Institut für Tierwissenschaften, Abt. Tierzucht und Tierhaltung
der Rheinischen Friedrich – Wilhelms – Universität Bonn

Identification of positional and functional candidate genes for meat and carcass quality in F_2 Duroc x Pietrain resource population

I n a u g u r a l – D i s s e r t a t i o n
zur Erlangung des Grades

Doktor der Agrarwissenschaft
(Dr. agr.)
der
Hohen Landwirtschaftlichen Fakultät
der
Rheinischen Friedrich – Wilhelms – Universität
zu Bonn

vorgelegt im Februar 2011

von

Autchara Kayan

aus

Chiang Mai, Thailand

Referent:	Prof. Dr. K. Schellander
Korreferent:	Prof. Dr. B. Petersen
Tag der mündlichen Prüfung:	31 March 2011
Erscheinungsjahr:	2011

Dedicated with reverence to my beloved parents and all members of my family

Identifizierung von positionellen und funktionellen Kandidatengenen für Fleisch- und Schlachtkörperqualitätsmerkmale in einer F_2 Duroc x Pietrain Ressource Population

Für die Analyse wurden fünf Gene, *AMBP, GC, PPP1R3B, TNC* und *IFI6*, ausgewählt. Die Auswahl dieser Gene erfolgte aufgrund ihrer merkmalsabhängigen unterschiedlichen Expressionsmuster und/oder ihrer Merkmals korrelierten Expression. Zusätzlich wurde für Assoziationsanalysen das Gen *HNF1A* ausgewählt. Eine F1 DuPi Population wurde durch Sequenzierung auf SNPs untersucht sowie genotypisiert. Für alle Gene, wurden die SNPs für 423 Tiere aus zwei Schweinepopulationen einer F2 Duroc x Pietrain (DuPi) (n = 313) und einer kommerziellen Herden von Pietrain (Pi) (n = 110) genotypisiert. Durch vergleichende und genetische Kartierung konnten die Positionen von *AMBP* und *TNC* auf SSC1, *GC* auf SSC8, *PPP1R3B* auf SSC15, *IFI6* auf SSC6 und *HNF1A* auf SSC14 lokalisiert werden. In diesen Regionen konnten ebenfalls QTL für Fleisch- und Schlachtkörperqualitätsmerkmale beobachtet werden. Nur der Polymorphismus im *IFI6* zeigte Assoziation mit den Merkmalen Tropfsaftverlust, pH-Wert sowie einigen anderen Schlachtkörper-und Fleischqualitätsmerkmalen. Die SNPs in den Genen *AMBP, GC, PPP1R3B, TNC* und *HNF1A* konnten mit dem Merkmal pH-Wert assoziiert werden. *AMBP* konnte mit den Merkmalen Leitfähigkeit, Schlachtkörperlänge, Rückenspeckdicke und Fettfläche assoziiert werden. Der Polymorphismus im GC zeigte Assoziationen mit der Fettfläche und der Schlachtkörperlänge. Die SNP von *PPP1R3B* zeigten eine Assoziation mit der Fleischfarbe, Scherkraft und der Schlachtkörperlänge. Bei den SNP von *TNC* hingegen konnte eine Assoziation mit der Fleischfarbe, der Leitfähigkeit, mit der Fleischfläche und dem Schinkengewicht festgestellt werden. Darüber hinaus konnten die Fleischqualitätsmerkmale wie Fleischanteil, Fleisch- und Fettfläche mit den Polymorphismen des *HNF1A* assoziiert werden. Jedoch zeigte keines der Kandidatengene eine signifikante Assoziation zu einem bestimmten Merkmal in beiden Schweinepopulationen. Der Grund dafür liegt wahrscheinlich in den Rasse-spezifischen Effekten die in Verbindung mit den Unterschieden in Schlachtkörper- und Fleischqualität dieser Schweinerassen steht. Die Expressionsanalyse der mRNA sowie der Proteine des Gens *IF16* erbrachten eine runter Regulierung in Proben mit niedrigem Tropfsaftverlust. *HNF1A* hingegen war höher in Muskel mit niedrigem pH-Wert exprimiert. Durch statistische Untersuchungen zeigte diese Studie eine Verbindung der

genetischen Variation in diesen Loci zu phänotypischen Variationen. Diese sechs Gene, *AMBP*, *GC*, *PPP1R3B*, *TNC*, *IFI6* and *HNF1A,* können somit als funktionelle und/oder positionelle Kandidatengene für Schlachtkörper- und Fleischqualitätsmerkmale angesehen werden.

Identification of positional and functional candidate genes for meat and carcass quality in F_2 Duroc x Pietrain resource population

Five genes, *AMBP*, *GC*, *PPP1R3B*, *TNC* and *IFI6*, whose candidacy for meat and carcass quality traits arises from their trait-dependent differential expression and/or trait correlated expression, were selected for analysis. Additionally, *HNF1A* was selected for association analysis. We were screening SNPs in F_1 DuPi population by sequencing and used for genotyping. For all genes, the SNPs were genotyped in 423 animals from two pig populations including F_2 Duroc x Pietrain (DuPi) (n = 313) and a commercial herd of Pietrain (Pi) (n = 110). Comparative and genetic mapping established the location of *AMBP* and *TNC* on SSC1, *GC* on SSC8, *PPP1R3B* on SSC15, *IFI6* on SSC6 and *HNF1A* on SSC14, respectively coinciding with QTL regions for meat and carcass quality traits. Only, the polymorphism of *IFI6* revealed association with drip loss, pH and also with several other measures of carcass and meat quality traits. SNP of *AMBP*, *GC*, *PPP1R3B*, *TNC* and *HNF1A* were associated with pH. *AMBP* was associated with conductivity, carcass length, backfat thickness and fat area. Polymorphism of *GC* was associated with fat area and carcass length. SNP of *PPP1R3B* was associated with meat colour, shear force and carcass length. SNP of *TNC* revealed association with meat colour, conductivity, muscle area and ham weight. Moreover, carcass quality traits such as meat percentage, muscle area and fat area were associated with *HNF1A* polymorphism. However, none of the candidate genes showed a significant association to a particular trait across all populations. This may be due to breed specific effects that are related to the differences in carcass and meat quality of these pig breeds. The mRNA and protein expression of *IFI6* were up regulated in low drip loss. The expression of *HNF1A* was higher in low pH muscle. This study reveals statistic evidence for a link of genetic variation of the region of these loci with phenotypic variation and promotes *AMBP*, *GC*, *PPP1R3B*, *TNC*, *IFI6* and *HNF1A* genes as functional and/or positional candidate genes for carcass and meat quality traits.

Content **Page**

List of abbreviations

ATP	: Adenosine triphosphate
ADP	: Adenosine diphosphate
AMBP	: Alpha-1-microglobulin/bikunin precursor
BLUP	: Best linear unbiased prediction
cDNA	: Complementary DNA
$Conductivity1_L$	: Conductivity at 45 min p.m. in loin
$Conductivity24_L$	: Conductivity at 24 hour p.m. in loin
$Conductivity24_H$	: Conductivity at 24 hour p.m. in loin
CPK	: Creatine phosphor-kinase
CSA	: Cross-sectional areas
DBP	: Vitamin D-binding protein
ddH_2O	: Deionized Millipore water
DFD	: Dark, firm and dry
DHA	: Docosahexaenoic acid
DNA	: Deoxyribonucleic acid
dNTP	: Deoxyribonucleotide triphosphate
DuPi	: Duroc x Pietrain population
EBV	: Estimated breeding values
EC	: Conductivity
ECM	: Extracellular matrix
eQTL	: Expression quantitative trait loci
EU	: European union
GC	: Group-specific component (vitamin D binding protein)
GH	: Growth hormone
GLUT-2	: Glucose metabolism
GP	: Glycogen phosphorylase
HNF1A	: HNF1 homeobox A
IFI6	: Interferon, alpha-inducible protein 6
IMF	: Intramuscular fat
LEA	: Loin eye area
LOD	: Logarithm of the odds (to the base 10)

LSM	: Least square means
MAS	: Marker-assisted selection
mRNA	: Messenger ribonucleic acid
MODY	: Maturity onset diabetes of the young
PCR	: Polymerase chain reaction
$pH1_L$	: pH value at 45 minute p.m. in loin
$pH24_L$	: pH value at 24 hour p.m. in loin
$pH24_H$	: pH value at 24 hour p.m. in ham
p.m.	: Post-mortem
Pi	: Pietrain population
Pop	: Population
PPP1R3B	: Protein phosphatase 1, regulatory (inhibitor) subunit 3B
PRKAG3	: Protein kinase adenosine monophosphate-activated γ3-subunit
PSE	: Pale, soft and exudative
PSS	: Porcine stress syndrome
OPTO	: Meat colour 24 hour p.m. in Mld at $13^{th}/14^{th}$ rib; OPTO star
QTL	: Quantitative trait loci
RN (PRKAG3)	: Rendement Napole (Protein kinase AMP activated, γ3 subunit)
RSE	: Red, soft and exudative
SNP	: Single nucleotide polymorphism
TNC	: Tenascin C
UTR	: Untranslated region
WHC	: Water holding capacity

List of tables

List of Figures

1. Introduction

Pig production is basically an enterprise which is able to convert inedible or inferior quality food into the food rich in animal protein. Pigs are much more efficient at converting grain, pasture and other low quality feed into pork which is used as human food. A majority of meat consumer also do not consume pork. In spite of these drawbacks, the consumption of pigs has greatly increased in recent years due to nutritional awareness in people. Swine producers must raise pigs which are high in lean meat and low in fat if they wish to keep the consumer market. The ultimate goal in all pig production is to produce efficient and profitable pig that yield a carcass high in the cuts of pork desired by the consumer. Moreover, the most important one is the weight loss caused by the drip during storage and retailing. This gives rise to considerable monetary loss. Some studies showed that drip loss had high correlations to the meat quality traits such as pH, conductivity and meat colour (Lee et al. 2000, Otto et al. 2004). Drip loss and related traits had low to moderate heritability ranging from 0.10 to 0.37, indicating that the environmental factors had strong effects on the traits (Hoveniera et al. 1992, Sellier 1998, Sonesson et al. 1998). However, genetic factors are among leading to the cause of the traits that affect on meat quality (de Oliveira Band et al. 2005). Up to now, the researchers are trying to approach this problem by using molecular genetic tools. Identification of major genes and molecular markers is a promising approach for improving economic trait such as drip loss in breeding animals. Improving meat quality is not only changing levels of traits like drip loss, tenderness or marbling but it is also about increasing uniformity (de Vries et al. 2000).

In this study, we used F_2 Duroc x Pietrain (DuPi) and commercial Pietrain (Pi) populations for association study, and only DuPi for mRNA and protein expression. Pietrain breed is used as a terminal sire due to exceptional muscularity and leanness, although Pietrain animals have relatively poor growth performance, low intramuscular fat content and meat quality. The Duroc has complementary features of Pietrain including lower carcass grade, high intramuscular fat content, fast growth, high prolificacy, resistance to stress and superior meat quality (Rohrer et al. 2006). Also overall fatness of Duroc pigs is greater than Pietrain pigs. Therefore, the study of the genetic diversity between Duroc, Pietrain and their crossbred generation will generate important new information of new candidate genes and potential DNA markers related

to meat quality traits. However, genes and mutations affecting economic traits have already been proposed in marker assisted selection plans in pigs and other successful applications might be derived from the identification of new mutations and their inclusion in advanced selection approaches (van der Steen et al. 2005). Among the new fields of genomics recently developed, functional genomics that allow considering many genes at the same time are very useful tools for a better understanding of the function and regulation of genes, and how these participate in complex networks controlling the phenotypic characteristics of a trait. In particular, global gene expression profiling at the mRNA can provide a better understanding of gene regulation that underlies biological functions and physiology related to the delivery of a better pig meat quality (Davoli and Braglia 2008). Previously, the study of gene expression profiles reported a large number of gene expressed differentially in porcine meat with high/low drip loss and low/high pH. All genes were suggested to be a good source of candidate genes for meat and carcass quality traits based on their biological pathway (Ponsuksili et al. 2008a, Ponsuksili et al. 2008b).

In order to gain more knowledge and provide useful data for marker assisted breeding programs and finally to improve pig production in the future, this study was undertaken by selecting candidate genes from previous studies (Ponsuksili et al. 2008a, Ponsuksili et al. 2008b, Fan et al., 2010a) based on their functions and/or location on QTL regions related to meat or carcass quality traits. Therefore, the objective of this study is to identify and analyze loci that may contain genetic variation underlying the meat quality and carcass traits in pigs.

2. Literature review

The importance of meat and carcass quality traits in the pork industry is beyond question. The quality of carcasses, meat and meat products is of predominant importance in a competitive market where consumers tend to have a preconceived idea about the criteria that define meat quality such as flavour, tenderness, juiciness, odour, colour and texture. The carcass evaluation could be interesting as a precocious classification of the final quality of meat coming from each carcass. Today the quality characteristics of the meat must be different according to its utilisation (supermarket, butcher, catering, refectory, etc.) and so it is very important to choose very early the final destination of the carcass. Obviously, the carcass classification must correlate with meat quality characteristics required by the final consumer (Lazzaroni et al. 2007). However, their practical improvement from a genetic standpoint is limited by the fact that no industry-wide programme has been established for their improvement. Indirectly however, through positive genetic associations between growth rate, live weight and eye muscle area, it appears likely that breeders have been selecting for improved lean meat yield already. Other traits such as tenderness, meat colour and fat colour are more difficult to improve through selection (Sellier 1998). In addition, there are negative genetic correlations between meat quality traits, for example improving marbling can lead to a decrease in lean meat yield. There are several important indicator traits for meat quality, such as pH, water holding capacity and colour. Their phenotypic and genetic correlations with performance traits and their estimated heritabilities are needed to combine meat quality with performance traits in a breeding program (Sonesson et al. 1998). Several studies have estimated heritabilities and correlation coefficients between these traits. Heritabilities for meat quality traits ranged from 0.20 for ultimate pH (Hoveniera et al. 1992). Water holding capacity traits had average heritability estimates of about 0.10-0.37 (Sellier 1998, Sonesson et al. 1998) and colour of 0.19-0.46 (Sonesson et al. 1998, Tholen et al. 2005). Pork tenderness is a highly heritable trait (0.45) in both Duroc and Landrace pigs (Lo et al. 1992, Suzuki et al. 2005). Although the results of these studies are fairly consistent, the range of the available heritability estimates is wide for some of the traits, due to differences in the mean values of breeds and the small sizes of recorded pig samples that were used in these studies. Also, different recording methods resulted in large differences in heritability estimates,

especially for the water holding capacity traits (Sonesson et al. 1998). Confidence in these estimates is constrained somewhat by the small numbers were used. It is well recognised that the expression of meat and carcass traits is critically influenced by the post-slaughter conditions. In view of the need to develop improved understanding of the relevance and magnitude of genetic influenced on meat and carcass quality traits.

2.1 Determinants of meat quality

The meat so produced is expected to be of good quality and appealing to the consumers. Many factors determine the quality in meat. It includes requirements of food safety and animal welfare. It also includes the sensory appeal of meat such as palatability and perceived healthiness, especially in relation to the amount and type of fat and other fatty components. Meat quality describes how much meat is attractive to consumers. Meat must look good to consumers before satisfying their palate when they decide to buy it. Once the meat is bought, cooked, and served, the aroma, tenderness, juiciness, and flavour must meet the expectations. The aroma and juiciness can be improved using spices and cooking method. However, the tenderness and flavour depend on textural characteristics, composition of meat, and many other factors.

2.1.1 Water holding capacity

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 water holding capacity (WHC) (Cheng and Sun 2008). It is clear that early post-mortem events including rate and extent of pH decline, proteolysis and even protein oxidation are key in influencing the ability of meat to retain moisture (Huff-Lonergan and Lonergan 2005). WHC is a measure of the ability of fresh meat to retain inherent water and is traditionally determined by gravimetric methods (Honikel 1998). The drip loss reveals the amount of free water that exudes under the force of gravity from the muscle fibres (Brøndum et al. 2000). In post-rigor muscle, the supply of ATP is depleted, resulting in the actomyosin bonds becoming more or less permanent. Within the structure of the muscle, there are several “compartments” were drip could originate. These could include the space within the myofibril, the

intracellular space outside the myofibril and the extracellular space, including the space between the muscle bundles. Loss of water from each of these compartments may involve slightly different mechanisms. In addition, loss of water from each of these compartments may occur at different times during storage (Huff-Lonergan and Lonergan 2005). The degradation of the cytoskeleton during ageing would increase WHC of meat by removing inter-myofibrillar and costameric connections and thereby reduce or remove the linkage between the rigor-induced lateral shrinkage of myofibrils and shrinkage of the whole muscle fibre. The rigor processes which promote both longitudinal and lateral contraction in muscle cells are normally completed by 24 h post-mortem (Kristensen and Purslow 2001). Ultimately, the entire meat production system, from the selection of genetics through storage and handling of the meat product can influence the water holding capacity of meat. It is therefore incumbent upon each segement of the production chain to optimize their operation to reduce moisture loss from the products. This coordinated effort is needed to ensure production of an economically viable, desirable, high quality fresh meat product.

2.1.2 pH and conductivity

Before slaughter, stress and exercise use up the animal's glycogen reserves, and, therefore, post-mortem lactic acid production through anaerobic glycolysis is diminished. In post-mortem muscle, the tissue attempts to maintain homeostasis by preserving cellular ATP concentrations, but due to circulatory failure following exsanguinations, muscle lacks the oxygen required for oxidative metabolism. Consequently, muscle glycogen is metabolized via anaerobic glycolysis, thus phosphorylating ADP to replenish ATP (Figure 1). Anaerobic glycolysis is less efficient than aerobic metabolism at generating ATP (Pelicano et al. 2006). Thus, as post-mortem metabolism continues, glycogen and ATP levels decline, and lactic acid accumulates with consequently lowering the muscle pH. This process results in an overall pH decline to an ultimate pH (pHu) of about 5.4 to 5.7 at 24 h in pig *longissimus dorsi* muscle (Bowker et al. 2002).

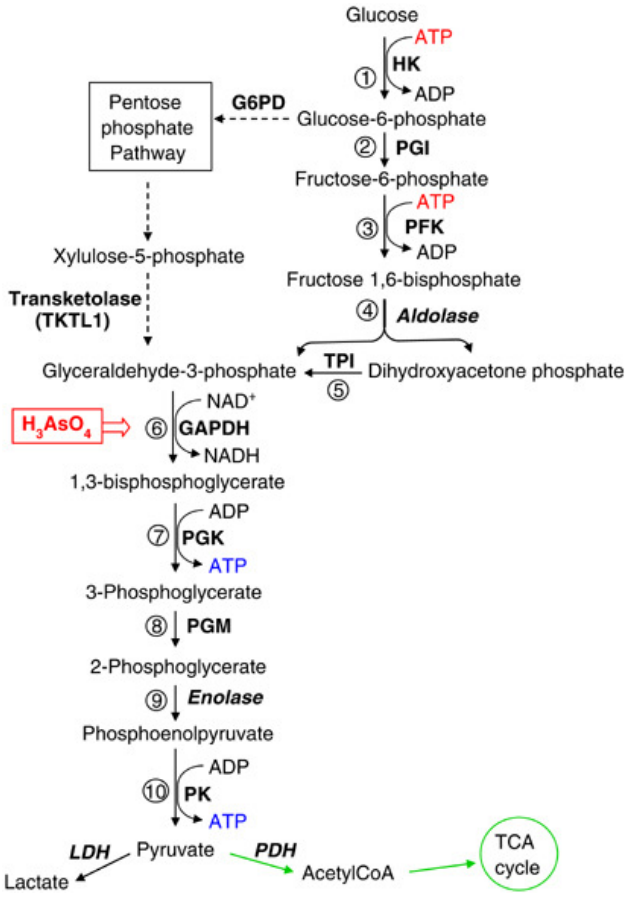

Figure 1: Glycolytic pathway and its metabolic interconnection with the pentose phosphate pathway. The solid arrows indicate glycolytic reactions, whereas the dashed arrows show the pentose phosphate pathway. The green arrows indicate further metabolism of pyruvate downstream of glycolysis (Adapted from Pelicano et al. 2006)

Conductivity (EC) seemed to be an accurate predictor of WHC in pork muscle when measured at 24 h post-mortem. It is conceivable that EC can be used independently, or with even better success in combination with pH, to classify WHC of pork carcasses (Lee et al. 2000). In post-mortem muscle with developing PSE characteristics the rapid decline of pH and diminishing ATP reserves damage the myofiber membrane and its insulating function is lost. The disrupted membranes allow continuity of extracellular and intracellular fluid and cause a generalized reduction of resistivity and a corresponding increase in conductivity. The typical development of pH values resulting in different meat quality conditions in swine is shown in Figure 2. Due to the curve characteristics measurement of pH at 45 minutes post-mortem and 24 hours post-mortem, respectively can be used to differentiate between normal and poor meat

quality, whereas pH values below 5.8 at 45 minutes post-mortem indicate the development of PSE (pale, soft and exudative) meat, DFD (dark dry and firm) and acid meat can only be differentiated from normal meat on the basis of ultimate pH values (DFD: > 6.2; Acid meat: < 5.4). Red, soft, exudative meat (RSE) however, is indistinguishable from normal meat by means of either early or ultimate pH values (Lengerken et al. 2002). A higher level of acidity in the muscle (lower pH) causes muscle proteins to denature and lose their ability to hold water. Therefore, meat with higher pH will tend to have more desirable characteristics such as darker colour, less drip loss, more firmness, and higher tenderness (Malek et al. 2001). Measurement of pH assesses chemical changes, and low frequency electrical measurements may provide a means of assessing physical changes in post-mortem muscle. Early post-mortem measurement of changes in electrical impedance and phase angle combined with pH may best predict the physicochemical changes that determine the ultimate water holding capacity of meat (Whitman et al. 1996). Both criteria could be a very important factor to determine in meat quality.

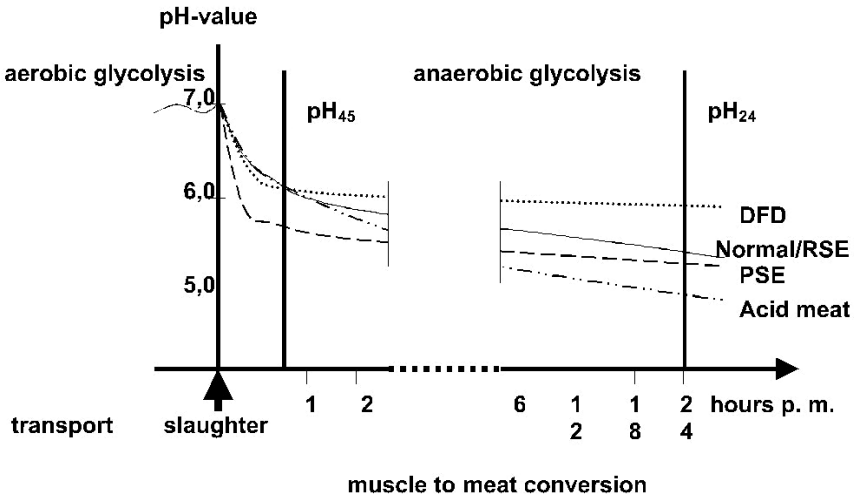

Figure 2: Typical development of the muscle pH values resulting in different meat quality in pigs (Adapted from Lengerken et al. 2002)

2.1.3 Meat colour

Consumers select meat cuts primarily for leanness and then for appearance and freshness, with judgments for the latter two attributes based primarily on brightness of colour. Because visual appearance of meat is important to the consumer when making

the decision to purchase, retailers discount or discard product that is not acceptable in colour, resulting in a substantial economic loss for the retailer. Meat colour varies between muscles due to differences in pigment content and muscle metabolism. Meat colour is defined by the extent of myoglobin oxygenation and the oxidative status of the haem iron (Satoh and Shikama 1981). Myoglobin is a water-soluble protein that stores oxygen for aerobic metabolism in the muscle. It consists of a protein portion and a nonprotein porphyrin ring with a central iron atom. The iron atom is an important player in meat colour. The defining factors of meat colour are the oxidation (chemical) state of the iron and which compounds (oxygen, water or nitric oxide) are attached to the iron portion of the molecule (Ramos et al. 2005). Oxidation of deoxymyoglobin or oxymyoglobin to metmyoglobin accounts for meat discoloration in retail display conditions (Liu et al. 1996). Oxidation of the haem iron from a ferrous (Fe^{2+}) to a ferric (Fe^{3+}) state give rise to the brown colour and is often associated to the release of oxygen radical. The concentration of residual oxygen and the radical formation are believed to be the major cause of colour deterioration in stored meat (Satoh and Shikama 1981). Oxymyoglobin, commonly known as the fresh meat colour, is the most desirable colour for fresh meats. The more oxidative nature of the *semimembranosus* muscle reveals in more dark, red and yellow surface independent on aging time compared with the colour of the *longissimus dorsi* muscle (Tikk et al. 2006). Thus, myoglobin can change from a dark purple colour to a bright red colour simply from oxygenation or to a brown colour by losing electrons. The pigments myoglobin, oxymyoglobin and metmyoglobin can be changed from one to the other, depending on the conditions at which the meat is stored. After cooking, a brown pigment called denatured metmyoglobin is formed, which normally cannot be changed to form another pigment. In the antemortem sector of research, meat colour is being related to genomic quantitative loci, numerous pre-harvest nutritional regimens, and housing and harvest environment (Mancini and Hunt 2005). Lighter coloured pork is often associated with more drip loss, poorer water holding capacity, and lower pH. The negative correlations of ultimate pH can be observed with Hunter L^* values (lightness) and drip loss (Malek et al. 2001).

2.1.4 Tenderness

Meat quality is a term used to describe a range of attributes of meat. Consumer research suggests that tenderness is a very important element of eating quality and that variations in tenderness affect the decision to repurchase. Actually, the meat tenderising process is unanimously recognized to be enzymatic in nature and the most studied proteolytic systems were cathepsins, calpains and, although more recently, the 20S proteasome (Sentandreu et al. 2002). In the immediate post-mortem period, as the muscle attempts to maintain homeostasis, muscle glycogen is metabolised via anaerobic glycolysis, thus phosphorylating ADP to supply ATP. Anaerobic glycolysis generates lactate that accumulates, lowering the intracellular pH, so that by 24 h post-mortem the pH has fallen to an ultimate pH (pHu) of about 5.4–5.7 (Bowker et al. 2000). Muscle is highly sensitive to both ATP and Ca^{2+}, which are both involved in the contraction–relaxation process. Consequently, as ATP levels are reduced and Ca^{2+} levels rise post-mortem irreversible cross-bridges between myosin heads and actin, and rigor mortis occurs in the tissue. Formation of rigor bonds is associated with an increase in toughness. Under normal post-mortem conditions these even occur over a periods of hours. However, carcass temperature fall below 10-15 ºC in early post-mortem period when lactate accumulation has not led to a major reduction in pH (6.0-6.4) and if there is sufficient residual ATP, the muscle will shorten and lead to toughening (Maltin et al. 2003). Another considerable muscular factor is muscle types. The ratio of slow-twitch oxidative (type I) and fast-twitch glycolytic (type II) muscle fibers also evidently influence meat tenderness. The ratio varies among individual animals of the same breed, breeds, and crosses. Tenderness is positively related to type I muscle and negatively related to the others. These differences are linked to higher ratio of protein turnover in tender muscle and a higher level of calpain which plays an important role in protein degradation, so in the meat tenderness (Lawrence and Fowler 2002, Neath et al. 2007).

2.2 Genetics affecting meat and carcass quality

Genetic factors are among the traits that affect pork quality, and the identification of major genes and molecular markers is a promising approach for improving economic traits, such as pork quality, that are not measurable in breeding animals. The quick evolution of the techniques and the possible reduction of assay costs will allow their

introduction into selection schemes (Nardone and Valfrè 1999). Genetic effects play a crucial role in 'designing' pig carcass composition and quality, although the heritability of quality traits is quite low (Sellier 1998). Genetic improvement of carcass traits is possible given the moderate to strong genetic correlations (Reverter et al. 2003). Lo et al. (1992) indicated genetic correlations between real-time ultrasonic and carcass measures of backfat thickness (0.85) and *longissimus dorsi* muscle area (0.87). The selection based on real-time ultrasonic data will result in effective improvement in carcass characteristics. The genetic and phenotypic correlations among meat quality traits have been reported. Borchers et al. (2007) reported the genetic correlations between the recorded meat quality traits in Table 1. The highest genetic correlations to drip loss are estimated for pH24 h, conductivity and meat brightness. Genetic correlations were estimated for intramuscular fat content with water holding capacity (-0.70) when intramuscular fat content increases, the water holding capacity improves (Suzuki et al. 2005). Sellier (1998) reported a mean genetic correlation of 0.15 between tenderness and intramuscular fat content. Hoveniera et al. (1992) reported that genetic and phenotypic correlations between intramuscular fat content and drip loss were almost zero (–0.07 and –0.03, respectively).

Table 1: Genetic correlations between the recorded meat quality traits (Adapted from Borchers et al. 2007)

Trait		2	3	4	5	6	7
Drip loss	1	-.66(.11)	-.72(.09)	-.50(.10)	.74(.09)	-.64(.11)	.13(.13)
$pH1_L$	2		.49(.09)	.10(.11)	-.57(.11)	.32(.12)	-.22(.11)
$pH24_L$	3			.84(.05)	-.14(.12)	.91(.04)	-.05(.11)
$pH24_H$	4				-.03(.12)	.86(.07)	-.13(.12)
$Conductivity24_L$	5					-.20(.12)	.55(.12)
Meat brightness	6						-.24(.13)
reflectace	7						

2.2.1 Heritabilities

Heritability is a concept that summarizes how much of the variation in a trait is due to variation in genetic factors. Often, this term is used in reference to the resemblance between parents and their offspring. In this context, high heritability implies a stronger importance of genetic factors between parents and offspring with regard to a specific trait, while low heritability implies a stronger influence from environmental factors (Wray and Visscher 2008). Heritability is a simple dimensionless measure of the importance of genetic factors in explaining the differences between individuals, and it allows an immediate comparison of the same trait across populations and of different traits within a population. Such comparisons can lead to insights into the biology of the phenotype, or can have practical consequences for plant and animal breeding programmes (Visscher et al. 2008). The heritability (h^2) estimates for pig meat quality traits were reported in many studies. Average values of h^2 estimates for twelve meat and carcass quality traits were calculated from the available studies and are given in Table 2.

Table 2: Heritability estimates for meat and carcass quality traits

Trait	Heritability (h^2)	References
$pH1_L$	0.16-0.44	Tholen et al. (2005)
$pH24_L$	0.14-0.38	Tholen et al. (2005)
$pH24_H$	0.15-0.35	Hoveniera et al. (1992)
$Conductivity1_L$	0.11-0.16	Tholen et al. (2005)
$Conductivity24_L$	0.07-0.37	Sellier (1998), Sonesson et al. (1998), Tholen et al. (2005)
Water holding capacity	0.10-0.37	Sellier (1998), Sonesson et al. (1998)
Meat colour	0.19-0.46	Sonesson et al. (1998), Tholen et al. (2005)
Tenderness	0.45	Lo et al. (1992)
Carcass length	0.62	Lo et al. (1992)
Backfat thickness	0.56	Lo et al. (1992)
Intramuscular fat	0.25	Fernández et al. (2003)
Ham weight	0.22	Hermesch et al. (2000)

2.2.2 The candidate gene approach for meat and carcass quality

The use of genomic markers to help in the selection of pork quality is one of the most promising developments in the pig industry. The major genes affecting pig meat quality are known: Ryanodine receptor (*HAL* or *RYR1* gene) that regulates Ca^{2+} transport across muscle cell membranes (Fujii et al. 1991), Rendement Napole gene (*RN*) that affects glycogen content of muscle (Milan et al. 2000). PSE has been known to be associated with variations in the recessive halothane gene or porcine stress syndrome (*PSS*) gene, and the availability of a test based on the identification of the causative mutation in the *PSS* gene was a key step in marker-assisted screening of pork quality. The test allows breeders to accurately separate all three *PSS* genotypes, instead of just reactors (*nn*) from non-reactors (*NN* and *Nn*), and has allowed more detailed studies of the effect of this mutation on pork quality (Band et al. 2005). The *RN* gene frequency ranged among abattoirs from 54 to 8%. Mean lean content was 58.9% for *nn*, 57.3% for *Nn*, and 55.8% for *NN* pigs, though a difference of 2.5% lean was observed between two abattoirs with the same *RN* gene frequency (Gispert et al. 2000). The negative effects of the halothane and *RN* genes can be found on fresh pork quality. The combination the detrimental effects of the two genes are additive for ultimate pH, objective colour, and water holding capacity (Hamilton et al. 2000). Genetic factors influencing basal metabolism clearly have the potential to similarly affect lactate accumulation and extent of pH decline (Huff-Lonergan and Lonergan 2005). A non-conserved substitution in protein kinase adenosine monophosphate-activated γ_3-subunit gene (*PRKAG3*) has explained the dominant mutation (denoted RN^-) that accounted for large differences in meat quality and processing yield in the Hampshire pig breed. The substitution in the *PRKAG3* gene causes a 70% increase in muscle glycogen in RN^- homozygous and heterozygous pigs. This increase in glycogen directly results in greater production of lactate in post-mortem muscle, a lower ultimate pH and poorer water holding capacity in fresh pork (Milan et al. 2000).

IGF2, insulin-like growth factor 2, is implicated in myogenesis and lean meat content. A mutation, a single base (A for G substitution) of *IGF2* (position 3072 in the intron 3), was the cause of a major QTL effect on muscle growth and fat deposition in pigs (Van Laere et al. 2003). The genotyping of pig populations for *IGF2* could be an important part of breeding programs in the future because mutation in *IGF2* may have potential

influence on meat quality and quantity. The A/G mutation has been selected for in-breeding schemes based on production performance and/or lean meat deposition. Carrodeguas et al. (2005) evaluated a rapid assay based on real time PCR (RT-PCR) to detection this mutation in three populations of purebred Iberian or Iberian × Duroc crosses, and in cured meat products and wild boars. Penetrance of the A mutation is about 80% in the commercial population. Purebred Iberian pigs were all homozygous G/G whereas crosses of Iberian pigs were heterozygous (90%) or homozygous A/A (10%). Besides the above mentioned major genes, genes in the leptin pathway are proving profitable in association studies with growth and backfat thickness, e.g. the *MC4R* gene. The most interesting polymorphism reported to date in the pig is p.Asp298Asn, which is significantly associated with variation in growth and fatness traits in most breeds and crosses. It was clearly associated with reduced feed intake, faster growth and less backfat thickness (Fan et al. 2009b, Kim et al. 2000, Meidtner et al. 2006). Several missense and silent mutations were identified in *CAST* and haplotypes covering most of the coding region were constructed and used for association analyses with meat quality traits. The calpastatin (*CAST*) gene in pork was associated with sensory tenderness, juiciness and other meat quality traits (Ciobanu et al. 2004, Meyers and Beever 2008).

Thus it is necessary to identify other genes affecting the phenotypic differentiation between animals with respect to these traits. The understanding of the metabolic processes taking place in the muscle post-mortem may suggest the way to identify sources of candidate genes. It might help to improve meat and carcass quality especially in terms of water holding capacity.

2.3 Molecular approaches in pig breeding program to improve meat and carcass quality

The advances in molecular genetics have led to the identification of genes and markers associated with meat quality in pig (Dekkers 2004). The development of a considerable number of annotated livestock genome sequences represents an incredibly rich source of information that can be used to identify candidate genes responsible for complex traits and quantitative trait loci effects. In particular, global gene expression profiling at the mRNA can provide a better understanding of gene regulation that underlies biological

functions and physiology related to the delivery of a better pig meat quality. Moreover, the possibility to realize an integrated approach of genomics with bioinformatics tools is essential to obtain a complete exploitation of the available molecular genetics information (Davoli and Braglia 2008). This has provided opportunities to enhance response to selection, in particular for traits that are difficult to improve by conventional selection (low heritability or traits for which measurement of phenotype is difficult, expensive, only possible late in life, or not possible on selection candidates) (Dekkers 2004). The identification of QTL related to production traits that are relevant for the pig industry has been mostly performed by using divergent crosses. Commercial purebred populations retain a significant amount of genetic variation, even for traits that have been selected for many generations. Many studies found QTL for meat and carcass quality traits (Liu et al. 2007, Vidal et al. 2005). Moreover, DNA marker technology can play an important role in improving meat quality. The other advantage of the DNA technology is that markers close to relevant genes or tests that identify mutations in candidate genes allow us to also exploit genes with smaller effects (de Vries et al. 2000). Candidate gene analyses have identified a number of important chromosomal regions and individual genes associated with growth rate, leanness, feed intake, meat quality, litter size and disease resistance. In the past few years, the commercial pig industry is actively incorporating the gene markers and traditional performance information to improve traits of economic importance in pig production using marker-assisted selection (MAS) (Rothschild 2004). Recently, a genome-wide association study (GWAS) has revolutionized the search for genetic influences on complex traits (Manolio 2010). An advanced progress on GWAS using SNP chip has been performed in livestock, including dairy with milk production traits (Jiang et al. 2010), pig with boar taint traits (Duijvesteijn et al. 2010), whereas in pig with meat quality is underway. The best approach to genetically improve meat quality is to find relevant DNA-markers directly in the populations under selection. For this reason, meat quality measurements should be performed continuously on the nucleus populations of breeding organizations. Since a full assessment of meat quality can only be done after slaughter, the data have to be collected on culled animals and cannot be obtained on potential breeding animals. The phenotypic meat quality data will not only enable the detection of relevant DNA markers, but will also be used to validate markers from experimental populations or to test candidate genes (de Vries et al. 1998). Significant markers or genes will be included

straight away in the selection process. An advantage of the molecular information is that we can obtain it already at very young age, which means that animals can be preselected based on DNA markers before the growing performance test. This is a great advantage for the overall testing and selection system. The continuous collection of meat quality data from nucleus lines is expensive. However, its use is not limited to DNA marker research. The data also allow the breeding organization to monitor their nucleus lines, which is important for optimizing the breeding direction. Furthermore, the data can be used directly in the selection process as phenotypic information on relatives (culled litter mates and half-sibs) of potential breeding animals (de Vries et al. 2000).

2.3.1 Quantitative trait loci (QTL)

Meat quality traits are controlled by an unknown number of genes mapping in QTL regions and some of these individual genes may have large effects on a specific trait. The main goal of genome research in farm animals in general is to map and to characterize trait loci (Andersson 2001). It can provide a useful bridge to link genome information with phenotype. In recent years the linkage and physical maps of the pig genome have developed considerably (Doerge 2002, Ovilo et al. 2002, Rothschild and Plastow 2008). Much statistical genetics work has been conducted on methods for linkage mapping of QTL in livestock (Malek et al. 2001, Ovilo et al. 2002, Rohrer et al. 2006, Rothschild 2004, van Wijk et al. 2005, Wimmers et al. 2006). The QTL and major genes affecting drip loss or water holding capacity were located on SSC 1, 2, 4, 6, 11, 13, 14, 15 and 18 (http://www.animalgenome.org/QTLdb/) (Jennen et al. 2007). In addition, Thomsen et al. (2004) described evidence QTL for drip loss and water holding capacity on SSC5 and 9, respectively. De Koning et al. (2000) and (2001) detected four QTL for drip loss, some with imprinted effects, on SSC 4, 6 (maternal), 14 (Mendelian), and 18 (paternal). Chromosome SSC14 at 66–72 cM was associated with drip loss (Rohrer et al. 2006). QTL with significant influences on meat quality were located on almost every porcine chromosome. Liu et al. (2007) reported that a QTL affecting pH value in loin, meat colour and conductivity was detected on SSC1 (Table 3). Malek et al. (2001) also reported a suggestive QTL for pH on SSC 5, 6, 14 and 15. The same QTL location on SSC14 for pH in the ham was found by De Koning et al (2000) and (2001). Geldermann et al. (1996) demonstrated a QTL for pH on SSC 6 near the *HAL*

gene but using *HAL*-positive pigs. Andersson-Eklund et al. (1998) found some evidence that the proportion of wild boar alleles on SSC 2, 10, 12, and 15 was associated with QTL affecting meat colour in a cross between the Wild Boar and Large White breeds. De Koning et al. (2001) found a total of nine suggestive and three significant QTL at the genome-wise level for various measures of reflectance: five QTL, on SSC 1, 3, 4, 13, and 14 affecting colour L* (lightness). The QTL with the highest effect was identified on SSC4 at the telomeric end of the q arm for meat colour (Duthie et al. 2011). Taniguchi et al. (2010) suggested that QTL for meat colour is located on SSC6. Chromosome SSC5 at 60–67 cM and chromosome SSC15 at 4–17 cM had suggestive QTL for cooking loss and pork intensity flavour determined by the trained sensory panel (Rohrer et al. 2006). QTL on SSC6 at position 70 cM was identified for cooking loss (Markljung et al. 2008). However, Malek et al. (2001) did not detect QTLs for cooking loss in this region. QTL on cooking loss has been found on 7, 14 and 18 (Jennen et al. 2007). There are several chromosome regions with a considerable effect on carcass traits in pigs. QTL effects were found for percentage lean meat and percentage lean meat plus bone in various cuts, proportion of bone in relation to lean meat in ham, muscle area, and carcass length (Andersson-Eklund et al. 1998). QTL were found for carcass length on SSC4 (Wimmers et al. 2002), 6, 7 and X (Sato et al. 2003, Nezer et al. 2002). van Wijk et al. (2006) reported that QTL affecting ham primal or subprimal weights were found on SSC2, 4, 5, 6, 10, 13, and 14. QTL on chromosomes SSC5, 11 and 13 affected loin primal or subprimal weights. QTL for carcass composition was also found on SSC1. QTL for a trait backfat thickness were detected on SSC2 (Liu et al. 2007), 4 (Wimmers et al. 2002), 7 and X (Sato et al. 2003). Milan et al. (2002) reported QTL for both leanness and fatness traits in the telomeric regions of SSC 1, 2, 4, 7 and X. Additional significant QTL was identified for ham weight on SSC 5.

Table 3: Quantitative trait loci (QTL) analysis for meat quality traits of the Duroc x Pietrain resource population (Adapted from Liu et al. 2007)

SSC	Trait[b]	Flanking markers	Significance level[c]
1	pH24loin	*S0312–S0113*	***
1	pH24ham	*S0312–S0113*	***
1	pH1loin	*S0312–S0113*	***
1	Con24loin	*S0312–S0113*	*
1	Con24ham	*S0312–SW373*	**
1	OPTO	*S0312–SW1957*	***
2	pH24ham	*SW1564–S0226*	*
2	DRIP	*SW2623–S0141*	*
2	Shear force	*SW834–S0226*	*
3	DRIP	*SW72–S0164*	*
5	DRIP	*SW491–SW1482*	*
7	Cooking loss	*S0064–SW175*	*
15	pH24loin	*SW1111–SW1119*	*
18	DRIP	*S0062–SWR414*	*
18	Cooking loss	*S0062–SWR414*	*

1. [a]QTL results of first analysis are shown in italics.

2. [b]pH24loin, pH24ham = pH 24 h postmortem in loin and ham, respectively; pH1loin = pH 45 min postmortem in loin; Con24loin, Con24ham = conductivity 24 h postmortem in loin and ham, respectively; OPTO, meat colour; DRIP, drip loss (bag method).

3. [c]Significance levels: ***, 1% genome wide; **, 5% genome wide; *, 5% chromosome wide.

2.3.2 Gene expression analysis

Tracing of gene expression process of the investigated trait in different stages or genetic background, including signalling pathway, regulatory network and complex genome-wide transcriptional profiles can contribute to a better understanding of the molecular architecture and find out the detailed clues that candidate gene tells. Important biological features of traits are directly reflected by transcript pattern, and quantitative traits are usually the consequence of the structure of genetic regulatory networks and the parameters that control the dynamics of those networks (Frank 2003). The genetic analysis of variation in gene expression would provide valuable models for studying complex and quantitative traits. High throughput technologies including microarrays have been widely used, allowing researchers to analyze the expression level of series of genes in different stages or genetic background. Expression level of a highly variable gene in an individual is considered as the 'phenotype', which is possibly influenced by genetic determinants (Cheung and Spielman 2002). Genome wide microarrays covering up to more than 20,000 transcripts were used to elucidate mechanisms of control of carcass growth and meat quality traits (Davoli et al. 2007, Ponsuksili et al. 2008a, Ponsuksili et al. 2008b). A number of researchers used a gene expression analysis approach for identifying candidate genes in pigs, for eye muscle area (Ponsuksili et al. 2000), muscle growth and structure (Murani et al. 2007), intramuscular and subcutaneous adipocytes (Zhou et al. 2010), heat stress in the pig small intestine (Yu et al. 2010) and other candidate genes with causative allelic variant that may be of biomedical, economic and evolutionary interest. In the study of Ponsuksili et al. (2008a), the results have shown the successful identification of candidate genes for water holding capacity traits (e.g. drip loss and pH) using a gene expression profiling. A microarray analysis comparing the divergence of high and low drip loss and pH revealed numbers of differentially expressed genes that may be responsible for the phenotype (drip loss and pH) difference in the individuals. Comparative expression profiling by hybridization of the Affymetrix GeneChip porcine genome arrays revealed 789 differential expressions of transcripts between high and low drip loss/pH groups at $P < 0.05$. Significance analysis of microarrays using a statistical package revealed a corresponding false discovery rate of about 35%. Of 789 transcripts, 166 showed down-regulation in the high drip loss group. The function-dependent strategy resulted in the

functional candidate gene approach, in which a putative candidate gene is the one that could be statistically detected from the genes controlling large components of inheritable gene expression variation (Schwerin et al. 2003). The statistically analysis has led to the conclusion that differential regulation of the abundance of transcripts of these biological networks in pork seemed to affect post-mortem biochemical processes of meat maturation. Such knowledge of this functional link is indicative for the identification of candidate genes for improvement of meat quality (Ponsuksili et al. 2008a).

2.3.3 Genome-wide association study (GWAS)

During the past few decades, advances in molecular genetics have led to the identification of genes, or markers associated with genes, that affect meat quality. Work on sequencing farm animal genomes will help us to understand how genes function in various organisms and might be applied in the field to study the molecular control of meat quality. A genome-wide association study is an approach that involves rapidly scanning markers across the complete sets of DNA, or genomes. In human, hundreds of thousands of single-nucleotide polymorphisms (SNPs) are tested for association with a disease in hundreds or thousands of persons. Genome-wide association study has revolutionized the search for genetic influences on complex traits (Manolio 2010). Recently, the high-density 60K porcine SNP array has been developed that offers a much higher resolution (Ramos et al. 2009). A genome-wide association study (GWAS) was initiated using the SNP array to identify the chromosomal regions and specific SNPs influencing boar taint levels in a commercial breeding population (Walstra et al. 1998). GWAS with the Porcine 60K+SNP BeadChip has been using for pig production and feet and leg structure traits (Fan et al. 2009a) and pig reproductive traits (Onteru et al. 2009). Duijvesteijn et al. (2010) reported that the Illumina Porcine 60K+SNP Beadchip was genotyped on 987 pigs divergent for androstenone concentration from a commercial Duroc-based sire line. The association analysis with 47,897 SNPs revealed that androstenone levels in fat tissue were significantly affected by 37 SNPs on pig chromosomes SSC1 and SSC6. Recently, development of GWAS with meat quality traits in pig is underway. In the future, markers from across the genome can be tested for association with each of these traits faster and cheaper than ever before through use

of the newly developed genotyping method. These new SNP chips will revolutionize the way livestock are genotyped, moving industry from genotyping animals for tens of markers to genotyping for thousands of markers with minimal impact on costs.

2.4 Selected candidate gene for meat and carcass quality

The candidate gene can be approached from four major categories: association studies, linkage analyses, gene expression and literature search (Sun et al. 2009). Nowadays, genomics allows large-scale analysis of gene characteristics (structural genomics) and expression (functional genomics). Genome mapping, comparative genomics and identification of quantitative trait loci and polymorphisms are the subject of active investigation to gain a better knowledge of the structure and function of genes. The candidate gene approach can be relatively straightforward compared to the QTL approach. For example, we have used polymorphisms in candidate genes to look for associations across populations. When associations are identified, the resulting marker can potentially be used in breeding programmes (de Vries et al. 2000). Quantitative trait loci will potentially improve genetic progress, especially for traits difficult or expensive to measure, through a genome-wide association study (GWAS) (Pryce et al. 2010). Carcass composition and meat quality traits are among those that would benefit most from the use of genetic marker information (Casas et al. 2003). Functional genomics, including analysis of the transcriptomic and genomic, provides new opportunities for understanding the molecular processes in muscle and how these influence its conversion to meat. Van der Steen et al. (2005) have identified genes associated with variation in different aspects of raw material (muscle) quality and to then develop genetic tools that could be utilized to improve this quality. DNA polymorphisms identified in the porcine genes illustrate the impact that such tools can have in improving meat quality. The resources developed in pork quality genes provide the basis for identifying more of these tools. To date DNA markers have been identified using two basic approaches: quantitative trait loci (QTL) mapping or the “candidate gene approach” (Zhu and Zhao 2007). DNA markers have been applied in swine breeding up to this point in time. Markers explaining variation in growth, lean percent, litter size, meat quality, susceptibility to developmental abnormalities, and even disease resistance have been identified and incorporated into breeding programs. Importantly, genomic and statistical

tools have been developed to make use of the proliferation of genomic information that is now available (van der Steen et al. 2005). The main aims of the transcriptome analysis of muscle at the time of slaughter are that some of these genes will be expressed in muscle at the time of slaughter and that variation in expression levels would be associated with variation in quality parameters of meat derived from this muscle. When these genes are identified the resources developed, it will enable further analysis of them and also the search for variation in the sequence of the genes. Once sequence polymorphism is identified this can then be tested for association with variation in the traits of interest (Plastow et al. 2005).

2.5 Source of candidate genes for meat and carcass quality

Candidate genes for this thesis were selected from an expression study of Ponsuksili et al. (2008a) and (2008b) and an association study of Fan et al. (2010b). Expression profiles of *longissimus dorsi* were compared between the two extreme groups of six discordant sib pairs (selected from 572 F_2 animals of a cross of Duroc and Pietrain [DuPi]) with 4.14 ± 0.77 vs 0.9 ± 0.77% drip (mean ± standard deviation) ($P < 0.0001$) as well as between groups with high/low pH at 24 hour p.m. employing Affymetrix GeneChip porcine genome array and were validated by real-time PCR. Expression profiling revealed 789 differential expressions of transcripts between high and low water holding capacity (WHC) group at $P < 0.05$ (Ponsuksili et al. 2008b). Moreover, expression profiling and eQTL analysis conducted on 74 F_2 animals of the DuPi resource population showed 1,279 transcripts with trait correlated expression to WHC. 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 (Ponsuksili et al. 2008a). Therefore, the large number of genes expressed represents a source of candidate genes that could influence carcass and meat quality traits. In this thesis, five candidate genes were selected from an expression study of Ponsuksili et al. (2008a) and (2008b) and one candidate gene from an association study of Fan et al. (2010b). All genes in this thesis were selected based on (i) known function of the particular gene

and/or (ii) the position, giving preference to those genes located in QTL region for meat and carcass quality traits.

2.5.1 The alpha-microglobulin/bikunin precursor (*AMBP*)

Bikunin, a protease inhibitor, functions in stabilization of the extracellular matrix and originates from the precursor polypeptide α-1-microglobulin/bikunin precursor (*AMBP*). Functionally, *AMBP* 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, Zhuo et al. 2001). The extracellular matrix is composed of an organized network of proteins and polysaccharides, which are secreted by the muscle cells and localized in the connective layers surrounding the muscle fibers. The extracellular matrix is composed of proteins including collagens and proteoglycans. Certain extracellular matrix macromolecules, especially the proteoglycans, interact with growth factors and are required for the cell to elicit a response to the growth factor. Proteoglycans contain a central core protein with one or more attached carbohydrate residues called glycosaminoglycans. Glycosaminoglycans covalently attached to the core protein include chondroitin sulfate, keratan sulfate, dermatan sulfate, and heparan sulfate. Based on this definition, the proteoglycans are a diverse family of macromolecules that exhibit developmental and tissue specificity in terms of their expression (Velleman 2007). Velleman (2000) reported that the extracellular matrix have functions in water holding capacity, elasticity, growth factor regulation and cellular growth properties. If the amount of proteoglycans have been reduced in animals selected for growth rate and muscling, the reduced water holding capacity would directly affect on juiciness and drip loss (Velleman 2002). The positioned of *AMBP* on SSC1 where QTL for meat quality traits (pH, colour, conductivity, fat area and backfat thickness) are reported in the same population (www.animalgenome.org/cgi-bin/QTLdb/SS/) (Liu et al. 2007). Moreover, this mapped position is coincided with the chromosomal location of *AMBP* in the porcine genome (NCBI, Ensembl).

2.5.2 The group-specific component protein (*GC*)

The group-specific component protein (*GC*), also known as vitamin D-binding protein 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. This gene is also expressed in pancreatic tissue. *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). The contraction of actin influences on the amount of water that can be held by the muscle (Huff-Lonergan and Lonergan 2007). Probably, *GC* may be involved with glucose or insulin metabolism since there are evidences that cells of the obese pig are mildly resistant to insulin action. The obese pig also exhibited a reduced growth hormone (GH) status, as indicated by lowered fasting GH levels (Wangsness et al. 1977). The reduced GH secretory potential in obese pigs is consistent with reduced muscle development and growth rate (Wangsness et al. 1980). The *GC* position is not mapped yet in the porcine genome but it is mapped on HSAP4 of NCBI database which is homologous to SSC8 (https://www-lgc.toulouse.inra.fr/pig/compare/SSCHTML/SSC8S.HTM). The chromosomal region of *GC* location on SSC8 did incorporate several QTL related to meat colour (Malek et al. 2001, Ovilo et al. 2002, Thomsen et al. 2004), backfat thickness, loin eye area, lean content and dressing percentage (Liu et al. 2007) (www.animalgenome.org/cgi-bin/QTLdb/). According to its position and function may be involved on meat and carcass quality traits.

2.5.3 The protein phosphate 1, regulatory (inhibitor) subunit 3B (*PPP1R3B*)

The *PPP1R3B* gene belongs to a family of proteins that target *PPP1R3B* to glycogen, it is expressed in muscle and liver. Moreover, *PPP1R3B* has effect on glycogen by stimulating the glycogen accumulation (Worby et al. 2008). The *PPP1R3B* gene is expressed in human skeletal muscle. Its effects on muscle glycogen metabolism are unknown. In human, the *PPP1R3B* gene has been examined as a candidate gene for the Type 2 diabetes and MODY (maturity onset diabetes of the young) (Dunn et al. 2006,

Montori-Grau et al. 2007). The interaction between glycogen phosphorylase (GP) and the liver glycogen targeting subunit (termed G_L) of PP1 (protein phosphatase 1) has emerged as a new potential anti-diabetic target, as the disruption of this interaction should increase glycogen synthesis, potentially providing an alternative approach to counteract the enhanced glycogenolysis without inhibiting GP activity (Zibrova et al. 2008). Above others also the specific regulatory role of the muscle-specific subunit G_L (*PPP1R3B*) in human muscle tissue is part of undergoing investigation. *PPP1R3B* is expressed in muscle and liver in humans at comparable levels, but only expressed in skeletal muscle of rodents and rabbits (Montori-Grau et al. 2007). According to the function of the *PPP1R3B* gene, muscles with low glycogen and lactate levels showed normal rates of post-mortem glycolysis and normal 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). The *PPP1R3B* gene can be considered as an promising positional candidate genes for meat quality traits due to its location in the QTL region for pH, meat colour, cooking loss and drip loss are reported on SSC15 (Edwards et al. 2003, Jennen et al. 2007).

2.5.4 The tenascin C (*TNC*)

The Tenascin C (*TNC*) gene is a member of a family of genes coding for extracellular matrix protein and this gene has function on extracellular matrix (ECM) and cell adhesion (Garrido et al. 1995). The ECM is a part of three connective tissue layers (endomysium, perimysium and epimysium) surrounding muscle fibers which are composed of fibrous and nonfibrous proteins including collagens and proteoglycans. The ECM is a major determinant of tissue water holding capacity (WHC), since proteoglycans have a negative charge density (high pH) which draws water into the tissue and creates a water compartment. This means an increased amount of proteoglycans contribute to increase WHC in tissue and to decrease drip loss (Velleman 2002). Extracellular space around muscle fibers continually increases up to 24 h post-mortem, but gaps between muscle fiber bundles decrease slightly between 9 and 24 h post-mortem, perhaps due to fluid outflow from these major channels. Thus, the rigor process could result in mobilization of water out not only out of the myofibril, but also

out of the extramyofibril spaces as the overall volume of the cell is constricted (Figure 3). In fact, reduction in the diameter of muscle cells has been observed in post-mortem muscle. This water that is expelled from the myofibril and ultimately the muscle cell eventually collects in the extracellular space. Several studies have shown that gaps develop between muscle cells and between muscle bundles during the post-rigor period. These gaps between muscle bundles are the primary channels by which purge flows from the meat; some investigators have actually termed them "drip channels" (Huff-Lonergan and Lonergan 2005). The post-mortem evolution of intra- and extracellular spaces has a relation with water movements in the muscle and with water holding capacity. This is confirmed by the very high correlation observed between the increase in extracellular space and muscle. Extracellular space reached its maximum value approximately 10 h post-mortem. Thus retraction of cells related to cellular death coincides with the period of polyphasic pH decrease and with progressive increase in extracellular space (Guignot et al. 1993). The positioned of *TNC* in the QTL region for pH, colour, conductivity, fat area and backfat thickness on SSC1 are reported in the same population (www.animalgenome.org/cgi-bin/QTLdb/SS/) (Liu et al. 2007). Moreover, this mapped position is coincided with the chromosomal location of *TNC* in the porcine genome (NCBI, Ensembl).

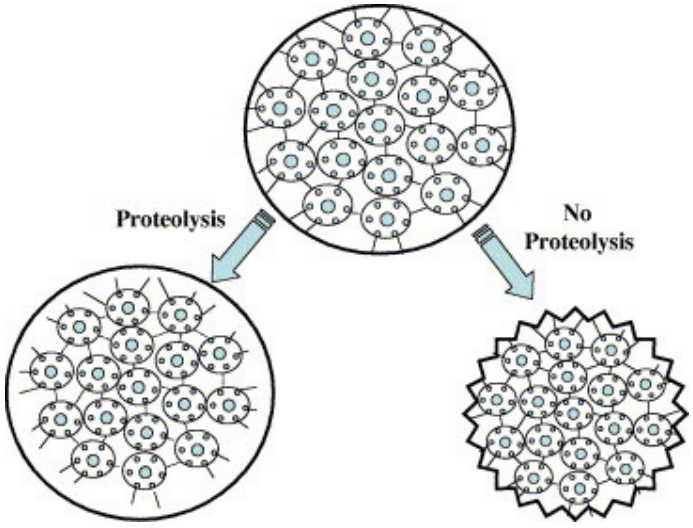

Figure 3: Schematic overview the potential changes in muscle cell diameter during post-mortem aging as influenced by proteolysis (Adapted from Huff-Lonergan and Lonergan 2005)

2.5.5 The interferon alpha inducible gene 6 (*IFI6*)

The Interferon inducible gene 6-16, *G1P3*, was first identified as one of the genes that are induced by interferon α and ß. *IFI6* is reported to have anti-apoptotic and immunomodulatory effects (Borden et al. 2000, Schaar et al. 2005, Tahara et al. 2005, Urabe 1994). Confronted to any type of stress (*e.g.* oxidative stress) cells react through chemical and hormonal signals and the cells received apoptosis-inducing signals are committed to apoptosis (Herrera-Mendez et al. 2006). Moreover, in case of post-mortem changes, apoptosis play vital roles for maintaining cellular integrity and structure. Cells in apoptosis are dissociated from others and 'shrink' which leads to a reduction in intracellular space and a parallel increase in extracellular space that alter muscle characteristics (Trump and Berezesky 1995). The anti-apoptotic mechanism of *IFI6* is not clear but it has been reported that *IFI6* plays its critical role in regulation of the apoptosis by controlling mitochondria and Ca^{2+} channels in endoplasmic reticulum (Tahara et al. 2005). Moreover, the calcium release has important effects on meat quality (Fujii et al. 1991, Lundström et al. 1989). The higher intracellular Ca^{2+} concentration stimulates different ATPases to an increase post-mortem metabolism, a fast decrease in pH and changes the water holding capacity of the muscle or drip loss (Honikel et al. 1986, Küchenmeister et al. 2000). *IFI6* is not mapped yet in pig but it is mapped on HSAP1p35 which is homologous to SSC6q22-26 (https://www-lgc.toulouse.inra.fr/pig/compare/SSC .htm). Several QTL for meat colour, water content, drip loss and pH, muscling and carcass length are mapped at a region on SSC6 (Edwards et al. 2008, Markljung et al. 2008, Taniguchi et al. 2010) which is homologous to HSAP1p35 where the human *IFI6* gene is located.

2.5.6 The hepatocyte nuclear factor-1 homobox A (*HNF1A*)

The *HNF1A* gene accounts for maturity-onset diabetes of the young type3 (MODY3), which is mainly caused by a defect in glucose-stimulated insulin secretion as a result of pancreatic ß-cell dysfunction (Sian 2000) and plays a key role in the regulation of pancreatic insulin secretion (Pearson et al. 2007). MODY is a group of monogenic forms of diabetes, characterized by early onset and autosomal dominant inheritance, caused by mutations in 6 known genes. The most common mutated genes responsible

for MODY2 and MODY3 are glucokinase (*GCK*) and hepatocyte nuclear factor-1 homeobox A (*HNF1A*), respectively (Fernando and André 2005). The most common disease in which glycogen metabolism becomes abnormal is diabetes, in which, because of abnormal amounts of insulin, liver glycogen can be abnormally accumulated or depleted. Restoration of normal glucose metabolism usually normalizes glycogen metabolism as well. In hypoglycemia caused by excessive insulin, liver glycogen level is high, but the high insulin level prevents the glycogenolysis necessary to maintain normal blood sugar levels. A study of Pearson et al. (2007) reported that birthweight and prevalence of neonatal hypoglycaemia were not increased in *HNF1A* mutation carriers. In human, *HNF1A* mutation has been demonstrated to have decreased transcriptional activity upon genes involved in glucose metabolism (GLUT-2). This transcriptional impairment is situated midway between normal and MODY causative mutations (Holmkvist et al. 2006). Fan et al. (2010b) reported that the SNPs of *HNF1A* were associated with intramuscular lipid and two meat quality sensory traits (juiciness and tenderness). Several QTL for marbling score (Edwards et al. 2008), tenderness (Harmegnies et al. 2006) and meat conductivity (Wimmers et al. 2006) are mapped at the region on SSC14 where *HNF1A* is located (http://www.animalgenome.org/cgi-bin/QTLdb/SS/draw_chromap?Chromos = 14).

3. Materials and methods

The aim of this study was to analyse the candidate genes for the meat and carcass quality in pigs. In total six functional candidate genes were investigated in this study. Genes were selected using two different strategies; in a first approach, genes differentially expressed between low/high drip loss and low/high pH of F_2 DuPi population, were identified by microarray analysis (Ponsuksili et al. 2008a, Ponsuksili et al. 2008b). Thus, first study to the selection of the five functional candidate genes (*AMBP*, *GC*, *PPP1R3B*, *TNC* and *IFI6*) based on Position and Function, to further screen for single nucleotide polymorphism (SNP) and association analysis with meat and carcass quality. Then the genes which give the association results with pH or drip loss in both DuPi and Pi populations were used for further functional analysis by mRNA and protein expression (Figure 4).

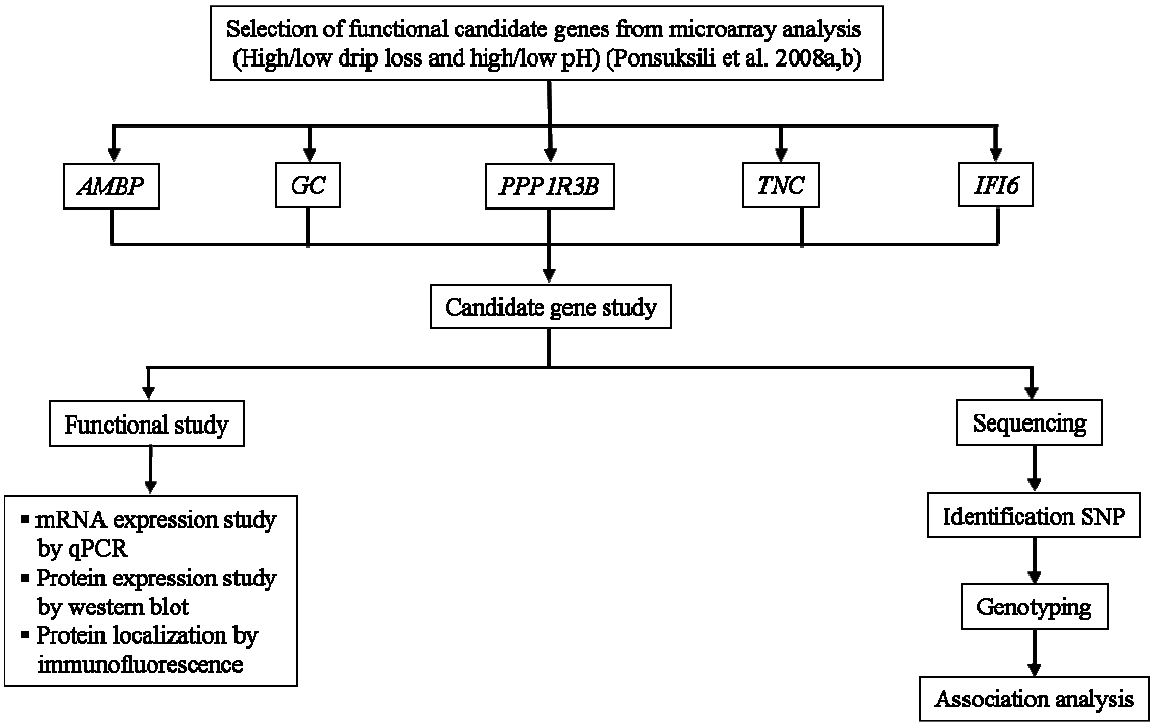

Figure 4: Overview of the first part of the experiment, the analysis of the selected genes from microarray analysis

Second, the *HNF1A* gene was selected based on the described function in the literature. Previously, the *HNF1A* gene was already identified as a positional and functional candidate genes, its role for meat and carcass quality in pig could be confirmed (Fan et al. 2010b). To complete the analysis of this gene regarding to the function, the functional analysis of this gene was also performed (Figure 5).

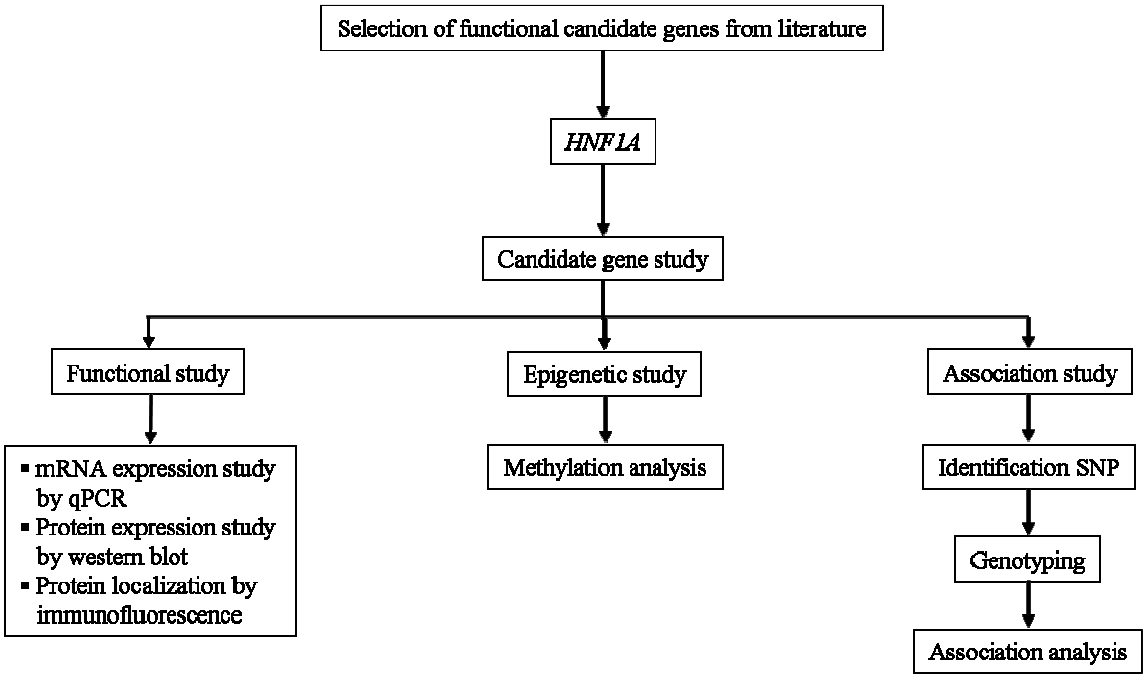

Figure 5: Overview of the second part of the experiment, the analysis of the selected gene from literature

3.1 Animals

3.1.1 Animals for association studies

For this study, a F_2 resource population based on the cross of Duroc and Pietrain pig (*n* = 313) and the commercial breed Pietrain (*n* = 110) were used for association study. The F_1 generation was produced by mating four Duroc boars to eight Pietrain sows and two Pietrain boars to five Duroc sows. The F_1 animals were reciprocally assigned to produce F_2 animals, with 13 Duroc x Pietrain (DuPi) F_1 females mated to two Pietrain x Duroc (PiDu) F_1 boars and 14 PiDu F_1 females mated to three DuPi F_1 boars (Figure 6) (Liu et al. 2007). The pigs from the commercial breed Pietrain (Pi) were described by Srikanchai et al. (2010). 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 animal were individually weighed at birth, at weaning, at the beginning and at the end of the testing, respectively. The pigs were given an *ad libitum* diet containing 16% crude protein, 1% lysine, 0.6% (methionine + cystine), 0.6 % threonine and 13 MJ metabolizable energy during the whole testing period from 10 to 22 weeks of age and slaughtered approximately at 85 kg slaughter weight, the average age at slaughter was 177.6 ± 15.6 days. The animals were tested and were found to be free of the mutation at the ryanodine receptor locus which is responsible for halothane susceptibility. All the pigs were slaughtered at the same commercial abattoir.

3.1.2 Animals for DNA methylation, mRNA, protein expression studies

The animals used in DNA methylation, mRNA and protein expressions were half siblings (all are males, 177 days old, 5 animals per group) of the Duroc and Pietrain crossbreeds that were born and raised in the same herd and under similar standard conditions. The mRNA and protein expressions of the *IFI6* gene were isolated from muscle (*longissimus dorsi*) of low (1.00 ± 0.37) and high (4.70 ± 0.57) drip loss animals. The DNA methylation, mRNA and protein expressions of the *HNF1A* gene were isolated from muscle (*longissimus dorsi*) of low (5.42 ± 0.02) and high (5.71 ±

0.03) pH muscle animals. Additionally, the muscle (*longissimus dorsi*) samples were collected from male, the 6 months old, Duroc and Pietrain cross breeds pigs (n = 3) for study in protein localization of the IFI6 and HNF1A protein by immunofluorescence. All pigs were born and raised in the same herd and under similar standard conditions.

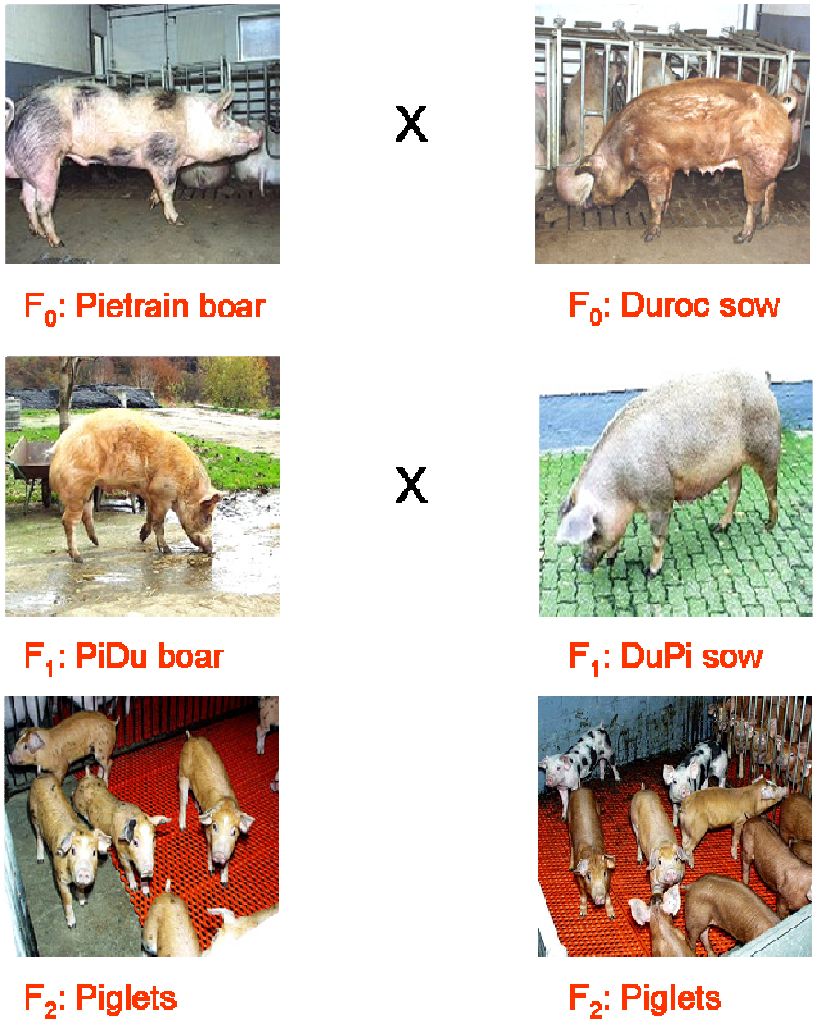

Figure 6: Structure of the Duroc x Pietrain resource population

3.1.3 Phenotypes

Meat quality traits analysed in this study cover the indicators water holding capacity including drip loss, thawing loss, cooking loss, pH at 45 min p.m. (pH1), pH at 24 h p.m. (pH24), conductivity at 45 min p.m. (Conductivity1) and conductivity at 24 h p.m. (Conductivity24). Conductivity and pH-value were measured by using Star-series equipment (Rudolf Matthaeus Company, Germany) both in the *longissimus dorsi*

between 13^{th}/14^{th} rib and in the *semimembranosus* muscle. Drip loss was scored based on a bag-method with a size-standardized sample from the *longissimus dorsi* collected at 24 h p.m. that was weighed, suspended in a plastic bag, held at 4 ºC for 48 h, and thereafter re-weighed (Honikel 1986). To determine cooking loss, a loin cube was taken from the *longissimus dorsi*, weighed, placed in a polyethylene bag and incubated in water at 75 ºC for 50 min. The bag was then immersed in flowing water at room temperature for 30 min and the solid portion was re-weighed. Thawing loss was determined similarly after at least 24 h freezing at -20 ºC. Drip loss, cooking loss, and thawing loss were calculated as a percentage of weight loss based on the start weight of a sample. Carcass quality traits were collected according to guidelines German performance test (ZDS 2004) (Table 4).

Table 4: Meat quality parameters in the DuPi and Pi populations

Variable	Pop.	N	Mean	Std	Pop.	N	Mean	Std
pH_{1L}	DuPi	313	6.58	0.21	Pi	110	6.21	0.49
pH_{24L}	DuPi	313	5.50	0.08	Pi	110	5.44	0.08
pH_{24H}	DuPi	313	5.64	0.12	Pi	110	5.60	0.14
$Conductivity_{1L}$	DuPi	313	4.36	0.63	Pi	110	5.97	3.11
$Conductivity_{24L}$	DuPi	313	2.76	0.81	Pi	110	5.15	2.95
$Conductivity_{24H}$	DuPi	313	4.78	2.06	Pi	-	-	-
Meat colour	DuPi	313	69.01	5.67	Pi	110	65.87	9.67
Drip loss	DuPi	313	2.08	0.95	Pi	110	4.65	4.14
Cooking loss	DuPi	313	24.67	1.96	Pi	-	-	-
Thawing loss	DuPi	313	8.20	1.84	Pi	-	-	-
Shear force	DuPi	303	35.10	6.43	Pi	-	-	-
Carcass length	DuPi	313	98.08	2.72	Pi	110	96.60	2.57
Backfat thickness	DuPi	313	2.12	0.29	Pi	110	1.71	0.47
Fat area	DuPi	313	16.02	2.90	Pi	110	10.84	2.01
Muscle area	DuPi	313	50.90	5.21	Pi	110	60.23	5.81
Shoulder weight	DuPi	313	7.34	0.51	Pi	110	7.67	0.30
Ham percentage	DuPi	-	-	-	Pi	110	34.61	1.07
Ham weight	DuPi	313	16.20	1.36	Pi	110	14.44	0.72

3.1.4 Chemicals and kits

Beckman Coulter (Krefeld):	CEQ™ 8000 Genetic Analysis System, Dye Terminator Cycle Sequencing (DTCS), Glycogen, sample loading solution (SLS)
Biomol (Hamburg):	Phenol
EZ DNA Methylation-Direct™Kit:	Proteinase K and storage buffer, M-digestion buffer (2×), CT conversion reagent, M-dilution buffer, M-solubilization buffer, M-reaction buffer, M-binding buffer, M-wash buffer, M-desulphonation buffer, M-elution buffer, Zymo-Spin™ IC columns, Collection tubes
Invitrogen Life Technologies (Karlsruhe):	DTT, SuperScriptTM II RNase H- Reverse Transcriptase, 5×first strand buffer, Random
MBI Fermentas (St. Leon-Rot):	Glycogen
Promega (Mannheim):	BSA, pGEM®-T vector, RQ1 RNase-free DNase, RNasin ribonuclease inhibitor, 2×rapid ligation buffer, T4 DNA ligase,
Qiagen Kit (Hilden):	RNeasy® Mini kit, GenEluteTM Plasmid Miniprep
Roth (Karlsruhe):	Acetic acid, Agar-Agar, Ampicillin, Bromophenol blue, Dimethyl sulfoxide (DMSO), Ethylenediaminetetraacetic acid (EDTA), Ethanol, Ethidium bromide, Hydrochloric acid, Isopropyl – Dthiogalactoside (IPTG), Nitric acid, Peptone, Sodium acetate,

	Sodium carbonate, Sodium chloride, Sodium hydroxide, TrisX-Gal (5 -bromo-4-chloro-3-indolylbeta-D-galactopyranoside), Yeast extract
Sigma-Aldrich Chemie GmbH (Munich):	Agarose, Ammonium acetate, Calcium chloride, Formaldehyde, Glutamine, Isopropanol, Magnesium chloride, β-Mercaptoethanol, Oligonucleotide primers, Penicillin, 10×PCR reaction buffer, Potassium chloride, Sodium dodecyl sulfate (SDS), Taq DNA polymerase, TRI Reagent
Stratagene (Amsterdam):	5 c DH *Escherichia coli* competent cells
USB (Ohio):	ExoSAP-IT

3.1.5 Reagents and media

All solutions used in the experiments were prepared with deionized millipore water (ddH_2O). The pH values were adjusted with either sodium hydroxide (NaOH) or hydrochloric acid (HCl).

Agarose loading buffer:	Bromophenol blue 0.0625 g Xylencyanol 0.0625 g Glycerol 7.50 ml ddH_2O added to 25.00 ml
Ampicillin (10 mg/ml):	Ampicillin powder 2.00 g Sterile, distilled water 40.00 ml Filtrate with 0.45 µl filter
Anode buffer I:	Tris-HCl (1 M, pH 10.4) 150.00 ml

	Methanol 50.00 ml Water added to 500.00 ml
Anode buffer II:	Tris-HCl (1 M, pH 10.4) 12.50 ml Methanol 50.00 ml Water added to 500.00 ml
0.3% BSA in PBS:	PSA 3 g 10×PBS: added to 1,000.00 ml
3% BSA in PBS:	PSA 30 g 10×PBS: added to 1,000.00 ml
Cathode buffer:	Tris-HCl (1 M, pH 9.4) 12.50 ml Methanol 50.00 ml 6-aminohexanoic acid 3.94 g Water added to 500.00 ml
DEPC-treated water:	DEPC 1.00 ml ddH_2O 1,000.00 ml Incubation at 37 °C and heat inactivated by autoclaving (120 °C for 30 min)
Digestion buffer:	NaCl 100 mM Tris-HCl 50 mM EDTA pH 8.0 1mM
dNTP solution:	dATP (100 mM) 10.00 µl dCTP (100 mM) 10.00 µl dGTP (100 mM) 10.00 µl dTTP (100 mM) 10.00 µl ddH_2O added to 400.00 µl
1M EDTA, pH 8.0:	EDTA 37.30 g

	ddH_2O added to 1,000.00 ml
10×FA buffer, pH 7.0:	MOPS 41.80 g Sodium acetate 4.10 g EDTA (0.5M) 20.00 ml ddH_2O added to 1000.00 ml
1.2% FA gel:	Agarose 1.20 g 10×FA buffer 10.00 ml DEPC ddH_2O 90.00 ml Ethidium bromide 2.00 µl Formaldehyde (37%) 1.80 ml
Guanidine:	Guanidine hydrochloride 1.43 g hydrochloride (0.3 M): 95% Ethanol added to 50.00 ml
IPTG solution:	IPTG 1.20 g ddH_2O added to 10.0 ml
LB-agar plate:	NaCl 8.00 g Peptone 8.00 g Yeast extract 4.00 g Agar-Agar 12.00 g Sodium hydroxide (40 mg/ml) 480.00 µl ddH_2O added to 800.00 ml
LB-broth:	NaCl 8.00 g Peptone 8.00 g Yeast extract 4.00 g NaOH (40 mg/ml) 480.00 µl ddH_2O added to 800.00 ml

10×PBS:	NaCl 87.67 g $Na_2HPO_4.2H_2O$ 15.00 g NaH_2PO_4 2.04 g Water added to 1,000.00 ml
1×PBS:	10×PBS 100.00 ml Water added to 1,000.00 ml
1×PBS-Tween (PBST):	1×PBS 999.50 ml Tween®20 0.50 ml Tris-HCl (1 M): Tris 121.14 g Water added to 1,000.00 ml
4% PFA (pH 7.3):	Paraformaldehyde 10.00 g 1×PBS added to 250.00 ml Bring to 65°C under ventilation hood, add 5 µl of 5 M NaOH for solution to become clear, store protected from light and use within 2 weeks
Phenol Chloroform:	Phenol : Chloroform 1 : 1 (v/v)
Proteinase K solution:	Protein K in 1×TE bufer 2% (w/v)
0.2% Porceau S:	Ponceau S 1.00 g Acetic acid 50.00 ml Water added to 500.00 ml
10×Running buffer:	Tris 30.30 g Glycine 144.00 g SDS 10.00 g Water added to 1,000.00 ml
4×Sample loading:	Tris (1 M, pH 6.8) 13.00 ml Buffer: SDS 6.00 g 2-Mercaptoethanol 10.00 ml

	Glycerine 20.00 ml Bromophenol blue 10.00 mg Water added to 50.00 ml
Seperation gel (15%):	Acrylamide/bis-acrylamide [30%/0.8% (w/w)] 7.50 ml Tris-HCl (1 M, pH 8.8) 5.60 ml SDS (10%) 0.15 ml Water 1.75 ml Temed 10.00 µl APS (20%) 30.00 µl
SDS solution:	Sodium dodecylsulfat in ddH_2O 10% (w/v)
3M Sodium Acetate, pH 5.2:	Sodium acetate 123.10 g ddH_2O added to 500.00 ml
Stacking gel:	Acrylamide/Bis-acrylamide [30%/0.8% (w/w)] 1.50 ml Tris-HCl (1 M, pH 6.8) 1.30 ml SDS (10%) 0.15 µl Water 7.05 ml Temed 10.00 µl APS (20%) 30.00 µl
TAE (50×) buffer, pH 8.0:	Tris 242.00 mg Acetic acid 57.10 ml EDTA (0.5 M) 100.00 ml ddH_2O added to 1000.00 ml
TBE (10×) buffer:	Tris 108.00 g Boric acid 55.00 g EDTA (0.5 M) 40.00 ml ddH_2O added to 1000.00 ml

TE (1×) buffer:	Tris (1 M) 10.00 ml EDTA (0.5 M) 2.00 ml ddH_2O added to 1000.00 ml
0.2% Triton-X100:	Triton 2.00 ml 10×PBS: added to 1,000.00 ml
X-gal:	X-gal 50.00 mg N, N'-dimethylformamide 1.00 ml

3.1.6 Used software

Comparative map of the pig:	https://www-lgc.toulouse.inra.fr/pig/compare/compare.htm
Correspondences between human and pig chromosomal segments:	Comparative cytogenetic map; http://www2.toulouse.inra.fr/lgc/pig/compare/compare.htm
General search, fragment comparision:	Basic local alignment search tool (BLAST); http://www.ncbi.nlm.nih.gov/BLAST/ http://www.ensembl.org/index.html
Manipulate and display a DNA sequence:	http://www.vivo.colostate.edu/molkit/manip/index.html
Multi amino acid alignment:	http://npsa-pbil.ibcp.fr/cgibin/npsa_automat.pl?page=/NPSA/npsa_multalinan. Html
Multi sequence alignment:	http://prodes.toulouse.inra.fr/multalin/multalin.html

Prediction to affect protein function (SIFT):	http://blocks.fhcrc.org/sift/SIFT.html
Primer design:	Primer3; http://frodo.wi.mit.edu/cgibin/ primer3 /primer3_www.cgi
Primer design for methylation:	http://www.urogene.org/methprimer/index1.html
Quantification tool for methylation analysis:	http://quma.cdb.riken.jp/
Restriction enzyme analysis:	http://tools.neb.com/NEBcutter2/index.php
Searching transcription factor binding site:	http://www.cbrc.jp/research/db/TFSEARCH.html
Statistical analysis:	SAS (version 9.1); SAS Institute Inc., NC, USA
Translation of a base sequence:	http://www.expasy.ch/tools/dna.html

3.1.7 Equipments

ABI PRISM® 7000 SDS:	Applied Biosystems, Foster city, USA
Centrifuge:	Hermle, Wehingen, Germany
CEQ™ 8000:	Genetic Analysis System Beckman Coulter GmbH, Krefeld, Germany
Cryostat CM 3050 S:	Leica, Nussloch, Germany
Electrophoresis (for agarose gels):	BioRad, Munich, Germany

Incubator:	Heraeus, Hanau, Germany
Millipore apparatus:	Millipore corporation, USA
PCR thermocycler (PTC100):	MJ Research, USA & BioRad, Germany
pH Meter:	Kohermann, Germany
Power supply PAC 3000:	Biorad, Munich, Germany
Spectrophotometer (DU-62):	Beckman, Unterschleissheim-Lohhof, Germany
Spectrophotometer, Ultrospec™ 2100 pro UV/Visible:	Amersham Bioscience, Munich, Germany
Thermalshake Gerhardt:	John Morris scientific, Melbourne, Australia
Nitrocellulose transfer:	Schleicher & Schuell BioScience (Dassel, Germany) membrane (Protran®, pore size 0.45 µm)
SuperFrost® Plus slide:	Menzel-Gläser, Braunschweig, Germany
Tuttnauer autoclave:	Connections unlimited, Wettenberg, Germany
Ultra low freezer (-80 ºC):	Labotect GmbH, Gottingen, Germany
UV Transilluminator (Uvi-tec):	Uni Equip, Martinsried, Germany

3.2 Methods

3.2.1 Methods for transcriptome analysis

3.2.1.1 RNA isolation and cDNA synthesis

Samples of different tissues and animals were used for RNA isolation using TRI Reagent (Sigma-Aldrich). The samples were first grinded in a mortar, then mixed and homogenized with 1 ml TRI Reagent. The samples were incubated for 10 min at room temperature to ensure the complete dissociation of the nucleoprotein complexes before adding 0.2 ml of chloroform. The mixtures were shaken and left at room temperature for 10 min and centrifuged at 7500 x *g* for 15 min and 4 °C. The upper aqueous phases were transferred to another sterilized tube and RNA was precipitated using 0.5 ml of isopropanol. After incubation at room temperature for 10 min, the samples were centrifuged at 7500 x *g* for 10 min and 4 °C. After this step the RNA pellet was visible, and subsequently washed using 75% (v/v) ethanol. Centrifugation was then performed and the RNA pellets were air-dried and resuspended in 40 µl of RNase free water.

In order to remove possible contaminating genomic DNA, the extracted RNA was treated with 5 µl RQ1 DNase buffer, 5 units DNase and 40 units RNase inhibitor in a 40 µl reaction volume. The mixture was incubated at 37 °C for 1 h followed by purification using chemicals and protocols of the RNeasy Mini Kit (Qiagen). The concentrations of the RNA after clean-up were determined spectrophotometrically at 260 and 280 nm; the purity of the RNA was estimated by the ratio A260/A280 with respect to contaminants that absorb the UV. Additional examination of integrity was done in a denaturing agarose gel electrophoresis and ethidium bromide staining. Finally, the purified RNA was stored at -80 °C for further analysis.

The individual RNA was used to synthesize first-strand cDNA using SuperScript II enzyme. First 1 µl of Oligo (dT)12 primer (100 µM) and 1 µl random primer were added to 1 µg of total RNA and incubated 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 for a final volume of 20 µl. The mix was incubated at 25 °C for 5 min followed by 42 °C for 1 h and stopped by heating at 70 °C for 15 min. The cDNA was diluted using 80 µl

RNase free water. The resulting cDNA was tested using housekeeping gene (*18S*) primers and kept at -20 °C until use.

3.2.1.2 Ligation and transformation

A fragment of genomic DNA derived from a PCR reaction was ligated into a plasmid pGEM-T Easy vector (Promega). In total 5 µl reaction mix containing 2.5 µl 2×ligation buffer, 0.5 µl pGEM-T (50 µg/ µl), 0.5 µl T4 DNA ligase (3 units/ µl) and 1.5 µl target template were used. The reaction was incubated at room temperature for 1 h or at 4 °C overnight. It was expected that most of the vectors had inserted a DNA fragment after the incubation period. The entire ligation reaction was added to 100 µl volume of competent JM109 *E. coli* cells and incubated on ice for 30 min. This mixture was further heat-shocked in a 42 °C water bath for 90 sec and immediately returned to ice for 2 min. Eight hundred microlitres of nutrient medium (LB-broth) were added and the mix was incubated at 37 °C for 90 min in a thermal 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'-dimethylformamide) and 20 µl of IPTG were prepared. At the end of incubation period, each transformation culture was plated on two of the prepared LB-agar plates and incubated at 37 °C overnight. After the incubation, the colonies were screened by blue white screening test based on the activity of β-galactosidase as white and blue for the presence and absence of inserted DNA fragments. White bacteria colonies and blue colonies were picked up from the plates and suspended in 30 µl 1×buffer for further testing. Each blue colony was used as a control of the length of the amplified DNA fragment in comparison to the vector without fragment from each plate. The same colonies were cultured in 5 ml ampicillin/LB-broth (5 mg/100 ml) in a shaking incubator at 37 °C for further plasmid isolation.

To confirm the insertion of the right fragments into the plasmid, a M13 PCR was performed. The 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 10×PCR buffer, 10 µl lysed bacterial solution, 0.5 µl dNTP (10 mM), 0.5 µl (10 µM) of each M13 primer (forward: 5'-TTGTAAAACGACGGCCAG T-3'; reverse: 5'-CAGGAAACAGCTATGACC-3') and 0.1 U 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 1.5% (w/v) agarose gel with 0.8 µg/ml ethidium bromide (4 µl) in 1×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 Quick Start Kit (Beckman Coulter) including DNA polymerase, pyrophosphatase, buffer, dNTP, dye terminator (ddNTP) and either the gene specific forward or reverse primer. After the sequencing PCR, 3 M NaOAc, 100 mM EDTA and glycogen were added to stop the reaction. To each sample, 60 µl of 98% ethanol (Roth) was added and mixed well by vortexing and then centrifuged for 15 min at 12000 x *g* at 4 °C. All liquid was removed and replaced with 200 µl 70% ethanol without mixing and centrifuged again for 15 min at 12000 x *g* at 4 °C. The ethanol was then removed and the sample was air dried for 10 min. The sample was then resuspended in 40 µl SLS (Beckman Coulter) then transferred manually to a CEQ sample plate and overlaid with mineral oil. Samples were sequenced using CEQ™ 8000 Genetic Analysis System (Beckman Coulter). The sequence method was based on a chain-reaction method. The sequencing PCR was performed to amplify the target fragment, by addition of the labeled base (ddNTP) the amplification reaction stipped after that particular length. Using this method it can be suggested that all possible fragment length are amplified in the product and can further be detected by sequencing, for example using a capillary sequencer. The results from sequence analysis were compared with published sequences using the program BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Plasmids from those clones with identity percentage higher than 90% were considered as the target gene fragment. Plasmids were then isolated for downstream application.

3.2.1.3 Plasmid DNA isolation

The GenEluteTM Plasmid Miniprep Kit was used to isolate plasmid DNA from the bacteria including the insert after the transformation. The plasmid DNA of the bacterial culture was centrifuged at 12000 x *g* for 1 min for harvesting cells, the supernatant was discarded. These cells were resuspended in 200 µl of resuspension solution, vortexed, 200 µl of lysis solution were added and subsequently mixed by inversion of tubes until it became a clear and viscous solution. After incubating at room temperature for 4 min 350 µl neutralization/binding buffer was added for cell precipitation, mixed gently and centrifuged at 12000 x *g* for 10 min. At the same time, the GeneElute Miniprep column was prepared by adding 500 µl preparation solution, centrifuging shortly and discarding the flow-through. After that, the clear supernatant was transferred to this binding column and centrifuged at 12000 x *g* for 1 min. The flow-through was discarded and the column was washed by adding 750 µl wash solution followed by centrifugation at 12000 x *g* for 1 min. To elute the DNA, the column was transferred to a fresh collection tube; 50 µl ddH_2O was added and centrifuged at 12000 x *g* for 1 min. The column was discarded, the DNA plasmid was collected in the water in the tube. For the determination of plasmid size and quality, 5 µl of plasmid DNA was checked together with 2 µl loading buffer using agarose gel electrophoresis. In addition, the quantity of plasmid was also measured by reading the absorbance at 260 nm in a spectrophotometer. An aliquot of the DNA plasmid solution was used to ckeck the fragment using sequencing. The remain part was stored at -20 °C and further used as template for setting up the standard curve in the real-time PCR (qPCR).

3.2.1.4 Quantitative RT-PCR study (qPCR)

cDNA were synthesised by reverse transcription PCR using 2 µg of total RNA, SuperScript II reverse transcriptase (Invitrogen) and oligo(dT)12 primer (Invitrogen). Gene specific primers of qPCR for *IFI6* gene (Fw: 5´-AAGGCGGTATCGCTCTTCTT-3´ and Rv: 5´-TTCTGTTTTGCTTGGTTTTGTTT-3´) and for *HNF1A* gene (Fw: 5´-AGGCTCGTGATTCTGCACTT-3´ and Rv: 5´- CACCAGCCTCACCTCTCTTC-3´) were designed by using the Primer3 software (Rozen & Skaletsky, 2000). Quantitative RT-PCR (qPCR) was set up using 2 µl first-strand cDNA template, 7.6 µl deionized

H_2O, 0.2 µM of upstream and downstream primers, and 10 µl 1×Power SYBR Green I master mix with ROX as reference dye (Applied Biosystems). The thermal cycling conditions were 3 min at 94 °C followed by 20 sec at 94 °C (40 cycles) and 1 min at 60 °C. Experiments were performed using the ABI prism® 7000 (Applied Biosystems) qPCR system. An amplification-based threshold and adaptive baseline were selected as algorithms. The expression profiles of the *IFI6* and *HNF1A* gene were normalized using TATA-box binding protein (*TBP*) as a reference gene (GenBank accession: DQ178129; Fw: 5´-GATGGACGTTCGGTTTAGG-3´ and Rv: 5´-AGCAGCACAGTACGAGCAA -3´). For the final analysis, the relative abundances were extracted for subsequent statistical analysis. Differences in *IFI6* and *HNF1A* gene expression were analyzed with the paired *t* tests. Values of $P < 0.05$ were considered to indicate statistically significant differences.

3.2.2 Methods for genome analysis

3.2.2.1 DNA extraction

For the DNA isolation, the tissue sample (approximately 100 mg) was cut into small pieces and placed in a 1.5 ml tube. 700 µl digestion buffer, 70 µl 10% SDS and 18 µl proteinase K were added for protein digestion. Samples were incubated overnight at 37 °C, 90 rpm. Seven hundred microlitres of phenol-chloroform were added and then the tube was shaken until an emulsion was formed and centrifuged at 10,000 rpm for 10 min. The upper part was transferred into a 2 ml tube, 700 µl of chloroform were added and shook gently. Samples were centrifuged at 10,000 rpm for 10 min. The upper part was carefully collected into 1.7 ml tube, 700 µl of isopropanol and 70 µl of sodium acetate were added into the tube and the samples were shaken gently until precipitation of DNA and then centrifuged at 10,000 rpm for 5 min. The aqueous phase was discarded, then 200 µl of 70% ethanol were added, the pellet liberated from the tube surface then centrifuged at 10,000 rpm for 5 min. The aqueous phase was discarded and the pellet left to dry. Fivehundred microlitres of 1×TE were added in each tube and the DNA samples were left overnight at room temperature. The DNA samples were diluted to a concentration of 50 ng/µl and stored at 4 °C for further analysis.

3.2.2.2 Polymerase chain reaction

To identify polymorphisms within candidate genes, specific primers were designed based on published sequences by using Primer3 software (Rozen and Skaletsky 2000). A list of primers used in this study is given in Table 4. Sequencing for SNP was performed in twenty F_1 DuPi animals (five boars and fifteen sows) where 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. Polymerase chain reactions (PCR) were performed in a 20 µl volume containing 2 µl of genomic DNA, 1×PCR buffer (with 1.5 mM $MgCl_2$), 0.25 mM of dNTP, 5 pM of each primer and 0.1 U of Taq DNA polymerase (GeneCraft). The PCR were performed under the following condition: initial denaturing at 95 ºC for 5 min followed by 35 cycles of 30 sec at 95 ºC, 30 sec at 58.7 ºC and 1 min at 72 ºC and a final elongation of 10 min at 72 ºC. The PCR products were sequenced and screening SNP by using the CEQ™ 8000 Genetic Analysis System (Beckman Coulter).

3.2.2.3 Sequencing

Comparative sequencing was done to screen for polymorphisms within the PCR fragment amplified using specific primers. The PCR product was checked on 1.5% agarose gel, 5 µl PCR product was incubated with 1 µl ExoSAP-IT at 37 °C for 30 min followed by ExoSAP-IT inactivation at 80 °C for 15 min. After clean up, the PCR product was mixed with 2 µl of either forward or reverse primer, 4 µl DTCS master mix (DNA polymerase, pyrophosphatase, buffer, dNTPs, and dye terminators) and 8 µl ddH_2O. The sequence PCR reaction run for 30 cycles, starting with a denaturation step at 96 °C for 20 sec followed by annealing at 50 °C for 20 sec and an extension step at 60 °C for 4 min. A stop solution including 2 µl 3 M NaOAc (pH 5.2), 2 µl of 100 mM EDTA (pH 8.0) and 1 µl glycogen (20 mg/ml) were added to the product after the sequencing PCR. 60 µl cold 95% ethanol were added. The solution was mixed throughly and centrifuged at 12000 x *g* for 15 min at 4 °C. The supernatant was removed and the pellet was rinsed two times with 200 µl cold 70% ethanol with centrifugation steps at 12000 x *g* at 4 °C for 5 min in between. The supernatant was removed and the pellet was air dried or vacuum dried for 10 min. The pellet was

resuspended with 40 µl of sample loading solution, transferred to the wells of the sample plate and overlaid with one drop of light mineral oil. The separation buffer was prepared in the buffer plate, both plates were loaded into the CEQ™ 8000 Genetic Analysis System (Beckman Coulter). A fragment specific sequencing program was started. A open source software program for comparative sequencing was used to polymorphism screening.

3.2.2.4 Genotyping

The PCR-RFLP method was used for genotype the DuPi (n = 313) and Pi (n = 110) population. The restriction enzymes were selected according to the recognition (http://tools.neb.com/NEBcutter2/index.php) of the polymorphic sites. The fragments with the detected mutation were amplified using different annealing temperature to get the PCR products (Table 5). An aliquot of the PCR product of each reaction was checked on 1.5% agarose gel (Fisher Scientific Ltd.) before digestion using different endonucleases. The digested products were separated using 3.0% agarose gel. The fragments were visualized under ultraviolet light, and the sizes and the number of fragments analysed using the molecular analyst software (BioRad Laboratories, Molecular Bioscience Group). PCR-RFLP patterns for six candidate genes were given in Table 6.

Table 5: Detailed information about 8 SNPs from six candidate genes analyzed in this study

Gene	Location	Sequence	Tm (ºC)	Size (bp)	Enzyme	Acc no
AMBP	intron10	F:GTGCAGGGGAAGTGTGTTCT R:GCAGCAGCTCTTCATCCTCT	58.7	730	SmaI	NC_010443; g.22229C>T
GC	intron7	F:CAAATGCTGTGACTCTGC R:CCAGGACTTTGGGGTTTC	58.7	346	BccI	GU065652; g.398C>T
PPP1R3B	exon2	F:CTTCCAGATCTCACCGAAGC R:TGTCCCAGTACGTCTGTCCA	58.9	602	BstuI	BW971859; c.479A>G
TNC	intron10	F: TGGGCATTTCACACAGGTAA R: CAAGAGCGAGAGGTCACACA	58.7	528	SacI	NC_010443; g.44488C>T
TNC	exon12	F:CTCAGGACGCTTCCATCTCT R:GTCCAGAGCTCGTGCAATTT	58.7	443	AciI	NC_010443; g.68794A>G
TNC	intron12	F:CTCAGGACGCTTCCATCTCT R:GTCCAGAGCTCGTGCAATTT	58.7	443	CviAII	NC_010443; g.68841C>T
IFI6	intron2	F: AAGGCGGTATCGCTCTTCTT R: AGGCAGCCACAGAGTTGG	58.7	639	HphI	GU931791 g.370A>G
HNF1A	intron1	F: ACGCTTGCTTCCATCCAC R: TGTTGAGGTGCTGGGACAG	60	240	HphI	NC_010456 g.8260A>G

Table 6: Genotype patterns of six candidate genes

Gene	Position	Genotype pattern	Fragment (bp)
AMBP	g.22229C>T	CC	529+201
		TT	730
GC	g.398C>T	CC	267+79
		TT	346
PPP1R3B	c.479A>G	AA	485+117
		GG	275+210+117
TNC	g.44488C>T	CC	432+96
		TT	528
TNC	g.68794A>G	AA	395+29+11
		GG	317+78+29+19
TNC	g.68841C>T	CC	443
		TT	414+29
IFI6	g.370A>G	AA	378+184+77
		GG	562+77
HNF1A	g.8260A>G	AA	144+96
		GG	240

3.2.2.5 Association study

Allele and genotype frequencies of each population were determined to detect SNP in the six candidate genes. Only for *TNC* gene, the haplotype phase between three SNPs was inferred using the expectation-maximization algorithm (PROC HAPLOTYPE, SAS Version 9.1). Only those animals whose haplotype pairs were assigned with the probability 1 were used. The traits and genotypes and its diplotypes were statistically analyzed by least squares analysis of variance using the General Linear Model procedure (PROC GLM). The association of the genotypes from six candidate genes with meat and carcass quality traits were calculated by analyzing variance of quantitative traits. For these analyses a generalized linear model of SAS (SAS Inst. Inc., Cary, NC) was used. The model was as follows:

$$Y_{ijklm} = \mu + \text{Genotype}_i + \text{Sire}_j + \text{Sex}_k + \text{YS}_l + \beta_{\text{SW}} + e_{ijklm}$$

where Y_{ijklm} is the observation of the trait (meat and carcass quality); μ is the population mean; Genotype_i is the effect of i-th genotype (i = 1, 2 and 3); Sire_j is the effect of j- th

sire (j = 1, 2, 3, 4 and 5); Sex_k is the effect of k-th sex (k = 1 for male or 2 for female); YS_l is the effect of l-th season of slaughter (l = 1 through 12) and β_{SW} is the linear effect of slaughter weight as covariate and e_{ijklm} is the random residual error. Least square mean values for the genotypes of each six candidate genes were compared by *t*-test and *P*-values were adjusted by the Tukey-Kramer correction.

3.2.2.6 Linkage mapping

Additional relative positions of the candidate genes were assigned using genetic mapping. The relative positions of the markers were assigned using the build, two point, chrompic and fixed options of CRIMAP (version 2.4) (Green et al. 1990). In the first step, the genes were assigned to the chromosome using information from different published databases. Candidate genes were assigned to SSC1, SSC6, SSC8, SSC14 and SSC15, respectively. In the first step, the genes were assigned to the chromosome using information from different published databases such as NCBI and Ensembl. The genotype of each gene was further analysed against 3 to 4 microsatellite markers on the assigned chromosome.

3.2.2.7 Promoter prediction, CpG island identification

The web based Primer3 (Rozen and Skaletsky 2000) program was used for designing primers of *HNF1A* gene (Fw: 5´-TAGTAGGTGTAAGGAGTTTGGTTGG-3´ and Rv: 5´- ACCAAAAAATAAAACCCTAACTCAC-3´). Genomic DNA was amplified by PCR and sequencing of amplified PCR fragments was performed by the CEQ™ 8000 sequencer system (Beckman Coulter). A sequence of transcription start site (TSS) was predicted using TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html). The published sequences (NC_010456.1) of the 5´-flanking regions which contained the promoters of the *HNF1A* gene were submitted to CpGProd software (Ponger and Mouchiroud 2002) in order to identify the CpG islands.

3.2.2.8 Genomic bisulfite PCR and sequencing

Only for *HNF1A* gene, genomic DNA was extracted from 5 animals with high pH muscle and 5 animals with low pH muscles, the same used for gene expression analysis. Each DNA (1 µg) was modified with the bisulfite conversion reaction according to the manufacturer protocol (EZ DNA Methylation Kit, Zymo Research). The converted DNA was diluted with 15 µl of ddH_2O. Each converted DNA (2 µl) was used as a template for PCR with primers designed using the MethPrimer program (Li and Dahiya 2002). The PCR products amplified from the bisulfite-treated DNA were further analyzed through cloning and sequencing. Each of PCR products were purified using QIAquick PCR purification kit (Qiagen), and then individually cloned into the pGEM-T Easy vector (Promega). In total 5 different clones of each animal were randomly selected for sequencing with M13 primers. Methylation sites were visualized and quality controlled by using QUMA (Kumaki et al. 2008) software. Sequences with a conversion rate below 90% were excluded from analysis.

3.2.3 Methods for protein analysis

3.2.3.1 Protein extraction

Protein was extracted from muscle (*longissimus dorsi*) using TRI Reagent following the manufacturer's instruction. The tissue samples were taken out from storage at -80 ºC just before isolation. All centrifugation steps were performed at 4 °C. Twenty milligram of tissue was ground by using mortal and pestle and incubated with 0.5 ml of TRI Reagent for 5 minutes at room temperature in a microcentrifuge tube. The clear homogenate was then mixed with 0.1 ml of chloroform, vigorously shaked by hand for 15 sec and centrifuged at 12,000 x *g* for 15 min. A colourless upper aqueous phase was moved out and the DNA from the interphase and organic phase was precipitated by adding 150 µl of 100% ethanol. The mixture was mixed by inversion, allowed to stand for 2 to 3 min at room temperature and centrifuged at 2,000 x *g* for 5 min. The supernatant was transferred to a new 2.0 ml tube, 750 µl of isopropanal was then added and allowed to stand at room temperature for 10 min followed by centrifugation at 12,000 x *g* for 10 min. The protein pellet was washed three times in 1 ml of 0.3 M guanidine hydrochloride/95% ethanol solution. During each wash, the sample was stored in wash

buffer for 20 min at room temperature and centrifuged at 7,500 x *g* for 5 min. After the three washes, 1 ml of 100% ethanol was added and the mixture was allowed to stand for 20 min and then centrifuged at 7,500 x *g* for another 5 min. Finally, the protein was dried under a vacuum for 5 min and dissolved in 200 µl of sample buffer containing 1% protease inhibitor cocktail followed by boiling for 5 min at 95 °C and storing at -20 °C.

3.2.3.2 Protein expression study by western blot

Protein was extracted from muscles (*longissimus dorsi*) which were also used for mRNA expression study. The protein samples were loaded on each well of a gradient gel (4-18% of SDS-PAGE), transferred to nitrocellulose membrane (Bio-Rad), blocked in blocking buffer (20 mM Tris pH 7.5, 150 mM NaCl, 0.05% Tween-20 and 1% polyvinylpyrolidone) for 1 h, and the membrane was incubated overnight at 4 °C with anti-G1P3 antibody (diluted 1:800; sc-82176, Santa Cruz) and anti-HNF1A antibody (diluted 1:500; sc-8986, Santa Cruz) in the blocking medium. Non-specific binding of antibody was washed off with six changes of 0.1% PBST, followed by detection with 1:5000 diluted HRP-conjugated secondary anti-goat IgG for anti-G1P3 antibody (Santa Cruz) and HRP-conjugated secondary anti-rabbit IgG for anti-HNF1A antibody (Santa Cruz) at room temperature for 1 h using the ECL Plus Detection System (Amersham Biosciences) and visualized by using Kodak BioMax XAR film (Kodak). GAPDH antibody was used as a loading control and for normalization. Relative band intensities were compared by determining the ratio of the area densities of target protein to reference protein bands for each lane using Image-J software (National Institute of Mental Health, Bethesda, Maryland, USA).

3.2.3.3 Protein localization by immunofluorescence

Muscle (*longissimus dorsi*) samples were collected. Immediately after collection, the samples were snap frozen in isopentane cooled in liquid nitrogen, and cryostat sections were cut (7-10 µm thick). To block unspecific staining, sections were incubated for 30 min with 5% bovine serum albumin in PBS (50 nM sodium phosphate, pH 7.4; 0.9% NaCl). Sections were incubated overnight at 4 ºC with the primary antibody (anti-G1P3) diluted at 1:50 in PBST and primary antibody (anti-HNF1A) diluted at 1:50 in PBST,

then followed by six times (10 min per time) washing with PBS. Then, the sections were incubated 1 h at room temperature with fluorescein-labeled donkey anti-goat IgG (1:200) (Santa Cruz) for the IFI6 protein and goat anti-rabbit IgG (1:200) (Santa Cruz) for the HNF1A protein followed by six times (10 min per time) washing with PBS. Finally, the sections were examined in a Zeiss Axioplan fluorescence microscope (Carl Zeiss). In case of negative controls, PBS was used instead of primary antibody.

4. Results

4.1 SNP screening

4.1.1 The *AMBP* gene

The comparative analysis between human (GenBank accession: NC_00009) and porcine (GenBank accession: NC_010443) sequence of the *AMBP* gene showed that these two mammalian species have highly conserved gene sequence (81%). The porcine *AMBP* gene was consisted of 11 exons and 10 introns. The SNP screening was found one SNP in the intron 10 (g.22229 C>T; GenBank accession: NC_010443). This SNP was selected for genotyping in DuPi and Pi populations. PCR-RFLP patterns for *AMBP* alleles were as follows: genotype AA resulted in three fragments (378, 184 and 77 bp) while genotype GG resulted in two fragments (562 and 77 bp) (Table 6).

4.1.2 The *GC* gene

The comparison of human (GenBank accession: NC_000004) and porcine (GenBank accession: AY710291) sequence of the *GC* gene revealed that these two sequences had highly conserved gene sequence (95%). From the comparative sequence, it could be estimated that this gene consisted of 13 exons and 12 introns. One polymorphic site was detected within the intron 7 (g.398 C>T; GenBank accession: GU065652). PCR-RFLP patterns for *GC* alleles were as follows: genotype CC resulted in two fragments (267 and 79 bp) while genotype TT resulted in one fragment (346 bp) (Table 6).

4.1.3 The *PPP1R3B* gene

The structure of the porcine *PPP1R3B* gene could be deduced by comparison of full-length human *PPP1R3B* gene (GenBank accession: NC_000008) and porcine EST (GenBank accession: BW971859). The comparative analysis revealed that this gene consisted of 2 exons and 1 intron. The SNP screening was detected one SNP within a coding region of exon 2 (c.479 A>G; GenBank accession: BW971859). PCR-RFLP patterns for *PPP1R3B* alleles were as follows: genotype AA resulted in two fragments

(485 and 117 bp) while genotype GG resulted in three fragments (275, 210 and 117 bp) (Table 6).

4.1.4 The *TNC* gene

Analysis of the sequencing data revealed that the porcine *TNC* gene contains 16 exons and 15 introns (GenBank accession: NC010443). Three polymorphism sites were detected. Two SNPs were located in the intron 10 (g.44488 C>T; GenBank accession: NC010443) and intron 12 (g.68841 C>T; GenBank accession: NC010443), respectively. One SNP was located in a coding region of exon 12 (g.68794 A>G; GenBank accession: NC010443). PCR-RFLP patterns for *TNC* at g.44488 C>T were as follows: genotype CC resulted in two fragments (432 and 96 bp) while genotype TT was resulted in one fragment (528 bp) (Table 6). PCR-RFLP patterns for *TNC* at g.68794 A>G were as follows: genotype AA resulted in three fragments (395, 29 and 11 bp) while genotype GG resulted in four fragments (317, 78, 29 and 19 bp). PCR-RFLP patterns for *TNC* at g.68841 C>T were as follows: genotype CC resulted in one fragment (443 bp) while genotype TT resulted in two fragments (414 and 29 bp).

4.1.5 The *IFI6* gene

The structure of the porcine *IFI6* gene was deduced by comparison of full-length human mRNA sequence and a porcine EST. The comparative analysis between human (GenBank accession: NC_000001) and porcine (GenBank accession: BN000123) sequence of the *IFI6* gene showed that this gene consisted of 5 exons and 4 introns. Screening for SNP showed one SNP located in the intron 2 (g.370 A>G; GenBank accession: GU931791). PCR-RFLP patterns for g.370 A>G were as follows: genotype AA resulted in three fragments (378, 184 and 77 bp) while genotype GG resulted in two fragments (562 and 77 bp) (Table 6).

4.1.6 The *HNF1A* gene

The porcine *HNF1A* gene (GenBank accession: NC_010456) consists of 19,504 bp. The structure of the porcine *HNF1A* gene contains 10 exons and 9 introns. A SNP was located within the intron 1 (g.8260 A>G; GenBank accession: NC_010456). PCR-RFLP patterns for g.8260 A>G were as follows: genotype AA resulted in two fragments (144 and 96 bp) while genotype GG resulted in one fragment (240 bp) (Table 6).

4.2 Association of the candidate genes with meat and carcass quality traits

4.2.1 Association of the *AMBP* gene with meat and carcass quality

Significant associations of *AMBP* (g.22229 C>T) are displayed in Table 8. The analysis revealed significant association of *AMBP* with $pH1_L$ ($P < 0.01$), $pH24_L$ ($P < 0.05$), $pH24_H$ ($P < 0.01$), $conductivity24_L$ ($P < 0.01$) and carcass length ($P < 0.05$) in the DuPi population. In the Pi population, association was observed with $pH24_H$ ($P < 0.05$), backfat thickness ($P < 0.05$) and fat area ($P < 0.05$). The genotyping of *AMBP* revealed that the homozygous "TT" (0.22) and "CC" (0.17) were found in a lower frequency compared to the heterozygous "CT" (0.61) in the DuPi population. Moreover, the heterozygous "CT" (0.58) was found in higher frequency than the homozygous "CC" and "TT" (0.17 and 0.25, respectively) in the Pi population (Table 7). In the DuPi population, the "C" allele occurred with higher frequency (0.52) than the Pi population (0.46). The "T" allele (0.48) in the DuPi population was observed with lower frequency than the Pi population (0.54). In the DuPi population, animals with the genotype "TT" tended to produce meat with higher $pH1_L$, $pH24_L$, $pH24_H$ and carcass length. The homozygous genotype "CC" led to produce meat with a higher $conductivity24_H$ than the homozygous genotype "TT" in the DuPi animals. In the Pi population, animals with the genotype "TT" had the lowest $pH24_H$ and fat area. Additionally, the heterozygous genotype "CT" had the lower backfat thickness than the homozygous genotype "CC" in the Pi population.

Table 7: Genotypic and allelic frequencies of six candidate genes in pigs

Gene	Position	Genotype	Population		Allele	Population	
			DuPi	Pi		DuPi	Pi
AMBP	g.22229C>T	CC	0.22	0.17	C	0.52	0.46
		CT	0.61	0.58	T	0.48	0.54
		TT	0.17	0.25			
GC	g.398C>T	CC	0.44	0.57	C	0.72	0.78
		CT	0.55	0.41	T	0.28	0.22
		TT	0.01	0.02			
PPP1R3B	c.479A>G	AA	0.69	0.89	A	0.84	0.94
		AG	0.30	0.09	G	0.16	0.06
		GG	0.01	0.02			
TNC	g.44488C>T	CC	0.02	0.02	C	0.31	0.47
		CT	0.58	0.90	T	0.69	0.53
		TT	0.4	0.08			
TNC	g.68794A>G	AA	0.35	0.06	A	0.64	0.21
		AG	0.57	0.3	G	0.36	0.79
		GG	0.08	0.64			
TNC	g.68841C>T	CC	0.31	1	C	0.64	1
		CT	0.66	--	T	0.36	--
		TT	0.03	--			
IFI6	g.370A>G	AA	0.42	0.05	A	0.66	0.30
		AG	0.48	0.49	G	0.34	0.70
		GG	0.10	0.46			
HNF1A	g.8260A>G	AA	--	0.03	A	0.07	0.14
		AG	0.14	0.22	G	0.93	0.86
		GG	0.86	0.75			

4.2.2 Association of the *GC* gene with meat and carcass quality

The results of *GC* showed a small effect on $pH24_H$, $conductivity1_L$ and thawing loss but none was statistically significant in the DuPi population ($P < 0.10$) (Table 9). In term of carcass quality, fat area showed significant association in the DuPi population ($P < 0.05$). However in the Pi population, the analysis revealed significant association of *GC* on $pH24_H$ and carcass length ($P < 0.05$). The genotype "CC" (0.44) and "CT" (0.55) had higher frequency compared to the homozygote "TT" (0.01) in the DuPi animals (Table 7). On the other hand in the Pi animals, the genotype "CC" (0.57) had higher frequency compared to the genotype "CT" and "TT" (0.41 and 0.02, respectively). Both, DuPi and Pi populations were showed the lowest frequencies of the homozygous "TT". The presence of the allele "C" was very high across both DuPi and Pi populations (0.72 and 0.78, respectively). Animals carrying the homozygous genotype "TT" had lower fat area than the homozygous genotype "CC" in the DuPi population. In the Pi animals, the genotype "TT" tended to produce meat with lower $pH24_H$ than the homozygous genotype "CC". In addition, animals with the genotype "TT" had higher carcass length than the homozygous genotype "CC".

Table 8: Least square means (LSM) and standard error (SE) for meat and carcass quality traits of the *AMBP* gene in the DuPi and Pi population

Trait	Population	Least Square Means (SE)			P-value	Effect (mean±SE)	
		CC	CT	TT		Additive	Dominance
Meat colour	DuPi	67.55 (0.77)	69.66 (0.45)	69.39 (0.88)	0.069	1.84±1.17 $^{n.s.}$	1.14±0.74*
$pH1_L$	DuPi	6.51^{a} (0.03)	6.60 (0.02)	6.63^{b} (0.03)	0.005	0.12±0.04**	0.03±0.03 $^{n.s.}$
$pH24_L$	DuPi	5.47^{a} (0.01)	5.50 (0.01)	5.51^{b} (0.01)	0.029	0.04±0.02*	0.01±0.01 $^{n.s.}$
$pH24_H$	DuPi	5.59^{a} (0.02)	5.65 (0.01)	5.66^{b} (0.01)	0.005	0.07±0.02**	0.02±0.02 $^{n.s.}$
$Conductivity24_L$	DuPi	3.05^{a} (0.10)	2.68 (0.06)	2.64^{b} (0.12)	0.007	-0.41±0.16**	-0.16±0.10 $^{n.s.}$
Carcass length (cm)	DuPi	97.73 (0.38)	97.80^{a} (0.23)	99.14^{b} (0.46)	0.033	-0.70±0.31*	0.63±0.37 $^{n.s.}$
Backfat thickness (cm)	DuPi	1.64 (0.05)	1.65^{a} (0.03)	1.49^{b} (0.06)	0.061	0.07±0.04 $^{n.s.}$	-0.08±0.05 $^{n.s.}$
Shoulder weight (kg)	DuPi	7.38 (0.05)	7.33^{a} (0.03)	7.50^{b} (0.06)	0.058	-0.06±0.04 $^{n.s.}$	0.11±0.05*
$pH24_H$	Pi	5.44^{a} (0.03)	5.44^{a} (0.03)	5.37^{b} (0.02)	0.028	0.03±0.02 $^{n.s.}$	-0.04±0.03 $^{n.s.}$
Backfat thickness (cm)	Pi	1.55^{a} (0.06)	1.38^{b} (0.03)	1.51 (0.05)	0.035	0.03±0.04 $^{n.s.}$	0.06±0.05 $^{n.s.}$
Fat area (cm^2)	Pi	12.32^{a} (0.53)	10.74 (0.29)	10.73^{b} (0.45)	0.031	0.70±0.32 $^{n.s.}$	0.07±0.42 $^{n.s.}$
Ham percentage (%)	Pi	1.78^{a} (0.06)	1.62^{b} (0.03)	1.73 (0.05)	0.063	-0.34±0.16*	-0.26±0.22 $^{n.s.}$

[1]pH24: pH 24 h post-mortem; [2]Conductivity1: Conductivity 45 min post-mortem; [3]Conductivity24: Conductivity 24 h post-mortem.
DuPi: Duroc x Pietrain population; Pi : Pietrain population
^{a,b,*} $P < 0.05$; ^{c,d,**} $P < 0.01$; ^{e,f,***} $P < 0.001$

Table 9: Least square means (LSM) and standard error (SE) for meat and carcass quality traits of the *GC* gene in the DuPi and Pi population

Trait	Population	Least Square Means (SE)			P-value	Effect (mean±SE)	
		CC	CT	TT		Additive	Dominance
$pH24_H$	DuPi	5.67 (0.01)	5.64 (0.01)	5.50 (0.01)	0.097	-0.58±0.06 $^{n.s.}$	0.08±0.06 $^{n.s.}$
$Conductivity1_L$	DuPi	4.3 (0.05)	4.46 (0.06)	5.10 (0.01)	0.090	0.35±0.32 $^{n.s.}$	-0.40±0.32 $^{n.s.}$
Thawing loss	DuPi	7.87 (0.16)	8.34 (0.16)	6.90 (0.01)	0.088	-0.96±0.92 $^{n.s.}$	0.48±0.91 $^{n.s.}$
Fat area (cm^2)	DuPi	17.11^a (0.35)	16.27 (0.30)	11.51^b (2.78)	0.034	2.78±1.40*	-1.94±1.41 $^{n.s.}$
$pH24_H$	Pi	5.61^a (0.30)	5.57 (0.03)	5.34^b (0.10)	0.033	-0.03±0.08 $^{n.s.}$	0.02±0.08 $^{n.s.}$
Carcass length (cm)	Pi	96.10^a (0.31)	97.50 (0.39)	97.10^b (1.94)	0.020	-0.42±0.90 $^{n.s.}$	-0.79±0.99 $^{n.s.}$
Ham weight (kg)	Pi	14.41 (0.09)	14.21 (0.11)	13.41 (0.55)	0.086	0.55±0.20*	-0.44±0.22 $^{n.s.}$

[1]pH24: pH 24 h post-mortem; [2]Conductivity1: Conductivity 45 min post-mortem; [3]Conductivity24: Conductivity 24 h post-mortem.
DuPi: Duroc x Pietrain population; Pi : Pietrain population
^{a,b,*} $P < 0.05$; ^{c,d,**} $P < 0.01$; ^{e,f,***} $P < 0.001$

4.2.3 Association of the *PPP1R3B* gene with meat and carcass quality

The *PPP1R3B* gene was significantly associated with meat colour, $pH1_L$, $pH24_H$, shear force value, carcass length ($P < 0.001$) and $pH24_L$ ($P < 0.01$) in the DuPi population (Table 10). This study could not observe association of *PPP1R3B* with meat and carcass quality traits in the Pi population ($P > 0.05$). The homozygous "AA" was the most frequent genotype (0.69) followed by the heterozygous "AG" (0.30) and homozygous "GG" (0.01) in the DuPi population. Moreover, the genotype "AA" (0.89) had higher frequency compared to the genotype "AG" and "GG" (0.09 and 0.02, respectively) in the Pi population (Table 7). The allele frequency of "G" was the lowest both in DuPi and Pi populations (0.16 and 0.06, respectively), whereas, the allele frequency of "A" was 0.84 and 0.94 in DuPi and Pi populations, respectively. In the DuPi animals, the presence of the homozygous genotype "AA" tended to produce meat with lower meat colour value, $pH24_L$ and $pH24_H$ than the homozygous genotype "GG". Additionally, the heterozygous genotype "AG" was the highest $pH1_L$ and carcass length but the lowest in shear force value.

4.2.4 Association of the *TNC* gene

4.2.4.1 Genotype and association analysis of the *TNC* gene with meat and carcass quality

In the DuPi population, the SNP of the porcine *TNC* gene, located on g.44488 C>T, revealed a significant association with meat colour and ham weight ($P < 0.05$) (Table 11). The genotype "CT" (0.58) had higher frequency compared to the genotype "TT" and "CC" (0.40 and 0.02, respectively) in the DuPi population (Table 7). Additionally, the allele "C" (0.31) had lower frequency than the allele "T" (0.69). The animals of the genotype "CC" had a lower meat colour value than those of the genotype "TT". Moreover, the animals with the homozygous "CC" had significantly heavier ham weights than the heterozygous "CT" animals. There are significant associations between the polymorphism g.68794 A>G with $pH24_H$ ($P < 0.01$) and muscle area ($P < 0.05$). The genotype "AG" (0.57) had higher frequency compared to the genotype "AA" and "GG" (0.35 and 0.08, respectively). The allele "A" (0.64) showed higher frequency than

the allele "G" (0.36). The homozygous "AA" tended to produce meat with lower muscle area and higher $pH24_H$. The SNP g.68841 C>T was not associated with any meat and carcass quality traits ($P > 0.05$) (data not shown). Also for the genotype frequency it was found that the genotype "CC" (0.31) and "CT" (0.61) had higher frequency compared to the homozygote "TT" (0.03) (Table 7). The allele "C" (0.64) had higher frequency than the allele "T" (0.36).

In the Pi population, the SNP genotyped at g.44488 C>T was significant associated with $pH24_H$ ($P < 0.01$) (Table 11). The genotype "CT" (0.90) had higher frequency compared to the genotype "CC" and "TT" (0.02 and 0.08, respectively). The allele "T" (0.53) showed higher frequency than the allele "C" (0.47). The animals of the genotype "TT" had the lowest $pH24_H$. The polymorphism g.68794 A>G showed association with conductivity1_L ($P < 0.05$) and backfat thickness ($P < 0.01$). For the genotype frequency, it was found that the genotype "GG" (0.64) and AG (0.30) had higher frequency compared to the homozygote "AA" (0.06) (Table 7). The presence of the allele "G" was more frequent compared with the allele "A" (0.79 and 0.21, respectively). The homozygous "GG" had the lowest conductivity1_L and highest backfat thickness. This study found that SNP at locus g.68841 C>T did not segregate in the Pi population.

4.2.4.2 Haplotype analysis of the *TNC* gene with meat and carcass quality

Eight different haplotypes were identified in the DuPi population: H1 (CAC), H2 (CAT), H3 (CGC), H4 (CGT), H5 (TAC), H6 (TAT), H7 (TGC) and H8 (TGT). The frequencies of eight haplotypes are 0.02, 0.05, 0.12, 0.11, 0.38, 0.18, 0.12 and 0.02, respectively. Additionally, four haplotypes segregated in the Pi population: H1 (CAC), H3 (CGC), H5 (TAC) and H7 (TGC). Frequencies of four haplotypes are 0.04, 0.43, 0.17 and 0.36, respectively. Among the three SNPs, those haplotype pairs assigned with the probability 1, were used for haplotype determination. The diplotypes both in DuPi and Pi populations showed a significant effect on $pH24_H$ ($P < 0.05$) (Table 12). Six haplotype combinations (diplotypes) in the DuPi population were detected, of which H5H6 had higher pH24 ham than H2H6 diplotype. In the Pi population, seven diplotypes were detected. Diplotype H5H7 had lower $pH24_H$ than H1H5, H3H3, H3H7, H5H3 and H7H7 ($P < 0.01$).

Table 10: Least square means (LSM) and standard error (SE) for meat and carcass quality traits of the *PPP1R3B* gene in the DuPi and Pi population

		Least Square Means (SE)				Effect (mean±SE)	
Trait	Population	AA	AG	GG	P-value	Additive	Dominance
Meat colour	DuPi	68.18[a] (0.46)	71.73 (0.68)	73.52[b] (4.33)	0.001	5.90±3.19 [n.s.]	0.03±1.70 [n.s.]
$pH1_L$	DuPi	6.55 (0.02)	6.66[b] (0.03)	6.33[a] (0.16)	0.001	-0.12±0.12 [n.s.]	0.16±0.06**
$pH24_L$	DuPi	5.49[a] (0.01)	5.52 (0.01)	5.61[b] (0.07)	0.009	0.05±0.05 [n.s.]	-0.003±0.02 [n.s.]
$pH24_H$	DuPi	5.62[a] (0.01)	5.65 (0.02)	6.02[b] (0.01)	0.001	0.19±0.07***	-0.07±0.04 [n.s.]
Shear force	DuPi	36.23 (0.44)	33.13[b] (0.71)	38.69[a] (2.96)	0.001	10.42±3.69**	-6.07±1.96**
Carcass length (cm)	DuPi	97.59 (0.19)	99.16[b] (0.28)	96.51[a] (1.78)	<0.001	0.54±0.91 [n.s.]	-2.10±0.94*
$pH24_L$	Pi	5.43 (0.01)	5.40 (0.03)	5.56 (0.06)	0.052	-0.06±0.06 [n.s.]	0.07±0.07 [n.s.]

[1]pH24: pH 24 h post-mortem; [2]Conductivity1: Conductivity 45 min post-mortem; [3]Conductivity24: Conductivity 24 h post-mortem.
DuPi: Duroc x Pietrain population; Pi : Pietrain population
[a,b,*] $P < 0.05$; [c,d,**] $P < 0.01$; [e,f,***] $P < 0.001$

Table 11: Least square means (LSM) and standard error (SE) for meat and carcass quality traits of the *TNC* gene in the DuPi and Pi population

			Least Square Means (SE)				Effect (mean±SE)	
Position	Traits	Population	CC	CT	TT	P-value	Additive	Dominance
g.44488 C>T	Meat colour	DuPi	65.40^{a} (3.16)	68.73 (0.54)	70.67^{b} (0.65)	0.043	-2.63±1.65 $^{n.s.}$	-0.68±1.69 $^{n.s.}$
	Ham weight (kg)	DuPi	17.48^{b} (0.60)	16.07^{a} (0.10)	16.30 (0.15)	0.029	0.59±0.32 $^{n.s.}$	0.82±0.32**
	Meat colour	Pi	82.86 (9.08)	62.86 (2.15)	58.43 (4.24)	0.071	12.28±5.22*	7.79±5.13 $^{n.s.}$
	$pH24_H$	Pi	5.75^{d} (0.11)	5.58 (0.03)	5.43^{c} (0.05)	0.008	0.16±0.06**	0.01±0.06 $^{n.s.}$
	Ham weight (kg)	Pi	15.80 (0.95)	17.90 (0.11)	18.33 (0.34)	0.056	-1.26±0.52*	-0.83±0.52 $^{n.s.}$
	Muscle area (cm^2)	Pi	51.74 (4.23)	60.94 (0.50)	59.69 (1.53)	0.087	-3.97±2.32 $^{n.s.}$	-5.22±2.32*
			AA	AG	GG			
g.68794 A>G	$pH24_H$	DuPi	5.67^{d} (0.01)	5.63 (0.01)	5.56^{c} (0.03)	0.003	0.05±0.02**	-0.01±0.02 $^{n.s.}$
	Muscle area (cm^2)	DuPi	49.63^{a} (0.64)	51.58^{b} (0.50)	50.44 (1.26)	0.035	-0.41±0.69 $^{n.s.}$	-1.55±0.83*
	$Conductivity1_L$	Pi	5.67 (0.96)	5.98^{b} (0.47)	4.79^{a} (0.48)	0.037	0.44±0.45 $^{n.s.}$	-0.74±0.61 $^{n.s.}$
	Backfat thickness (cm)	Pi	0.88 (0.11)	0.73^{c} (0.05)	0.90^{d} (0.03)	0.008	-0.01±0.05 $^{n.s.}$	0.17±0.07*

[1]pH24: pH 24 h post-mortem; [2]Conductivity1: Conductivity 45 min post-mortem; [3]Conductivity24: Conductivity 24 h post-mortem.
DuPi: Duroc x Pietrain population; Pi : Pietrain population
^{a,b,*} $P < 0.05$; ^{c,d,**} $P < 0.01$; ^{e,f,***} $P < 0.001$

Table 12: Least square means (LSM) and standard errors (SE) for carcass and meat quality traits across diplotypes of the *TNC* gene in the DuPi and Pi population

		Least square means (SE)									
Trait	Population	H1H5	H2H6	H3H3	H3H7	H5H3	H5H5	H5H6	H5H7	H7H7	P-Value
$pH24_H$	DuPi	5.73(0.09)	5.46(0.07)[a]	--	5.66(0.05)	--	5.72(0.05)	5.78(0.04)[b]	5.66(0.05)	--	0.015
$pH24_H$	Pi	5.69(0.08)[a]	--	5.91(0.13)[a]	5.61(0.03)[a]	5.70(0.14)[a]	5.50(0.11)	--	5.24(0.11)[b]	5.77(0.17)[a]	0.047

[1]pH24: pH 24 h post-mortem
DuPi: Duroc x Pietrain population; Pi : Pietrain population
[a, b] $P < 0.05$; [c, d] $P < 0.01$

4.2.5 Association of the *IFI6* gene with meat and carcass quality

Association analysis of g.370 A>G with meat and carcass quality traits revealed significant ($P < 0.05$) association with meat colour, $pH24_L$, $pH24_H$, $conductivity24_H$, $conductivity1_L$, drip loss and carcass length in the DuPi population. Meat colour, muscle area and ham percentage were found significantly ($P < 0.05$) associated with g.370 A>G genotype in the Pi population (Table 13). The genotype frequencies for "AA", "AG" and "GG" were 0.42, 0.48 and 0.10 in the DuPi animals and 0.05, 0.49 and 0.46 in the Pi animals, respectively. The allele frequencies for "A" and "G" were 0.66 and 0.34 in the DuPi animals and 0.30 and 0.70 in the Pi animals, respectively. The allele "A" was the most frequent allele in the DuPi animals whereas "G" was the most frequent allele in the Pi animals. The genotype "AA" was associated with higher $pH24_L$, $pH24_H$, $conductivity24_H$ and drip loss in the DuPi population (Table 7). In the DuPi and Pi population, animals with the homozygous "AA" genotype were associated with higher meat colour values which can cause more reddish meat. In the Pi population, the heterozygous "AG" had higher ham percentage and heavier ham weight than the homozygous animals.

4.2.6 Association of the *HNF1A* gene with meat and carcass quality

The *HNF1A* gene revealed significant differences with $pH24_H$, meat percentage and muscle area in the DuPi population ($P < 0.05$) (Table 14). Moreover, statistical analysis revealed significant differences with $pH24_L$, fat area and backfat thickness in the Pi population ($P < 0.05$). Only two of three genotypes were observed for the *HNF1A* gene in the DuPi population which were the genotype "AG" and "GG". In the Pi animals, all of three genotypes were present. There was a large range in the genotype frequency "GG" in DuPi and Pi populations (0.86 and 0.75, respectively) (Table 7). The frequency of the allele "G" was higher than the allele "A" in both DuPi (0.93 and 0.07, respectively) and Pi populations (0.86 and 0.14, respectively). The homozygous genotype "GG" animals had higher $pH24_H$, meat percentage and muscle area than the heterozygous "AG" animals in the DuPi population. The Pi population, the homozygous genotype "GG" animals had lower $pH24_L$ and fat area than the heterozygous "AG" and homozygous genotype "AA", respectively. The homozygous genotype "AA" animals had lower backfat thickness than the homozygous genotype "GG" animals.

Table 13: Least square means (LSM) and standard error (SE) for meat and carcass quality traits of the *IFI6* gene in the DuPi and Pi population

		Least Square Means (SE)				Effect (mean±SE)	
Trait	Population	AA	AG	GG	*P*-value	Additive	Dominance
Meat colour	DuPi	70.01[a] (0.51)	68.63 (0.48)	66.39[b] (1.29)	0.015	-3.61±1.39**	0.43±0.84 [n.s.]
pH 24 loin	DuPi	5.51[a] (0.01)	5.50 (0.01)	5.46[b] (0.02)	0.055	-0.05±0.02*	0.01±0.01 [n.s.]
pH 24 ham	DuPi	5.67[a] (0.01)	5.63 (0.01)	5.60[b] (0.03)	0.024	-0.07±0.03*	0.004±0.02 [n.s.]
Conductivity 1 loin	DuPi	4.46 (0.56)	4.30[c] (0.53)	4.77[d] (0.14)	0.003	0.31±0.15*	-0.32±0.09***
Conductivity 24 ham	DuPi	5.28[c] (0.19)	4.48 (0.18)	4.16[d] (0.47)	0.003	-0.34±0.20 [n.s.]	0.03±0.12 [n.s.]
Drip loss (%)	DuPi	2.25[a] (0.08)	1.94[b] (0.08)	2.23 (0.22)	0.024	-0.02±0.23 [n.s.]	-0.30±0.14*
Carcass length (cm)	DuPi	98.46[a] (0.26)	97.64 (0.24)	97.06[b] (0.55)	0.024	0.70±0.29*	0.12±0.40 [n.s.]
Meat colour	Pi	73.99[a] (3.36)	64.14[b] (1.87)	64.96 (1.87)	0.049	4.51±1.90*	5.33±2.42*
Muscle area (cm^2)	Pi	63.33 (2.82)	61.29[a] (0.92)	57.58[b] (0.98)	0.018	2.15±1.51 [n.s.]	-0.91±1.72 [n.s.]
Ham weight (kg)	Pi	13.68 (0.29)	14.36 (0.10)	14.29 (0.10)	0.089	-0.01±0.19 [n.s.]	-0.18±0.22 [n.s.]

[1]pH24: pH 24 h post-mortem; [2]Conductivity1: Conductivity 45 min post-mortem; [3]Conductivity24: Conductivity 24 h post-mortem.
DuPi: Duroc x Pietrain population; Pi : Pietrain population
[a,b,*] $P < 0.05$; [c,d,**] $P < 0.01$; [e,f,***] $P < 0.001$

Table 14: Least square means (LSM) and standard error (SE) for meat and carcass quality traits of the *HNF1A* gene in the DuPi and Pi population

Trait	Population	Least Square Means (SE)			P-value	Effect (mean±SE)	
		AA	AG	GG		Additive	Dominance
$pH24_H$	DuPi	--	5.59[a] (0.03)	5.65[b] (0.01)	0.042	--	--
Meat percentage (%)	DuPi	--	59.51[a] (0.32)	60.30[b] (0.12)	0.021	--	--
Muscle area (cm^2)	DuPi	--	49.37[a] (0.96)	51.83[b] (0.36)	0.017	--	--
Backfat thickness (cm)	DuPi	--	2.25(0.06)	2.14(0.02)	0.069	--	--
$pH24_L$	Pi	5.41(0.05)	5.47[c] (0.02)	5.42[d] (0.01)	0.026	-0.01±0.02 [n.s.]	-0.05±0.03 [n.s.]
Fat area (cm^2)	Pi	12.02[c] (1.26)	11.96 (0.44)	10.58[d] (0.24)	0.015	0.72±0.64 [n.s.]	-0.66±0.76 [n.s.]
Backfat thickness (cm)	Pi	1.20(0.14)[c]	1.67 (0.05)	1.65[d] (0.03)	0.011	-0.24±0.08**	-2.22±0.07**

[1]pH24: pH 24 h post-mortem.
DuPi: Duroc x Pietrain population; Pi : Pietrain population
[a,b,*] $P < 0.05$; [c,d,**] $P < 0.01$; [e,f,***] $P < 0.001$

4.3 Genetic mapping of the candidate genes

The genetic mapping (data not shown) detected the location of the *AMBP* gene in between the markers *SW1311* and *SW195* on SSC1. The *GC* gene was assigned to SSC8 between the markers *SW2160* and *S0144*. The *PPP1R3B* gene was mapped between the markers *SW936* and *SW1119* on SSC15. Also, the *TNC* gene was mapped on SSC1 between the markers *SW1957* and *SW373*. Genetic mapping of the *IFI6* gene revealed a close linkage to the loci *S0220* and *S0059* on SSC6. Finally, the *HNF1A* gene was mapped on SSC14 between the markers *SW857* and *S0007*. All genetic mapping was using data from DuPi population which coincided with their location in database (NCBI, Ensembl, https://www-lgc.toulouse.inra.fr/pig/ compare/ SSC.htm).

4.4 mRNA expression study by qPCR

4.4.1 The *IFI6* gene

The mRNA expression of the *IFI6* gene on skeletal muscle, qPCR was performed. mRNA expression was investigated and compared between high and low drip loss animals. There was significant ($P < 0.05$) difference in the mRNA expression of the *IFI6* gene between high and low drip loss (Figure 7). mRNA expression was up regulated in low drip loss animals compared to high drip loss animals.

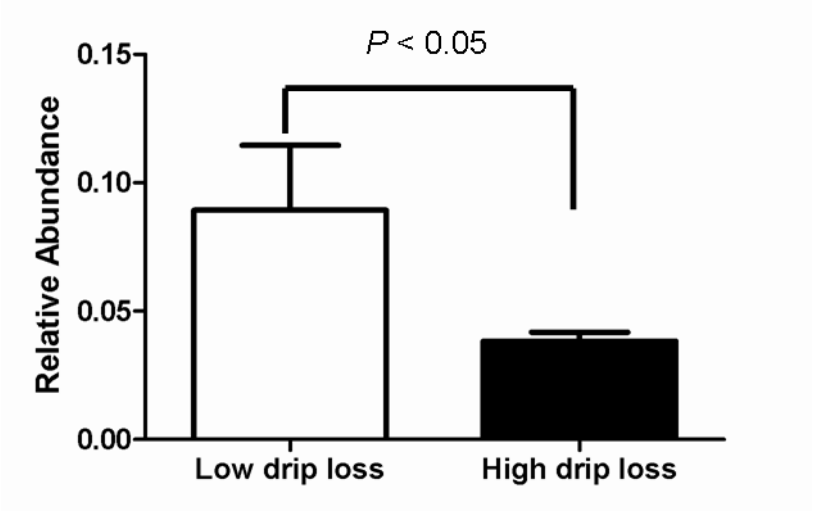

Figure 7: Expression analysis of the *IFI6* gene in pigs (n = 5 per group). Relative abundance of the porcine *IFI6* gene transcript in skeletal muscle between low and high drip loss animals

4.4.2 The *HNF1A* gene

The expression of mRNA level was analysed and compared between animals with high and low pH value in muscle. There was a significant ($P < 0.05$) difference in the mRNA expression of the *HNF1A* gene between animals with high and low pH value in muscle (Figure 8). mRNA expression was up regulated in animals with low pH value in muscle compared to animals with high pH value in muscle.

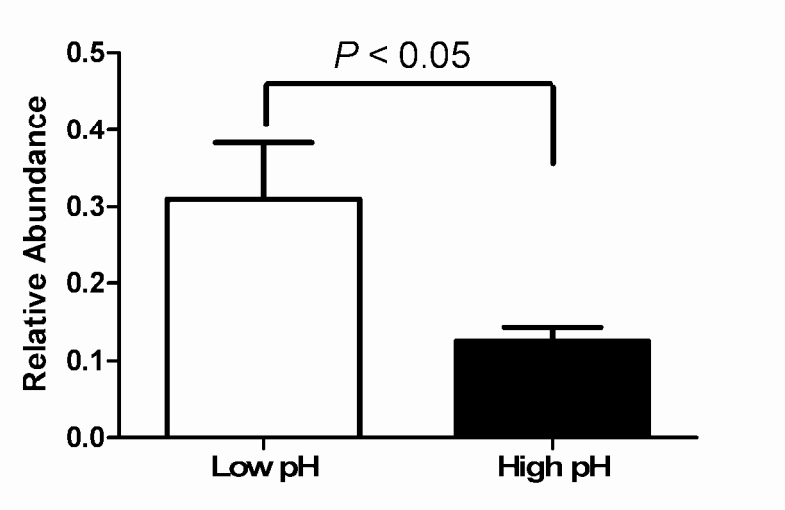

Figure 8: Expression analysis of the *HNF1A* gene in pigs ($n = 5$ per group). Relative abundance of the porcine *HNF1A* gene transcript in skeletal muscle between animals with low and high pH value in muscle

4.5 Protein expression study by western blot analysis

4.5.1 The IFI6 protein

The protein expression was investigated and compared between high and low drip loss animals. The western blot analysis showed significant ($P < 0.01$) difference between high and low drip loss samples (Figure 9). The result revealed that the expression of the IFI6 protein was up regulated in low drip loss samples compared with high drip loss samples.

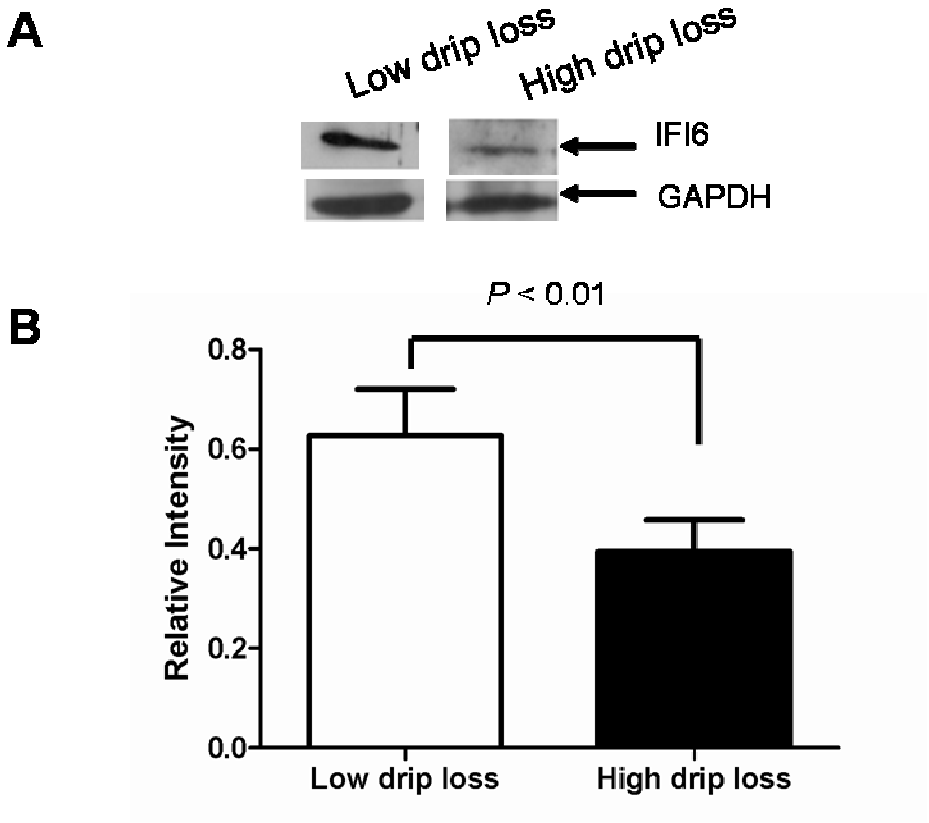

Figure 9: Expression analysis of IFI6 protein in pigs (n = 5 per group). (A) Western blot analysis of IFI6 and GAPDH protein expression in low drip loss and high drip loss animals. (B) The relative intensity graph of low drip loss and high drip loss animals normalized by GAPDH

4.5.2 The HNF1A protein

The protein expression was investigated and compared between animals with high and low pH value in muscle. The western blot analysis showed significant ($P < 0.05$) difference between animals with high and low pH value in muscle (Figure 10). The result revealed the expression of the HNF1A protein was up regulated in animals with low pH value in muscle compared to animals with high pH value in muscle.

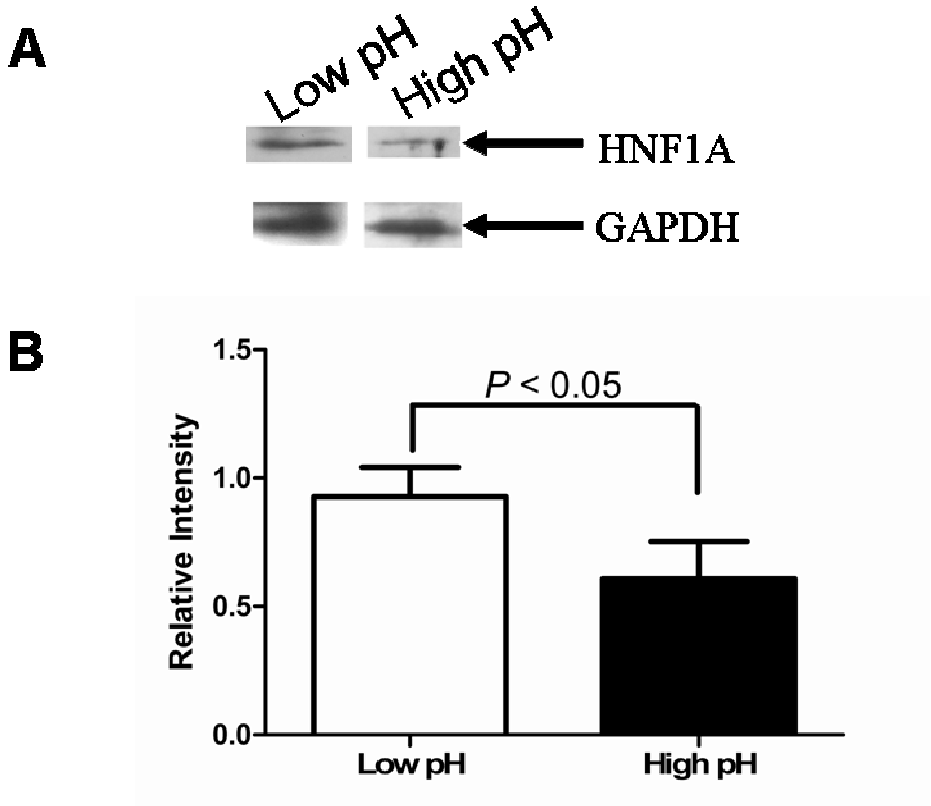

Figure 10: Expression analysis of HNF1A protein in pigs (n = 5 per group). (A) Western blot analysis of HNF1A and GAPDH protein expression in animals with low pH value in muscle and animals with high pH value in muscle. (B) The relative intensity graph of animals with low pH value in muscle and animals with high pH value in muscle normalized by GAPDH

4.6 Protein localization by immunofluoresence analysis

4.6.1 The IFI6 protein

The IFI6 protein was detected by immunofluorescence in the muscle fiber samples. The higher signal intensity was observed on the muscle fiber membrane and cytoplasm (Figure 11).

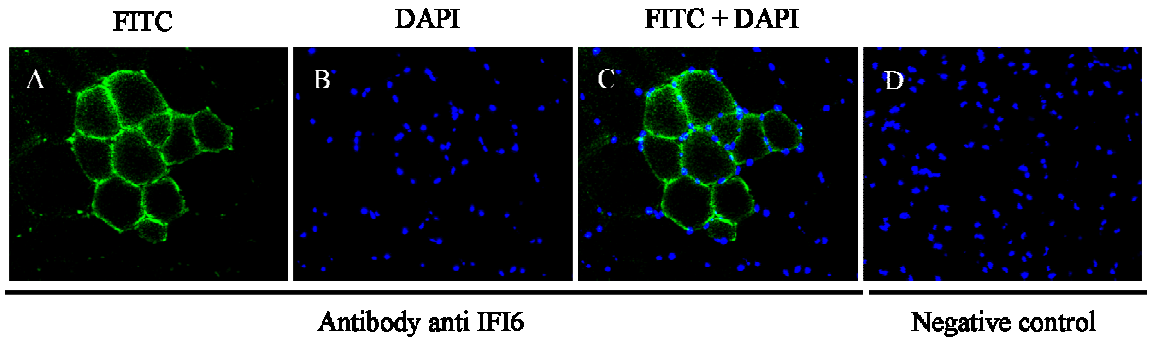

Figure 11: Immunolocalization of IFI6 protein in muscle cells of 6-month-old-pigs. Staining for IFI6 was detected on the muscle cell membrane and cytoplasm (A), nuclear staining (B), IFI6 protein + nuclear staining (C) and negative control (D)

4.6.2 The HNF1A protein

The HNF1A protein was detected by immunoflorescence in the muscle fiber samples. A higher signal intensity was observed in the nucleus of the muscle (Figure 12).

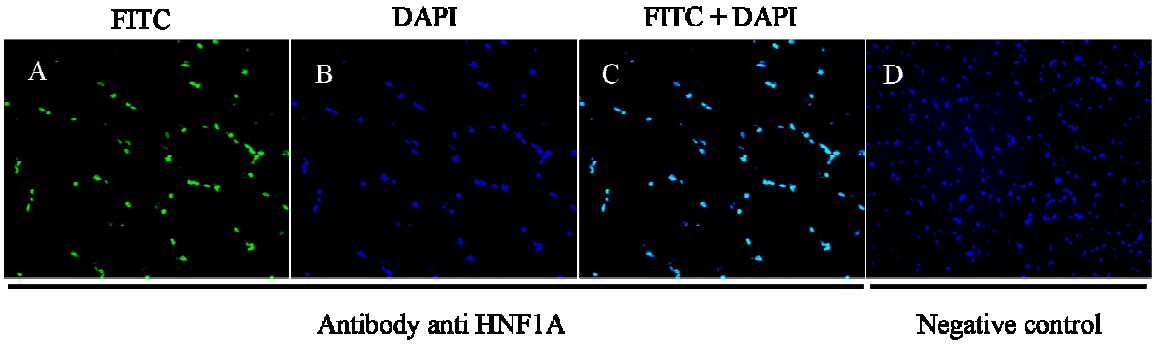

Figure 12: Immunolocalization of HNF1A protein in muscle cells of 6-month-old-pigs. Staining for HNF1A was detected on the nucleus of the muscle (A), nuclear staining (B), HNF1A protein + nuclear staining (C) and negative control (D)

4.7 DNA methylation analysis

4.7.1 DNA methylation pattern of the *HNF1A* gene

This study analyzed the methylation status of the computationally predicted CpG island using genomic DNA isolated from animals with high pH muscle and low pH muscle from the DuPi population. The use of DNA from these animals allowed us to minimize possible effects of genetic background expect pH value in muscle on the methylation status of the analyzed animals. The MethPrimer program predicted that the CG number was 16 in the CpG island which was validated in the sequence of PCR product of the porcine *HNF1A* gene derived from bisulfate-converted DNA. The sequence of the *HNF1A* gene was analyzed using TFSEARCH (http://molsun1.cbrc.aist.go.jp/research /db/TFSEARCH.html). The five CG sites were identified which include putative transcription factor binding sites for StuAp at CG number 2 and 3, USF at CG number 8, ADR1 at CG number 10 and cap at CG number 14. The bisulfate sequencing analysis revealed that the total methylation percentages were not differed between animals with high pH muscle and low pH muscle ($P = 0.053$) but it tended to be higher in animals with low pH muscle (Figure 13 and 14). Within the individual CG sites were no significant difference between animals with high pH muscle and low pH muscle ($P > 0.05$) (Figure 15).

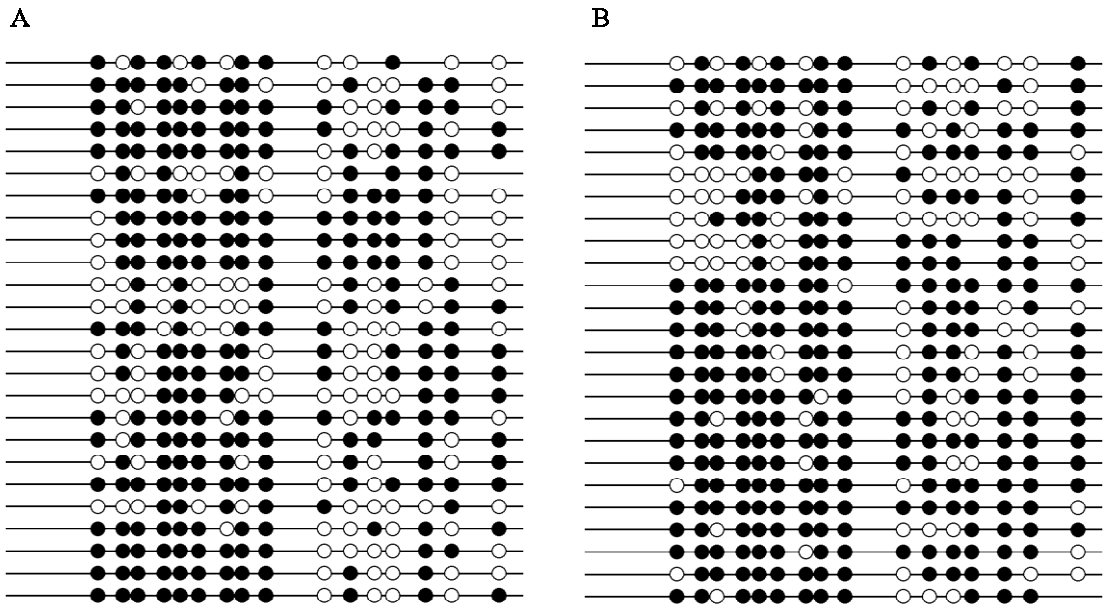

Figure 13: Methylation profile of the *HNF1A* gene, black and white circles represent methylated and unmethylated repectively. (A) Animals with high pH muscle (B) Animals with low pH muscle

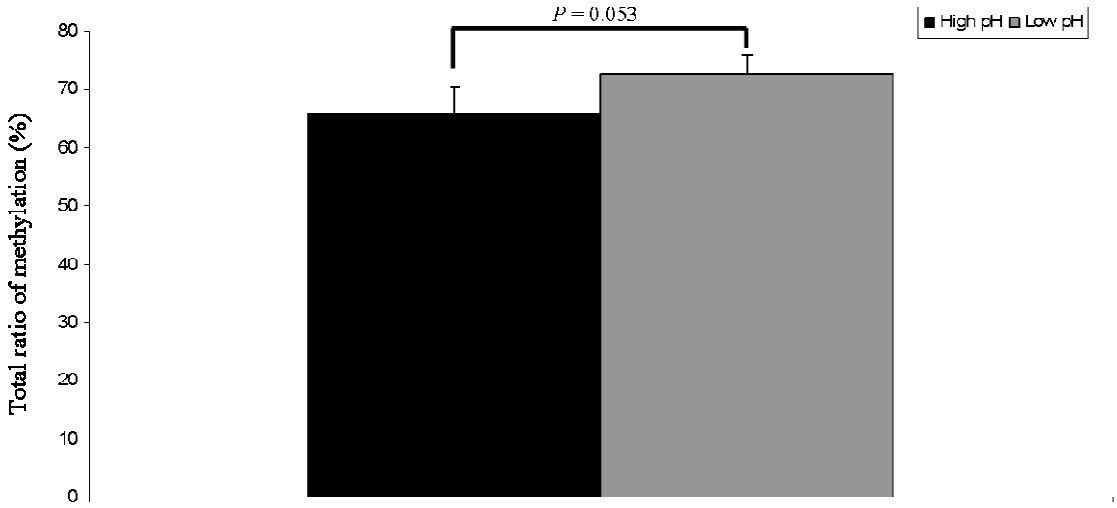

Figure 14: Total ratio of methylation percentage compared between animals with high pH muscle and low pH muscle

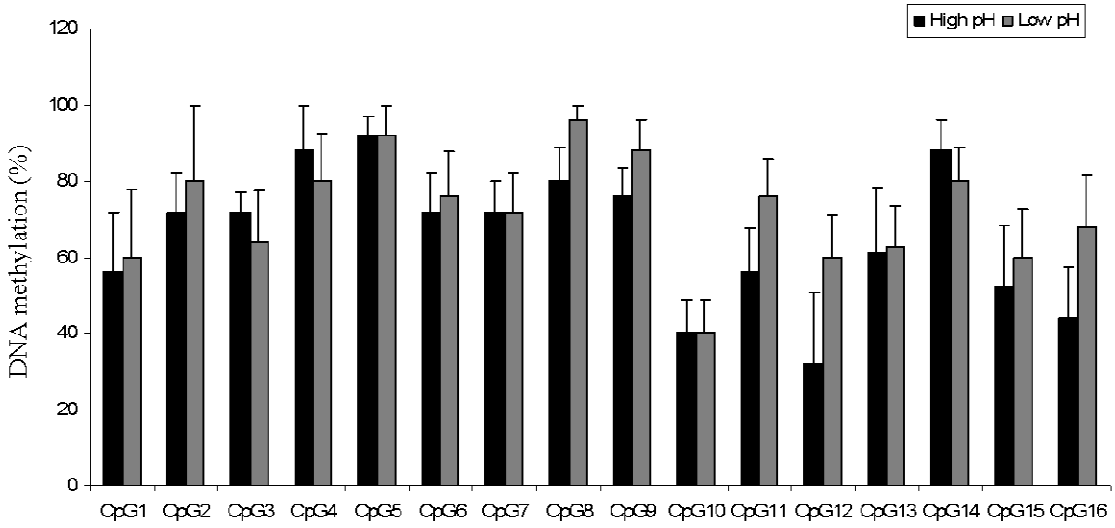

Figure 15: Methylation percentage of the *HNF1A* gene compared between animals with high pH muscle and low pH muscle

5. Discussion

5.1 The analysis of the *AMBP* gene

The *AMBP* gene codes for a polypeptide that gives rise to two different secreted proteins upon endopeptidase cleavage in hepatocytes (Akerstrom et al. 2000). One of the two proteins after cleavage, α_1-microglobulin (α_1m) is a glycoprotein that associates with other plasma proteins, and binds a chromophore formed during heme-binding and degradation (Allhorn et al. 2002). α_1m belongs to the Lipocalin family (Flower 1996), has immunoregulatory properties and is involved in heme-catabolism (Allhorn et al. 2002). Bikunin, the second protein, is a Kunitz-type proteinase inhibitor of the inter-α-inhibitor family (Salier et al. 1996). Although proteolysis regulation is the presumed role of bikunin, other functions have been proposed (Fries and Blom 2000). The expression of the *AMBP* gene was found in myocytes and bikunin in cardiac muscle, nervous system microvasculature, and connective tissue in mouse (Sanchez et al. 2002).
The analysis in this study revealed significant association of *AMBP* on $pH1_L$, $pH24_L$, $pH24_H$, $conductivity24_L$ and carcass length in the DuPi population. Association was observed on $pH24_H$, backfat thickness and fat area in the Pi population. A previous study has shown a negative correlation between pH and conductivity (Byrne et al. 2000). Moreover, conductivity can be used independently, or with even better success in combination with pH, to classify water holding capacity (WHC) of pork carcasses (Lee et al. 2000).
Bikunin a protease inhibitor, functions in stabilization of the extracellular matrix originates from a precursor polypeptide named α-1-microglobulin/bikunin precursor (*AMBP*) (Tyagi et al. 2002). Functionally, *AMBP* plays a role during the regulation of development, cell growth, metabolism, immune response and modulate extracellular matrix protein as well as the level of intracellular calcium (Grewal et al. 2005). The extracellular matrix is reported to influence on water holding capacity, elasticity, growth factor regulation and cellular growth properties (Velleman 2000). This study observed effect of the *AMBP* gene on pH and conductivity. It may be related to the extracellular matrix composition. The extracellular matrix is composed of proteins including collagens and proteoglycans and has various functions in skeletal muscle. Proteoglycans are important for growth and muscling in animals, since proteoglycans have a negative

charge density (high pH) which draws water into the tissue and creates a water compartment (Velleman 2002). Yoon and Halper (2005) reported that proteoglycans are negatively charged hydrophilic molecules that can retain water 50 times their weight. They are mostly entrapped within and between collagen fibrils and fibers. Genetic mapping in this study revealed the position of *AMBP* on SSC1 where QTL for pH, colour, conductivity, fat area and backfat thickness are reported (www.animalgenome .org/cgi-bin/QTLdb/SS/) (De Koning et al. 2001, Liu et al. 2007, Milan et al. 2002). Moreover, this mapped position coincides with the chromosomal location of *AMBP* in the porcine genome (NCBI, Ensembl).

5.2 The analysis of the *GC* gene

The *GC* gene is forms three domains, the first of which contains the sterol binding site. The three domains share limited sequence homology, with each other and to similar repeats in the human serum albumin (Cooke et al. 1988). The rat *GC* gene is expressed at high levels in liver and moderate levels in kidney, testis, abdominal fat, and yolk sac. Very low levels of *GC* can be detected in all other tissues (Cooke et al. 1991). In this study, the *GC* gene showed a significant association with $pH24_H$ and carcass length in the Pi population. Sun et al. (2009) reported that the group-specific component protein or vitamin D-binding protein (*DBP*) plays an important role in the clearance of cellular actin from the extracellular space. Actin is one of the most abundant and highly conserved proteins involved in cell motility, control of cell shape and muscle contraction (Otterbein et al. 2002). This gene is also the major carrier for vitamin D_3 metabolites (D_3) in a variety of mammalian species (Goldschmidt-Clermont et al. 1988). A functional role for vitamin D in muscle was appreciated more with the discovery of vitamin D receptors in skeletal muscle tissue (Simpson et al. 1985). Vitamin D deficiency has been known to cause proximal (Al-Said et al. 2009), muscle weakness (Russell 1994), hypotonia (Rimaniol et al. 1994), and prolonged time to peak muscle contraction, as well as prolonged time to muscle relaxation (Rodman and Baker 1978) and generalized musculoskeletal pain (Reginato and Falasca 1992). A study of Foote et al. (2004) reported a relationship between vitamin D_3 and calcium that vitamin D_3 would increase the Ca^{2+} concentration of blood and muscle. It has been suggested that raised Ca^{2+} in muscle may underlie the link between ante-mortem muscle pH

(glycolysis), rate of pH decline after death and muscle proteolysis, fibre fragmentation and reduced water holding capacity (Sandercock et al. 2009). Genetic mapping in this study mapped *GC* on SSC8. The *GC* position is not mapped yet in porcine genome but it is mapped on HSAP4 (www.ncbi.nlm.nih.gov/) which is homologous to SSC8 (www-lgc.toulouse.inra.fr/pig/ compare/). The location of *GC* did incorporate the flanking regions of several QTL related to meat colour (Malek et al. 2001, Ovilo et al. 2002, Thomsen et al. 2004), backfat thickness, loin eye area, lean content and dressing percentage (Liu et al. 2007) (www.animalgenome.org /cgi-bin/QTLdb) (Ovilo et al. 2002, Thomsen et al. 2004). Moreover, a QTL for pH1 is identified very close to the marker *S0144* in Duroc and Berlin Miniature crosses (Wimmers et al. 2006).

5.3 The analysis of the *PPP1R3B* gene

The *PPP1R3B* gene belongs to a family of proteins that target *PPP1R3B* to glycogen, it is expressed in muscle and liver (Worby et al. 2008). The *PPP1R3B* gene is expressed in human, but not expressed in rodent skeletal muscle. In human, the *PPP1R3B* gene has been examined as a candidate gene for the Type 2 diabetes and MODY (maturity onset diabetes of the young) (Dunn et al. 2006, Montori-Grau et al. 2007). Considering these results, the *PPP1R3B* gene was significantly associated with meat colour, $pH1_L$, $pH24_H$, shear force value and carcass length and $pH24_L$ in the DuPi population. A significance association could not be observed in meat and carcass quality trait of the Pi population. *PPP1R3B* has an effect on glycogen by stimulating the glycogen accumulation (Worby et al. 2008) and decreases muscle GP (glycogen phosphorylase) phosphatase activity (Doherty et al. 1995). The interaction between glycogen phosphorylase (GP) and the liver glycogen targeting subunit (termed G_L) of PP1 (protein phosphatase 1) has emerged as a new potential anti-diabetic target, as the disruption of this interaction should increase glycogen synthesis, potentially providing an alternative approach to counteract the enhanced glycogenolysis without inhibiting GP activity (Zibrova et al. 2008). Muscle with high glycogen and lactate content showed rapid post-mortem glycolysis, paler surface colour, high drip loss and higher extents of protein denaturation (Choe et al. 2008). These data support my finding that *PPP1R3B* is associated with meat quality parameters such as $pH1_L$, $pH24_L$, $pH24_H$, meat colour and shear force. Moreover, a number of QTL for pH, meat colour, cooking

loss and drip loss are reported on SSC15 (Edwards et al. 2003, Jennen et al. 2007) where the *PPP1R3B* gene position is detected by genetic mapping in pig in this study.

5.4 The analysis of the *TNC* gene

The *TNC* gene is a member of a family of genes coding for extracellular matrix protein (Garrido et al. 1995) with a very restricted expression in normal musculoskeletal tissues, but it is expressed abundantly during regenerative processes of these tissues and embryogenesis (Jarvinen et al. 2003). In this study, the association studies revealed statistical evidence to link genetic variation at these loci or their close regions with meat and carcass quality traits. The individual loci of the *TNC* gene showed different effects to a specific trait such as pH, conductivity, meat colour, muscle area, ham weight and backfat thickness. It has been widely reported that final pH as one of the most important parameters to explain the differences in colour, lipid oxidation and drip loss during storage of pork (Maribo et al. 1998). Additionally, a strong correlation between pH and colour has been shown in a previous study (Qiao et al. 2007). However, no variations of this gene showed significant association with the drip loss trait. This might be due to breed specific effects that are related to the extreme muscle phenotype of the pig breeds or may be due to incomplete linkage disequilibrium with causal mutations and/or to effects in the context of DNA variation at other interacting loci (Srikanchai et al. 2010). On the other hand, it may be a consequence from gene function itself on the different traits (Le Flem et al. 1999). However, haplotypes, which are specific combinations of nucleotides on the same chromosome, will provide more information for the complex relationship between DNA variation and phenotypes than that in any single SNP (Grindflek et al. 2004, Stephens et al. 2001). This study indicated that the unique interactions of multiple SNPs within a haplotype ultimately can affect on phenotype and have more predictive power. The *TNC* gene has functions on extracellular matrix (ECM) and cell adhesion. The ECM plays a key role in the transmission of forces generated by the organism (e.g. muscle contraction) and externally application (e.g. gravity). Cell-matrix adhesion sites are good candidates to transmit forces from the ECM to the cytoskeleton (Sarasa-Renedo and Chiquet 2005). The EMC supports the cellular elements and maintains the structure integrity of multicellular organisms (Takala and Virtanen 2000). The ECM is a major determinant in tissue water holding

capacity (WHC), since proteoglycans have a negative charge density (high pH), elasticity, migration, adhesion, growth factor regulation and cellular growth properties (Velleman 2002). For the associated traits *TNC* was considered as a positional candidate gene, supported by the genetic mapping of the *TNC* gene on SSC1, which is closely linked to QTL area for carcass length, 10^{th} rib backfat thickness, average backfat thickness, leaf fat, loin eye area and intramuscular fat content (Paszek et al. 2001) and also for meat colour, pH and conductivity (Liu et al. 2007).

5.5 The analysis of the *IFI6* gene

The Interferon inducible gene 6-16, *G1P3*, was first identified as one of the genes that are induced by interferon α and ß (Borden et al. 2000, Schaar et al. 2005, Takala and Virtanen 2000, Urabe 1994). The *IFI6* gene is expressed in human keratinocytes (Szegedi et al. 2010) and gastric cell lines and tissues (Tahara et al. 2005). This is the first report for association of *IFI6* with meat and carcass quality trait in pigs. The present study found an association of this locus with meat colour, $pH24_L$, $pH24_H$, $conductivity24_H$, $conductivity1_L$, drip loss, carcass length, muscle area, ham weight and ham percentage. This gene showed significant associations only for meat colour trait in both populations. Drip loss depends on the extent of the pH fall after slaughter and both pH and drip loss lead to the changes of meat colour (Fischer 2007, Lopez-Bote and Warriss 1988). Low level pH has been reported to have high relationship with high drip loss (Warner et al. 1997). This SNP was obtained on intron 2 and it is difficult to conclude how this genetic variation affects muscle activity. Though 'silent', it could affect *IFI6* function by altering the mRNA stability (Capon et al. 2004). Turri et al. (1995) reported that the splice donor site of the human *IFI6* gene was found in exon 2 by 12 or 24 nt and inserting four or eight amino acids respectively into the predicted gene product. The mRNA splicing is linked to variation elsewhere in the coding sequence that subsequently affects the amino acid sequence (Hickford et al. 2009).
IFI6 is not mapped yet in pig but it is mapped on HSAP1p35 which is homologous to SSC6q22-26 (https://www-lgc.toulouse.inra.fr/pig/compare/SSC.htm). Genetic mapping of *IFI6* revealed a very close linkage to the marker *S0059* (SSC6q25-226) (Robic et al. 1995) and several QTLs and candidate genes have been reported at the vicinity of this region on SSC6 in pigs affecting meat colour, water content, drip loss and pH

(Markljung et al. 2008, Taniguchi et al. 2010). The result of the present study confirmed that the position of *IFI6* is in a region that affects meat and carcass quality traits. Furthermore, a QTL detected on SSC6 close to this region is reported to influence more muscling in ham and loin and for carcass length in pigs (Edwards et al. 2008). These results are in good agreement with our finding of genetic mapping.

In term of expression in this study, both mRNA and protein was highly expressed in low drip loss. These results revealed that *IFI6* might involve in maintaining meat quality in pigs. Moreover, IFI6 was highly localized in the muscle cell (myocyte) membrane. Cells die primarily by one of two major mechanisms, i.e. necrosis or apoptosis (Mesires and Doumit 2002). *IFI6* is reported to inhibit cytochrome c release from mitochondria by inhibiting the depolarization of mitochondrial membrane and by regulating the Ca^{2+} channel which consequently attenuate the apoptosis (Cheriyath et al. 2007, Tahara et al. 2005). Alterations in intracellular Ca^{2+} homeostasis are involved in the alteration of apoptosis since a sustained increase in cytosolic Ca^{2+} concentration accompanies with apoptosis in cells (Cerella et al. 2003). The higher intracellular Ca^{2+} concentration stimulates different ATPases that leads to an increase of post-mortem metabolism, combined with a fast decrease in pH, resulting in inferior meat quality and changes the water holding capacity of the muscle (Honikel et al. 1986, Küchenmeister et al. 2000). Therefore, it might be suggested that *IFI6* plays vital roles in altering myocytes structure during apoptosis as well as in contributing in Ca^{2+} pathway for maintaining muscle integrity and drip loss. The protein degradation has also been implicated in apoptosis in both invertebrates and mammals (Fearnhead et al. 1995). Furthermore, increased oxidative stress after slaughter leads to the accumulation of damaged proteins, which are not eliminated properly resulting in aggregation of disturbed proteolytic enzymes activities (Floyd and Carney 1992) and leads to the activation of the mitochondria-associated apoptotic pathway (Combaret et al. 2009). In apoptosis process, proteins associated with muscle properties are reported to be degenerated (Argilés et al. 2008). A significant correlation between the degradation of protein and water holding capacity is reported in pork especially to maintain fluidity within the cells (Huff-Lonergan and Lonergan 2007). The post-mortem proteolysis could be predicted by either pH or calcium concentration in pork (Hopkins and Thompson 2002). The early and unregulated release of Ca^{2+}, myosin denaturation and a reduced water holding capacity contributes to the incidence of pale, soft and exudative

resulting in tough meat and high economic loss (Monin et al. 1999, Penny 1969). Therefore, the present result indicated that *IFI6* might play a critical role as an enhancing factor for protecting muscle from apoptosis and might function as a cell survival protein which is involved in meat quality especially water holding capacity.

5.6 The analysis of the *HNF1A* gene

The *HNF1* gene is a transcriptional activator of many hepatic genes including albumin, alpha1-antitrypsin, and alpha- and beta-fibrinogen. It is related to the homeobox gene family and is predominantly expressed in liver and kidney (Pontoglio et al. 1996). This study found that the *HNF1A* gene is associated with meat and carcass quality traits such as $pH24_H$, meat percentage and muscle area in the DuPi population. Moreover, statistical analysis revealed significant differences with $pH24_L$, fat area and backfat thickness in the Pi population. This supports previous studies which reported that *HNF1A* was associated with fatness, intramuscular lipid content, sensory juiciness and loin muscle area in a Yorkshire pig experiment population and in a Berkshire x Yorkshire resource population (Fan et al. 2010a, Fan et al. 2010b). More than 200 exonic SNPs and truncating mutation have been identified in the human *HNF1A* gene and they are distributed throughout all of the 10 exons (Ellard and Colclough 2006, Ryffel 2001, Winckler et al. 2005). The *HNF1A* protein is composed of three major domains, that is, the dimerization domain (encoded by exon 1), DNA-binding domain (encoded by exons 2 to 4) and the transactivation domain (exons 6 to 10) (Ellard and Colclough 2006, Ryffel 2001). In addition, the *HNF1A* gene encodes for three variant isoforms, which are possibly relevant to the relatively high-mutation occurrences in the first six exons (Bellanne-Chantelot et al. 2008). A previous study in pig reported that no mutation was detected in the first six exons in the porcine *HNF1A* gene (Fan et al. 2010a). The exact molecular mechanisms of MODY3 caused by the *HNF1A* gene are still being studied (Rhoads and Levisky 2008, Weedon and Frayling 2007). The *HNF1A* gene acts as a key transcription factor involved in the regulation of genes critical for the liver, pancreas and kidney, such as the insulin and *IGF1* genes (Kitanaka 2008). The *HNF1A* gene knockout mice appeared to have severe reduction in the insulin secretary response (Shih et al. 2001). The mutations in humans could bring about reduced insulin secretion and eventually impaired glucose tolerance and diabetes (Rhoads and Levisky

2008). The present study, the *HNF1A* gene was found to be up regulated in animal with low pH muscles. The *HNF1A* gene plays an important role in glucose activation and insulin secretion (Vayro et al. 2001). The *HNF1A* gene regulated the expression of miRNA194, which is usually highly expressed in different intestinal epithelial cells and possibly involved in the activation of specific pathways such as Notch and Wnt (Hino et al. 2008). Chafey et al. (2009) suggested that defects in Wnt signalling may also determine a metabolic switch in fuel utilization towards glycolysis. Glycogen is a metabolic substrate that fuels post-mortem lactate production and thus enables the pH decline (Lawrie et al. 1958). Anagnostou and Shepherd (2009) demonstrated that alterations in glucose availability can induce autocrine activation of the Wnt/β-catenin pathway in macrophages and that this is dependent on the hexosamine pathway. Although much still remains to be addressed (Sethi and Vidal-Puig 2008), the possibility clearly exists whereby Wnt signals may be directly affected by glucose availability. This study observed the methylation percentage in difference pH muscle, although these two groups do not differ in the methylation percentage and CG site. It is probably that other mechanisms may differentially regulate the expression level of the *HNF1A* gene in these two groups. The present study found that methylation status did not differ substantially between low and high pH muscle, suggesting that methylation of the *HNF1A* gene may not play a major role in glycolysis pathway. Harries et al. (2009) concluded that alterations to expression of *HNF1A* isoforms may underlie some of the phenotypic variation caused by mutations in the *HNF1A* gene. The *HNF1A* gene was mapped close to *SWC6* on SSC14 (Fan et al., 2010; NCBI) where QTL for marbling score (Edwards et al. 2008), tenderness (Harmegnies et al. 2006) and meat conductivity (Wimmers et al. 2006) are reported in the previous studies.

5.7 A hypothesis to explain the role of candidate genes in meat quality

The individual candidate genes showed different effects on a particular trait such as pH, conductivity and drip loss. It has been reported that drip loss has a strong negative correlation with pH and a positive correlation with conductivity (Estévez et al. 2004, Lee et al. 2000, Suzuki et al. 2005). In general, most of these parameters are correlated with or dependent on each other (Lee et al. 2000, Ponsuksili et al. 2009). The selection for increased lean weight would result in increased muscle moisture content but

decreased muscle pH and intramuscular fat content (Cameron 1990). In term of carcass traits such as loin, ham, shoulder, belly weight and loin eye area are reported to be correlated with drip loss (Otto et al. 2006). This is not surprising since all traits are quantitative traits controlled by several loci and/or several traits are influenced by the same or linked loci (Haley et al. 1994, Liu et al. 2007). Previously, Ponsuksili et al. (2008b) reported a number of transcripts with trait-correlated expression to drip loss. The transcripts being up-regulated at high drip loss belong to groups of genes functionally categorized as genes of membrane proteins, signal transduction, cell communication, response to stimulus, and cytoskeleton. Among genes down-regulated with high drip loss, functional groups of apoptotic pathways, oxidoreductase activity, lipid metabolism and electron transport were identified. Many studies have shown that the degradation of the cytoskeleton and other structural proteins play an important role in drip loss at the post-mortem stage (Lonergan and Lonergan 2005, Melody et al. 2004, Scheffler and Gerrard 2007, Zhang et al. 2006). Moreover, higher Ca^{2+} concentration present in muscle fibers early post-mortem is a source for the activation of Ca^{2+} dependent protease, phosphatases and phospholipases like the calpain system which influences drip production. Increased cytoplasmic Ca^{2+} levels are also observed due to excessive exercises. This may initiate vicious cycles of cell degradation because of the Ca^{2+} dependent activation of proteolytic enzymes such as calpain that by themselves digest structural elements of the muscle fibers leading to membrane damage, leakage of intracellular water and proteins and further accumulation of Ca^{2+} (Armstrong 1990). In case of post-mortem changes, apoptosis is important because cells in apoptosis are dissociated from others and ‘shrink’ which leads to a reduction in intracellular space and a parallel increase in extracellular space that alters the cellular integrity and structure as well as muscle characteristics (Trump and Berezesky 1995). The hypothesis is that if the genes in the extracellular matrix receptor interaction pathway promote muscle proteins, it can better withstand degradation during the post-mortem stage. If the genes in the calcium signaling or the oxidative phosphorylation pathway maintain the ATP levels in muscle post-mortem and reduce the rate of the pH decline, it might help to improve the meat quality, especially in case of water holding capacity.

In the present study, the candidate genes can be separated into two groups. The first group, *AMBP*, *GC*, *PPP1R3B*, *TNC* and *HNF1A*, was found to be significantly associated with pH in at least one pig population ($P < 0.05$). High pH can be assigned to

the same group of genes negatively correlated with drip loss, since it is known that drip loss is negative correlated with pH. The second group, *IFI6* was significantly associated with drip loss in at least one pig population ($P < 0.05$). In total, one out of six genes was associated with drip loss in this study. Some gene expression levels showed correlation with pH but did not show association with drip loss trait, indicating that not all differentially expressed genes are polymorphic or the direct cause for a trait. Those gene effects may be strongly dependent on the environmental effects that can mask an association. In general, heritability estimates for drip loss are quite low and varying from 0.08- 0.30 depending on the method of drip measurement or the breed effects (Hermesch et al. 2000, Sellier 1998, Sonesson et al. 1998, Suzuki et al. 2005, van Wijk et al. 2005). Moreover, the individual candidate genes can be assigned to 3 main groups (structural properties, metabolic properties and apoptosis) according to their functions as described in the previous section. The 'structural properties' group includes *AMBP*, *GC* and *TNC*. The 'metabolic properties' group includes *PPP1R3B* and *HNF1A* which are both involved in the glucose and glycogen metabolism. The 'apoptosis' group includes *IFI6*. In the early post-mortem stage, muscle cells are confronted with oxidative stress and increased Ca^{2+} and radical oxygen species levels, which can destroy the cell structure/membrane (Lambert et al. 2001, Sanoudou et al. 2004). The higher intracellular Ca^{2+} concentration stimulates different ATPases that leads to an increase of post-mortem metabolism, combined with a fast decrease in pH, resulting in inferior meat quality and changes of the water holding capacity of the muscle (Honikel et al. 1986, Küchenmeister et al. 2000). Therefore, the positive or negative correlation between the candidate genes expression levels and drip loss may indicate that their cellular functions are connected to the response to oxidative stress and leading to apotosis. Muscle structural and metabolic properties expressed during life affect meat quality at post-mortem. Although, most of these six candidate genes were selected based on their function and position that may be related to meat quality, also many significant associations with carcass quality traits were detected. This is not surprising, because there are many studies reporting the correlation between carcass and meat quality traits (Estévez et al. 2004, Kušec et al. 2003, Ponsuksili et al. 2009, Suzuki et al. 2005, Warner et al. 1997). Drip loss for example had strong positive correlations with loin eye area and conductivity24 and strong negative correlations with average backfat thickness, fat area, meat colour and pH24 (Ponsuksili et al. 2009). However, none of the

genes showed significant associations for a particular trait across all populations. This may be due to breed-specific effects that are related to the extreme muscle phenotypes of the pig breeds or may be due to incomplete linkage disequilibria with causal mutations and/or to effects in the context of DNA variation at other interacting loci (Srikanchai et al. 2010) or might be due to breed specific effect and the different population size of animals should be considered (Fan et al. 2010b).

During the past few decades, advances in molecular genetics have led to the identification of multiple genes or genetic markers associated with genes that affect traits of interest in livestock, including genes for single-gene traits and QTL or genomic regions that affect quantitative traits. This has provided opportunities to enhance response to selection, in particular for traits that are difficult to improve by conventional selection (low heritability or traits for which measurement of phenotype is difficult, expensive, only possible late in life, or not possible on selection candidates) (Dekkers 2004). Moreover, these candidate genes could be recommended for further study or integrated in selection for particular trait. The current association studies revealed statistic evidence for a link between the genetic variation at these loci or their vicinity and carcass and meat quality traits. This study also used the knowledge about their role in physiology and/or their mapping to support the findings. The results of this study give strong evidence for the potential for marker assisted selection and provide evidence for molecular mechanisms of candidate genes on meat and carcass quality traits in pigs.

5.8 Future prospects

During the past few decades, advances in molecular genetics have led to the identification of multiple genes or genetic markers associated with genes that affect traits of interest in livestock, including single genes of large effect and QTL (genomic regions that affect quantitative traits). Quantitative trait loci (QTL) alleles for traits of economic importance can be identified which have significant physiological associations with meat quality, these may be combined with estimated breeding values (EBV's) and incorporated into best linear unbiased prediction (BLUP) models in a process known as marker-assisted selection (MAS) (Kuhn et al. 2005). MAS has particular advantages for traits that challenge traditional selection, including difficult to measure traits, those that are measured for only one sex and traits that can only be

assessed after the end of an individual's reproductive period, such as lifetime fecundity or those that must be measured post-mortem, such as many meat quality traits (Dekkers 2004). Recently, a genome-wide association study (GWAS) is an approach that involves rapidly scanning markers across the complete sets of DNA, or genomes. GWAS has revolutionized the search for genetic influences on complex traits (Manolio 2010). The Illumina Porcine 60K+SNP Beadchip was genotyped on 987 pigs divergent for androstenone concentration from a commercial Duroc-based sire line. The association analysis with 47,897 SNPs revealed androstenone levels in fat tissue (Duijvesteijn et al. 2010). Knowledge gained from these approaches can be beneficial in defining and optimising management systems for quality, providing assurance of meat quality and in tailoring quality to suit market needs. In the future, markers from across the genome can be tested for association with each of these traits faster and cheaper than ever before through use of the newly developed genotyping method.

This study revealed many significant effects on carcass and meat quality traits that could be integrated in the SNP array for further study. Therefore, future research in pig genetics and meat quality will be the availability of the sequenced genome and large-scale DNA arrays or SNP chips to perform low cost genome scan. It is foreseeable that the emerging functional genomics technologies will allow the identification and mapping of functional allelic variants affecting meat quality and animal performance in commercial populations. The increasing value of genomics and the potential of genomics to increase the control both of qualitative characteristics of meat and of many economically important physiological functions are expected to further contribute to improve meat and carcass quality in pig.

6. Summary

The present work was carried out to analyse candidate genes derived from their differential expression and/or trait correlated expression with water holding capacity and their association with meat and carcass quality traits in previous studies. Six genes, *AMBP*, *GC*, *PPP1R3B*, *TNC*, *IFI6* and *HNF1A* were selected based on their known function and/or their mapping to QTL regions for carcass and meat quality traits. For the identification of polymorphisms, screening for SNP was performed by sequence alignment using the F_1 DuPi population in total twenty animals. For the association studies, the SNPs of the six genes were genotyped in a total of 413 animals from 2 pig populations including F_2 Duroc x Pietrain (DuPi) population (n = 313) and a commercial herd of Pietrain (Pi) (n = 110). The genetic mapping established the location of *AMBP* and *TNC* on SSC1, of *GC* on SSC8, of *PPP1R3B* on SSC15, of *IFI6* on SSC6 and of *HNF1A* on SSC14 respectively, coinciding with QTL regions for meat and carcass quality traits. In particular, only one gene (*IFI6*) was associated with the trait drip loss. Five genes (*AMBP*, *GC*, *PPP1R3B*, *TNC* and *HNF1A*) were associated with muscle pH. In detail, the association analysis of *AMBP* showed effects ($P < 0.05$) on $pH1_L$, $pH24_L$, $pH24_H$, $conductivity24_L$ and carcass length in DuPi; $pH24_H$, backfat thickness and fat area in Pi. The SNP of *GC* was associated ($P < 0.10$) with $pH24_H$, $conductivity1_L$, thawing loss and backfat thickness in DuPi; $pH24_H$ and carcass length ($P < 0.05$) in Pi. The SNP of *PPP1R3B* was associated ($P < 0.05$) with meat colour, $pH1_L$, $pH24_L$, $pH24_H$, shear force and carcass length in DuPi; $pH24_L$ ($P < 0.10$) in Pi. Moreover, three SNPs were detected at g.44488 C>T, g.68794 A>G and g.68841 C>T in the *TNC* gene. In DuPi, g.44488 C>T was associated with meat colour and ham weight ($P < 0.05$); g.68794 A>G was associated with $pH24_H$ ($P < 0.01$) and muscle area ($P < 0.05$) but g.68841 C>T was not statistically associated with any traits. The genotyping in Pi showed that g.44488 C>T was significantly associated with $pH24_H$ ($P < 0.01$), whereas g.68794 A>G was associated with $conductivity1_L$ ($P < 0.05$) and backfat thickness ($P < 0.01$). However, no segregation could be observed for g.68841C>T in Pi. Diplotypes showed significant effects on $pH24_H$ ($P < 0.05$) in both populations. SNP of *IFI6* was detected which was significantly ($P < 0.05$) associated with meat colour, $pH24_L$, $pH24_H$, $conductivity24_H$, $conductivity1_L$, drip loss and carcass length in DuPi; meat colour, muscle area and ham percentage in the Pi. For the *HNF1A*

gene significant differences ($P < 0.05$) with $pH24_H$, meat percentage and muscle area in DuPi were found. Moreover, statistical analysis revealed significant differences ($P < 0.05$) with $pH24_L$, fat area and backfat thickness in Pi.

According to the association results, we selected the *IFI6* and *HNF1A* genes to deeply study into the function of their roles. According to the association of the *IFI6* gene was with the drip loss trait. We focused on the role of the *IFI6* gene on drip loss. Both qPCR and western blot revealed that the *IFI6* gene and protein expressed significantly ($P < 0.05$) higher in skeletal muscle of low drip loss animals compared to that of high drip loss animals. In the localization study, the IFI6 protein was detected in the myocytes membrane. In addition, association of the *HNF1A* gene was significant associated with the pH muscle trait. The highest expression mRNA of the *HNF1A* gene was detected in animals with low pH muscle. Both qPCR and western blot revealed that the *HNF1A* gene and protein expressed significantly ($P < 0.05$) higher in animals with low pH muscle compared to animals with high pH muscle. In the localization study, the HNF1A protein was detected in the nucleus of muscle myocyte. The total methylation percentages and individual CG sites were not differed between animals with high pH muscle and low pH muscle ($P > 0.05$).

The individual candidate genes showed different effects on a particular trait, which, as is reported, are correlated with each other. However, none of the genes showed significant associations for a particular trait across all populations. This may be due to breed-specific effects that are related to the extreme muscle phenotypes of the pig breeds or may be due to incomplete linkage disequilibria with causal mutations and/or to effects in the context of DNA variation at other interacting loci; this is not unexpected as the traits analysed are quantitative traits controlled by several loci.

In conclusion, six candidate genes were investigated, the polymorphisms were detected, also the regional assignments were performed and functional aspects of the candidate genes were investigated. The study used knowledge about their physiological roles to support their putative involvement in the genetic regulation of meat and carcass quality traits. The association study of six candidate genes revealed statistic evidence for a link between the genetic variation at these loci or their vicinity and meat and carcass quality traits. Moreover, the functional study of two candidate genes (*IFI6* and *HNF1A*) showed their vital roles on meat quality traits as well.

6. Zusammenfassung

Die vorliegende Arbeit wurde durchgeführt, um Kandidatengene zu analysieren, die auf Grund ihrer differentiellen Expression und / oder merkmalskorrelierten Expression mit Wasserbindevermögen ausgewählt wurden und in früheren Studien einen Zusammenhang mit Fleisch- und Schlachtkörperqualitätsmerkmalen zeigten. Sechs Gene, *AMBP*, *GC*, *PPP1R3B*, *TNC*, *IFI6* und *HNF1A*, wurden basierend auf ihrer bekannten Funktion und / oder deren Zuordnung zu QTL Regionen für Schlachtkörper- und Fleischqualitätsmerkmalen ausgewählt. Für die Identifizierung von Polymorphismen, erfolgte das SNP-Screening mit Hilfe von Sequenz-Alignment in einer F_1 DuPi Population von insgesamt zwanzig Tieren. Für die Assoziationsstudien wurden die SNPs der sechs Gene in insgesamt 413 Tiere von zwei Schweinepopulationen einschließlich einer F2 Duroc x Pietrain (DuPi) Population (n = 313) und einer kommerziellen Herde von Pietrain (Pi) (n = 110) genotypisiert. Durch genetische Kartierung konnte *AMBP* und *TNC* auf SSC1, *GC* auf SSC8, *PPP1R3B* auf SSC15, *IFI6* auf SSC6 und *HNF1A* auf SSC14 lokalisiert werden. Diese Positionen stimmten mit QTL Regionen für Fleisch- und Schlachtkörperqualitätsmerkmale überein. Nur eins der sechs Gene (*IFI6*) konnte mit dem Merkmal Tropfsaftverlust assoziiert werden. Die anderen fünf Gene (*AMBP*, *GC*, *PPP1R3B*, *TNC* und *HNF1A*) zeigten einen Zusammenhang mit dem Merkmal Muskel pH-Wert. Im Einzelnen erbrachte die Assoziationsanalyse das dass Gen *AMBP* einen Effekt ($P < 0.05$) auf den $pH1_L$, $pH24_L$, $pH24_H$, die Leitfähigkeit 24_L und die Schlachtkörperlänge in der DuPi Population sowie auf den $pH24_H$, Rückenspeck und das Fettfläche in der Pi Population hat. Der SNP von *GC* zeigte eine Assoziation ($P < 0.10$) mit den $pH24_H$, der Leitfähigkeit1_L, dem Auftauverlust sowie den Rückenspeck in der DuPi Population und mit dem Merkmal $pH24_H$ und Schlachtkörperlänge ($P < 0.05$) in der Pi Population. Der SNP von *PPP1R3B* erbrachte einen Zusammenhang ($P < 0.05$) mit der Fleischfarbe, den $pH1_L$, $pH24_L$, $pH24_H$, der Scherkraft und Schlachtkörperlänge in der DuPi Population. Mit den Merkmal $pH24_L$ ($P < 0.10$) konnte eine Assoziation in der Pi Population festgestellt werden. Darüber hinaus wurden drei SNPs bei g.44488 C>T, g.68794 A>G und g.68841 C>T im Gen *TNC* erkannt. In der DuPi Population konnte der SNP bei g.44488 C>T mit dem Merkmal Fleischfarbe und Schinkengewicht ($P < 0.05$) assoziiert werden; das SNP bei g.68794 A>G zeigte einen Zusammenhang mit den Merkmalen $pH24_H$ ($P <$

0.01) und im Muskelbereich ($P < 0.05$), hingegen konnte beim SNP g.68841 C>T statistisch keine Assoziation mit einem Merkmal des Schlachtkörpers und der Fleischqualität entdeckt werden. Die Genotypisierung in Pi Tieren zeigte, dass g.44488 C>T signifikant mit $pH24_H$ assoziiert ($P < 0.01$), während g.68794 A>G sowohl mit der $Leitfähigkeit1_L$ ($P < 0.05$) und Rückenspeckdicke ($P < 0.01$) assoziiert werden konnte. g.68841C>T segregierte in der Pi Population nicht. Diplotypen zeigten signifikante Effekte auf den $pH24_H$ ($P < 0.05$) in beiden Populationen. Der SNP von *IFI6* konnte in der DuPi Population signifikant ($P < 0.05$) mit der Fleischfarbe, den $pH24_L$, $pH24_H$, der $Leitfähigkeit24_H$, der $Leitfähigkeit1_L$, dem Tropfsaftverlust und der Schlachtkörperlänge assoziiert werden. In der Pi Population zeigte sich hingegen ein Zusammenhang mit der Fleischfarbe, dem Fleischfläche und Schinkenanteil. *HNFIA* zeigte signifikante Unterscheide ($P < 0.05$) mit den Merkmalen $pH24_H$ und dem Fleisch- und Muskelanteil in der DuPi Population. Des Weiteren konnte durch statistische Analysen signifikante Unterschiede ($P < 0.05$) zum $pH24_L$, dem Fettanteil und Rückenspeck in Pi ermittelt werden.

In Übereinstimmung mit den Assoziationsergebnissen wurden die Gene *IFI6* und *HNF1A*, für weitere funktionelle Analysen, selektiert. Entsprechend der Assoziationsergebnisse besteht ein signifikanter Unterschied zwischen *IFI6* und dem Merkmal Tropfsaftverlust. Durch dieses Ergebnis wurde der Focus dieser Studie auf das *IFI6* Gen und das Merkmal Tropfsaftverlust gelegt. Durch qPCR und Werstern Blot konnte eine signifikant ($P < 0.05$) höhere *IFI6* Gen- und Proteinexpression im Skelettmuskel bei Tieren mit niedrigem Tropfsaftverlust im Vergleich zu Tieren mit hohem Tropfsaftverlust ermittelt werden. In der Lokokalisationsstudie wurde das *IFI6* Protein in der Myozytenmembran identifiziert. Darüber hinaus war das Assoziationsergebnis des *HNF1A* Gens signifikant unterschiedlich in Verbindung mit dem pH-Wert im Muskel. Aufgrund dieses Ergebnisses lag der Focus darauf die Rolle des *HNF1A* Gens im Bezug zum pH-Wert des Muskels zu untersuchen. Die höchste mRNA Expression von *HNF1A* zeigten Tiere mit niedrigem pH-Wert im Muskel. Durch qPCR und Werstern Blot konnte eine signifikant ($P < 0.05$) höhere *HNF1A* Gen- und Proteinexpression bei Tieren mit niedrigem pH-Wert im Muskel im Vergleich zu Tieren mit hohem pH-Wert im Muskel festgestellt werden. In der Lokalisationsstudie wurde das HNF1A Protein im Nukleus der Muskelmyozyten identifiziert. Es konnten keine Unterschiede bei der gesamten Methylierung und dem individuellen CG-Bereich

zwischen Tieren mit hohem pH-Wert im Muskel und niedrigem pH-Wert im Muskel ermittelt werden ($P > 0.05$).

Die individuellen Kandidatengene zeigten unterschiedliche Effekte auf spezielle Merkmale, die miteinander korrelieren. Dennoch konnte bei keinem dieser Gene eine signifikante Assoziation mit einem speziellen Merkmal in beiden Populationen erwiesen werden. Der Grund dafür liegt wahrscheinlich in den rassenspezifischen Effekten die in Verbindung mit den extremen Muskel Phenotypen dieser Schweinerassen steht. Oder der Grund könnte bei unvollständigen Kopplungsungleichgewichten mit kausalen Mutationen und / oder bei Effekten im Kontext der DNA Variation mit anderen interagierenden Loci liegen. Das ist nicht unerwartet, da die analysierten Merkmale durch mehrere Loci gesteuert werden. Zusammenfassend wurden sechs Gene untersucht, deren Polymorphismen identifiziert, das regionale Assignment und auch die Funktion untersucht.

Diese Studie verwendete das Wissen über die physiologische Rolle der Gene um ihre vermutliche Beteiligung an der genetischen Regulierung von Fleisch- und Schlachtkörpermerkmalen zu unterstützen. Die Assoziationsstudie der sechs Kandidatengene lieferte statistische Belege für einen Zusammenhang zwischen der genetischen Variation dieser Loci oder deren Umgebung und der Fleisch- und Schlachtkörpermerkmalen. Darüber hinaus zeigte die funktionelle Studie die entscheidende Rolle zweier Kandidatengene (*IFI6* und *HNF1A*) im Bereich der Fleischqualitätsmerkmale.

7. References

Akerstrom B, Logdberg L, Berggard T, Osmark P, Lindqvist A (2000): Alpha(1)-microglobulin: a yellow-brown lipocalin. Biochim Biophys Acta 1482, 172-184

Allhorn M, Berggard T, Nordberg J, Olsson ML, Akerstrom B (2002): Processing of the lipocalin alpha(1)-microglobulin by hemoglobin induces heme-binding and heme-degradation properties. Blood 99, 1894-1901

Al-Said YA, Al-Rached HS, Al-Qahtani HA, Jan MM (2009): Severe proximal myopathy with remarkable recovery after vitamin D treatment. Can J Neurol Sci 36, 336-339

Anagnostou SH, Shepherd PR (2008): Glucose induces an autocrine activation of the Wnt/beta-catenin pathway in macrophage cell lines. Biochem J 416, 211-218

Andersson-Eklund L, Marklund L, Lundstrom K, Haley CS, Andersson K, Hansson I, Moller M, Andersson L (1998): Mapping quantitative trait loci for carcass and meat quality traits in a wild boar x Large White intercross. J Anim Sci 76, 694-700

Andersson L (2001): Genetic dissection of phenotypic diversity in farm animals. Nat Rev Genet 2, 130-138

Argilés JM, López-Soriano FJ, Busquets S (2008): Apoptosis signalling is essential and precedes protein degradation in wasting skeletal muscle during catabolic conditions. Int J Biochem Cell Biol 40, 1674-1678

Armstrong RB (1990): Initial events in exercise-induced muscular injury. Med Sci Sports Exerc 22, 429-435

Band GO, Guimarães SEF, Lopes PS, Schierholt AS, Silva KM, Pires AV, Benevenuto Júnior AA, Gomide LAM (2005): Relationship between the Porcine Stress Syndrome gene and pork quality traits of F2 pigs resulting from divergent crosses. Genet Mol Biol 28, 88-91

Bellanne-Chantelot C, Carette C, Riveline JP, Valero R, Gautier JF, Larger E, Reznik Y, Ducluzeau PH, Sola A, Hartemann-Heurtier A, et al. (2008): The type and the position of HNF1A mutation modulate age at diagnosis of diabetes in patients with maturity-onset diabetes of the young (MODY)-3. Diabetes 57, 503-508

Borchers N, Otto G, Kalm E (2007): Genetic relationship of drip loss to further meat quality traits in purebred Piétrains. Arch Tierz, Dummerstorf 50, 84-91

Borden EC, Lindner D, Dreicer R, Hussein M, Peereboom D (2000): Second-generation interferons for cancer: clinical targets. Semin Cancer Biol 10, 125-144

Bowker BC, Grant AL, Forrest JC, Gerrard DE (2002): Muscle metabolism and PSE pork. J Anim Sci 79, 1-8

Brøndum J, Munck L, Henckel P, Karlsson A, Tornberg E, Engelsen SB (2000): Prediction of water-holding capacity and composition of porcine meat by comparative spectroscopy. Meat Sci 55, 177-185

Byrne CE, Troy DJ, Buckley DJ (2000): Postmortem changes in muscle electrical properties of bovine M. longissimus dorsi and their relationship to meat quality attributes and pH fall. Meat Sci 54, 23-34

Cameron ND (1990): Genetic and phenotypic parameters for carcass traits, meat and eating quality traits in pigs. Livest Prod Sci 26, 119-135

Capon F, Allen MH, Ameen M, Burden AD, Tillman D, Barker JN, Trembath RC (2004): A synonymous SNP of the corneodesmosin gene leads to increased mRNA stability and demonstrates association with psoriasis across diverse ethnic groups. Hum Mol Genet 13, 2361-2368

Carrodeguas JA, Burgos C, Moreno C, Sánchez AC, Ventanas S, Tarrafeta L, Barcelona JA, Otilia López M, Oria R, López-Buesa P (2005): Incidence in diverse pig populations of an IGF2 mutation with potential influence on meat quality and quantity: An assay based on real time PCR (RT-PCR) Meat Sci 71, 577-582

Casas E, Shackelford SD, Keele JW, Koohmaraie M, Smith TPL, Stone RT (2003): Detection of quantitative trait loci for growth and carcass composition in cattle. J Anim Sci 81, 2976-2983

Cerella C, D'Alessio M, De Nicola M, Magrini A, Bergamaschi A, Ghibelli L (2003): Cytosolic and endoplasmic reticulum Ca^{2+} concentrations determine the extent and the morphological type of apoptosis, respectively. Ann N Y Acad Sci 1010, 74-77

Chafey P, Finzi L, Boisgard R, Cauzac M, Clary G, Broussard C, Pegorier JP, Guillonneau F, Mayeux P, Camoin L, et al. (2009): Proteomic analysis of beta-catenin activation in mouse liver by DIGE analysis identifies glucose metabolism as a new target of the Wnt pathway. Proteomics 9, 3889-3900

Cheng Q, Sun D-W (2008): Factors affecting the water holding capacity of red meat products: A review of recent research advances. Crit Rev Food Sci Nutr 48, 137 - 159

Cheriyath V, Glaser KB, Waring JF, Baz R, Hussein MA, Borden EC (2007): G1P3, an IFN-induced survival factor, antagonizes TRAIL-induced apoptosis in human myeloma cells. J Clin Invest 117, 3107–3117

Cheung VG, Spielman RS (2002): The genetics of variation in gene expression. Nat Genet 32 Suppl, 522-525

Choe JH, Choi YM, Lee SH, Shin HG, Ryu YC, Hong KC, Kim BC (2008): The relation between glycogen, lactate content and muscle fiber type composition, and their influence on postmortem glycolytic rate and pork quality. Meat Sci 80, 355-362

Ciobanu DC, Bastiaansen JW, Lonergan SM, Thomsen H, Dekkers JC, Plastow GS, Rothschild MF (2004): New alleles in calpastatin gene are associated with meat quality traits in pigs. J Anim Sci 82, 2829-2839

Combaret L, Dardevet D, Bechet D, Taillandier D, Mosoni L, Attaix D (2009): Skeletal muscle proteolysis in aging. Curr Opin Clin Nutr Metab Care 12, 37-41

Cooke NE, McLeod JF, Wang XK, Ray K (1991): Vitamin D binding protein: genomic structure, functional domains, and mRNA expression in tissues. J Steroid Biochem Mol Biol 40, 787-793

Cooke NE, Murgia A, McLeod JF (1988): Vitamin D-binding protein. Structure and pattern of expression. Ann N Y Acad Sci 538, 49-59

Davoli R, Braglia S (2008): Molecular approaches in pig breeding to improve meat quality. Brief Funct Genomic Proteomic 6, 313-21

Davoli R, Colombo M, Schiavina S, Fontanesi L, Buttazzoni L, Russo V (2007): Transcriptome analysis of porcine skeletal muscle: differentially expressed genes in Italian Large White pigs with divergent values for glycolytic potential. Ital J Anim Sci 6, 113-115

de Koning DJ, Harlizius B, Rattink AP, Groenen MA, Brascamp EW, van Arendonk JA (2001): Detection and characterization of quantitative trait loci for meat quality traits in pigs. J Anim Sci 79, 2812-2819

de Koning DJ, Rattink AP, Harlizius B, van Arendonk JA, Brascamp EW, Groenen MA (2000): Genome-wide scan for body composition in pigs reveals important role of imprinting. Proc Natl Acad Sci U S A 97, 7947-7950

de Oliveira Band G, Guimarães SEF, Lopes PS, Schierholt AS, Silva KM, Pires AV, Júnior AAB, de Miranda Gomide LA (2005): Relationship between the Porcine Stress Syndrome gene and pork quality traits of F2 pigs resulting from divergent crosses. Genet Mol Biol 28, 88-89

de Vries AG, Faucitano L, Sosnicki A, Plastow GS (2000): The use of gene technology for optimal development of pork meat quality. Food Chem 69, 397-405

de Vries AG, Sosnicki A, Garniera JP, Plastow GS (1998): The role of major genes and DNA technology in selection for meat quality in pigs. Meat Sci 49, S245-S255

Dekkers JCM (2004): Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. J Anim Sci 82, E313-328

Doerge RW (2002): Mapping and analysis of quantitative trait loci in experimental populations. Nat Rev Genet 3, 43-52

Doherty MJ, Moorhead G, Morrice N, Cohen P, Cohen PT (1995): Amino acid sequence and expression of the hepatic glycogen-binding (GL)-subunit of protein phosphatase-1. FEBS Lett 375, 294-298

Duijvesteijn N, Knol EF, Merks JW, Crooijmans RP, Groenen MA, Bovenhuis H, Harlizius B (2010): A genome-wide association study on androstenone levels in pigs reveals a cluster of candidate genes on chromosome 6. BMC Genet 11, 42

Dunn JS, Mlynarski WM, Pezzolesi MG, Borowiec M, Powers C, Krolewski AS, Doria A (2006): Examination of PPP1R3B as a candidate gene for the type 2 diabetes and MODY loci on chromosome 8p23. Ann Hum Genet 70, 587-593

Duthie CA, Simm G, Doeschl-Wilson A, Kalm E, Knap PW, Roehe R (2011): Quantitative trait loci for meat quality traits in pigs considering imprinting and epistatic effects. Meat Sci 87, 394-402

Edwards DB, Bates RO, Osburn WN (2003): Evaluation of Duroc- vs. Pietrain-sired pigs for carcass and meat quality measures. J Anim Sci 81, 1895-1899

Edwards DB, Ernst CW, Raney NE, Doumit ME, Hoge MD, Bates RO (2008): Quantitative trait locus mapping in an F2 Duroc x Pietrain resource population: II. Carcass and meat quality traits. J Anim Sci 86, 254-266

Ellard S, Colclough K (2006): Mutations in the genes encoding the transcription factors hepatocyte nuclear factor 1 alpha (HNF1A) and 4 alpha (HNF4A) in maturity-onset diabetes of the young. Hum Mutat 27, 854-869

Estévez M, Ventanas J, Cava R (2004): Lipolytic and oxidative changes during refrigeration of cooked loin chops from three lines of free-range-reared Iberian pigs slaughtered at 90 kg live weight and industrial genotype pigs. Food Chem 87, 367-376

Fan B, Onteru SK, Garrick D, Stalder KJ, Rothschild MF (2009a): A genome-wide association study for pig production and feet and leg structure traits using the PorcineSNP60 BeadChip. Pig Genome III Conference, November 2-4, Hinxton, Cambridge, UK. Abstr No. 6

Fan B, Onteru SK, Plastow GS, Rothschild MF (2009b): Detailed characterization of the porcine MC4R gene in relation to fatness and growth. Anim Genet 40, 401-409

Fan B, Du Z-Q, Rothschild MF (2010a): The hepatocyte nuclear factor-1 alpha (HNF1A) gene is associated with fatness and loin muscle area in the pig. Animal 4, 1619-1627

Fan B, Lkhagvadorj S, Cai W, Young J, Smith RM, Dekkers JC, Huff-Lonergan E, Lonergan SM, Rothschild MF (2010b): Identification of genetic markers associated with residual feed intake and meat quality traits in the pig. Meat Sci 84, 645-650

Fearnhead HO, MacFarlane M, Dinsdale D, Cohen GM (1995): DNA degradation and proteolysis in thymocyte apoptosis. Toxicol Lett 82-83, 135-141

Fernández A, de Pedro E, Núñez N, Silió L, García-Casco J, Rodríguez C (2003): Genetic parameters for meat and fat quality and carcass composition traits in Iberian pigs Meat Sci 64, 405-410

Fernando MAG, André FR (2005): Genetic and clinical characteristics of maturity-onset diabetes of the young. Diabetes Metab 7, 318-326

Fischer K (2007): Drip loss in pork: influencing factors and relation to further meat quality traits. J Anim Breed Genet 124, 12-18

Flower DR (1996): The lipocalin protein family: structure and function. Biochem J 318 (Pt 1), 1-14

Floyd RA, Carney JM (1992): Free radical damage to protein and DNA: mechanisms involved and relevant observations on brain undergoing oxidative stress. Ann Neurol 32, S22-27

Foote MR, Horst RL, Huff-Lonergan EJ, Trenkle AH, Parrish FC, Jr., Beitz DC (2004): The use of vitamin D3 and its metabolites to improve beef tenderness. J Anim Sci 82, 242-249

Frank SA (2003): Genetic variation of polygenic characters and the evolution of genetic degeneracy. J Evol Biol 16, 138-142

Fries E, Blom AM (2000): Bikunin. Not just a plasma proteinase inhibitor. Int J Biochem Cell Biol 32, 125–137

Fujii J, Otsu K, Zorzato F, de Leon S, Khanna VK, Weiler JE, O'Brien PJ, MacLennan DH (1991): Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science 253, 448-451

Garrido JJ, Lahbib-Mansais Y, Geffrotin C, Yerle M, Vaiman M (1995): Localization of the tenascin-C gene to pig Chromosome 1. Mamm Genome 6, 221-221

Geldermann H, Mueller E, Beeckman P, Knoor C, Yue G, Moser G (1996): Mapping quantitative trait loci by means of marker genes in F2 generations of Wild Boar, Pietrain and Meishan pigs. J Anim Breed Genet 113, 381-387

Gispert M, Faucitano L, Oliver MA, Guàrdia MD, Coll C, Siggens K, Harvey K, Diestre A (2000): A survey of pre-slaughter conditions, halothane gene frequency, and carcass and meat quality in five Spanish pig commercial abattoirs. Meat Sci 55, 97-106

Goldschmidt-Clermont PJ, Van Baelen H, Bouillon R, Shook TE, Williams MH, Nel AE, Galbraith RM (1988): Role of group-specific component (vitamin D binding protein) in clearance of actin from the circulation in the rabbit. J Clin Invest 81, 1519-1527

Green P, Falls K, Crook S (1990): Documentation for CRIMAP, ver. 2.4. Washington University School of Medicine, St Louis

Grewal JS, Tsai JY, Khan SR (2005): Oxalate-inducible AMBP gene and its regulatory mechanism in renal tubular epithelial cells. Biochem J 387, 609-616

Grindflek E, Hoen N, Sundvold H, Rothschild MF, Plastow G, Lien S (2004): Investigation of a peroxisome proliferator-activated receptor gamma haplotype effect on meat quality and carcass traits in pigs. Anim Genet 35, 238-241

Guignot F, Vignon X, Monin G (1993): Post mortem evolution of myofilament spacing and extracellular space in veal muscle. Meat Sci 33, 333-347

Hamilton DN, Ellis M, Miller KD, McKeith FK, Parrett DF (2000): The effect of the Halothane and Rendement Napole genes on carcass and meat quality characteristics of pigs. J Anim Sci 78, 2862-2867

Haley CS, Knott SA, Elsen JM (1994): Mapping quantitative trait loci in crosses between outbred lines using least squares. Genetics 136, 1195-1207

Harmegnies N, Davin F, De Smet S, Buys N, Georges M, Coppieters W (2006): Results of a whole-genome quantitative trait locus scan for growth, carcass composition and meat quality in a porcine four-way cross. Anim Genet 37, 543-553

Hermesch S, Luxford BG, Graser HU (2000): Genetic parameters for lean meat yield, meat quality, reproduction and feed efficiency traits for Australian pigs 1. Description of traits and heritability estimates. Livest Prod Sci 65, 239-248

Herrera-Mendez CH, Becila S, Boudjellal A, Ouali A (2006): Meat ageing: Reconsideration of the current concept. Trends Food Sci Technol 17, 394-405

Harries LW, Brown JE, Gloyn AL (2009): Species-specific differences in the expression of the HNF1A, HNF1B and HNF4A genes. PLoS One 4, e7855

Hickford JG, Forrest RH, Zhou H, Fang Q, Han J, Frampton CM, Horrell AL (2009): Polymorphisms in the ovine myostatin gene (MSTN) and their association with growth and carcass traits in New Zealand Romney sheep. Anim Genet 41, 64-72

Hino K, Tsuchiya K, Fukao T, Kiga K, Okamoto R, Kanai T, Watanabe M (2008): Inducible expression of microRNA-194 is regulated by HNF-1alpha during intestinal epithelial cell differentiation. RNA 14, 1433-1442

Hirai M, Suzuki S, Hinokio Y, Hirai A, Chiba M, Akai H, Suzuki C, Toyota T (2000): Variations in vitamin D-binding protein (group-specific component protein) are associated with fasting plasma insulin levels in Japanese with normal glucose tolerance. J Clin Endocrinol Metab 85, 1951-1953

Holmkvist J, Cervin C, Lyssenko V, Winckler W, Anevski D, Cilio C, Almgren P, Berglund G, Nilsson P, Tuomi T, et al. (2006): Common variants in HNF-1 α and risk of type 2 diabetes. Diabetologia 49, 2882-2891

Honikel KO (1998): Reference methods for the assessment of physical characteristics of meat. Meat Sci 49, 447-457

Honikel KO (1986): Wasserbindungsvermögen von Fleisch. Mitteilungsblatt der BAFF 94, 7150 - 7154

Honikel KO, Kim CJ, Hamm R, Roncales P (1986): Sarcomere shortening of prerigor muscles and its influence on drip loss. Meat Sci 16, 267-282

Hopkins DL, Thompson JM (2002): The relationship between post-mortem calcium concentration or pH and indicators of proteolysis in ovine muscle. Meat Sci 61, 411-414

Hoveniera R, Kanisa E, van Asseldonkb T, Westerink NG (1992): Genetic parameters of pig meat quality traits in a halothane negative population. Livest Prod Sci 32, 309-321

Huff-Lonergan E, Lonergan SM (2005): Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Sci 71, 194-204

Huff-Lonergan E, Lonergan SM (2007): New frontiers in understanding drip loss in pork: recent insights on the role of postmortem muscle biochemistry. J Anim Breed Genet 124, 19-26

Jarvinen TA, Jozsa L, Kannus P, Jarvinen TL, Hurme T, Kvist M, Pelto-Huikko M, Kalimo H, Jarvinen M (2003): Mechanical loading regulates the expression of tenascin-C in the myotendinous junction and tendon but does not induce de novo synthesis in the skeletal muscle. J Cell Sci 116, 857-866

Jennen DG, Brings AD, Liu G, Jungst H, Tholen E, Jonas E, Tesfaye D, Schellander K, Phatsara C (2007): Genetic aspects concerning drip loss and water-holding capacity of porcine meat. J Anim Breed Genet 124 Suppl 1, 2-11

Jiang L, Liu J, Sun D, Ma P, Ding X, Yu Y, Zhang Q (2010): Genome wide association studies for milk production traits in Chinese Holstein population. PLoS One 5, e13661

Jiang H, Xiong DH, Guo YF, Shen H, Xiao P, Yang F, Chen Y, Zhang F, Recker RR, Deng HW (2007): Association analysis of vitamin D-binding protein gene polymorphisms with variations of obesity-related traits in Caucasian nuclear families. Int J Obes 31, 1319-1324

Kim KS, Larsen N, Short T, Plastow G, Rothschild MF (2000): A missense variant of the porcine melanocortin-4 receptor (MC4R) gene is associated with fatness, growth, and feed intake traits. Mamm Genome 11, 131-135

Kitanaka S (2008): Role of HNF-1α and HNF-1ß on insulin, IGF-1 and other potential target genes. Expert Rev Endocrinol Metab 3, 441-452

Kristensen L, Purslow PP (2001): The effect of ageing on the water-holding capacity of pork: role of cytoskeletal proteins. Meat Sci 58, 17-23

Küchenmeister U, Kuhn G, Ender K (2000): Seasonal effects on Ca2+ transport of sarcoplasmic reticulum and on meat quality of pigs with different malignant hyperthermia status. Meat Sci 55, 239-245

Kuhn C, Leveziel H, Renand G, Goldammer T, Schwerin M, Williams JL (2005): Genetic markers for beef quality, In J. F. Hocquette, & S. Gigli (Eds.), Indicators of milk and beef quality. EAAP publication No.112

Kumaki Y, Oda M, Okano M (2008): QUMA: quantification tool for methylation analysis. Nucleic Acids Res 36, W170-175

Kušec G, Kralik G, Petričević A, Gutzmirtl H, Grgurić D (2003): Meat quality indicators and their correlation in two crosses of pigs. Agric Conspec Sci 68, 115-119

Lambert IH, Nielsen JH, Andersen HJ, Ortenblad N (2001): Cellular model for induction of drip loss in meat. J Agric Food Chem 49, 4876-4883

Lawrence TLJ, Fowler VR (2002): Growth of farm animals. CABI Publishing

Lawrie RA, Gatherum DP, Hale HP (1958): Abnormally low ultimate pH in pig muscle. Nature 182, 807-808

Lazzaroni C, Gigli S, Gabina D (2007): Evaluation of carcass and meat quality in ruminants. EAAP Scientific Series 123, pp.1-11

Le Flem L, Picard V, Emmerich J, Gandrille S, Fiessinger JN, Aiach M, Alhenc-Gelas M (1999): Mutations in promoter region of thrombomodulin and venous thromboembolic disease. Arterioscler Thromb Vasc Biol 19, 1098-1104

Lee S, Norman JM, Gunasekaran S, van Laack RLJM, Kim BC, Kauffman RG (2000): Use of electrical conductivity to predict water-holding capacity in post-rigor pork. Meat Sci 55, 385-389

Lengerken G, Maak S, Wicke M (2002): Muscle metabolism and meat quality of pigs and poultry. Vet Zootech 20, 82-86

Li LC, Dahiya R (2002): MethPrimer: designing primers for methylation PCRs. Bioinformatics 18, 1427-1431

Liu G, Jennen DG, Tholen E, Juengst H, Kleinwachter T, Holker M, Tesfaye D, Un G, Schreinemachers HJ, Murani E, et al. (2007): A genome scan reveals QTL for growth, fatness, leanness and meat quality in a Duroc-Pietrain resource population. Anim Genet 38, 241-252

Liu Q, Scheller KK, Arp SC, Schaefer DM, Williams SN (1996): Titration of fresh meat color stability and malondialdehyde development with Holstein steers fed vitamin E-supplemented diets. J Anim Sci 74, 117-126

Lo LL, McLaren DG, McKeith FK, Fernando RL, Novakofski J (1992): Genetic analyses of growth, real-time ultrasound, carcass, and pork quality traits in Duroc and Landrace pigs: II. Heritabilities and correlations. J Anim Sci 70, 2387-2396

Lonergan EH, Lonergan SM (2005): Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Sci 71, 194-204

Lopez-Bote C, Warriss PD (1988): A note on the relationships between measures of water holding capacity in the M. longissimus dorsi and total drip loss from butchered pig carcasses during storage. Meat Sci 23, 227-234

Lundström K, Essén-Gustavsson B, Rundgren M, Edfors-Lilja I, Malmfors G (1989): Effect of halothane genotype on muscle metabolism at slaughter and its relationship with meat quality – a within-litter comparison. Meat Sci 25, 251–263

Malek M, Dekkers JCM, Lee HK, Baas TJ, Prusa K, Huff-Lonergan E, Rothschild MF (2001): A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. II. Meat and muscle composition. Mamm Genome 12, 637-645

Maltin C, Balcerzak D, Tilley R, Delday M (2003): Determinants of meat quality: tenderness. Proc Nutr Soc 62, 337-347

Mancini RA, Hunt MC (2005): Current research in meat color. Meat Sci 71, 100-121

Manolio TA (2010): Genomewide association studies and assessment of the risk of disease. N Engl J Med 363, 166-176

Maribo H, Olsen EV, Barton-Gade P, Møller AJ, Karlsson A (1998): Effect of early post-mortem cooling on temperature, pH fall and meat quality in pigs. Meat Sci 50, 115-129

Markljung E, Braunschweig MH, Karlskov-Mortensen P, Bruun CS, Sawera M, Cho IC, Hedebro-Velander I, Josell A, Lundstrom K, von Seth G, et al. (2008): Genome-wide identification of quantitative trait loci in a cross between Hampshire and Landrace II: meat quality traits. BMC Genet 9, 22

Meidtner K, Wermter AK, Hinney A, Remschmidt H, Hebebrand J, Fries R (2006): Association of the melanocortin 4 receptor with feed intake and daily gain in F2 Mangalitsa x Pietrain pigs. Anim Genet 37, 245-247

Melody JL, Lonergan SM, Rowe LJ, Huiatt TW, Mayes MS, Huff-Lonergan E (2004): Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. J Anim Sci 82, 1195-1205

Mesires NT, Doumit ME (2002): Satellite cell proliferation and differentiation during postnatal growth of porcine skeletal muscle. Am J Physiol Cell Physiol 282, C899-906

Meyers SN, Beever JE (2008): Investigating the genetic basis of pork tenderness: genomic analysis of porcine CAST. Anim Genet 39, 531-543

Milan D, Bidanel JP, Iannuccelli N, Riquet J, Amigues Y, Gruand J, P. LR, Renard C, Chevalet C (2002): Detection of quantitative trait loci for carcass composition traits in pigs. Genet Sel Evol 34, 705–728

Milan D, Jeon J-T, Looft C, Amarger V, Robic A, Thelander M, Rogel-Gaillard C, Paul S, Iannuccelli N, Rask L, et al. (2000): A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. Science 288, 1248-1251

Monin G, Larzul C, Le Roy P, Culioli J, Mourot J, Rousset-Akrim S, Talmant A, Touraille C, Sellier P (1999): Effects of the halothane genotype and slaughter weight on texture of pork. J Anim Sci 77, 408-415

Montori-Grau M, Guitart M, Lerin C, Andreu AL, Newgard CB, Garcia-Martinez C, Gomez-Foix AM (2007): Expression and glycogenic effect of glycogen-targeting protein phosphatase 1 regulatory subunit GL in cultured human muscle. Biochem J 405, 107-113

Murani E, Muraniova M, Ponsuksili S, Schellander K, Wimmers K (2007): Identification of genes differentially expressed during prenatal development of skeletal muscle in two pig breeds differing in muscularity. BMC Dev Biol 7, 109

Nardone A, Valfrè F (1999): Effects of changing production methods on quality of meat, milk and eggs. Livest Prod Sci 59, 165-182

Neath KE, Del Barrio AN, Lapitan RM, Herrera JRV, Cruz LC, Fujihara T, Muroya S, Chikuni K, Hirabayashi M, Kanai Y (2007): Difference in tenderness and pH decline between water buffalo meat and beef during postmortem aging. Meat Sci 75, 499-505

Nezer C, Moreau L, Wagenaar D, Georges M (2002): Results of a whole genome scan targeting QTL for growth and carcass traits in a Pietrain x Large White intercross. Genet Sel Evol 34, 371-387

Onteru SK, Fan B, Garrick D, Stalder KJ, F. RM (2009): Whole genome analyses for pig reproductive traits using the PorcineSNP60 BeadChip. Pig Genome III Conference. November 2-4, Hinxton, Cambridge, UK. Abstr No. 5

Otterbein LR, Cosio C, Graceffa P, Dominguez R (2002): Crystal structures of the vitamin D-binding protein and its complex with actin: Structural basis of the actin-scavenger system. Proc Natl Acad Sci U S A 99, 8003-8008

Otto G, Roehea R, Looft H, Thoelking L, Henning M, Plastow GS, Kalm E (2006): Drip loss of case-ready meat and of premium cuts and their associations with earlier measured sample drip loss, meat quality and carcass traits in pigs. Meat Sci 72, 680-687

Otto G, Roehe R, Looft H, Thoelking L, Kalm E (2004): Comparison of different methods for determination of drip loss and their relationships to meat quality and carcass characteristics in pigs. Meat Sci 68, 401-409

Ovilo C, Clop A, Noguera JL, Oliver MA, Barragan C, Rodriguez C, Silio L, Toro MA, Coll A, Folch JM, et al. (2002): Quantitative trait locus mapping for meat quality traits in an Iberian x Landrace F2 pig population. J Anim Sci 80, 2801-2808

Paszek AA, Wilkie PJ, Flickinger GH, Miller LM, Louis CF, Rohrer GA, Alexander LJ, Beattie CW, Schook LB (2001): Interval mapping of carcass and meat quality traits in a divergent swine cross. Anim Biotechnol 12, 155-165

Pearson ER, Boj SF, Steele AM, Barrett T, Stals K, Shield JP, Ellard S, Ferrer J, Hattersley AT (2007): Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. PLoS Med 4, e118

Pelicano H, Martin DS, Xu RH, Huang P (2006): Glycolysis inhibition for anticancer treatment. Oncogene 25, 4633-4646

Penny IF (1969): Protein denaturation and water-holding capacity in pork muscle. Int J Food Sci Technol 4, 269-273

Plastow GS, Carrión D, Gil M, GarcIa-Regueiro JA, i Furnols MF, Gispert M, Oliver MA, Velarde A, Guàrdia MD, Hortós M, et al. (2005): Quality pork genes and meat production. Meat Sci 70, 409-421

Ponger L, Mouchiroud D (2002): CpGProD: identifying CpG islands associated with transcription start sites in large genomic mammalian sequences. Bioinformatics 18, 631-633

Ponsuksili S, Chomdej S, Murani E, Blaser U, Schreinemachers HJ, Schellander K, Wimmers K (2005): SNP detection and genetic mapping of porcine genes encoding enzymes in hepatic metabolic pathways and evaluation of linkage with carcass traits. Anim Genet 36, 477-483

Ponsuksili S, Jonas E, Murani E, Phatsara C, Srikanchai T, Walz C, Schwerin M, Schellander K, Wimmers K (2008a): Trait correlated expression combined with expression QTL analysis reveals biological pathways and candidate genes affecting water holding capacity of muscle. BMC Genomics 9, 367

Ponsuksili S, Murani E, Phatsara C, Jonas E, Walz C, Schwerin M, Schellander K, Wimmers K (2008b): Expression profiling of muscle reveals transcripts differentially expressed in muscle that affect water-holding capacity of pork. J Agric Food Chem 56, 10311-10317

Ponsuksili S, Murani E, Phatsara C, Schwerin M, Schellander K, Wimmers K (2009): Porcine muscle sensory attributes associate with major changes in gene networks involving CAPZB, ANKRD1, and CTBP2. Funct Integr Genomics 9, 455-471

Ponsuksili S, Wimmers K, Schmoll F, Robic A, Schellander K (2000): Porcine ESTs detected by differential display representing possible candidates for the trait “eye muscle area“. J Anim Breed Genet 117, 25-35

Pontoglio M, Barra J, Hadchouel M, Doyen A, Kress C, Bach JP, Babinet C, Yaniv M (1996): Hepatocyte nuclear factor 1 inactivation results in hepatic dysfunction, phenylketonuria, and renal Fanconi syndrome. Cell 84, 575-585

Pryce JE, Bolormaa S, Chamberlain AJ, Bowman PJ, Savin K, Goddard ME, Hayes BJ (2010): A validated genome-wide association study in 2 dairy cattle breeds for milk production and fertility traits using variable length haplotypes. J Dairy Sci 93, 3331-3345

Qiao J, Wang N, Ngadi MO, Gunenc A, Monroy M, Gariépy C, Prasher SO (2007): Prediction of drip-loss, pH, and color for pork using a hyperspectral imaging technique. Meat Sci 76, 1-8

Ramos AM, Crooijmans RP, Affara NA, Amaral AJ, Archibald AL, Beever JE, Bendixen C, Churcher C, Clark R, Dehais P, et al. (2009): Design of a high density SNP genotyping assay in the pig using SNPs identified and characterized by next generation sequencing technology. PLoS One 4, e6524

Ramos EM, Gomide LAM, Fontes PR, Ramos ALS, Peternelli LA (2005): Meat color evaluation and pigment levels in bullfrog (Rana catesbeiana) slaughtered by different methods. Aquaculture 245, 175-182

Reginato AJ, Falasca GF (1992): Can vitamin D deficiency produce an unusual pain syndrome?. Arch Intern Med 152, 1720–1721

Reverter A, Johnston DJ, Perry D, Goddard ME, Burrow HM (2003): Genetic and phenotypic characterisation of animal, carcass, and meat quality traits from temperate and tropically adapted beef breeds. 2. Abattoir carcass traits. Aust J Agric Res 54, 119-134

Rhoads DB, Levisky LL (2008): Function of HNF1 in the pathogenesis of diatetes. Expert Rev Endocrinol Metab 3, 391-403

Rimaniol JM, Authier FJ, Chariot P (1994): Muscle weakness in intensive care patients: initial manifestation of vitamin D deficiency. Intensive Care Med 20, 591-592

Robic A, Parrou JL, Yerle M, Goureau A, Dalens M, Milan D, Gellin J (1995): Pig microsatellites isolated from cosmids revealing polymorphism and localized on chromosomes. Anim Genet 26, 1-6

Rodman JS, Baker T (1978): Changes in the kinetics of muscle contraction in vitamin D-depleted rats. Kidney Int 13, 189-193

Rohrer GA, Thallman RM, Shackelford S, Wheeler T, Koohmaraie M (2006): A genome scan for loci affecting pork quality in a Duroc-Landrace F2 population. Anim Genet 37, 17-27

Rothschild MF (2004): Porcine genomics delivers new tools and results: This little piggy did more than just go to market. Genet Res 83, 1-6

Rothschild MF, Plastow GS (2008): Impact of genomics on animal agriculture and opportunities for animal health. Trends Biotechnol 26, 21-25

Rozen S, Skaletsky H (2000): Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 132, 365-386

Russell JA (1994): Osteomalacic myopathy. Muscle Nerve 17, 578-580

Ryffel GU (2001): Mutations in the human genes encoding the transcription factors of the hepatocyte nuclear factor (HNF)1 and HNF4 families: functional and pathological consequences. J Mol Endocrinol 27, 11-29

Salier JP, Rouet P, Raguenez G, Daveau M (1996): The inter-alpha-inhibitor family: from structure to regulation. Biochem J 315 (Pt 1), 1-9

Sanchez D, Martinez S, Lindqvist A, Akerstrom B, Falkenberg C (2002): Expression of the AMBP gene transcript and its two protein products, alpha(1)-microglobulin and bikunin, in mouse embryogenesis. Mech Dev 117, 293-298

Sandercock DA, Barker ZE, Mitchell MA, Hocking PM (2009): Changes in muscle cell cation regulation and meat quality traits are associated with genetic selection for high body weight and meat yield in broiler chickens. Genet Sel Evol 41, 8

Sanoudou D, Kang PB, Haslett JN, Han M, Kunkel LM, Beggs AH (2004): Transcriptional profile of postmortem skeletal muscle. Physiol Genomics 16, 222-228

Sarasa-Renedo A, Chiquet M (2005): Mechanical signals regulating extracellular matrix gene expression in fibroblasts. Scand J Med Sci Sports 15, 223-230

Sato S, Oyamada Y, Atsuji K, Nade T, Kobayashi E, Mitsuhashi T, Nirasawa K, Komatsuda A, Saito Y, Terai S, et al. (2003): Quantitative trait loci analysis for growth and carcass traits in a Meishan x Duroc F2 resource population. J Anim Sci 81, 2938-2949

Satoh Y, Shikama K (1981): Autoxidation of oxymyoglobin. A nucleophilic displacement mechanism. J Biol Sci 256, 10272-10275

Schaar CG, Kluin-Nelemans HC, Te Marvelde C, le Cessie S, Breed WP, Fibbe WE, van Deijk WA, Fickers MM, Roozendaal KJ, Wijermans PW (2005): Interferon-alpha as maintenance therapy in patients with multiple myeloma. Ann Oncol 16, 634-639

Scheffler TL, Gerrard DE (2007): Mechanisms controlling pork quality development: The biochemistry controlling postmortem energy metabolism. Meat Sci 77, 7-16

Schwerin M, Czernek-Schafer D, Goldammer T, Kata SR, Womack JE, Pareek R, Pareek C, Walawski K, Brunner RM (2003): Application of disease-associated differentially expressed genes--mining for functional candidate genes for mastitis resistance in cattle. Genet Sel Evol 35 Suppl 1, S19-34

Sellier P (1998): Genetics of meat and carcass traits. In: M Rothschild, A. Ruvinsky (eds),The Genetics of the Pig. CAB International, Wallingford, Oxon, UK, pp. 463-510.

Sentandreu MA, Coulis G, Ouali A (2002): Role of muscle endopeptidases and their inhibitors in meat tenderness. Trends Food Sci Technol 13, 400-421

Sethi JK, Vidal-Puig AJ (2008): Wnt signalling at the crossroads of nutritional regulation. Biochem J 416, e11-13

Sheehy PA, Riley LG, Raadsma HW, Williamson P, Wynn PC (2009): A functional genomics approach to evaluate candidate genes located in a QTL interval for milk production traits on BTA6. Anim Genet 40, 492-498

Shih DQ, Bussen M, Sehayek E, Ananthanarayanan M, Shneider BL, Suchy FJ, Shefer S, Bollileni JS, Gonzalez FJ, Breslow JL, et al. (2001): Hepatocyte nuclear factor-1alpha is an essential regulator of bile acid and plasma cholesterol metabolism. Nat Genet 27, 375-382

Sian E (2000): Hepatocyte nuclear factor 1 alpha (HNF-1α) mutations in maturity-onset diabetes of the young. Hum Mutat 16, 377-385

Simpson RU, Thomas GA, Arnold AJ (1985): Identification of 1,25-dihydroxyvitamin D3 receptors and activities in muscle. J Biol Chem 260, 8882-8891

Sonesson AK, de Greef KH, Meuwissen THE (1998): Genetic parameters and trends of meat quality, carcass composition and performance traits in two selected lines of large white pigs. Livest Prod Sci 57, 23-32

Srikanchai T, Murani E, Wimmers K, Ponsuksili S (2010): Four loci differentially expressed in muscle tissue depending on water-holding capacity are associated with meat quality in commercial pig herds. Mol Biol Rep 37, 595-601

Stephens JC, Schneider JA, Tanguay DA, Choi J, Acharya T, Stanley SE, Jiang R, Messer CJ, Chew A, Han JH, et al. (2001): Haplotype variation and linkage disequilibrium in 313 human genes. Science 293, 489-493

Sun J, Jia P, Fanous AH, Webb BT, van den Oord EJCG, Chen X, Bukszar J, Kendler KS, Zhao Z (2009): A multi-dimensional evidence-based candidate gene prioritization approach for complex diseases-schizophrenia as a case. Bioinformatics 25, 2595-6602

Suzuki K, Irie M, Kadowaki H, Shibata T, Kumagai M, Nishida A (2005): Genetic parameter estimates of meat quality traits in Duroc pigs selected for average daily gain, longissimus muscle area, backfat thickness, and intramuscular fat content. J Anim Sci 83, 2058-2065

Szegedi K, Sonkoly E, Nagy N, Nemeth IB, Bata-Csorgo Z, Kemeny L, Dobozy A, Szell M (2010): The anti-apoptotic protein G1P3 is overexpressed in psoriasis and regulated by the non-coding RNA, PRINS. Exp Dermatol 19, 269-278

Tahara E, Tahara H, Kanno M, Naka K, Takeda Y, Matsuzaki T, Yamazaki R, Ishihara H, Yasui W, Barrett JC, et al. (2005): G1P3, an interferon inducible gene 6-16, is expressed in gastric cancers and inhibits mitochondrial-mediated apoptosis in gastric cancer cell line TMK-1 cell. Cancer Immunol Immunother 54, 729-740

Takala TE, Virtanen P (2000): Biochemical composition of muscle extracellular matrix: the effect of loading. Scand J Med Sci Sports 10, 321-325

Taniguchi M, Hayashi T, Nii M, Yamaguchi T, Fujishima-Kanaya N, Awata T, Mikawa S (2010): Fine mapping of quantitative trait loci for meat color on Sus scrofa chromosome 6: analysis of the swine NUDT7 gene1. J Anim Sci 88, 23-31

Tholen E, Jüngst H, Schulze-Langenhorst C, Schellander K (2005): Genetic foundation of meat quality traits of station tested slaughter pigs in North Rhine-Westphalia (Germany). A status report. Arch Tierz, Dummerstorf 48, 123-130

Thomsen H, Lee HK, Rothschild MF, Malek M, Dekkers JC (2004): Characterization of quantitative trait loci for growth and meat quality in a cross between commercial breeds of swine. J Anim Sci 82, 2213-2228

Tikk K, Tikk M, Karlsson AH, Andersen HJ (2006): The effect of a muscle-glycogen-reducing finishing diet on porcine meat and fat colour. Meat Sci 73, 378-385

Trump BF, Berezesky IK (1995): Calcium-mediated cell injury and cell death. FASEB J 9, 219-228

Turri MG, Cuin KA, Porter AC (1995): Characterisation of a novel minisatellite that provides multiple splice donor sites in an interferon-induced transcript. Nucleic Acids Res 23, 1854-1861

Tyagi S, Salier J-P, Lal SK (2002): The liver-specific human [alpha]1-microglobulin/bikunin precursor (AMBP) is capable of self-association. Arch Biochem Biophys 399, 66-72

Urabe A (1994): Interferons for the treatment of hematological malignancies. Oncology 51, 137-141

van der Steen HAM, Prall GFW, Plastow GS (2005): Application of genomics to the pork industry. J Anim Sci 83, E1-8

van Laere AS, Nguyen M, Braunschweig M, Nezer C, Collette C, Moreau L, Archibald AL, Haley CS, Buys N, Tally M, et al. (2003): A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature 425, 832-836

van Wijk HJ, Arts DJ, Matthews JO, Webster M, Ducro BJ, Knol EF (2005): Genetic parameters for carcass composition and pork quality estimated in a commercial production chain. J Anim Sci 83, 324-333

van Wijk HJ, Dibbits B, Baron EE, Brings AD, Harlizius B, Groenen MA, Knol EF, Bovenhuis H (2006): Identification of quantitative trait loci for carcass composition and pork quality traits in a commercial finishing cross. J Anim Sci 84, 789-799

Vayro S, Wood IS, Dyer J, Shirazi-Beechey SP (2001): Transcriptional regulation of the ovine intestinal Na+/glucose cotransporter SGLT1 gene. Role of HNF-1 in glucose activation of promoter function. Eur J Biochem 268, 5460-5470

Velleman SG (2007): Muscle development in the embryo and hatchling. Poult Sci 86, 1050-1054

Velleman SG (2002): Role of the extracellular matrix in muscle growth and development. J Anim Sci 80, E8-13

Velleman SG (2000): The role of the extracellular matrix in skeletal development. Poult Sci 79, 985-989

Vidal O, Noguera JL, Amills M, Varona L, Gil M, Jimenez N, Davalos G, Folch JM, Sanchez A (2005): Identification of carcass and meat quality quantitative trait loci in a Landrace pig population selected for growth and leanness. J Anim Sci 83, 293-300

Visscher PM, Hill WG, Wray NR (2008): Heritability in the genomics era--concepts and misconceptions. Nat Rev Genet 9, 255-266

Walstra P, Claudi-Magnussen C, Chevillon P, Von Seth G, Diestre A, Matthews K, Homer D, Bonneau M (1998): An international study on the importance of androstenone and skatole for boar taint: levels of androstenone and skatole by country and season. Livest Prod Sci 62, 15–28

Wangsness PJ, Martin RJ, Gahagan JH (1977): Insulin and growth hormone in lean and obese pigs. Am J Physiol 233, E104-108

Wangsness PJ, Martin RJ, Gatchel BB (1980): Insulin induced growth hormone response in fast-growing, lean and in slow-growing, obese pigs. Growth 44, 318-326

Warner RD, Kauffman RG, Greaser ML (1997): Muscle protein changes post mortem in relation to pork quality traits. Meat Sci 45, 339-352

Warriss PD, Brown SN, Gade PB, Santos C, Costa LN, Lambooij E, Geers R (1998): An analysis of data relating to pig carcass quality and indices of stress collected in the European union. Meat Sci 49, 137-144

Weedon MN, Frayling TM (2007): Insights on pathogenesis of type 2 diabetes from MODY genetics. Curr Diab Rep 7, 131-138

Whitman TA, Forrest JC, Morgan MT, Okos MR (1996): Electrical measurement for detecting early postmortem changes in porcine muscle. J Anim Sci 74, 80-90

Wimmers K, Fiedler I, Hardge T, Murani E, Schellander K, Ponsuksili S (2006): QTL for microstructural and biophysical muscle properties and body composition in pigs. BMC Genet 7, 15

Wimmers K, Murani E, Ponsuksili S, Yerle M, Schellander K (2002): Detection of quantitative trait loci for carcass traits in the pig by using AFLP. Mamm Genome 13, 206-210

Winckler W, Burtt NP, Holmkvist J, Cervin C, de Bakker PI, Sun M, Almgren P, Tuomi T, Gaudet D, Hudson TJ, et al. (2005): Association of common variation in the HNF1alpha gene region with risk of type 2 diabetes. Diabetes 54, 2336-2342

Worby CA, Gentry MS, Dixon JE (2008): Malin decreases glycogen accumulation by promoting the degradation of protein targeting to glycogen (PTG). J Biol Chem 283, 4069-4076

Wray N, Visscher P (2008): Estimating Trait Heritability. Nature Education 1, 1

Yoon JH, Halper J (2005): Tendon proteoglycans: biochemistry and function. J Musculoskelet Neuronal Interact 5, 22-34

Yu J, Yin P, Liu F, Cheng G, Guo K, Lu A, Zhu X, Luan W, Xu J (2010): Effect of heat stress on the porcine small intestine: a morphological and gene expression study. Comp Biochem Physiol A Mol Integr Physiol 156, 119-128

ZDS (2004): Zentralverband der Deutschen Schweineproduktion e.V. Richtlinie fuer die Stationspruefung auf Mastleistung, Schlachtkoerperwert und Fleischbeschaffenheit Beim Schwein. Ausschuss fuer Leistungspruefung und Zuchtwertschaetzung Bonn, Germany

Zhang WG, Lonergan SM, M.A. G, E.H. L (2006): Contribution of postmortem changes of integrin, desmin and μ-calpain to variation in water holding capacity of pork. Meat Sci 74, 578-585

Zhu M, Zhao S (2007): Candidate gene identification approach: progress and challenges. Int J Biol Sci 3, 420-427

Zhang WG, Lonergan SM, Gardner MA, Lonergan EH (2006): Contribution of postmortem changes of integrin, desmin and μ-calpain to variation in water holding capacity of pork. Meat Sci 74, 578-585

Zhuo L, Yoneda M, Zhao M, Yingsung W, Yoshida N, Kitagawa Y, Kawamura K, Suzuki T, Kimata K (2001): Defect in SHAP-hyaluronan complex causes severe female infertility. J Biol Chem 276, 7693-7696

Zibrova D, Grempler R, Streicher R, Kauschke SG (2008): Inhibition of the interaction between protein phosphatase 1 glycogen-targeting subunit and glycogen phosphorylase increases glycogen synthesis in primary rat hepatocytes. Biochem J 412, 359-366

Acknowledgments

I would like to express my sincere and greatest appreciation to my supervisor Prof. Dr. Karl Schellander, Animal Breeding and Husbandry Group from the Institute of Animal Science of University of Bonn, for offering me the opportunity to pursue a Ph.D. degree, for his invaluable advices during the realization of this study. Prof. Schellander always encourages and supports me, especially in the difficult period of this study, give me the idea to get further progress; pays attention to my study and life in this Institute, solves my personal and technical problems worth of more mention; Prof. Schellander also has great patience for me. Here is lack of words to express my deepest thanks to Prof. Schellander.

I am also very grateful to Prof. Dr. Brigitte Petersen, the Institute of Animal Science of University of Bonn, for her willingness to be a co-supervisor in this study.

I would like to thank my supervisor in Chiang Mai University, Thailand, Prof. Dr. Sanchai Jaturasitha, since I was study in Master degree in Meat Science of Chiang Mai University. He always give me a suggestion for the ideas about life and research, give me a knowledge during my study. I remember this sentence from him as well “Life is not smooth but be strong and patient”, this sentence make me have a power when I feel disappoint with my experiment and get me rid of any obstacle. I believe that now he is looking for my success and always beside me when I need his support.

There were lager difficulties at the beginning of my Ph.D. study, not only language, but also laboratory work. Our senior of Thai group in the Institute of Animal Science guided me for the whole process of the beginning of laboratory work as well. Without their help, I could not fulfill my Ph.D. study.

I am very grateful to Prof. Dr. Brigitte Schmitz at Institute of Biochemistry, University of Bonn for the utilization of laboratory facilities. Moreover, special thanks will go to Mr. Bernhard Gehrig, institute of Biochemistry, University of Bonn for a guideline, prepared all chemicals for western blotting and help me to solve the problems during I

was working there. He is the excellent technician for me, without him I could not finished my experiment.

I would like to thanks Dr. Chirawath Phatsara and Dr. Dawit Tesfaye for their kind cooperation. Thanks for their technical help in various aspects, valuable comments and making available all facilities for carrying out this work.

I would like to thank Dr. Heinz Jungst and their staff members at the research station Frankenforst for the experimental animals and samples which were used for all experiments in this study.

My grateful thanks go to Mohammed Ulas Cinar and Mohammed Jassin Uddin for his helping and correction of this manuscript. I would like to thank Maren Pröll and Christiane Neuhoff for their kind cooperation to write in the german abstract and summary parts of my dissertation.

I would like to thank all administrative members of the Institute of Animal Science, particularly Ms. Bianca Peters and Ms. Ulrike Schröter for their kind to help with all documents. Thanks also go to Mr. Peter Müller for his really useful help in computer technique, and Ms. Nadine Leyer for offering me everything during lab work of this work.

I would like to thank all my Thai friends in Bonn and all member of Animal Breeding and Husbandry group, Institute of Animal Science, University of Bonn. They make me feel like “I’m not alone in Germany”. Many Thanks to Ms. Saowaluck Yammuen-art, my senior in our institute, for teaching a basic of molecular genetic. Also many thanks to Ms. Kanokwan Kaewmala who always stay with me at night during I’m working in Biochemistry institute and helping me in many lab works, without her I will feel lonely.

I deeply thank my parents, Mr. Veerasak and Mrs. Lamai Kayan, who encourage and inspiration all the time and support me to concentrate on my study in Germany. I also thank all member of my family who are my best brothers (Mr.Jetsada Kayan and Mr.Apichart Kayan) and always support me to study in the higher education. My special

thanks will go though Dr. Sadachai Nittayarumphong who always beside me in difficult time, give me a hug when I feel disappoint, give me the smiling and force me to complete my work as soon as possible. Thanks for waiting me to come back to Thailand.

Finally, I am very much grateful to all people who have contributed in one way or another for the realization of this work.