCTGCCTGGAGAAC Rv: CACGTACCAGCAGGGAAAGT
RBP4	Ssc.15695.1.S1	NM_214057	14	249	Fw: GAGAACTTCGACAAGGCTCG Rv: GGGTCCTCGGTGTCTGTAAA
SERPINA3-2	Ssc.15773.1.S1	NM_213787	7q	158	Fw: ATGTCCCTCTTCCTGGCTCT Rv: GCAGCTTCTTGATCTGGTCC
TBP		DQ178129		151	Fw: TCGGTTTAGGTTGCAGCAC Rv: GCAGCACAGTACGAGCAACT
TF	Ssc.4222.1.S1	X12386	13q31	172	Fw: AGAACTGCTGGCTGGAA Rv: GCCGATACACAGAGCACAGA
TNC	Ssc.16209.1.S1	NM_214230	1q	300	Fw: CTCATTGTGTACACGCCCAC Rv: ACGTTTCGAAAGCAATGTCC
TTR	Ssc.640.1.S1	NM_214212	6	346	Fw: ATGGTCAAAGTCCTGGATGC Rv: AGAGTAGGGGCTAAGCAGGG
HMBS		NM_001097412		179	Fw: AAGGTGCCAAGAACATCCTG Rv: CTGGGGTAATCACTCCCTGA
TYROBP	Ssc.507.1.A1	NM_214202		137	Fw: AGCCCAAATCAGGACAGTCA Rv: CTGTGGATCCGTATCCTGGT

3.3 Statistical analysis

3.3.1 Statistical analysis of QTL

3.3.1.1 Characterization of markers

The heterozygosity is a widely used measure of allelic diversity or infortiveness of a genetic marker. The informativeness of a genetic marker increases as heterozygosity increases. The heterozygosity of a genetic marker is estimated by:

$$H = 1 - \sum_{k=0}^{k} p_i^2$$

Where p_i is the frequency of the i^{th} allele and k is the number of alleles (Nei 1987; Otto and Goldstein 1992)

Alternative measure of the informativeness of a genetic marker in outbred species is the polymorphic information content (Botstein et al. 1980). The PIC of a genetic marker is estimated by:

$$PIC = 1 - \sum_{i=1}^{k} p_i^2 - \sum_{i=1}^{k} \sum_{j=1+1}^{k} p_i^2 p_j^2$$

Where p_i is the frequency of the i^{th} allele and k is the number of alleles (Botstein et al. 1980; Otto and Goldstein 1992). Regarding to codominant marker PIC was developed for ascertaining the allele transmitted by an affected heterozygous parent carrying a dominant disease allele (Otto and Goldstein 1992). PIC estimates the probability that a co-dominant marker genotype of an offspring can be used to deduce which of two marker alleles were transmitted by a parent carrying a dominant disease allele. The term polymorphic information contetnt is alternatively and frequently used for heterozygosity and possibly other measures of marker informativeness.

3.3.3.2 Linkage analysis and genetic map construction

The data obtained from genotyping were used to construct linkage maps. The data were firstly checked for any genotyping errors by using Pedcheck software (v. 1.1) (O'Connell and Weeks 1998). Then multi point linkage analyses were carried out for males and sex average with the CRI-Map software (v. 2.4) (Green 1990). Various Cri-MAP options were used to determine the marker orders and marker distances within linkage groups. Recombination units were converted into map distances using Kosambi (Kosambi 1944) mappig function for eQTL mapping. The generated sex-average recombination units can be converted to Kosambi centimorgan by:

M = ¼ ln (1+2R/1-2R),

Where;
M = map distance in Morgan
R = recombination

3.3.3.3 eQTL analysis

eQTL analysis was performed using the regression approach implemented in "QTL Express" internet based publicly available software http://qtl.cap.ed.ac.uk/ (Seaton et al. 2002). This program is designed for the analyses of three generation pedigrees derived from a cross between outbred lines. This approach assumes that the founder populations are fixed for alternative QTL alleles in F_2 population. These two alleles will be denoted Q for the Duroc allele and q for the Pietrain allele. Under this assumption, the probability (P) of a F2 individual being one of four possible QTL genotypes p(QQ), p(Qq), p(qQ) or p(qq), conditional on the marker genotypes at any putative location in the genome, were computed as described Haley et al. (1994). Sex average distances were used in all analysis, since Knott et al. (1998) showed that using sex-specific map had limited effects on the results. The different hypothesis (linked QTLs, genomic imprinting) were tested by computing at every cM of the whole genome, the reduction in sum of squares (F-ratio test) caused by adding new components to a no-QTL and to one QTL models. By this procedure, the additive and dominance coefficients and the F-

ratio values were calculated. The proportions of F_2 phenotypic variance that was explained by QTL effects was calculated as reduction of the residual mean square within the F_2 generation. The following equation was used:

$$Var\% = \frac{MS_R - MS_F}{MS_R} \text{ x } 100$$

Where, MS_R was the mean of square of the reduced model; MS_F was the mean of square of the full model.

The QTL Express program including backcross/F_2 dataset was used following an additive and dominant model with permutated chromosome-wide permutations at a total of 1000 iterations. The chromosome-wide analysis was done by measuring QTL for all traits at the same time. Relevant fixed effects for gene expression values including class effects and covariates were tested using GLM procedure of SAS (v. 9.2). Among fixed effects, sex as class effect and slaughter weight as co-variate were found to be non significant on expression of genes therefore they were excluded from the final model.

The single QTL analysis was performed by F_2 outbred design in QTL Express. The single QTL model with additive and dominance effects was fitted at 1-cM intervals along every single chromosome. A permutation method was used to obtain the empirical distribution of the test statistics with 1000 times permutation under the null hypothesis (no linked QTL) (Churchill and Doerge 1994). The 5% chromosome-wide threshold was considered the suggestive level and obtained as in the paper de Koning et al. (1999). The 95% confidence intervals (CI_{95}) of eQTL were calculated by a bootstrap method based on 2000 replacement resampling (Visscher et al. 1996).

Based on a robust two-step procedure for QTL mapping in the QTL Express program, marker genotypes were used to estimate the identity-by-descent (IBD) probabilities at 1-cM intervals through chromosomes. These probabilities are used to calculate additive and dominance coefficients for a putative QTL at each position and the trait values are regressed onto these coefficients to calculate F-ratios testing the existence of a QTL at given position. Linear models are fitted to phenotypic data using a general linear model. For the genetic component in the linear model, a single or a two QTL model is fitted (Green et al. 1990).

One QTL regression model

The regression analysis of the backcross population calculates the transmission probabilities using simple algorithm. The estimable allele substitution effect is defined by the QQ-qq, which contains both an additive and dominance part. If the effect of recurrent QTL genotype is larger than the effect of heterozygous genotype, the value is positive. The QTL regression model for single analysis was:

$$Y_i = \mu + S_i + c_{ai}a + c_{di}d + \varepsilon_i \text{ [Model 1]}$$

where:

Y_i = phenotype (gene expression) of the i^{th} offspring

μ = overall mean

S_i = Fixed effect of slaughter day, i = 1-12

c_{ai} = additive coefficient of the i^{th} individual at a putative QTL in the genome

c_{di} = dominant coefficient of the i^{th} individual at a putative QTL in the genome

a = additive effects of a putative QTL

d = dominant effects of a putative QTL

ε_i = residual error

Two Mendelian QTL model

To distinguish between the presence of one QTL and with large effect and two linked QTL with smaller effects, a two dimensional QTL search at 1-cM grid was carried out for those linkage groups, where significant evidence for one QTL was detected by one QTL model, also by the cofactor analysis. The presence of two QTL in the same linkage group was tested by adding additive and dominance effects for a second QTL in the model:

$$Y_i = \mu + S_i + c_{ai1}a_1 + c_{di1}d_1 + c_{ai2}a_2 + c_{di2}d_2 + \varepsilon_{ij} \text{ [Model 2]}$$

Where, y_i, μ, S_i and ε_i have the same meaning as in model 1. a_1, d_1, a_2 and d_2 are the additive and dominance effects for QTL1 and QTL2 respectively. c_{ai1}, c_{ai2}, c_{di1} and c_{di2}

are corresponding coefficients. Coefficients c_{a1}, c_{a2}, c_{d1} and c_{d2} were calculated conditional upon the markers, as follows:

$$c_{a1}=p_1(QQ)-p_1(qq)$$
$$c_{d1}=p_1(Qq)$$
$$c_{a2}=p_2(QQ)-p_2(qq)$$
$$c_{d2}=p_2(Qq)$$

where: p1 and p2 are the probabilities for configurations QQ, Qq and qq in location1 and location 2. A 1-cM grid search was performed in QTL Express by fitting model 2 to estimate the effects of two QTL at separate positions within the same linkage group simultaneously, examinig all possible pairs of locations, to test whether the two-QTL model explained significantly more variation than the best QTL from the one-QTL analysis. Two F-statistics were computed. The first F-value was obtained by contrasting model 2 with no QTL model with 4 df in the numerator (F_{4df}). When F-ratio (F_{4df}) reached the suggestive level threshold, a second F-value was calculated by contrasting model 2 with QTL model 1 with 2 df in the numerator (F_{2df}). The presence of two QTL on the linkage group was concluded only when both F-statistics reached significant threshold.

One QTL model with imprinting

The presence of imprinting effects (i) was tested by considering the paternal or maternal origin of grandparental (Duroc or Pietrain) alleles, including the difference between two classes of heterozygotes in the model as suggested by Knott et al. (1998):

$$Y_i = \mu + S_i + c_{ai}a+c_{di}d+ c_{ii}i+ \varepsilon_i \text{ [Model 3]}$$

Where y_i, μ, S_i, a, d, c_{ai}, c_{di} and ε_i have the same meaning as in model 1. i is the imprinting effect, and c_{ii} = p(Q(from sire)q) – p(qQ(from dam)) is the corresponding coefficient. Model 3 was first contrasted to a no QTL model with 3 degrees of freedom in the numerator (F_{3df}). When significant, model 3 was contrasted to the best one QTL

model (model 1) with 1 degree of freedom in the numerator (F_{1df}), in order to test the significance of the imprinting effects.

3.3.3.4 Significant threshold

Detection of QTL was based on a F statistics that was computed from sums of squares explained by the additive and dominance (also inclusive imprinting effects if exists) coefficients for the QTL. The significant thresholds were determined empirically by data permutation test (Churchill and Doerge 1994). This empric method uses distribution of data from genotypes and phenotypes. In order to determine significant thresholds, a total of 1000 permutations were performed for each chromosome x trait combination. The chromosome-wide 1% and 5% significance threshold were calculated by QTL Express. The 1% and 5% experiment-wide significant thresholds were calculated by transformation with Bonferoni correction for 18 autosomes of the haploid porcine genome. As there were no markers genotyped on the X chromosome, transformation was done only for an experiment-wide, not for a genome-wide significant threshold level. The experiment-wide significance level was calculated by the following term:

$$\mathrm{Pr} = \frac{1-(1-Pc)}{r}$$

where:
r = length of a specific chromosome / sum of length of all chromosomes
Pc = chromosome-wide significance threshold

3.3.3.5 Gene expression association with meat quality traits

Analysis of variance was performed to investigate the effects of gene expression on different meat quality traits. Descriptive statistics was calculated by using MEAN procedure of SAS (v. 9.2). Association of gene expression levels with meat quality traits were based on mixed model which was performed with the MIXED procedure of SAS. The fixed effect part of the model was derived using GLM procedure of SAS (v. 9.2).

Relevant fixed effects for meat quality traits as well as the corresponding proportion of variation explained by these effects (R^2) were calculated as well. In the fixed effects, slaughter date and sex of the animal were accounted as class effects. Slaughter weight and gene expressions were included as a linear co-variable for meat quality traits. Family (Dam x Sire) were considered random affects for the mixed model. Sire and dam effects were also tested. However, according to fit statistics option of PROC MIXED based on the smaller value is better; sire and dam showed always highest values among sire, dam and sire x dam (family) factors. Thus, dam and family were considered as random effects for the mixed model. The regression between normalized gene expressions and meat quality traits was tested using mixed model (PROC MIXED) in SAS (v. 9.2). The following model was used:

$$Y_{jkif} = \mu + sex_j + slaughter\ date_k + COV\ gene\ expression_i + COV\ slaughter\ weight_f + \varepsilon_{ijkf}$$

where:

Y_{ijkf}	= observation ijkf for meat quality traits (pH_1 loin, pH_{24} loin, drip loss, cook loss, thaw loss and shear force)
μ	= overall mean
sex_j	= the fixed effect of sex j (j =1, 2)
slaughter $date_k$	= the fixed effect of slaughter date k (k = 1 to 12 levels)
COV gene $expression_j$	= the covariate of covariate i (i = expression of 20 genes)
COV slaughter $weight_f$	= the covariate of slaughter weight f (1 level)
ε_{ijkf}	= the residual error.

4 Results

4.1 Meat quality traits

Descriptive statistics for meat quality parameters, used for this study are shown in table 4.1. The mean of 45 min pH post-mortem was 6.56 ± 0.21. After 24 hours post-mortem pH was measured as 5.49 ± 0.08 in loin. The ultimate pH in ham was measured slightly higher (5.63 ± 0.12) compared to pH_{24L}. While measuring the pH, conductivity was also measured in two time points but only in loin; conductivity 45 min and 24 h post-mortem were recorded as 4.35 ± 0.62 and 2.79 ± 0.82, respectively. In DUPI population drip loss was measured only in loin. Drip loss was recorded as 2.07 ± 0.96 in loin using the bag method. Thereafter, thawing loss and cooking loss were measured and recorded as 8.20 ± 1.86 and 24.68 ± 1.97, respectively. Shearforce was measured in three replications, and mean of these three values were recorded as population mean. It was detected as 35.18 ± 6.62.

Table 4.1: Descriptive statistics of meat quality parameters

Trait	N	Mean	SE	Minimum	Maximum
pH_{1L}	331	6.56	0.21	5.91	7.02
pH_{24L}	331	5.49	0.08	5.30	5.84
pH_{24H}	331	5.63	0.12	5.38	6.37
$Conductivity_{1L}$	331	4.35	0.62	2.80	6.00
$Conductivity_{24L}$	331	2.79	0.82	1.60	9.20
Drip loss	331	2.07	0.96	0.50	5.60
Cooking loss	331	24.68	1.97	17.20	29.40
Thaw loss	331	8.20	1.86	3.30	13.60
Shear force	320	35.18	6.62	21.96	61.21
Meat colour	331	69.03	5.76	49.00	85.00

4.2 Transcript abundance

In order to compare the expression level of 20 genes in muscle tissue samples derived from phenotypically differentiated for drip loss and muscle pH in 300 pigs, we analyzed the mRNA levels of longissimus dorsi muscle using the multi-plex gene quantification method. Descriptive statistics regarding AUC values of 20 genes is given in Table 4.2. The expression levels of all genes obtained from multi-plex were normalized to

geometric mean of actin, beta (*ACTB*) and TATA box-binding protein (*TBP*). The relative expressions of 20 genes are given in figure 4.1. The expression values are clustred as high, moderate and low. According to clustering, gene *A2M*, *ANGPTL4*, *TYROBP*, *TNC* and *CAPNS1* were found to be in high gene expression group. *SERPINA3-2*, *PPARGC1* and *RBP4* were found in middle gene expression cluster. *APOC3*, *ALB*, *PI*, *GC*, *PPP1R3B*, *GSTA2*, *ATF4*, *TTR*, *APOA1*, *CYP2C33*, *TF* and *AMBP* were measured in low expression group. Figure 4.1 shows the three gene expression cluster among 20 genes.

Table 4.2: Descritive statistics of *A2M*, *ACTB*, *ALB*, *AMBP*, *ANGPTL4*, *APOA1*, *APOC3*, *ATF4*, *CAPNS1*, *CYP2C33*, *GC*, *GSTA2*, *HMBS*, *PI*, *PPARGC1*, *PPP1R3B*, *RBP4*, *SERPINA3-2*, *TBP*, *TF*, *TNC*, *TTR* and *TYROBP* genes

Gene	N	Mean	SD	Minimum	Maximum
A2M	300	51.11	90.90	0.83	728.49
*ACTB**	300	1570.83	2605.89	5.41	22.154.19
ALB	300	3.85	2.59	0.11	25.90
AMBP	300	2.70	1.94	0.08	17.86
ANGPTL4	300	45.1	82.78	0.77	660.5
APOA1	300	17.36	15.99	0.14	141.66
APOC3	300	4.38	3.19	0.05	28.50
ATF4	300	12.75	27.41	0.09	274.76
CAPNS1	300	45.92	86.56	0.58	752.14
CYP2C33	300	13.58	22.26	0.53	176.91
GC	300	7.27	15.46	0.02	133.27
GSTA2	300	16.95	12.36	0.67	105.96
*HMBS**	300	1322.38	1384	93.95	17868.86
PI	300	19.40	33.62	0.27	259.35
PPARGC1	300	40.05	89.21	0.29	712.43
PPP1R3B	300	4.10	2.82	0.15	26.87
RBP4	300	28.4	39.41	0.56	372.95
SERPINA3-2	300	20.47	19.00	0.23	152.46
*TBP**	300	2212.72	3919.86	26.72	41207.26
TF	300	3.40	2.80	0.15	2.80
TNC	300	49.69	109.33	0.16	1042.17
TTR	300	2.72	2.24	0.05	22.00
TYROBP	300	51.93	90.85	0.74	700.42

* used as house keeping genes for gene expression normalization

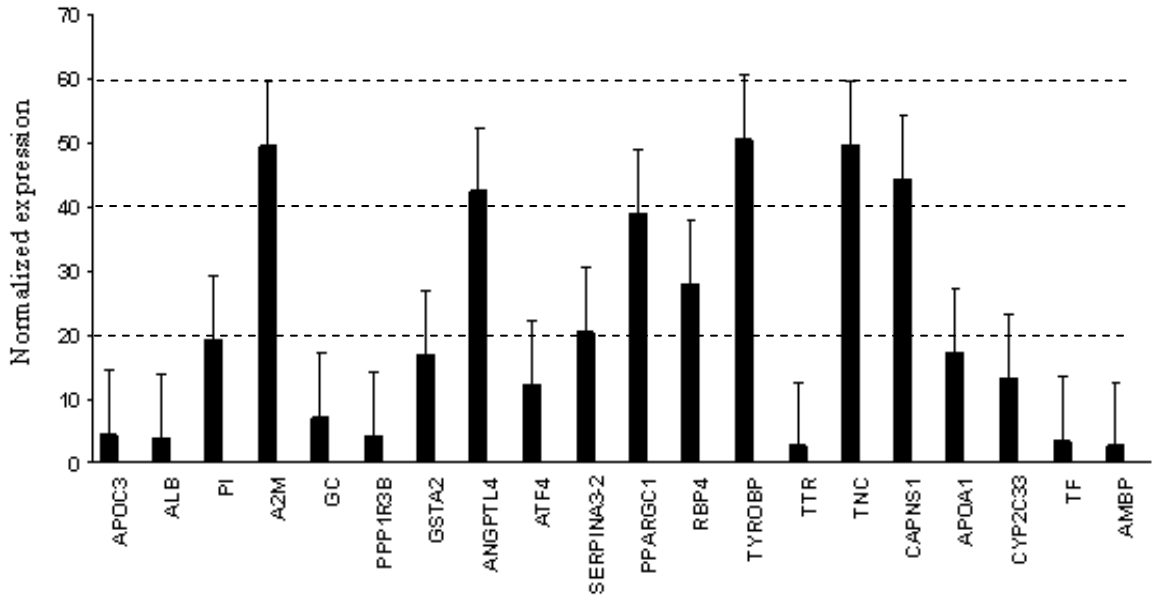

Figure 4.1: Expression value of *A2M*, *ACTB*, *ALB*, *AMBP*, *ANGPTL4*, *APOA1*, *APOC3*, *ATF4*, *CAPNS1*, *CYP2C33*, *GC*, *GSTA2*, *HMBS*, *PI*, *PPARGC1*, *PPP1R3B*, *RBP4*, *SERPINA3-2*, *TBP*, *TF*, *TNC*, *TTR* and *TYROBP* genes based on the AUC of GenomeLab GeXP instrument. Figure shows low, moderate and high gene expression

4.3 Validation of microarray results in *A2M*, *ACTB*, *ALB*, *AMBP*, *ANGPTL4*, *APOA1*, *APOC3*, *ATF4*, *CAPNS1*, *CYP2C33*, *GC*, *GSTA2*, *HMBS*, *PI*, *PPARGC1*, *PPP1R3B*, *RBP4*, *SERPINA3-2*, *TBP*, *TF*, *TNC*, *TTR* and *TYROBP* genes

The validation of gene expression in high vs. low drip loss and muscle pH 24 h post-mortem in loin confirmed the result of microarray. However, couple of genes showed different regulation between two systems. The genes *APOC3*, *ALB*, *PI*, *A2M*, *PPP1R3B*, *GSTA2*, *ATF4*, *SERPINA3-2*, *PPARGC1*, *RBP4*, *TTR*, *CAPNS1*, *APOA1*, *CYP2C33*, *TF* and *AMBP* were validated in high vs low drip loss group of animals. However, the genes *GC*, *ANGPTL4*, *TYROBP* and *TNC* were not validated in GenomeLab GeXP. The result of validation in drip loss is given in figure 4.2.

In high vs low pH 24 h post-mortem in loin, *APOC3*, *ALB*, *PI*, *A2M*, *GC*, *GSTA2*, *ANGPTL4*, *ATF4*, *SERPINA3-2*, *PPARGC1*, *RBP4*, *TYROBP*, *TNC*, *CAPNS1*, *CYP2C33* and *AMBP* were validated by GenomeLab GeXP. On the other hand, *PPP1R3B*, *TTR*, *APOA1* and *TF* were not validated with the same system. The result of validation in drip loss is given in figure 4.3.

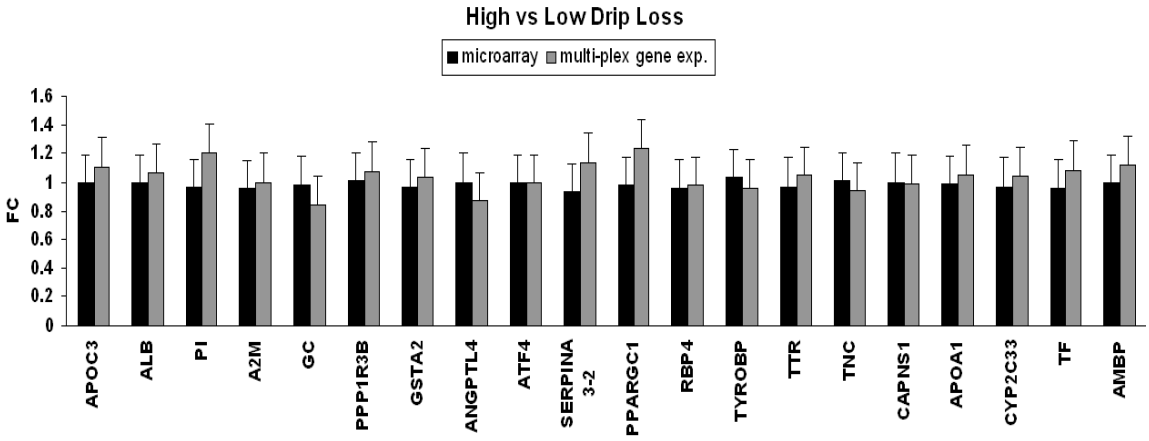

Figure 4.2: Validation of microarray results in high vs low drip loss animals

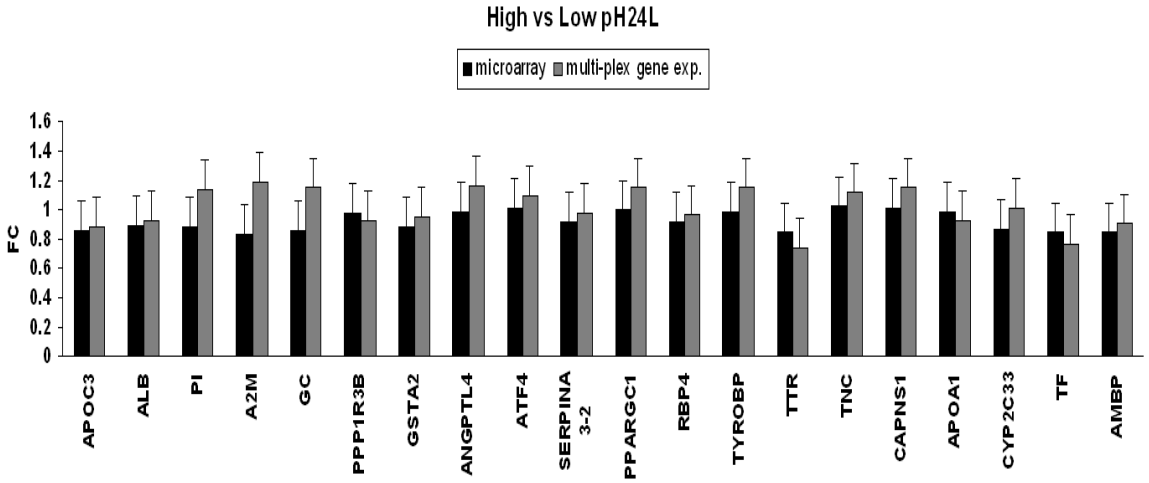

Figure 4.3: Validation of microarray results in high vs low muscle pH animals

4.4 Regression analysis between meat quality traits and expression of *A2M*, *ALB*, *AMBP*, *ANGPTL4*, *APOA1*, *APOC3*, *CAPNS1*, *CYP2C33*, *GC*, *PI*, *PPARGC1*, *PPP1R3B*, *RBP4*, *SERPINA3-2*, *TF*, *TNC*, *TTR* and *TYROBP* genes

Regression analysis between meat quality traits and gene expressions showed that among twenty genes, 18 genes showed association with drip loss, thawing loss, cooking loss, pH_{24L}, pH_{1L} and shear force with different significancy levels (Table 4.3). The genes *ALB*, *AMBP*, *CAPNS1*, *TNC* and *TYROBP* were found to be associated with more than one trait (Table 4.3). Twelve genes including *APOA1* ($p = 0.06$), *ANGPTL4* ($p =$

0.09), *APOC3* (p = 0.01), *SERPINA3-2* (p = 0.06), *RBP4* (p = 0.09), *TYROBP* (p = 0.07), *TNC* (p = 0.07), *CAPNS1* (p = 0.05), *CYP2C33* (p = 0.08), *PI* (p = 0.05), *A2M* (p = 0.05), and *PPARGC1* (p = 0.03) were showed association with drip loss trait. Among them *APOC3* and *PPARGC1* showed highly significant association with drip loss (p = 0.01 and 0.03 respectively). Moreover, *PPARGC1* gene was also detected to be up regulated in high drip loss group compared to low drip loss ($p<0.05$) (see figure 4.4). Additionally, three of genes, GC (p = 0.009), *TYROBP* (p = 0.04), *TNC* (p = 0.03) and *CAPNS1* (p = 0.07) were associated with cooking loss beside drip loss as well. The gene expression of *PPP1R3B* (p = 0.01), *ALB* (p = 0.04) and *AMBP* (p = 0.04) were found to be associated with shear force. *ALB* (p = 0.06) was also found to be associated with thawing loss. Moreover, expression of *AMBP* was also associated with pH_{24L} as detected also with shear force. An additional association between pH_{24} loin and expression of *TF* has been estimated (p = 0.06). The only association for pH_1 loin has been found with the expression of TTR (p = 0.04).

Table 4.3: Regression analysis between meat quality traits and expression of *A2M*, *ALB*, *AMBP*, *ANGPTL4*, *APOA1*, *APOC3*, *CAPNS1*, *CYP2C33*, *GC*, *PI*, *PPARGC1*, *PPP1R3B*, *RBP4*, *SERPINA3-2*, *TF*, *TNC*, *TTR* and *TYROBP* genes

Traits	Fixed effects						Random effect
	R^2	Gene	Gene expression	Slaughter date	Sex	Slaughter weight	Family
pH_{24LD}	0.27	*TF*	* (0.06)	***	*	*	√
pH_{24LD}	0.27	*AMBP*	* (0.03)	***	*	*	√
pH_{1LD}	0.22	*TTR*	* (0.04)	***	ns	*	√
Drip loss	0.11	*PPARGC1*	* (0.03)	ns	ns	**	√
Drip loss	0.10	*APOC3*	* (0.01)	ns	ns	**	√
Drip loss	0.10	*APOA1*	* (0.06)	ns	ns	**	√
Drip loss	0.10	*SERPINA3-2*	* (0.06)	ns	ns	**	√
Drip loss	0.10	*RBP4*	* (0.09)	ns	ns	**	√
Drip loss	0.10	*TYROBP*	* (0.07)	ns	ns	**	√
Drip loss	0.10	*TNC*	* (0.07)	ns	ns	**	√
Drip loss	0.10	*CAPNS1*	* (0.05)	ns	ns	**	√
Drip loss	0.10	*CYP2C33*	* (0.08)	ns	ns	**	√
Drip loss	0.10	*PI*	* (0.05)	ns	ns	**	√
Drip loss	0.10	*A2M*	* (0.05)	ns	ns	**	√
Drip loss	0.10	*ANGPTL4*	* (0.09)	ns	ns	**	√
Thaw loss	0.12	*ALB*	* (0.06)	**	*	*	√
Cook loss	0.24	*GC*	** (0.009)	***	*	***	√

Cook loss	0.23	*CAPNS1*	* (0.07)	***	ns	***	√
Cook loss	0.23	*TNC*	* (0.03)	***	ns	***	√
Cook loss	0.24	*TYROBP*	* (0.04)	***	ns	***	√
Shear force	0.17	*PPP1R3B*	* (0.01)	**	**	ns	√
Shear force	0.16	*ALB*	* (0.04)	**	*	ns	√
Shear force	0.16	*AMBP*	* (0.04)	**	*	ns	√

ns not significant
*** ($p<0.001$), ** ($p<0.01$) and * ($p<0.1$)
R-square values derived from general linear model.
P values derived from mixed model.

4.5 Step-wise regression analysis between meat quality traits and gene expression values

In order to understand the relation between gene expression values and meat quality phenotypes stepwise regression was done additionally. Separate multiple regression models based on independent contrasts investigating the effects of gene expression and fixed effects on five different meat quality traits were done. The models were constructed by sequentially removing variables, keeping those with $p<0.15$. Each column contains one best regression model relating to that specific meat quality trait. Dashes indicate variables entered the model but excluded from the final best model. Stepwise regression tests showed that drip loss was positively correlated with slaughter weight and expression of the *ALB* gene (Table 4.4). Nevertheless, in regression analysis done by the MIXED procedure, no association could be detected in the same gene (Table 4.3). The pH_{1L} was positively correlated with sex, season, slaughter weight and the expression of *ANGPTL4*, *TF*, *ALB* and *AMBP* genes. pH_{24H} and pH_{24L} were only positively correlated with sex, season and slaughter weight. Thawing loss was negatively correlated with sex and expression of *GC*, *ALB* and *APOA1* whereas positively correlated with *ANGPTL4* (Table 4.4).

Table 4.4: Stepwise multiple regression models: relation of gene expressions with meat quality traits

	Meat quality traits (dependent variables)				
Independent variable included in the best model	pH_{1L}	pH_{24L}	Cooking loss	Thawing loss	Drip loss
Sex	--	C(p)=26.72 R^2=0.15 P<0.0001	C(p)=12.429 R^2=0.27 P=0.05	C(p)=-1.368 R^2=0.03 P=0.06	--
Slaughter date	C(p)=27.40 R^2=0.0808 P<.0001	C(p)=18.999 R^2=0.15 P<.0001	C(p)=18.062 R^2=0.24 P<.0001	--	--
Slaughter weight	C(p)=23.21 R^2=0.0196 P=0.017	C(p)=4.503 R^2=0.214 P=0.005	C(p)=14.209 R^2=0.26 P=0.01	--	C(p)=1.460 R^2=0.037 P=0.0015
ALB	C(p)=20.78 R^2=0.0141 P=0.04	--	--	C(p)=0.111 R^2=0.01 P=0.03	C(p)=0.010 R^2=0.05 P=0.108
AMBP	C(p)=20.78 R^2=0.0141 P=0.04	--	--	C(p)=-1.943 R^2=0.03 P=0.10	--
TF	C(p)=2.638 R^2=0.16 P=0.06	--	--	C(p)=-2.025 R^2=0.04 P=0.14	--
APOA1	--	--	--	C(p)=-4.940 R^2=0.06 P=0.02	--
ALB	C(p)=11.114 R^2=0.117 P=0.02	--	--	--	--
AMBP	C(p)=5.748 R^2=0.14 P=0.007	--	--	--	--
SERPINA3-2	--	--	--	--	C(p)=0.559 R^2=0.04 P=0.08
PI	--	--	--	--	C(p)=-6.865 R^2=0.08 p=0.002
A2M	C(p)=-6.889 R^2=0.09 P=0.14	--	--	--	--
Whole model	p<.0001	p<.0001	p<.0001	p<.0001	P=0.0031

4.6 Differentially regulation of genes in high vs low drip loss, pH_1 and pH_{24}

Gene expression analyses using GenomeLab GeXP system showed that expression of some genes significantly differed between samples high and low drip loss (high drip loss ≥ 1.8 %; low drip loss <1.8 %) and pH_{24L} (high $pH_{24L} \leq 5.49$; low $pH_{24L} > 5.49$) (Figure 4.4 and 4.5). Among twenty genes, *PPARGC1* was found to be significantly

differentially expressed between high vs. low drip loss (p = 0.03; figure 4.4). Moreover, for the high and low drip loss group, the expression level of *AMBP* gene was also found to be differentially regulated (p = 0.06; figure 4.4). Both, *PPARGC1* and *AMBP* were up regulated in the group of animals with higher drip loss. Although the expression of other genes was not significantly (p>0.1) different, expression of these genes was slightly higher in high drip loss class.

In the high pH_{24L} class, expression of *TTR* and *TF* (p = 0.06 and p = 0.07, respectively; figure 4.5) found to be differentially regulated. The expression of both genes was slightly higher in the high pH_{24L} group. Interestingly, no difference was observed in the mRNA level of genes between high and low pH_{1L} in the DUPI population (Figure 4.6).

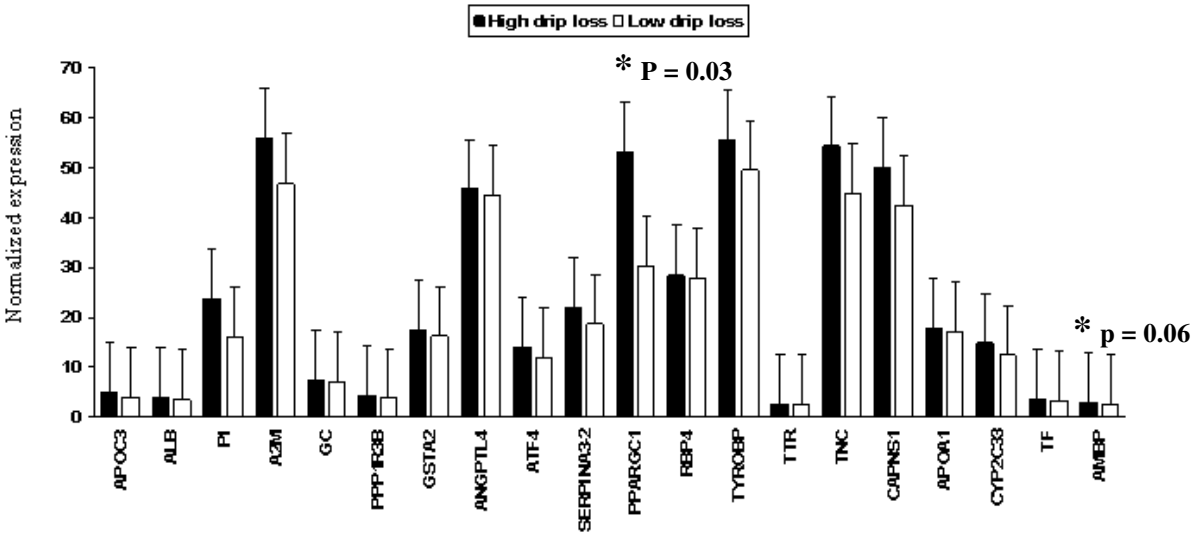

Figure 4.4: Differentially gene expression between high vs. low drip loss.

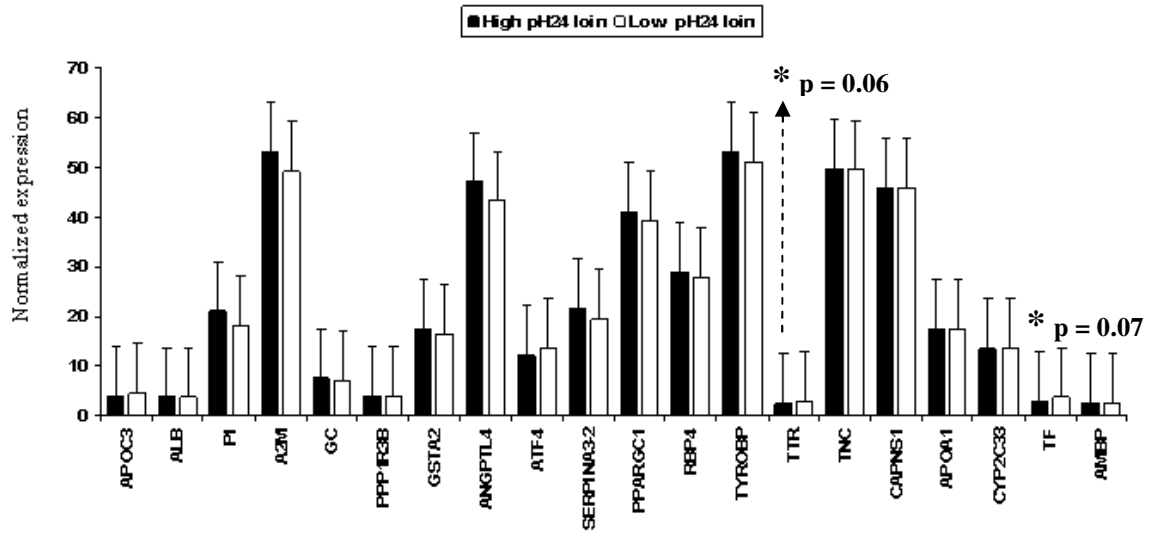

Figure 4.5: Differentially gene expression between high vs. low pH_{24L}.

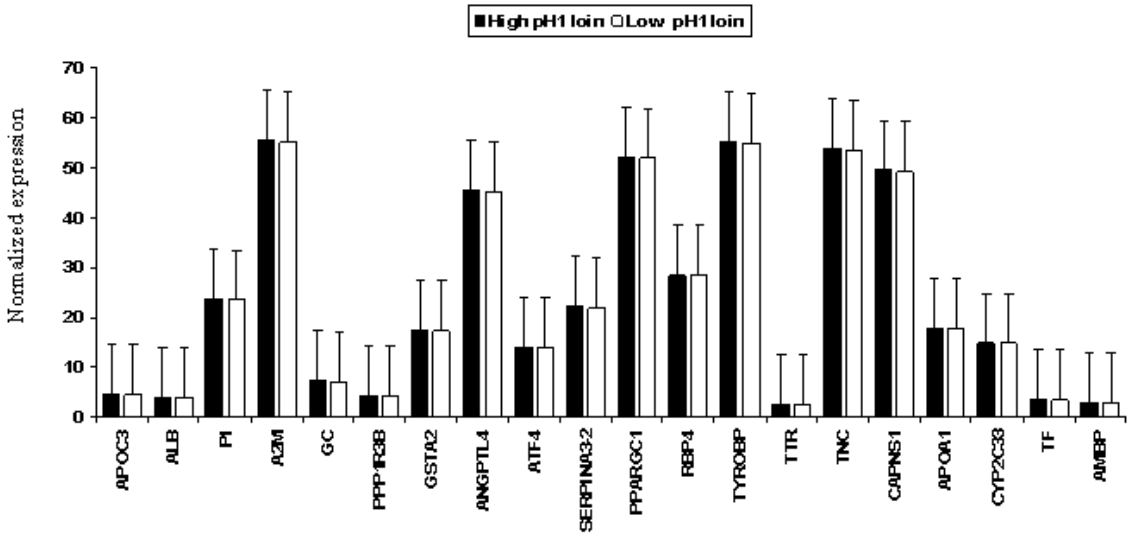

Figure 4.6: Differentially gene expression between high vs. low pH_{1L}

4.7 Result of eQTL under the line-cross model

In total, 25 eQTL were identified on porcine autosomes 1, 2, 3, 4, 6, 7, 8, 9, 10, 11 and 13 (Table 4.5). Among 20 traits, eQTL of 13 traits (*PPARGC1*, *ALB*, *SERPINA3-2*, *PPP1R3B*, *GSTA2*, *APOC3*, *RBP4*, *APOA1*, *TF*, *ATF4*, *PI*, *CAPNS1* and *TNC*) reached the acceptable LOD score threshold (1.8). Among those eQTL, 17 eQTL were detected significant at 5% chromosome-wide level (CW) (Table 4.5). An eQTL for *ALB* on SSC2 was detected as 1% chromosome-wide significant level. Moreover, on SSC2, eQTL for *SERPINA3-2* was detected as 5% genome-wide level (GW) which showed the highest F-value in the analysis. On SSC1, a chromosome-wide suggestive eQTL for *PPARGC1* was found between the markers *SW373* and *SW1301* at 187 cM. The most promising eQTL were detected on SSC2 for 6 traits proximal to marker *S0141*. On SSC2, two eQTL were found for *AMBP* and GC at 118 cM close to marker *SW240*. On SSC3, a suggestive ($p<0.05$; CW) eQTL was detected for *PPP1R3B* at 86 cM close to marker *S0002* On SSC4, two eQTL for *RBP4* and *APOA1* were detected at 67 and 72 cM, respectively close to marker *S0214*. On SSC6, only one suggestive ($p < 0.05$; CW) eQTL was detected for *PPARGC1* at 30 cM between the markers *S0035* and *S0087*. Five eQTL were detected on SSC7. Among them three for *APOC3*, *ALB* and *TF* were found close to the marker *S0064* at 33, 36 and 38 cM, respectively. On SSC7, an additional suggestive ($p<0.05$; CW) eQTL was detected for TNC at 103 cM close to marker *S0115*. An eQTL was detected on SSC8 for *GSTA2* at 19 cM close to marker

SW2611. Additionally, 2 eQTL for *GC* and *AMBP* were detected at 22 and 26 cM, respectively between the markers *SW2611* and *S0086* on SSC8. On SSC9, an eQTL for *SERPINA3-2* at 27 cM was found between the markers *SW911* and *SW54*. The only eQTL for *PI* and *CAPNS1* were detected on SSC10 and 11, respectively. eQTL for *PI* was detected at 118 cM close to marker *SW951* and eQTL for *CAPNS1* was detected at 0 cM proximal to marker *SW2008*. On SSC13 two suggestive ($p<0.05$; CW) eQTL were detected, one at 31 cM and the other at 120 cM, close to markers *SW344* and *S0289*, respectively.

4.8 Result of eQTL under the two-QTL model

The two-QTL model was used to identify the presence of possible two-eQTL regions on the same chromosome. Results for the two-QTL model conducted with QTL Express are presented in table 4.10. Significant evidence for an additional eQTL under a two-QTL model was found on SSC1 and SSC2. On SSC1, it was detected for *PPARGC1* with a distance of 153 cM between two loci. The two loci were detected at 35 and 188 cM, respectively. SSC1 was genotyped with 18 microsatellite markers and the average marker distance was 11.2 cM. In this case several markers were located between two-QTL regions and one of the two chromosomal regions was identified in the single QTL approach (Table 4.6). The two loci on SSC1 for *PPARGC1* in this study were in repulsion phase. The eQTL affecting *PPARGC1* at 35 cM and 188 cM jointly explained 4.02 % of the phenotypic variation.

On SSC2, 5 two-eQTL pairs were detected for *ALB*, *PPP1R3B*, *TTR*, *APOC3* and *APOA1* close to marker *S0141*. There was a distance of 90 cM between these two-eQTL and two microsatellite markers were located between the eQTL loci. Average marker density for SSC2 was given as 15.9 cM. For the all given traits, the first eQTL was detected close to marker *S0141* where this region was also detected with single-eQTL model. The second-eQTL of two-QTL model was detected close to marker *SW1564*. The phenotypic variation explained by the two loci is given in table 4.6.

4.9 Result of eQTL under imprinting model

In total 8 imprinting eQTL were detected on SSC2 and 9 with the imprinting QTL model of QTL Express software. On SSC2, seven imprinting QTL were detected, of which 6 were paternally imprinted (maternally expressed) and one was maternally imprinted (paternally expressed). The identified imprinting eQTL on SSC2 were found to be close to marker *S0141* at around 33 cM which also detected with the line-cross QTL model. On SSC9, the only paternally imprinted eQTL was mapped for *SERPINA3-2* at 26 cM close to marker *SW911*. The phenotypic variation explained by the imprinting QTL model is given in table 4.7.

Table 4.5: Summary of line-cross eQTL locations and their relative additive and dominant effects on gene expression values in the DUPI F_2 resource population

SSC	Trait	Position (cM)	F - value	Var (%)	Add	SE	Dom	SE	Closest marker
1	*PPARGC1*	187	5.92*	3.42	-5.56	2.4	-11.99	4.82	SW1301
2	*ALB*	32	7.68**	3.7	1.24	0.36	-1.87	0.52	S0141
	PPP1R3B	31	7.55*	3.26	1.46	0.42	-2.16	0.64	S0141
	GSTA2	29	5.46	3.15	3.48	1.30	-7.1	2.34	S0141
	SERPINA3-2	32	8.51***	4.01	5.94	1.64	-8.68	2.34	S0141
	RBP4	33	4.17	3.16	4.02	1.73	-6.54	2.38	S0141
	APOC3	31	4.83	3.27	0.89	0.31	-1.31	0.49	S0141
	AMBP	118	3.68	1.86	0.55	0.20	-0.30	0.33	SW240
	GC	118	3.87	1.81	0.54	0.19	-0.0007	0.32	SW240
3	*PPP1R3B*	86	5.10*	3.37	0.28	0.10	0.41	0.23	S0002
4	*RBP4*	67	4.83*	3.3	3.8	1.22	0.29	2.0	S0214
	APOA1	72	5.24*	3.24	2.25	0.8	1.74	1.45	S0214
6	*PPARGC1*	30	4.86*	3.67	7.19	2.95	-17.76	7.04	S0035-S0087
7	*APOC3*	33	5.6*	3.27	-0.41	0.2	0.73	0.29	S0064
	ALB	36	5.22*	3.17	-0.41	0.25	0.97	0.38	S0064
	TF	38	4.48	3.05	-0.36	0.26	1.06	0.41	S0064
	TNC	103	4.92*	2.48	0.17	0.12	-0.64	0.21	S0115 – S0101
8	*GSTA2*	19	5.33*	3.28	0.64	1.41	-10.58	3.47	SW2611-S0086
	AMBP	26	3.74	2.05	0.38	0.35	2.33	0.88	SW2611 – S0086
	GC	22	3.40	1.61	0.15	0.33	2.17	0.83	SW2611 – S0086
9	*SERPINA3-2*	27	4.97*	3.14	2.19	1.27	-6.24	2.31	SW911-SW54
10	*PI*	118	5.75*	3.47	-2.07	1.00	4.32	2.01	SW951
11	*CAPNS1*	0	4.97*	2.55	-3.76	1.87	-5.64	2.75	SW2008
13	*PPP1R3B*	31	4.91*	3.37	-0.46	0.14	0.52	0.41	S0219 – SW344
	TNC	120	5.77*	3.10	-0.11	0.13	-0.80	0.24	S0289

- *significant at the 5% chromosome-wide level
- **significant at the 1% chromosome-wide level
- ***significant at the 5% genome-wide level
- SE: standart error
- Add: Additive effect and standard error. Positive values indicate the Duroc alleles result in higher values than Pietrain alleles; negative values indicate that Duroc alleles result in lower values than Pietrain alleles.
- Dom: Dominance effect and standard error.
- Chromosomal position in Kosambi cM
- Var: The percentage of phenotypic variance explained by the eQTL
- SSC: Sus scrofa chromosome
- The closest markers were those markers around the peak, as near as possible

Table 4.6: Summary of the two linked eQTL for gene expression in the DUPI F2 resource population

SSC	Trait	Position (cM)		F -value		Var (%)	Effect A		Effect B		Closest marker	
		eQTL A	eQTL B	2vs0	2vs1		Add±SE	Dom±SE	Add±SE	Dom±SE	eQTL A	eQTL B
1	*PPARGC1*	35	188	4.0	2.0	4.02	-0.49± 2.1	-7.56± 3.7	-5.59± 2.4	-13.3±4.9	SWR2300	SW1301
2	*ALB*	39	124	6.4*	5.0	4.17	1.88± 0.4	-2.79± 0.6	-0.93± 0.3	1.53±0.5	S0141	SW1564
	PPP1R3B	42	124	5.5	3.3	3.98	2.16± 0.5	-3.27± 0.8	-0.87± 0.4	1.60±0.6	S0141	SW1564
	TTR	30	125	4.7	5.4	4.16	1.35± 0.4	-2.02± 0.6	-1.11± 0.3	1.17±0.5	S0141	SW1564
	APOC3	33	123	6.3*	7.5	4.09	1.20± 0.3	-1.53± 0.4	-0.72± 0.2	1.56±0.4	S0141	SW1564
	APOA1	33	122	4.5	5.5	3.12	3.49± 1.1	-3.84± 1.4	-1.95± 0.9	3.69±1.4	S0141	SW1564

- *significant at the 5% chromosome-wide level
- SE: standart error
- Chromosomal position in Kosambi cM
- Add: Additive effect and standard error. Positive values indicate the Duroc alleles result in higher values than Pietrain alleles; negative values indicate that Duroc alleles result in lower values than Pietrain alleles
- Dom: Dominance effect and standard error
- Var: The percentage of phenotypic variance explained by the eQTL
- SSC: Sus scrofa chromosome
- Chromosomal position in Kosambi cM
- The closest markers were those markers around the peak, as near as possible

Table 4.7: Summary of imprinting eQTL locations and their relative additive, dominant and imprinting effects on gene expression values the DUPI F_2 resource population

SSC	Trait	Position (cM)	F – Value	V (%)	Add	SE	Dom	SE	Imp	SE	Inferred mode of expression	Closest marker
2	*APOC3*	31	3.22	2.78	0.89	0.32	-1.34	0.54	-0.04	0.3	Maternal	S0141
	ALB	33	5.57*	3.45	1.27	0.36	-2.1	0.56	-0.41	0.35	Maternal	S0141
	PPP1R3B	33	5.45*	3.48	1.48	0.41	-2.25	0.63	-0.50	0.39	Maternal	S0141
	GSTA2	33	5.53*	3.56	3.55	1.28	-7.46	1.97	-3.20	1.23	Maternal	S0141
	SERPINA3-2	31	5.72*	3.60	5.91	1.66	-8.97	2.76	0.76	1.58	Paternal	S0141
	RBP4	33	2.96	3.05	4.09	1.74	-7.42	2.67	-1.22	1.66	Maternal	S0141
	TTR	33	4.4*	3.15	0.76	0.37	-1.92	0.57	-0.92	0.35	Maternal	S0141
9	*SERPINA3-2*	26	4.83*	3.46	2.15	1.24	-5.77	2.25	-2.18	1.32	Maternal	SW911

- *significant at the 5% chromosome-wide level
- SE: standart error
- Add: Additive effect and standard error. Positive values indicate the Duroc alleles result in higher values than Pietrain alleles; negative values indicate that Duroc alleles result in lower values than Pietrain alleles
- Dom: Dominance effect and standard error
- Var: The percentage of phenotypic variance explained by the eQTL
- SSC: Sus scrofa chromosome
- The closest markers were those markers around the peak, as near as possible
- Chromosomal position in Kosambi cm

5 Discussion

5.1 eQTL analyses of *PPARGC1*, *ALB*, *PPP1R3B*, *GSTA2*, *SERPINA3-2*, *RBP4*, *APOC3*, *AMBP*, *GC*, *APOA1*, *TF*, *ATF4*, *TNC*, *PI*, and *CAPNS1*

Analysis of expression quantitative trait loci (eQTL) provides a means for detecting transcriptional regulatory relationships at a genome-wide scale (Michaelson et al. 2009). Many investigations have reported the successful mapping of eQTL in rats, mice, humans or pigs with different sample sizes (Hubner et al. 2005; Morley et al. 2004; Ponsuksili et al. 2010; Stranger et al. 2005). But experimental data from genetic investigations of gene expression in farm animals are still limited (Ponsuksili et al. 2010). Mapping of eQTL to the specific gene indicates that *cis*-changes are responsible for the different expression levels, whereas mapping positions of eQTL that are different from the positions of the corresponding genes indicate *trans*-regulation. In this study, 25 eQTL on 11 different porcine autosomes were detected that shows *trans*-regulation of these genes which were consistent with the previous reports for pigs (Ponsuksili et al. 2008a) and other species (Bystrykh et al. 2005; Morley et al. 2004). Ponsuksili et al. (2010) stated that the expression of genes associated with traits that have low heritability (that is, the high dependency on non-genetic factors), like drip loss or meat pH is coincides with the fact that they are under the control of several *trans*-regulated genes, that is, genes that are controlled by other genes. *Trans*-eQTL usually reflect genetic regulation that is dispersed across many loci with small effects means that they explain small phenotypic variance (Gibson and Weir 2005) and it was shown that the expression level of the trans-regulated genes was less heritable than that of *cis*-regulated genes (Petretto et al. 2006). *Trans*-regulated genes appear to be more complex (i.e., under polygenic control) than the *cis*-regulated genes and likely to reflect the additive outcome of genetic, epigenetic, and environmental regulation (Petretto et al. 2006).

A total of 25 eQTL for gene expression of 15 candidate genes were detected on 11 different porcine automes. A LOD score of 1.8 assumed as cut-off for the given eQTL. Seventeen of all eQTL on SSC 1, 2, 3, 4, 6, 7, 8, 9, 10, 11 and 13 were detected at suggestive level ($p<0.05$; CW). The rest 8 eQTL could not reach the 5% CW suggestive level. Among them, only an eQTL for *SERPINA3-2* on SSC2 could reach the genome-wide 5% significance level. The basic suggestive level is accepted as 5% chromosome-wide significant value. From the results of the linkage analysis using gene expressions,

all detected eQTL are likely to be *trans*-regulated since gene positions and detected eQTL did not overlap within 20 Mb (general consideration for *cis*-regulated genes) intervals. In the same animal population (DUPI), eQTL by microarray expression profile revealed that 96 of 104 trait associated eQTL were reported as *trans*-regulated (Ponsuksili et al. 2008a). This result is consistent with the previous result of Ponsuksili et al. (2008a) since they also reported eQTL of these genes as *trans*-regulated. On SSC1 an eQTL was detected for *PPARGC1* close to marker *SW1301* at 187 cM. In the same marker position, neither drip loss nor muscle pH QTL was detected in the same population (Liu et al. 2007). However, three QTL for skeletal muscle pH were detected between the markers *SW1828* and *SW1301* (Sanchez et al. 2006). Beeckman et al. (2003) also identified a significant QTL for conductivity 24 h pm in the same marker interval in Meishan, Pietrain and European Wild Boar cross population. On the other hand, by using a two-QTL model, an additional eQTL was detected for *PPARGC1* at 35 cM between the markers *SWR2300* and *SW1653*. The detected eQTL in our study overlaps with several other eQTL in the same marker region (Ponsuksili et al. 2008a). No QTL was detected for drip loss and pH in the DUPI population. Although, several significant QTL were detected for muscle pH in this population between the markers *S0312* and *S0113*, this region is not close to our detected eQTL region. However, significant QTL for longissimus dorsi muscle pH and conductivity 24 h pm were detected in Duroc and Berlin Miniature (DUMI) cross between the markers *SW1515* and *SW1851* (Ponsuksili et al. 2005; Wimmers et al. 2006). In total 8 eQTL were detected on SSC2 of which 6 eQTL were detected vicinity of marker *S0141*. In the DUPI population a suggestive QTL for drip loss was also detected between the markers *SW2623* and *S0141* at 20.1 cM (Liu et al. 2007). Malek et al. (2001) estimated a QTL for drip loss on SSC2 between the markers *SWR2157* and *SWR345* in a Berkshire and Yorkshire cross overlapping with our detected eQTL. A significant QTL for pH 24 h pm was found at the marker site of *SW240* in a Meishan, Pietrain and European wild boar cross population (Lee et al. 2003). Heuven et al. (2009), observed also ultimate pH QTL between the markers *SW1686* and *SW2167* (65 cM) close to our estimated eQTL. Several other QTL for drip loss and muscle pH have been identified in different experimental populations. Van Wijk et al. (2006) identified a significant QTL for drip loss on SSC2 at 32 cM (*SWR738 – SW240*) in a cross of Pietrain / Large White boar line and commercial sow. In a similar experimental population an additional QTL for drip

loss has been reported by Kim et al. (2005b) at 35 cM (*SW2445 – SW1686*). Another QTL was also observed for drip loss at 46.2 cM (*SW2445 – SW766*) by Thomsen et al. (2004). Several other QTL for longissimus dorsi pH at 0-10 cM have been identified by other groups (de Koning et al. 2003; Evans et al. 2003; Rohrer et al. 2006). The positions of our detected eQTL are interesting not only because of coinciding QTL and eQTL regions, but also while different chromosomal positions were mapped with the two-eQTL model and imprinting eQTL model. With the two-eQTL model, beside the eQTL detected for *ALB*, *PPP1R3B*, *TTR*, *APOC3* and *APOA1* close to marker *S0141*, the second eQTL was detected proximal to marker *SW1564*. Malek et al. (2001), reported a significant QTL for drip loss at 114.4 cM between the markers *SWR2157* and *SWR308*. In the same marker region, several other muscle pH QTL were also mapped which is coinciding with our detected eQTL (Qu et al. 2002; Rohrer et al. 2006; Thomsen et al. 2004). Harmegnies et al. (2006) reported a significant QTL for cooking loss between the markers *SWR2157* and *SW2513* in Landrace x Large White x Pietrain cross population. In the similar region, QTL for tenderness score in pigs are coinciding with our detected eQTL for 5 genes which were mapped in different pig populations (Ciobanu et al. 2004; Kim et al. 2005a; Meyers et al. 2007; Stearns et al. 2005). Moreover, our detected imprinting eQTL on SSC2 showed maternally (*APOC3*, *ALB*, *PPP1R3B*, *GSTA2*, *RBP4* and *TTR*) and paternally expression (*SERPINA3-2*). Although, in pig very few imprinting genes has been identified, evidence of imprinting QTL have been reported for some specific chromosomes including SSC5 and SSC9 (Rohrer et al. 2006; Thomsen et al. 2004). Previously, de Koning et al. (2000) identified a QTL for body composition in pigs, they detected four QTL out of five on SSC2, 6 and 7 were subjected to imprinting. In mouse, genomic imprinting as a form of epigenetic regulation has been shown to influence several sub-chromosomal areas (Hudson et al. 2010). Although, the mechanisms underlying the imprinting genes in pigs are not fully understood (Bischoff et al. 2009), the genes insulin-like growth factor 2 (*IGF2*) and *H19* express paternally and maternally, respectively, in humans, mice, sheep, cattle and as well as in pig (Amarger et al. 2002; Li et al. 2008). Moreover, *IGF2* is the major candidate for a paternally expressed QTL in the pig primarily affecting muscle development. Considering the complex regulation of *IGF2* it has been shown that there is close interaction between *IGF2*, *INS* and *H19* on SSC2p (Amarger et al. 2002). On SSC2 two eQTL were detected for *AMBP* and *GC* at 118 cM close to marker *SW240*. A

suggestive QTL for drip loss on SSC2 at 114.4 cM was reported by Malek et al. (2001) which is close to our detected eQTL for *GC* between the markers *SWR2157* and *SWR308*. Moreover, a QTL for water-holding capacity was found by Malek et al. (2001) between *SW1564* and *SW1370*. On SSC3 a 5% chromosome-wide suggestive level eQTL was identified for PPP1R3B close to marker S0002 at 86 cM. There is no any reported QTL vicinity of our detected eQTL in the same population (Liu et al. 2007). Only a QTL previously reported for pH 45 min pm was detected between the markers *SW2047* and *ACTG2* covering also our detected eQTL (Edwards et al. 2008). On SSC4 two suggestive eQTL were detected for *RBP4* and *APOA1* at 67 and 72 cM, respectively close to marker *S0214*. In DUPI population, a QTL for drip loss was mapped between the markers S0214 and S0097 (Liu et al. 2008). de Koning et al. (2001) reported a QTL for drip loss spanning the markers *S0301* and *S0001* in Meishan and commercial cross population. Moreover, different research groups found various QTL for pH for 24 hour post-mortem in loin between the markers *S0001* and *S0097*. (Mercade et al. 2005; Wimmers et al. 2006). Additionally, our detected eQTL for *RBP4* and *APOA1* was harbored with previously reported QTL for water-holding capacity on SSC4 in Large white and Meishan cross population (Su et al. 2004). On SSC6, an eQTL was detected for *PPARGC1* at 30 cM between the markers *S0035* and *S0087*. Liu et al. (2008) identified a drip loss QTL in the DUPI population between the same marker interval with our detected eQTL. Another QTL for drip loss was reported at 74 and 61.2 cM between the markers *SW1057* and *S0220* (de Koning et al. 2001; Markljung et al. 2008). Moreover, several pH 24 h pm in loin QTL were mapped to close region of our detected eQTL is also supporting our findings for the *PPARGC1* eQTL (de Koning et al. 2001; Kim et al. 2005b; Markljung et al. 2008). On SSC7, three eQTL for *APOC3*, *ALB* and *TF* were detected at 33, 36 and 38 cM, respectively, close to the marker S0064. Although, there is no reported drip loss QTL close to our detected eQTL, several significant and suggestive QTL for muscle pH and pH decline in different time points were mapped in vicinity of eQTL region (de Koning et al. 2003; Demeure et al. 2005; Duan et al. 2009b; Evans et al. 2003). On SSC7, three eQTL for *APOC3*, *ALB* and *TF* was detected at 33, 36 and 38 cM, respectively, close to marker *S0064*. Although, there is no reported drip loss QTL close to our detected eQTL, several significant and suggestive QTL for muscle pH and pH decline at different time points were mapped in vicinity of the eQTL region (de Koning et al. 2003; Demeure et al. 2005; Duan et al.

2009; Evans et al. 2003). The eQTL for TNC on SSC7 was suggestive ($p < 0.05$; CW); in the same marker interval (*SW175 – S0115*), an association was detected between the detected polymorphism of non-annotated EST (Affymetrix probe set ID: Ssc.25503.1.S1_at) and drip loss and pH 24 h pm in loin (Srikanchai et al. 2010). A significant QTL for pH 24 h pm in loin was detected between the markers S0115 and S0066 on SSC7 (Yue et al. 2003). Moreover, two meat color QTL were found close to marker *SW1806* on SSC7 (Ovilo et al. 2002; Thomsen et al. 2004).

On SSC8, a suggestive ($p < 0.05$; CW) eQTL for GSTA2 was found at 19 cM (*SW2611 – S0086*). No drip loss QTL was detected neither in the DUPI nor in another population. However, a QTL for longissmus dorsi pH was mapped between the markers *SW905* and *SW211* at 42 cM (Rohrer et al. 2006). Moreover, a significant conductivity 45 min pm QTL was detected in a Meishan x Pietrain x European Wild Boar cross population (Beeckmann et al. 2003). On chromosome 9, an eQTL for *SERPINA3-2* was detected at 27 cM close to marker *SW911*. Several QTL for muscle pH were reported in different populations (de Koning et al. 2003; Evans et al. 2003). Nevertheless, there is no QTL previously reported for drip loss or water-holding capacity in this region. Interestingly, a suggestive paternally imprinted eQTL was also detected for *SERPINA3-2* at 26 cM close to marker *SW911*. Moreover, based on the homology between SSC9 and HSAP7q21.3, the paternally expressed 10 (*PEG10*) gene was reported as an imprinted gene in pigs. However, *PEG10* is located on SSC9q, this region is far from our detected eQTL interval. Nevertheless, two maternally expressed QTL for drip loss and muscle pH were given by two different studies in pigs (de Koning et al. 2001; Thomsen et al. 2004). The only eQTL on SSC10 was mapped for *PI* at 118 cM close to marker *SW951*. Neither drip loss nor pH QTL was found at the similar marker region. However, in same animal population a significant conductivity QTL was found between the markers *S0070* and *SW951*. Moreover, two pH decline QTL were reported in the same marker interval (Duan et al. 2009b). The only eQTL for *CAPNS1* was found on SSC11 at 0 cM position at *SW2008* marker. A drip loss QTL was mapped in the vicinity of our detected eQTL at 7 cM in a Berkshire and Yorkshire cross population (Malek et al. 2001). Harmegnies et al. (2006) reported 24 h pm conductivity QTL in a close region to our detected eQTL. Although, different pH and conductivity traits were located on SSC11, none of them were close to the marker *SW2008*. In the DUPI population, there is no detected QTL for drip loss and muscle pH in that region. Two suggestive ($P<0.05$, CW)

eQTL for *PPP1R3B* and *TNC* were detected on SSC13. Of these, *PPP1R3B* was found between the markers *S0219* and *SW344* at 31 cM. On SSC13, there was no estimated QTL close to these markers in the same population. Our eQTL coincided with a previously repoted significant QTL for pH 45 min pm in a Pietrain-Large White boar and commercial sow cross at 36 cM between the markers *SWR428* and *SW864* (van Wijk et al. 2006). Moreover, Malek et al. (2001), reported a QTL for water-holding capacity at 43 cM close to our eQTL for *PPP1R3B*. The second eQTL on this chromosome was detected for *TNC* at 121 cm close to marker *S0289*. There was no detected QTL overlapping with this eQTL in the same population and in other population given in the literature.

5.2 Association of muscle specific gene expression profile with meat quality traits and contribution of the genes in meat quality

5.2.1 Peroxisome proliferator–activated receptor-γ coactivator-1 (*PPARGC1*)

Peroxisome proliferator–activated receptor-γ coactivator-1 (*PPARGC1*) was found to be associated with drip loss ($p = 0.03$) (Table 4.3). This gene also was found to be differentially regulated in high vs. low drip loss mentioned in the result part and it is significantly up-regulated in high drip loss (Figure 4.4). *PPARGC1* is a coactivator of PPAR-γ and other nuclear hormone receptors and plays an essential role in energy homeostasis (Esterbauer et al. 2002), body weight regulation and composition (Jacobs et al. 2006). The *PPARGC1A* gene is a coactivator of a subset of genes that control oxidative phosphorylation. *PPARGC1A* exerts its function through a whole range of nuclear hormone receptors and other transcription factors, and is primarily expressed in tissues with high energy demands (Larrouy et al. 1999). Besides having an important influence on the regulation and composition of the body weight, it also is an important factor in determining muscle fibre type composition (Dulloo and Samec 2001; Lin et al. 2002; Mortensen et al. 2006). It has been shown that *PPARGC1A* increases the amount of oxidative muscle fibres, and that it also is expressed at a higher level in these muscle fibres. Mutations in *PPARGC1* showed significant association with aerobic fitness related with endurance capacity in human (Lucia et al. 2005). Post-mortem metabolism in terms of pH decline of muscle is substantially affected from oxygen availability and

muscle temperature which causes of drip loss in meat. Wimmers et al. (2006) reported *PPARGC1A* as a functional candidate gene for muscle fibre traits. Associations have been found between mutations in the coding region of *PPARGC1A* and certain fat characteristics in the pig (Jacobs et al. 2006b; Kunej et al. 2005; Stachowiak et al. 2007). The vicinity of SSC8, where *PPARGC1A* is located, exhibits a significant QTL for longissimus dorsi muscle pH between the markers *SW905* and *SW211* (Rohrer et al. 2006). Corresponding with findings in humans (Larrouy et al. 1999), expression of *PPARGC1A* found to be higher in m. longissimus dorsi compare to backfat. It was reported that this gene as a interesting candidate gene for meat quality (Erkens et al. 2006). These findings are in accordance with our result that expression of this gene was found to be significantly associated with drip loss (Table 4.3). In our population this gene showed higher expression in high drip loss group compared to low drip loss group (Figure 4.4). These results suggest that *PPARGC1* is likely to be a putative candidate gene for drip loss in porcine meat.

5.2.2 Apolipoprotein C-III (*APOC3*) and Apolipoprotein A-I (*APOA1*)

Apolipoprotein C-III (*APOC3*) mapped to SSC9p13 in accordance human-porcine comparative map. *APOC3* has a in central role in fat cell differentiation and muscle fibre type determination (Kunej et al. 2005). It is a strong inhibitor of lipoprotein lipases (LPL), a key enzyme in fatty acid delivery to muscle and adipose tissue. Thomsen et al. (2004) detected a significant drip loss QTL detected in Duroc and Pietrain F_2 population *SW2401* and *SW174* markers which is close to the position of *APOC3* (Ensembl). Furthermore, on q arm of SSC9, 3 QTL were identified for post-mortem muscle pH in different significancy levels (de Koning et al. 2001; Glenn et al. 2007; Harmegnies et al. 2006). The *APOC3* gene is located in the confidence interval of lipid content, stearic acid percentage and fat percentage QTLs reported by different researchers (Edwards et al. 2008; Nii et al. 2006; Rohrer et al. 2006). Negative phenotypic correlation exists between the carcass traits related to fatness (back fat, fat area, as well as fat-to-meat ratio) and drip loss in the DUPI population (-0.19, -0.27, -0.31, respectively). Moreover, genes of the GO category of lipid metabolism were also enriched in the low drip loss group (Ponsuksili et al. 2008b). In our study, although expression of *APOC3* was found to be associated with drip loss, the expression of this gene was found slightly higher in

high drip loss group compared to low drip loss group (Figure 4.4). Furthermore, in pig and beef, lipid content was found to be correlated with muscle pH (Lonergan et al. 2007; Page et al. 2001).

Apolipoprotein A-I (*APOA1*) is the major apoprotein of high density lipoprotein (HDL) and is a relatively abundant plasma protein. This gene was mapped to position SSC9p1.1-1.2. Near position to this gene, a drip loss QTL was reported between the markers *SW2401* and *SW174* on this chromosome. Moreover, vicinity of *APOA1* gene, several QTL for post-mortem muscle pH was reported (Thomsen et al. 2004; de Koning et al. 2001; Harmegnies et al. 2006; Evans et al. 2003; de Koning et al. 2003). Abundance of pH and drip loss QTL where *APOA1* is located, found to be in accordance with our gene expression and meat quality association results.

5.2.3 Calpain, small subunit 1 (*CAPNS1*)

Calpain, small subunit 1 (*CAPNS1*), encodes the small 28-kDa regulatory subunit which form heterodimers with *CAPN1* and *CAPN2* genes. Calpains are a family of Ca^{+2}-dependent intracellular cysteine. The *CAPNS1* gene is mapped to SSC6q1.1-1.2 (Drogemuller and Leeb 2002). The physiological roles and possible functional distinctions of calpains remain unclear, but suggested functions include participation in cell division and migration, integrin-mediated signal transduction, apoptosis, and regulation of cellular control proteins such as cyclin D1 and p53. It has been well documented that these proteases play an important role in proteolytic processes in the muscles, degrading quite a big number of myofibrillar proteins, but not actin, myosin and a-actinin (Juszczuk-Kubiak et al. 2009). Involvement of calpains in water-holding capacity has been shown by different researchers (Huff-Lonergan and Lonergan 2007). Greater activation of μ-calpain within the first 24h post-mortem should correspond to greater proteolysis and less myofibre shrinkage (Huff-Lonergan and Lonergan 2007). A QTL for drip loss on SSC6 harbouring the markers *SW1057* and *S0220* where *CAPNS1* is located (de Koning et al. 2001). On similar region several QTLs for 24h pm loin and ham pH was reported by different researcher. These findings are in accordance with our association analyses (Table 4.3).

5.2.4 Transferrin (*TF*)

The protein encoded by transferrin (*TF*) is a glycoprotein with an approximate molecular weight of 76.5 kDa. Transferrin carries iron from the intestine, reticuloendothelial system and liver parenchymal cells to all proliferating cells in the body. It carries iron into cells by receptor-mediated endocytosis. Over-expression of transferrin has been observed in hypertrophied lamb muscles (Hamelin et al. 2006; Sayd et al. 2006). This muscle group has higher glycolytic metabolism and less developed vascularisation which causes less blood flow through the muscle. Over-expression of transferrin has been observed in the light color meat group compared to dark meat group in pigs (Sayd et al. 2006). In present study, we detected up-regulation of *TF* in the high drip loss group (Figure 4.5) and down-regulation in the pH_{24L} group. It could be hypothesised that lower blood (less iron) supply can cause less O_2 supply and at the end hypoxia and pH decline in skeletal muscle. On chromosome 13q (Ensembl) different QTL for pH and water-holding capacity were detected. Two QTL for longissimus dorsi pH were obtained spanning the markers *S0282* to *SWR428* and *S0068* to *SWR48* respectively (Evans et al. 2003; Rohrer et al. 2006). A water-holding capacity QTL was also detected on SSC13 at 43 cM (Malek et al. 2001).

5.2.5 Alpha-1-microglobulin/bikunin precursor (*AMBP*)

Alpha-1-microglobulin/bikunin precursor (*AMBP*), encodes two plasma glycoproteins: A1M, an immunosuppressive lipocalin, and bikunin, a member of plasma serine proteinase inhibitor family with prototypical Kunitz-type domain (Grewal et al. 2005). A strong immunoreaction of α_1m was observed in developing myocytes in the myotome at early stages of mouse development. α_1m-positive myocytes are seen in every skeletal muscle (Sanchez et al. 2002). *AMBP* was assigned to SSC1q (Ensembl) close to marker S0354. where several QTL for carcass traits were identified (Nonneman et al. 2005). A QTL for drip loss was detected in the vicinity of the *S0056* marker on SSC1q (Malek et al. 2001). Additionally, a QTL for longissimus dorsi pH was detected between *SW1828* and SW2512 (Sanchez et al. 2006). Both QTL were detected close to the position of *AMBP* gene.

AMBP is a extracellular matrix protein and plays a role during the regulation of development, cell growth, metabolism, immune response and modulates extracellular matrix protein as well as the level of intracellular calcium (Grewal et al. 2005). The extracellular matrix is a part of three connective tissue layers (endomysium, perimysium and epimysium) surrounding muscle fibers. The extracellular matrix is composed of proteins including collagens and proteoglycans, having various functions in skeletal muscle. The reduced amount of proteoglycans in animals which are selected for growth rate and muscling, can lead to decreased water-holding capacity (Velleman 2002). Association between gene expression and meat quality traits were estimated with mixed model. A suggestive association was detected between *AMBP* expression with cooking loss ($p < 0.1$) (Table 4.5). However, existence of correlation with cooking loss and drip loss, no association was detected between expression of *AMBP* and pH_{1L}, pH_{24L}, drip loss, thaw loss and shear force.

5.2.6 Vitamin D binding protein (*GC*)

Vitamin D-binding protein (*GC*) is a multifunctional serum glycoprotein and it is the major serum transport protein for vitamin D sterols (Hirai et al. 2000; Jiang et al. 2007). Certain allelic variations within the sequence of *GC* have an effect on glucose tolerance and insulin secretion. *GC* plays also an important role in clearance of cellular actin from the extracellular space (Goldschmidt-Clermont et al. 1988). Actin is one of the most abundant and highly conserved proteins in eukaryotic cells and it is involved in different functions including cell motility, control of cell shape and muscle contraction (Otterbein et al. 2002). By this effect *GC* might also be a regulator for meat quality in pigs as the structure of muscle and its degree of contraction influences the amount of water that can be held by the muscle (Huff-Lonergan and Lonergan 2007). The expression of this gene was found to be associated with cooking loss ($p < 0.05$). According genetic mapping based on our genotyping results, this gene is located on SSC8 between the markers *SW2160* and *S0144* in the DUPI population. A QTL for pH_{45} close to marker S0144 was estimated in Duroc and Berlin Miniature (DUMI) resource population (Wimmers et al. 2006). Several other QTL for different meat pH parameters were detected in the same marker interval where *GC* is located (Duan et al. 2009).

5.2.7 Protein phosphatase 1, regulatory (inhibitor) subunit 3B (*PPP1R3B*)

The glycogen-targeting *PP1* (protein phosphatase 1) subunit (G_L) gene is expressed in muscle and liver in humans at comparable levels. Its effects on muscle glycogen metabolism are still unknown; however G_L has been shown to modulate the activity of PP1 on glycogen-metabolizing enzymes, either purified or in the context of hepatic cells (Montori-Grau et al. 2007; Munro et al. 2002). Purified G_L enhances the muscle GS (glycogen synthase) phosphatase activity of PP1, whereas it decreases muscle GP (glycogen phosphorylase) phosphatase activity (Doherty et al. 1995). Muscles with low glycogen and lactate levels showed normal rates of post-mortem glycolysis and optimal meat quality. On the other hand, muscle with high glycogen and lactate content showed rapid post-mortem glycolysis, paler surface colour, high drip loss and higher extents of protein denaturation than muscles with high glycogen and low lactate content (Choe et al. 2008). Expression of the *PPP1R3B* gene showed suggestive association ($p < 0.05$) with pH_{24L} (Table 4.3). *PPP1R3B* is locating on SSC15 (Ensembl) close to *SW1401*. Vicinity of the *SW1401*, several QTL for drip loss and post-mortem muscle pH was detected. Thomsen et al. (2004) was identified a QTL for drip loss close to marker *SW1263*. An additional significant QTL for drip loss was also found between the markers *SW964* and *SY1*. Moreover, Liu et al. (2008) detected a QTL for ham pH between the markers *SW1111* and *SW936* in the DUPI population.

5.2.8 Tenascin-C (*TNC*)

The *TNC* gene is a member of a family of genes coding for extracellular matrix protein (Garrido et al. 1995). It has a function in cell communication, extracellular matrix-receptor interaction and focal adhesion. *TNC* counterbalances cell adhesion to substrata, correlated with cytokinesis and motility, and prevents cells from adhering too tightly to other extracellular matrix proteins (Imanaka-Yoshida et al. 2001). The extracellular matrix is a part of three connective tissue layers surrounding muscle fibers. The connective tissue layers are the endomysium, perimysium and epimysium. The extracellular matrix is composed of fibrous and nonfibrous proteins including collagens and proteoglycans. If the amount of proteoglycan has been reduced in animals selected for growth rate and muscling, the reduced degree of water holding capacity would directly impact the meat juiciness and drip loss (Velleman 2002). Gene mapping for

TNC showed that this gene is located on SSC1q (Ensembl). Expression of *TNC* gene was found significantly associated ($p < 0.01$) with pH_{1L} and cooking loss ($p < 0.05$) (Table 4.3). Sanchez et al. (2006) reported three significant QTL for semispinalis dorsi, longissimus dorsi and biceps femoris on SSC1q. A significant QTL for pH_{24L} was also found in DUPI population in very close region to *TNC* location between the markers *S0312* and *S0113*.

5.2.9 Retinol binding protein 4 (*RBP4*)

This protein belongs to the lipocalin family and is the specific carrier for retinol (vitamin A) in the blood. It delivers retinol from the liver stores to the peripheral tissues. In plasma, the RBP-retinol complex interacts with transthyretin which prevents its loss by filtration through the kidney glomeruli. A deficiency of vitamin A blocks secretion of the binding protein posttranslationally and results in defective delivery and supply to the epidermal cells. *RBP4* is a novel adipokine that likely contributes to systemic insulin resistance and dyslipidemia (Shea et al. 2007; Vitkova et al. 2006). The role of genetic variations in *RBP4* on phenotypes of glucose and lipid metabolism is not clear in humans. However, Shea et al. (2009) detected five SNPs within *RBP4* (rs3758539, G/A 5' flanking region; rs61461737, A/G intron; rs10882280, C/A intron; rs11187545, A/G intron; and rs12265684, C/G intron). After correcting for multiple testing, they observed a significant association between the minor allele of two noncoding SNPs (rs10882280 and rs11187545) and higher serum HDL-C ($p = 0.043$ and 0.042, respectively). Different investigations showed function of *RBP4* in insulin resistance. Insulin resistance occurs under conditions of obesity, metabolic syndrome, and type 2 diabetes. It was found to be accompanied by down-regulation of the insulin responsive glucose transporter *GLUT4* gene. Decreased adipocyte *GLUT4* caused secretion by adipocytes of the serum retinol-binding protein *RBP4*. Enhanced levels of serum RBP4 appeared to be the signal for the development of systemic insulin resistance both in experimental animals and in humans (Wolf 2007). The blood level of glucose is maintained within a narrow range. In case of excess, such as after a meal, glucose is taken up by skeletal muscle; in case of insufficiency, as during starvation, glucose is produced and released by the liver. Glucose uptake is regulated by insulin: in case of a high level of glucose, insulin is secreted and stimulates uptake, storage, and metabolism in skeletal muscle

and adipose tissue (Shepherd and Kahn 1999). Suh et al. (2010) investigated the association between serum RBP4 concentrations and insulin resistance in perimenopausal women serum. They found, RBP4 concentrations positively correlated only with fasting glucose and serum RBP4 appears to identify age-induced insulin resistance by physiologic changes due to aging or menopause and by increasing hepatic glucose production. In pigs, *RBP4* was mapped on SSC14 close to marker *SWR925* (Ensembl). QTL studies showed that, a drip loss QTL was mapped close to *SW1081* and *SW1557* where *RBP4* is located. Different QTL positions have been also found for meat pH close to *RBP4* position (Malek et al. 2001; Wimmers et al. 2006).

6 Summary

The present study was carried out (i) to quantify transcript abundance of drip loss related genes and further figure out the expression profile of these genes in low and high drip loss and meat pH in the Duroc / Pietrain (DUPI) F_2 resource population, (ii) to understand the association of gene expression with meat quality traits (iii) to identify expression quantitative loci (eQTL) of drip loss related genes.

Twenty up or down regulated genes including three reference genes from the microarray results were selected for this purpose. In total 300 DUPI animals were subjected for the gene expression and eQTL analysis and all animals were genotyped using 122 microsatellite markers. In total, 25 eQTL were identified on porcine autosomes 1, 2, 3, 4, 6, 7, 8, 9, 10, 11 and 13. Among 20 traits, eQTL of 13 traits (*PPARGC1*, *ALB*, *SERPINA3-2*, *PPP1R3B*, *GSTA2*, *APOC3*, *RBP4*, *APOA1*, *TF*, *ATF4*, *PI*, *CAPNS1* and *TNC*) reached the acceptable LOD score threshold (1.8). Among those eQTL, 17 eQTL were detected significant at 5% chromosome-wide level (CW). An eQTL for ALB on SSC2 was detected as 1% chromosome-wide significant level. Moreover, on SSC2, eQTL for *SERPINA3-2* was detected as 5% genome-wide level (GW) which showed the highest F-value in the analysis. On SSC1, a chromosome-wide suggestive eQTL for *PPARGC1* was found between the markers *SW373* and *SW1301* at 187 cM. The most promising eQTL were detected on SSC2 for 6 traits proximal to marker *S0141*. On SSC2, two eQTL were found for *AMBP* and *GC* at 118 cM close to marker *SW240*. Moreover, on SSC2, 5 two-eQTL pairs were detected for *ALB*, *PPP1R3B*, *TTR*, *APOC3* and *APOA1* close to marker *S0141*. There was a distance of 90 cM between these two-eQTL and two microsatellite markers were located between the eQTL loci. Average marker density for SSC2 was given as 15.9 cM. For the all given traits, the first eQTL of two-eQTL model was detected close to marker *S0141* where this region was also detected with single-eQTL model. Another two-eQTL was detected close to marker *SW1564*. Additionally, on SSC2, seven imprinting eQTL were detected, of which 6 were paternally imprinted (maternally expressed) and one was maternally imprinted (paternally expressed). The identified imprinting eQTL on SSC2 were found very close to marker *S0141* at around 33 cM which also detected with the line-cross eQTL model. On SSC3, a suggestive ($p < 0.05$;CW) eQTL was detected for *PPP1R3B* at 86 cM close to marker *S0002*. On SSC4, two eQTL for *RBP4* and *APOA1* were detected at 67 and

72 cM, respectively close to marker *S0214*. On SSC6, only one suggestive ($p < 0.05$; CW) eQTL was detected for *PPARGC1* at 30 cM between the markers *S0035* and *S0087*. Five eQTL were detected on SSC7. Among them three for *APOC3*, *ALB* and *TF* were found close to the marker *S0064* at 33, 36 and 38 cM, respectively. On SSC7, an additional suggestive ($p < 0.05$; CW) eQTL was detected for *TNC* at 103 cM close to marker *S0115*. An eQTL was detected on SSC8 for *GSTA2* at 19 cM close to marker *SW2611*. Additionally, two eQTL for *GC* and *AMBP* were detected at 22 and 26 cM, respectively between the markers *SW2611* and *S0086* on SSC8. On SSC9, an eQTL for *SERPINA3-2* at 27 cM was found between the markers *SW911* and *SW54*. The only eQTL for *PI* and *CAPNS1* were detected on SSC10 and 11, respectively. eQTL for *PI* was detected at 118 cM close to marker *SW951* and eQTL for *CAPNS1* was detected at 0 cM close to marker *SW2008*. On SSC13 two suggestive ($p < 0.05$; CW) eQTL were detected for *PPP1R3B* and *TNC*, at 31 cM and at 120 cM, close to markers *SW344* and *S0289*, respectively.

Regression analysis between meat quality traits and gene expressions showed that among twenty genes, 18 genes showed association with drip loss, thawing loss, cooking loss, pH_{24L}, pH_{1L} and shear force with different significancy levels. The genes *ALB*, *AMBP*, *CAPNS1*, *TNC* and *TYROBP* were found to be associated with more than one trait. Twelve genes including *APOA1* ($p = 0.06$), *ANGPTL4* ($p = 0.09$), *APOC3* ($p = 0.01$), *SERPINA3-2* ($p = 0.06$), *RBP4* ($p = 0.09$), *TYROBP* ($p = 0.07$), *TNC* ($p = 0.07$), *CAPNS1* ($p = 0.05$), *CYP2C33* ($p = 0.08$), *PI* ($p = 0.05$), *A2M* ($p = 0.05$), and *PPARGC1* ($p = 0.03$) were showed association with drip loss trait. Among them *APOC3* and *PPARGC1* showed highly significant association with drip loss ($p = 0.01$ and 0.03, respectively). Moreover, *PPARGC1* gene was also detected to be up regulated in high drip loss group compared to low drip loss ($p < 0.05$). Additionally, three of genes, *GC* ($p = 0.009$), *TYROBP* ($p = 0.04$), *TNC* ($p = 0.03$) and *CAPNS1* ($p = 0.07$) were associated with cooking loss beside drip loss as well. The gene expression of *PPP1R3B* ($p = 0.01$), *ALB* ($p = 0.04$) and *AMBP* ($p = 0.04$) were found to be associated with shear force. *ALB* ($p = 0.06$) was also found to be associated with thawing loss. Moreover, expression of *AMBP* was also associated with pH_{24L} as detected also with shear force. An additional association between pH_{24} loin and expression of *TF* has been estimated ($p=0.06$). The only association for pH_{1L} has been found with the expression of *TTR* ($p = 0.04$).

Gene expression analyses showed that expression of some genes significantly differed between samples high and low drip loss (high drip loss ≥ 1.8 %; low drip loss <1.8 %) and pH_{24L} (high $pH_{24L} \leq 5.49$; low $pH_{24L} > 5.49$). Among twenty genes, *PPARGC1* was found to be significantly differentially expressed between high vs. low drip loss ($p = 0.03$). Moreover, for the high and low drip loss group, the expression level of AMBP gene was also found to be differentially regulated ($p = 0.06$). Both, *PPARGC1* and *AMBP* were up regulated in the group of animals with higher drip loss. Although the expression of other genes was not significantly ($p > 0.1$) different, expression of these genes was slightly higher in high drip loss class. In the high pH_{24L} class, expression of *TTR* and *TF* ($p = 0.06$ and $p = 0.07$, respectively) found to be differentially expressed. The expression of both genes was slightly higher in the high pH_{24L} group. Interestingly, no difference was observed in the mRNA level of genes between high and low pH_{1L} in the DUPI population.

As a summary, our eQTL results suggest that detected genomic regions for drip loss and pH putative candidate genes were coincided with QTL for drip loss and muscle pH on different porcine autosomes. However, our detected eQTL and gene positions were not coincided, this supports that the transcriptional regulation for these genes is likely to be *trans*-regulated. Beside these, on SSC2, the candidate genes underlying the eQTL peaks might be further investigated with the aim to understand gene-gene interactions and genetic pathways for meat quality traits in pigs.

7 Zusammenfassung

Die vorliegende Studie diente, zur (i) Quantifizierung der mRNA-Gehalte für Tropfsaftverlust relevante Gene und zur Erstellung der Expressionsprofile dieser Gene in Proben von Schweinen einer Duroc/Pietrain (DUPI) F2 Ressource Population mit hohem und niedrigem Tropfsaftverlust, des weiteren, (ii) zum Verständnis der Assoziation der Genexpression mit Fleischqualitätsmerkmalen und (iii) zur Identifizierung Expressions- Quantitative Trait Loci (eQTL) für die Tropfsaftverlust relevanten Gene.

Zwanzig hoch bzw. runter regulierten Gene inklusive drei Referenz Gene wurden aus der Microarrayanalyse für die weiteren Experimente ausgewählt. Insgesamt wurden 300 DUPI Tiere für Exppression- und eQTL- Analyse eingesetzt. Insgesamt konnten 25 eQTL auf den Schweine Autosomen 1, 2, 3, 4, 6, 7, 8, 9, 10, 11 und 13 identifiziert werden. Von 20 Genen, erreichten eQTL von 13 Genen (*PPARGC1, ALB, SERPINA3-2, PPP1R3B, GSTA2, APOC3, RBP4, APOA1, TE, ATF4, PI* und *CAPNS1*) einen akzeptablen LOD score Schwellenwert von 1.8. Bei 11 von diesen eQTL wurde mit einer Signifikanz von 5% ein Chromosomweites (CW) Level beobachtet. Für das Gen *ALB* wurde auf SSC2 ein signifikantes eQTL mit einer Chromosomweiten Irrtumswahrscheinlichkeit von 1% detektiert. Des weiterem, erreichten die eQTL auf SSC2 von den *SERPINA2-3* Genen einen genomweiten (GW) Wert von 5%, welcher den höchsten F-Statistik Wert in der Analyse darstellte. Die übrigen fünf eQTL die auch den akzeptablen LOD score Wert erreichten, zeigten weder einen 5% Chromosomweiten noch einen suggestiven 1igen% Chromosomweiten signifikanten Wert. Ein Chromosomweit suggestives eQTL für *PPARGC1* konnte zwischen den Marker *SW373* und *SW1301* bei 187 cM gefunden werden. Die meisten vielversprechenden eQTL für 6 Merkmale waren auf SSC2 detektiert, in der Nähe des Markers *S0141*. Auf dem Autosom SSC2 wurden zwei eQTL identifiziert, für *AMBP* und *GC*, diese befanden sich bei 118 cM in der Nähe des Markers *SW240*. Zudem, konnten auf SSC2 5 zwei-eQTL Paare für *ALB, PPP1R3B, TTR, APOC3* und *APOA1* in der Nähe des Markers *S0141* detektiert werden. Zwischen diesen zwei-eQTL befand sich eine Distanz von 90 cM so wie zwei Microsatelliten Marker. Die durchschnittliche Marker Dichte für SSC2 war vorgegeben mit 15.9 cM. Für alle angegebenen Merkmale, wurde das erste eQTL in der Nähe des Markers *S0141* detektiert, ebenso konnte mittels

des single-eQTL Modells diese Region ermittelt werden. Das zweite eQTL, nach dem zwei-eQTL Modell, konnte im Bereich des Markers *SW1564* festgestellt werden. Des Weiteren, konnte auf SSC2 sieben imprinting QTL gefunden werden, von diesen zeigten 6 paternales Imprinting (maternale Expression) und 1 maternales Imprinting (paternale Expression). Die identifizierten Imprinting eQTL auf SSC2 wurden in der Nähe des Markers *S0141* bei 33 cM gefunden, was mit dem Kreuzungs- QTL Modell ebenfalls detektiert wurde. Auf SSC3 wurde ein suggestiv ($p<0.05$; CW) eQTL für *PPP1R3B* bei 83 cM in der Nähe des Markers *S0002* detektiert. Für *RBP4* und *APOA1* konnten jeweils auf SSC4 ein eQTL bei 67 und 72 cM beim Marker *S0214* detektiert werden. Auf SSC6 wurde nur ein suggestiv ($p<0.05$; CW) eQTL für *PPARGC1* gefunden, dieses befand sich bei 30 cM zwischen den Markern *S0035* und *S0087*. Auf dem Autosom SSC7 konnten fünf eQTL identifiziert werden. Von diesen befanden sich drei für *APOC3, ALB* und *TF* in der Nähe des Markers *S0064* bei 33, 36 und 38 cM. Für *TNC* konnte auf SSC7 ein suggestives ($P<0.05$; CW) eQTL bei 103 cM in der Nähe des Markers *S0115* detektiert werden. Auf SSC8 wurde für *GSTA2* bei 19 cM und in der Nähe des Markers *SW2611* ein eQTL gefunden. Das Weitern, wurde ein eQTL für GC und ein eQTL für *AMBP* bei 22 und 26cM zwischen den Markern *SW2611* und *S0086* auf SSC8 identifiziert. Für *SERPINA3-2* konnte bei 27 cM auf SSC9 zwischen den Markern *SW911* und *SW54* ebenfalls ein eQTL detektiert werden. Die einzigen eQTL für *PI* und *CAPNS1* wurden auf SSC10 und 11 gefunden. Dabei befand sich das eQTL für PI bei 118 cM in der Nähe des Markers *SW951* und das eQTL für *CAPNS1 0* cM proximal zum Marker SW2008. Auf dem Autosom SSC13 konnten zwei suggestive ($p<0.05$; CW) eQTL detektiert werden, sie befanden sich bei 31 und bei 120 cM in der Nähe der Marker *SW344* und *S0289*.

Regressionsanalysen zwischen Fleischqualitätsmerkmalen und Genexpression zeigten das von 20 Genen, 18 mit Tropfsaftverlust, Tauverluste, Kochverluste, pH_{24L}, pH_{1L} und Scherkraft mit unterschiedlichen Signifikanzen assoziierten.. Die Gene *ALB, AMBP, CAPNS1, TNC* und *TYROBP* zeigten dabei eine Assoziation mit mehr als einem Merkmal.

Zwölf Gene, *APOA1 ($p = 0.06$), APOC3 ($p = 0.01$), SERPINA3-2 ($p = 0.06$), RBP4 ($p = 0.09$), TYROBP ($p = 0.07$), TNC ($p = 0.07$), CAPNS1 ($p = 0.05$), CYP2C33 ($p = 0.08$), PI ($p = 0.05$), A2M ($p = 0.05$), ANGPTL4 ($p = 0.09$) und PPARGC ($p = 0.03$)* zeigten eine Assoziation mit Tropfsaftverlust ($p<0.1$), zwei von ihnen, *APOC3* und

PPARGC1, sogar mit einer sehr hohen Signifikanz (p = 0.01 und p = 0.03). Zudem war das Gen *PPARGC1* im Vergleich von hohem Tropfsaftverlust mit niedrigem Tropfsaftverlust im hohen Tropfsaft runter reguliert. Des Weiteren, zeigten drei der Gene, *GC* (p = 0.009), *TYROBP* (p = 0.04), *TNC* (p = 0.03) und *CAPNS1* (p = 0.07) sowohl eine Assoziation mit Tropfsaftverlust als auch mit Kochverlust. Die Expression von *PPP1R3B* (p = 0.01), *ALB* (p = 0.04) und *AMBP* (p = 0.04) erbrachte eine Assoziation mit der Scherkraft. *ALB* (p = 0.06) zeigte ebenfalls eine Assoziation mit den Auftauverlust. Zu dem, erbrachte die Expression von *AMBP* eine Assoziation des Gens mit den Merkmalen pH_{24L} sowie mit der Scherkraft. Zwischen den Merkmal pH_{24} Lende und der Expression von *TF* konnte ebenfalls eine Assoziation beobachtet werden (p = 0.06). Die einzige Verknüpfung für das Merkmal pH_1 Lende konnte mit der Expression von TTR (p = 0.06) gezeigt werden.

Die Expressionsanalyse der Gene zeigte das die Expression einiger Gene signifikante Unterschiede zwischen den Proben mit hohem Tropfsaftverlust und niedrigem Tropfsaftverlust (hoher Tropfsaftverlust $\geq$1.8 %; niedriger Tropfsaftverlust <1.8 %) sowie zum pH_{24L} (hoher $pH_{24L} \leq 5.49$; niedriger $pH_{24L} > 5.49$) aufwiesen. Unter zwanzig Genen, war PPARGC1 eines der Gene welches eine signifikant unterschiedliche Expression zwischen hohem und niedrigem Tropfsaftverlust zeigt (p = 0.03). Ebenso wie das Gen *AMBP*, welches unterschiedlich exprimiert (p = 0.06) war zwischen diesen Gruppen. Beide Gene, *PPARGC1* und *AMBP*, waren runter reguliert in der Gruppe der Tiere mit hohem Tropfsaftverlust. Die Expression der anderen Gene hingegen wiesen keine signifikanten Unterschiede auf, ihre Expression war nur geringfügig höher in der Gruppe mit hohem Tropfsaftverlust. Im Hinblick auf das Merkmal pH_{24L} konnte bei *TTR* und *TF* (p = 0.06 und p = 0.07; Abbildung 4.5) eine unterschiedliche Regulation in der Expression beobachtet werden. Die Expression beider Gene, war in der Gruppe mit hohem pH_{24L} geringfügig höher. Interessanterweise, konnte bei der Betrachtung des Merkmals pH_{1L} keine Unterschiede im mRNA Level in der DUPI Population beobachtet werden.

Zusammenfassend zeigen die eQTL Ergebnisse, dass die entdeckten genomischen Regionen von vier mutmaßlichen Kandidatengenen für Tropfsaftverlust und pH-Wert sich mit QTL für Tropfsaftverlust und Muskel pH-Wert auf unterschiedlichen Schweine Autosomen decken. Hingegen, deuten die gefunden eQTL und Genregionen die sich nicht mit QTLs deckten auf eine trans-wirkende Transkriptionale Regulation hin. Des

weiteren, die auf SSC2 gefundenen Kandidatengene und die zugrundeliegenden eQTL sollten weiter untersucht werden, mit dem Ziel eines bessern Verständnisses der Gen-Gen Interaktionen sowie der genetischen Pathways von Fleischqualitätsmerkmalen in Schwein.

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