Institut für Tierwissenschaften, Abt. Tierzucht und Tierhaltung

der Rheinischen Friedrich–Wilhelms–Universität Bonn

Analysis of functional candidate genes for meat quality and carcass traits in pigs

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 Agrarwissenschaften

(Dr. agr.)

der

Hohen Landwirtschaftlichen Fakultät

der

Rheinischen Friedrich–Wilhelms–Universität

zu Bonn

vorgelegt im

Dezember 2009

von

Tiranun Srikanchai

aus

Chiang Mai, Thailand

Analysis of functional candidate genes for meat quality and carcass traits in pigs

Twelve genes, *BVES*, *SLC3A2*, *AHNAK*, *ZDHHC5*, *CS*, *LYZ*, *KERA*, *COQ9*, *UN* (a non-annotated EST), *EGFR*, *VTN* and *ZYX*, whose candidacy for traits related to water-holding capacity of meat arises from their trait-dependent differential expression and/or trait correlated expression, were selected for analysis. Based on *in silico* analysis SNPs were detected, confirmed by sequencing and used for genotyping. For the first eleven genes, the SNPs were genotyped in ca. 1,800 animals from 6 pig populations including commercial herds of Pietrain (PI(a/b)), Pietrain x (German Large White x German Landrace) (PIF1(a/b/c)), and German Landrace (DL(a/b)) and one experimental F_2-population Duroc x Pietrain (DUPI). For *ZYX*, the SNPs were genotyped in 870 animals from 4 pig populations including PI, DL, F1 and PIF1. Comparative and genetic mapping established the location of *BVES* on SSC1, of *SLC3A2*, *AHNAK* and *ZDHHC5* on SSC2, of *CS*, *LYZ* and *KERA* on SSC5, of *COQ9* on SSC6, of *UN* on SSC7, of *EGFR* on SSC9, of *VTN* on SSC12 and of *ZYX* on SSC18 respectively, coinciding with QTL regions for carcass and meat quality traits. *BVES*, *SLC3A2*, *AHNAK*, *CS*, *LYZ*, *UN*, *VTN* and *ZYX* revealed association with drip loss and also with several other measures of carcass and meat quality traits. *KERA* was associated with loin eye area and pH. Moreover, several carcass fatness traits and meat quality traits such as meat color and thawing loss were associated with *COQ9* and *EGFR*. 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. This study reveals statistic evidence for a link of genetic variation at these loci or close to them with phenotypic variation and promotes those twelve candidate genes as functional and/or positional candidate genes for carcass and meat quality traits

Analyse von funktionalen Kandidatengenen für Fleischqualität und Schlachtkörpermerkmale in Schweinen

Zwölf Gene, *BVES, SLC3A2, AHNAK, ZDHHC5, CS, LYZ, KERA, COQ9, UN* (ein unannotiertes EST), *EGFR, VTN* und *ZYX*, deren Kandidatenstatus für Merkmale des Wasserbindungsvermögens von Fleisch auf ihrer merkmalsabhängigen differentiellen Expression und/oder merkmalskorrelierten Expression beruht, wurden für die Analyse ausgewählt. Basierend auf *in silico*-Analysen wurden SNPs detektiert, die durch Sequenzierung bestätigt und dann zur Genotypisierung verwendet wurden. Für die ersten elf Gene wurden die SNPs in ca. 1.800 Tieren aus sechs Schweinepopulationen genotypisiert; dazu gehörten kommerzielle Herden der Rassen Pietrain (PI(a/b)), Deutsche Landrasse (DL(a/b)) und eine Drei-Rassen-Kreuzung aus Pietrain x (Deutsches Edelschwein x Deutsche Landrasse) (PIF1(a/b/c)) sowie eine experimentelle F2-Population aus Duroc x Pietrain (DUPI). Für *ZYX* wurden die SNPs in 870 Tieren aus vier Schweinepopulationen genotypisiert; dazu gehörten PI, DL, F1 und PIF1. Genetische Kartierung etablierte die Lage von *BVES* auf SSC1, von *SLC3A2, AHNAK* und *ZDHHC5* auf SSC2, von *CS, LYZ* und *KERA* auf SSC5, von *COQ9* auf SSC6, von *UN* auf SSC7, von *EGFR* auf SSC9, von *VTN* auf SSC12 und von *ZYX* auf SSC18, jeweils in Regionen, die mit QTL-Regionen für Schlachtkörper- und Fleischqualitätsmerkmale zusammenfallen. *BVES, SLC3A2, AHNAK, CS, LYZ, UN, VTN* und *ZYX* ließen sowohl Assoziationen mit Tropfsaftverlust als auch mit verschiedenen anderen Messgrößen für Schlachtkörper- und Fleischqualitätsmerkmale erkennen. *KERA* war mit der Kotelettfläche und dem pH-Wert assoziiert. Außerdem waren verschiedene Schlachtkörper-Fettmerkmale und Fleischqualitätsmerkmale wie z.B. OPTO und Auftauverlust mit *COQ9* und *EGFR* assoziiert. Allerdings zeigte keines der Kandidatengene eine signifikante Assoziation mit einem bestimmten Merkmal in allen Populationen. Dies könnte an den rassenspezifischen Effekten liegen, die die Unterschiede in der Schlachtkörper- und Fleischqualität dieser Schweinerassen bedingen. Diese Studie zeigt statistische Hinweise auf eine Verbindung von genetischer Variation an oder in der Nähe dieser Loci und phänotypischer Variation. Dies bestätigt die zwölf Kandidatengene als funktionale und/oder positionelle Kandidatengene für Schlachtkörper- und Fleischqualitätsmerkmale.

Contents

List of abbreviations

ABF	: average backfat thickness
ATP	: Adenosine triphosphate
ADP	: Adenosine diphosphate
AHNAK	: AHNAK nucleoprotein (desmoyokin)
BF1	: shoulder fat depth
BF2	: fat depth at 10th rib
BF3	: loin fat depth
BVES	: blood vessel epicardial substance
COOK	: cooking loss
CON1	: conductivity in *M.longissimus dorsi* at $13^{th}/14^{th}$ rib 45 minute p.m.
CON24	: conductivity in *M.longissimus dorsi* at $13^{th}/14^{th}$ rib 24 hour p.m.
COQ9	: Coenzyme Q9 homolog (*S. cerevisiae*)
CS	: citrate synthase
DL	: German Landrace
DNA	: Deoxyribonucleic acid
dNTP	: Deoxyribonucleotide triphosphate
DRIP	: drip loss
EGFR	: Epidermal growth factor receptor
eQTL	: expression quantitative trait loci
EST	: expressed sequence tag
ExoI	: Exonuclease I
FA	: fat area
HAL (RYR1)	: Halothane (ryanodine receptor)
HWE	: Hardy-Weinberg equilibrium
Indel	: insertion/deletion polymorphism
KERA	: Keratocan
LEA	: loin eye area
LOD	: logarithm of the odds (to the base 10)

LSM	: least square means
LYZ	: Lysozyme
MAS	: marker assisted selection
MFR	: meat to fat ratio
MH	: malignant hyperthermia
PCR	: polymerase chain reaction
pH1	: pH value in *M.longissimus dorsi* at $13^{th}/14^{th}$ rib 45 minute p.m.
pH24	: pH value in *M.longissimus dorsi* at $13^{th}/14^{th}$ rib 24 hour p.m.
p.m.	: post mortem
OPTO	: meat color 24 h p.m. in Mld at $13^{th}/14^{th}$ rib; OPTO star
PI	: Pietrian
PIF1	: Pietrian x (German Large White x German Landrace)
PSE	: pale, soft, exudative
PSS	: porcine stress syndrome
QTL	: quantitative trait loci
RN (PRKAG3)	: Rendement Napole (Protein kinase AMP activated, $\gamma 3$ subunit)
SAP	: shrimp alkaline phosphates
SBE	: single base extension
SF	: shear force
SLC3A2	: Solute carrier family 3, member 2
SNP	: single nucleotide polymorphism
SR	: sarcoplasmic reticulum
THAW	: thaw loss
UN	: unknown
UTR	: untranslated region
VTN	: Vitronectin
WHC	: water-holding capacity
X^2	: Chi-square
ZDHHC5	: Zinc finger, DHHC-type containing 5
ZYX	: Zyxin

List of tables

List of figures

1. Introduction

During the last decades, intensive selection for rapid lean growth in pigs induced a shift in muscle metabolism towards a more glycolytic and less oxidative fiber type. With regard to this, unfavorable genes correlated to altered meat quality are also selected (Cameron, 1990; Weiler et al. 1995). One of the most significant problems the pork industry has to face is a lack of consistency in water-holding capacity (WHC). Pork with the extreme defect PSE (pale, soft and exudative) can lose as much weight as 10% (Melody et al. 2004). Surveys conducted in the USA in 2003 indicate that 15.5% of the produced pork was PSE (Stetzer and McKeith, 2003), which is estimated to cost the pork industry $100 million annually due to the inferior quality of the PSE pork (Carr et al. 1997). However, the frequency of PSE pork has been reduced since the discovery of the major gene ryanodine receptor 1 (RYR1; the Halothane locus), which has been shown to cause PSE (Fujii et al. 1991). The selection against PSE meat was performed by eliminating the homozygous genotype 'nn' (stress susceptible) of the RYR1 gene. But since this allele also had positive effects on lean muscle deposition (Tor et al. 2001; Lengerken et al. 2002), the percentage of heterozygous animals still remained. Although the frequency of PSE pork is not a big problem anymore, the pig industry still needs to improve the trait WHC in pork. Since WHC can be measured directly via the measure "drip loss"; it has been a significant parameter of research attention. Interestingly, some studies showed that drip loss had high correlations to the routinely recorded meat quality traits such as pH and conductivity (Lee et al. 2000; Borchers et al. 2007), while moderate correlations were found to carcass fatness traits (Mörlein et al. 2007; Ponsuksili et al. 2008a). 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 (Sellier, 1998; van Wijk et al. 2005). 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 (Rothschild and Plastow, 1999; van der Steen et al. 2005). Previously, the studies of gene expression profiles revealed a large number of genes differentially expressed in groups

with high/low drip loss and high/low pH, or with expression correlated with drip loss. These studies revealed relevant biological pathways and were a source of candidate genes (Ponsuksili et al. 2008a, b).

In order to gain more knowledge and provide useful data for marker assisted breeding schemes and finally to improve pig production in the future, the present study was undertaken by selecting candidate genes from previous studies (Ponsuksili et al. 2008a, b) 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

Marketing of food in general and meat in particular has changed considerably during the last decades. In general, the price for pigs is based on the carcass quality (carcass weight and lean meat percentage), while no regard is paid to meat quality. Carcass and meat quality are considered to be reversely correlated (Huff-Lonergan et al. 2003), meaning that improving leanness would lead to the reduction of meat quality (e.g. high leanness is associated with undesired bright color, low pH and high drip loss). Since then meat quality has become more important while the carcass quality has been consistent. In the current pig price system in Germany, which supports the breeding and management aims, price additions are only allowed up to 58% of lean meat. More leanness is not paid for (Kallweit et al. 2007). Also, the amount of pork sold in butcher shops is decreasing while the amount of pre-packed pork in grocery stores and discounters is growing. This allows the customer to visualize meat quality by color and drip loss if the packages are transparent and have been stored in the counter for some time. Both carcass and meat quality are complex and multivariate properties which are influenced by multiple interacting factors. These include genetic and environmental factors (Rosenvolt and Andersen, 2003). In order to develop more comprehensive strategies for reducing the variation found in pork quality, the biochemistry underlying postmortem metabolism must be defined.

2.1 The conversion of muscle to meat

The main role of muscle is to provide a means of locomotion through carefully orchestrated contraction/relaxation cycles. This whole cycling process is modulated by fluctuations in cytosolic calcium (Ca^{2+}) levels. At contraction-induced levels, Ca^{2+} "instigates" conformational changes in the thin filaments and allows myosin to bind actin molecules. In order for muscle to relax, Ca^{2+} is removed from the sarcoplasm, via an ATP dependent Ca^{2+} pump, and is resequestered in the sarcoplasmic reticulum (SR), thereby removing the ability of Ca^{2+} to induce contraction. ATP is the source of energy for breaking actomyosin cross-bridges and for maintaining the Ca^{2+} pump of the sarcoplasmic reticulum, whereas Ca^{2+} is a primary regulator of contraction (Figure 1) (reviewed by

Bowker et al. 2000). Thus, ATP and Ca^{2+} can be considered two major players in muscle contraction and metabolism.
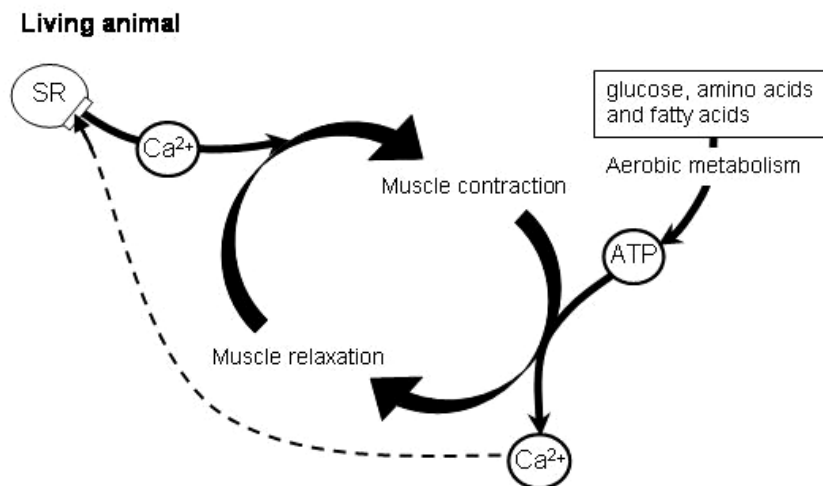
Living animal

Figure 1: The contraction/relaxation of muscle in living animal

In postmortem muscle, the tissue attempts to maintain homeostasis by maintaining cellular ATP concentrations, because ATP production is necessary to keep the muscle in the relaxed state but due to circulatory failure following exsanguination, muscle lacks the oxygen required for oxidative metabolism. Consequently, muscle glycogen is metabolized via anaerobic glycolysis, thus phosphorylating ADP to replenish ATP. Anaerobic glycolysis is less efficient at generating ATP than aerobic metabolism. Thus, as postmortem metabolism continues, glycogen and ATP levels decline, and lactic acid accumulates, lowering the muscle pH. Because less ATP is available, the formation of actomyosin bonds shortens sarcomeres and increases muscle tension, signaling the onset of *rigor mortis*. *Rigor mortis* is complete when the ATP supply is exhausted; thus, actomyosin crossbridges cannot be broken and the muscle is relatively inextensible (Figure 2). This process results in an overall pH decline from 7.4 in living muscle to an ultimate pH (pHu) of about 5.4 to 5.7 at 24 h *post mortem* in normal pig *longissimus* muscle (reviewed by Bowker et al. 2000; Melody et al. 2004). The rate and extent of pH decline during the conversion of muscle to meat significantly impact the development of fresh meat quality attributes. The onset of *rigor mortis* at high temperature and low pH causes

the denaturation of approximately 20% of the sarcoplasmic and myofibrillar protein. Abnormally low pH reduces the net charge of myofibrillar proteins, and the attraction moves filaments closer together and forces water out of the myofilament lattice. Moreover, sarcoplasmic protein solubility declines with decreasing pHu and contributes to paler pork color. The rate and extent of postmortem pH decline significantly influence protein characteristics and thus critically affect pork quality development (Bee et al. 2007; Scheffler and Gerrard, 2007).
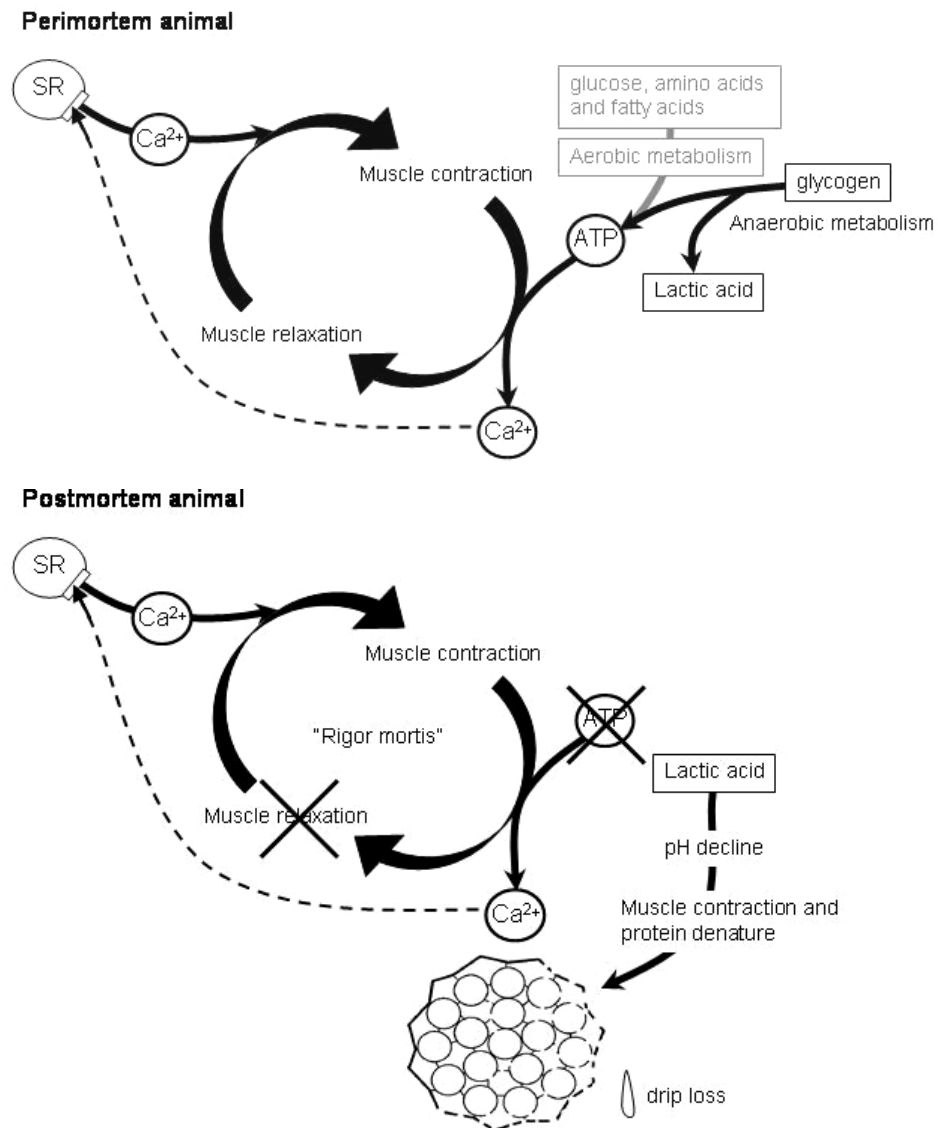
Figure 2: The conversion of muscle to meat

2.2 Major genes affecting pig meat quality

In pig the most frequently observed deviation from normal meat quality is the so-called PSE (pale, soft and exudative) meat. This tendency to produce PSE pork was closely associated with porcine stress syndrome (PSS), a condition synonymous with human malignant hyperthermia (MH). Pigs susceptible to MH display hypermetabolism, elevated body temperature, and muscle rigidity upon exposure to the anesthetic halothane (Lengerken et al. 2002; Melody et al. 2004).

The halothane (HAL) gene locus, the name referring to the exposure of halothane gas used to screen for stress-susceptible pigs, has two alleles: the normal dominant allele (N) and the mutant recessive allele (n). The causative polymorphism is the SNP at position 1843 (C>T) in the skeletal muscle ryanodine receptor (RYR1) that maps to SSC 6, leading to an amino acid change from Arginine to Cysteine (Fujii et al.1991). The RYR1, or Ca^{2+}-release channels in MH-susceptible pigs are hypersensitive to agents that stimulate opening, thus allowing longer open time probability and resulting in enhanced Ca^{2+}-release and greater twitch tension, resulting in sustained contraction and metabolism. This defect in Ca^{2+}-concentration has important consequences for production and meat quality traits. The HAL mutation may contribute to leanness and heavy muscling by causing spontaneous muscle contraction and greater energy utilization, leading to work-induced muscle hypertrophy and limiting fat deposition. However, the positive effects of the mutant allele on performance is negated by an increased risk for stress-induced death and high susceptibility to acute stress prior to slaughter, which may manifest in an accelerated rate of pH decline and the production of PSE pork (Essen-Gustavsson et al. 1992; Fernandez et al. 2002)

The second major gene effecting pig meat quality is the rendement napole (RN) gene, whose name referres to a method of estimating ham yield. It was first noticed in France that meat from Hampshire pigs often had extremely low pH and a much lower yield of a cured-cooked ham product called the "Paris Ham". Once termed the "Hampshire Effect," the effect was found to be dominant (Monin and Sellier, 1985). Further analysis has shown that the RN- allele increases the amount of glycogen in white muscle by about 70%. The existence of the RN locus was mapped to SSC 15 (Milan et al. 1996). The identification of

the causative mutation revealed that the gene involved is a new member of a gene family coding one of the regulatory subunits of the AMP-activated protein kinase complex (named PRKAG3). The test for the RN- mutation is being used to remove the defect from primarily Hampshire based lines (Le Roy et al. 2000; Milan et al. 2000). Interestingly, additional mutations within the gene have been discovered and are of importance to the industry (Ciobanu et al. 2001).

The HAL and RN genes are called major genes for carcass and meat quality traits in pigs as their strong effect on those properties (Figure 3) has been confirmed in studies on various breeds and lines.
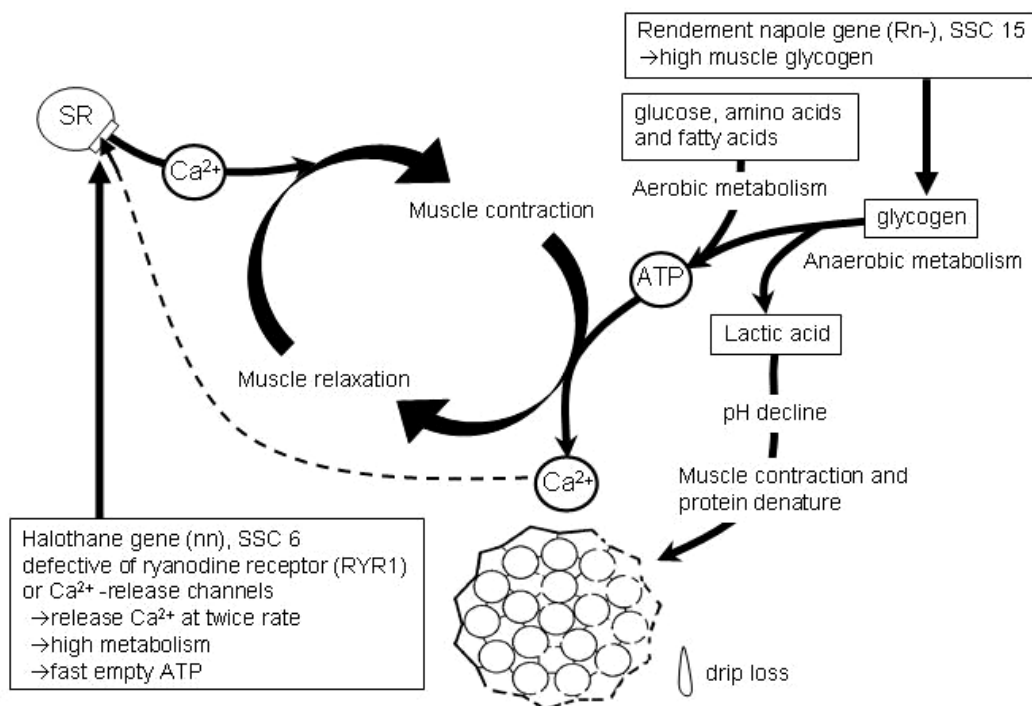
Figure 3: The effect of major genes (HAL and RN) on meat quality

The deleterious genotype of RYR1 was very quickly eliminated from many breeders' herds through the use of the HAL-1843™ molecular genetic test (Fujii et al. 1991). Despite this, considerable differences in meat and carcass quality are observed in animals of the same breed and the same genotype at the locus RYR1. This fact demonstrates that the RYR1 locus is indeed a major gene affecting carcass and meat quality, but additional

genetic factors are involved (Lengerken et al. 2002). Thus it is necessary to identify other genes affecting the phenotypic differentiation between animals with respect to these traits. The understanding of the previously explained metabolic processes taking place in the muscle *post mortem* may suggest the way to identify sources of candidate genes. The hypothesis is that if we can detect the genes in calcium signaling pathways or oxidative-glycolytic phosphorylation pathways that can reduce the rate of pH decline or the genes in extracellular matrix pathways that protect muscle proteins stronger against degradation during the postmortem stage, it might help to improve meat quality especially in terms of water-holding capacity.

2.3 The correlation between carcass and meat quality traits

Meat quality comprises factors relevant for the perception and nutritional and sensory properties. The latter can be measured by means of technological indicators such as pH, conductivity, color, drip loss, cooking loss, thaw loss, or shear force. Most of these parameters are correlated with or dependent on each other e.g. drip loss has a strong positive correlation with conductivity (Lee et al. 2000; Ponsuksili et al. 2009). Moreover, many studies report the correlation between carcass and meat quality traits, the same findings correspond to the positive phenotypic correlation that exists between the carcass fatness and water-holding capacity of meat (Estévez et al. 2004; Suzuki et al. 2005; Kušec et al. 2003; Ponsuksili et al. 2009) (Table 1).

Table 1: Correlation coefficients between meat and carcass quality traits[1]

	pH24	OPTO	CON24	DRIP	FA	ABF
OPTO	0.45***					
CON24	0.04	0.11**				
DRIP	−0.22***	−0.31***	0.27***			
FA	0.12**	0.09*	−0.12**	−0.27***		
ABF	0.05	0.02	−0.14**	−0.19***	0.82***	
LEA	0.02	−0.09*	0.16***	0.18***	−0.11**	−0.18***

*p<0.05; **p<0.01; ***p<0.001
[1]Ponsuksili et al. (2009)
LEA = loin eye area, FA = fat area, ABF = fverage back fat, OPTO = meat color, pH24 = pH at 24 hours p.m., CON24 = conductivity at 24 hours p.m., DRIP = drip loss

This is not surprising as all traits are quantitative traits controlled by several loci but several traits may also be influenced by the same or linked loci (Haley et al. 1994; Liu et al. 2007). Therefore, the association study of candidate genes for meat quality traits may reveal statistic evidence linked to them or related traits.

2.4 The candidate gene approach for carcass and meat quality

There are some major problems that breeders encountered when attempting to breed for improved carcass and meat quality: (i) these traits are to some extent negatively correlated (Huff-Lonergan et al. 2003), (ii) most of the traits can only be measured *post mortem*, which makes prior breeding selection difficult and (iii) the consumers' as well as the producers' conception of high meat quality is not uniform and their expectations are changing continually (Dekkers, 2004; Kallweit et al. 2007; Ponsuksili et al. 2009). The identification of genes that regulate carcass and meat quality traits, the so-called marker-assisted selection (MAS), will assist in efficient meat production and facilitate the resolution of existing production problems.

Candidate genes may be identified based on knowledge of physiology, biochemistry or pathology, which clearly indicates the mechanism of the trait ('direct candidate' approach). Indirect approaches to identify candidate genes are: (i) the 'positional candidate' approach, which combines linkage information for a particular trait and mapping information on genes exhibiting particular functional properties and/or patterns of expression ('functional candidates'); and (ii) the 'comparative mapping' approach, which combines the results of reverse genetics efforts and information on genes mapped in the corresponding synteny group in other species (Kim et al. 2000). To date, by using these techniques, many candidate genes have been investigated to identify genes affecting economic traits. Jennen et al. (2007) summarized a large number of potential candidate genes for meat quality traits based on their function regarding muscle development and metabolism in the QTL areas of the Duroc-Pietrain (DUPI) population (Table 2). In addition, more recent approaches to detect candidate genes are based on the analyses of differences in the expression profiles in particular subsets of cells/tissues and/or individuals with certain phenotypes (Te Pas et al. 2005; Cagnazzo et al. 2006; Wimmers et al. 2007; Ponsuksili et al. 2008a).

Table 2: Potential candidate genes mapped on porcine chromosomes containing meat quality quantitative trait loci (QTL) regions of the Duroc-Pietrain (DUPI) population[1]

SSC	Trait	Gene	Gene description
1	pH,	AK1	Adenylate kinase 1
	conductivity,	CAPN3	Calpain 3
	meat color	IGF1R	Insulin-like growth factor 1 receptor
		LHX3	LIM homeobox 3
		TPM1	Tropomyosin alpha, skeletal muscle
2	pH,	ACTN3	Actinin alpha 3
	conductivity,	CAST	Calpastatin
	drip loss,	CKMT2	Creatine kinase, mitochondrial 2
	meat color	FTH1	Ferritin, heavy polypeptide 1
		IGF2	Insulin-like growth factor 2
		MYOD1	Myogenic differentiation 1
3	drip loss	ACTB	Beta Actin
		ALDOA	Aldolase A
		ATP2A1	ATPase, Ca2+ transporting, cardiac muscle, fast twitch 1
		BCL7B	B-cell CLL/lymphoma 7B
		HUMMLC2B	Myosin regulatory light chain 2
5	drip loss	ATF4	Activating transcription factor 4
		ATP5B	ATP synthase, H+ transporting mitochondrial F1 complex
		LIMA1	LIM domain and actin binding 1
		IGF1	Insulin-like growth factor 1
		MYBPC1	Myosin-binding protein C
		MYF5	Myogenic factor 5
6	shear force	CKM	Muscle creatine kinase
		GYS1	Glycogen synthase 1
		RYR1	Ryanodine receptor 1
		TGFB1	Transforming growth factor, beta 1
		TNNT1	Troponin T type 1
7	cooking loss	CALM1	Calmodulin 1
		MYH7	Myosin heavy chain beta slow
		NDRG2	N-myc downstream-regulated gene 2
		PKM2	Pyruvate kinase, muscle
		RPS10	Ribosomal protein S10
15	pH	GDF8; MSTN	Growth differentiation factor 8; myostatin
		MYOM2	Myomesin (M-protein) 2
		SARCOSIN	Sarcomeric muscle protein
		DES	Desmin
		MYL1	Fast skeletal myosin alkali light chain 1
18	drip loss	CAPZA2	Capping protein (actin filament) muscle Z-line, alpha 2
		IFRD1	Interferon-related developmental regulator 1
		IGFBP3	Insulin-like growth factor binding protein 3
		PGAM2	Phosphoglycerate mutase 2
		PRKAG2	Protein kinase, AMP-activated, gamma 2 non-catalytic subunit

[1]Jennen et al. (2007)

Beside two major genes (RYR1 and RN), mutations in several other genes have been associated with meat quality parameters with side or direct effects also on lean meat deposition, carcass and growth traits for some of them (Ciobanu et al. 2004; Otto et al.

2007; Wimmers et al. 2007). Genes involved in the regulation of the energy balance, glycogen metabolism and glycolysis of the skeletal muscle have been reported to associate with carcass or meat quality parameters. Candidate genes coding for muscle enzyme isoforms involved in the glycolytic pathway were tested for their association with meat quality parameters and production traits. For example, significant association was observed for the muscle phosphoglycerate mutase 2 (PGAM2) gene and ham weight (Fontanesi et al. 2008). The muscle pyruvate kinase (PKM2) gene was associated with average daily gain, lean cuts, backfat thickness, feed:gain ratio (Fontanesi et al. 2008), glycolytic potential, pH and drip loss (Sieczkowska et al. 2009). The skeletal muscle glycogen synthase (GYS1) gene was associated with pH (Zuo et al. 2007). Moreover, it has been known that meat quality affected by genetic factors relates back to the prenatal formation of muscle tissue (myogenesis), which are regulated by the myogenic regulatory factors (MRF) gene family. The MRF gene family consists of four structurally related transcription factors myogenin; MYOD1(MYF3), MYF4, MYF5 and MYF6 regulate both skeleton muscle fiber development and postnatal hypertropic growth (TePas and Soumillion, 2001). Therefore, The MRF gene family is considered as strong candidate gene fore carcass and meat quality traits. For example, MYOD1 was significantly associated with intramuscular fat (Verner et al. 2007). MYF4 was related with carcass meat weight (loin and ham), loin eye area (CiesÍak et al. 2000). MYF5 was significantly associated with loin weight (Verner et al. 2007), drip loss, water holding capacity and meat color (Liu et al. 2007), intramuscular fat and pH (Liu et al. 2008).

2.5 The functional genomics approach

Functional genomics includes function-related aspects of the genome itself such as mutation and polymorphism (such as SNP) analysis, as well as measurement of molecular activities. Tracing of gene expression process of the investigated trait in different stages or genetic background can contribute to a better understanding of the molecular architecture and find out the detailed clues that candidate gene tells. In general, important biological features of traits are directly reflected by transcript pattern, and quantitative traits were 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 (Cheung and Spielman, 2002). Apparently, the variations of traits are directly depending on the variations of transcriptome and proteome rather than the variation of genomic DNAs. The genes responsible for the variation of gene expression process are also responsible for the variation of trait, and the candidate gene governing the major genetic component of trait variation can be mined from the pattern of gene expression profiles.

The functional genomics approach will provide the opportunity to investigate global changes in known or unknown genes expression in muscle and to associate them with phenotypic characteristics, and these new approaches will generate new candidate genes to be tested for marker-assisted selection to improve livestock production. Instead of focusing only on the discovery of a single gene or DNA markers that co-segregate with a qualitative traits, in recent times the researchers focused their interests on elucidating complex traits by the detection of the large-scale molecular gene expression profiles, gene clusters and networks that are characteristics of a biological process or of a specific phenotype (Tuggle et al. 2007).

To date, some researchers began to consider or use this approach for seeking candidate genes in different fields. For instances, by using this strategy, functional candidate genes for muscle development in bovine fetuses (Crosier et al. 2002), genetic resistance for *mastitis* in cows (Schwerin et al. 2003), nutrient transformation in cattle (Schwerin et al. 2006), responses for anabolic agents in heifers (Reiter et al. 2007), fat characteristics in pigs (Li et al. 2008), immune responsiveness in pigs (Ponsuksili et al. 2008c), levels of androstenone in boars (Moe et al. 2008), congenital splay leg syndrome in piglets (Maak et al. 2009), placental development in sows (Zhou et al. 2009) and *Haemephilus parasuis* infection in porcine spleen (Chen et al. 2009). Furthermore, gene expression profiling approach has been used up to now by some groups to better understand the changes in gene expression during porcine muscle growth and development using samples from different pig breeds (Te Pas et al. 2005; Zhao et al. 2005; Reecy et al. 2006; Cagnazzo et al. 2006). An example is the study of Wimmers et al. (2007), who reported an association of

functional candidate genes (ANK1, bR10D1, CA3, EPOR, HMGA2, MYPN, NME1, PDGFRA, ERC1, TTN) arising from their differential expression in prenatal muscle development depending on meat quality and carcass traits in several pig breeds. The identification of differentially expressed genes for muscle growth and development may be of high importance also for both genetic and physiological studies related to pig meat quality. The first aim when looking at gene expression in muscle is to get a better understanding of biochemical characteristics of the tissue (muscle type), which influence meat quality traits. Papers have been published recently on embryonic and reproductive tissues (Blomberg et al. 2006; Green et al. 2006) on porcine brain (Nobis et al. 2003) liver and adipose tissue (Hausman et al. 2006) and one study combined microarray analysis, SNP detection within expression candidates, and association and physical mapping analyses to find liver genes affecting carcass traits (Ponsuksili et al. 2005). The development of genomic application in animal science may allow the discovery of gene networks and classes of genes that affect and are key drivers of a specific physiological state or a specific phenotype of a quantitative trait.

Previously, the study in transcriptome profiles of *M. longissimus dorsi* of pigs providing meat with high and low water-holding capacity offers insight into the biological processes in the muscle and the maturing meat and their influence on meat quality (Ponsuksili et al. 2008a), further study by the same group combined trait correlated expression, expression QTL analysis to find biological pathways and candidate genes affecting water-holding capacity of muscle in pigs (Ponsuksili et al. 2008b), therefore the aim of the present study was to determine the association of sequence variation of selected candidate genes derived from their expression profiles with carcass and meat quality traits in pigs.

2.6 Source of candidate genes

Expression profiles of *M. longissimus dorsi* were compared between the two extreme groups of six discordant sib pairs (selected from 572 F2 animals of a cross of Duroc and Pietrain [DUPI]) with 4.14 ± 0.77 vs $0.9 \pm 0.77\%$ drip (mean $\pm$ standard deviation) (P<0.0001) as well as between groups with high/low pH at 24 hour *p.m.* (data not show) 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 WHC group at $p < 0.05$ (Ponsuksili et al. 2008a). Moreover, expression profiling and eQTL analysis conducted on 74 F2 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. 2008b). Therefore, the large number of genes expressed represents a source of candidate genes that could influence carcass and meat quality traits. In this study, a shortlist of 12 candidate genes was established based on (i) known function of the particular gene and/or (ii) the position, giving preference to those genes located in QTL regions for carcass and meat quality traits (Table 4).

Table 3: Candidacy of selected candidate genes for carcass and meat quality traits

Gene symbol	Description	Candidacy for carcass and meat quality traits		
		Expression (fold changes/correlation)	Function	Position (QTL regions)
BVES	blood vessel epicardial substance	negative correlated with drip loss (r=-0.81) (Ponsuksili et al. 2008b)	plays an important role in development of cardiac and skeletal muscle tissues (Smith and Bader, 2006)	SSC1 (backfat thickness, pH) (de Koning et al. 2001; Evans et al. 2003; Liu et al. 2007)
SLC3A2	solute carrier family 3, member 2	negative correlated with drip loss (r=-0.43) (Ponsuksili et al. 2008b)	encodes a cell surface, transmembrane protein, mediates integrin-dependent signaling related to normal cell growth (Feral et al. 2005)	SSC2 (backfat thickness, loin muscle area, drip loss) (Harmegnies et al. 2006; Thomsen et al. 2004; Liu et al. 2007)
AHNAK	AHNAK nucleoprotein	up-regulation in the high drip loss group (1.40) (Ponsuksili et al. 2008a) positive correlated with drip loss (r=0.53) (Ponsuksili et al. 2008b)	mediates cellular localization and interaction with L-type Ca^{2+} channels, calcium-binding S100B protein, as well as actin of thin filaments for muscle contraction (Hohaus et al. 2002; Haase et al. 2004)	SSC2 (backfat thickness, loin muscle area, drip loss) (Harmegnies et al. 2006; Thomsen et al. 2004; Liu et al. 2007)
ZDHHC5	zinc finger, DHHC-type containing 5	negative correlated with drip loss (r=-0.49) (Ponsuksili et al. 2008b)	required for palmitoyltransferase activity (Fukata et al. 2004)	SSC2 (backfat thickness, loin muscle area, drip loss) (Harmegnies et al. 2006; Thomsen et al. 2004; Liu et al. 2007)

Table 3: Candidacy of selected candidate genes for carcass and meat quality traits
(continued)

Gene symbol	Description	Candidacy for carcass and meat quality traits		
		Expression (fold changes/correlation)	Function	Position (QTL regions)
CS	citrate synthase	negative correlated with drip loss (r=-0.38) (Ponsuksili et al. 2008b)	a Krebs tricarboxylic acid cycle enzyme, found in nearly all cells capable of oxidative metabolism (Kohn et al. 2005)	SSC5 (backfat thickness, pH, drip loss, meat color, shear force) (Harmegnies et al. 2006; Thomsen et al. 2004; Milan et al. 2002)
LYZ	lysozyme	up-regulation in the high drip loss group (1.70) (Ponsuksili et al. 2008a)	anti-microbial agent (Gorbenko et al. 2007)	SSC5 (backfat thickness, pH, meat color) (Harmegnies et al. 2006; Thomsen et al. 2004; Milan et al. 2002)
KERA	Keratocan	up-regulation in the high pH24 group (2.34) (Ponsuksili et al. 2008a)	a member of the small leucine-rich proteoglycan (SLRP) family, may be important in developing and maintaining for the structure of extracellular matrix (Iozzo, 1998)	SSC5 (backfat thickness, pH, meat color) (Malek et al. 2001)
COQ9	coenzyme Q9 homolog (S. cerevisiae)	negative correlated with drip loss (r=-0.47) (Ponsuksili et al. 2008b)	involved in the biosynthesis of coenzyme Q, a crucial component of the oxidative phosphorylation process in mitochondria (Johnson et al. 2005)	SSC6 (backfat thickness, pH, drip loss, meat color) (Edwards et al. 2008; de Koning et al. 2001; Kim et al. 2005)
UN	non-annotated EST (probe set ID: Ssc.25503.1. S1_at)	down-regulation in the high drip loss group (1.51) (Ponsuksili et al. 2008a) negative correlated with drip loss (r=0.58) (Ponsuksili et al. 2008b)	unknown	SSC7 (backfat thickness, loin muscle area, loin weight, pH, meat color, shear force) (Liu et al. 2007; Harmegnies et al. 2006; Su et al. 2004)
EGFR	epidermal growth factor receptor	positive correlated with drip loss (r=0.67) (Ponsuksili et al. 2008b)	a receptor for members of the epidermal growth factor family, leads to cell proliferation (Herbst, 2004)	SSC9 (backfat thickness) (Kim et al. 2006)
VTN	vitronectin	down-regulation in the high drip loss group (2.86) (Ponsuksili et al. 2008a)	promotes cell adhesion and spreading, inhibits the membrane-damaging (Schar et al 2008)	SSC12 (loin muscle area, meat color) (Milan et al. 2002; Yue et al. 2003; Thomsen et al. 2004)
ZYX	zyxin	up-regulation in the high drip loss group (1.35) (Ponsuksili et al. 2008a)	modulates the cytoskeletal organization of actin bundles (Nix et al. 2001)	SSC18 (backfat thickness, pH, drip loss) (Malek et al. 2001; de Koning et al. 2001)

3. Materials and methods

3.1 Materials

3.1.1 Animals and phenotypes

Genomic DNA and phenotypic data were obtained from animals of commercial purebred and crossbred herds including German Landrace (DL) (line a; n=290 and b; n=192) and Pietrain (PI) (line a; n=259 and b; n=190), German Large White x German Landrace (F1; n=188) and PIF1 (line a; n=481, b; n=331 and c; n=338) as well as an experimental F2-population based on a reciprocal cross of Duroc x Pietrain (DUPI; n=417). In this study, samples and phenotypic data from nine pig herds were collected. The sampling of the first seven herds took place in commercial slaughter houses in the Netherlands and Germany in 2003-2004, including two herds (PI(a) and PIF1(a)) from the breeding company (Bundeshybridzuchtprogramm; BHZP) and five herds (DUPI, PIF1(b), PI(b), F1 and DL(b)) from the research farm at the university of Bonn, Germany. Samples and data from another two commercial herds (PIF1(c) and DL(a)) were collected at the slaughter house at the research institute for the biology of farm animals (FBN), Germany in 2005-2007. The carcass and meat quality data were collected according to the guidelines of the Zentralverband der Deutschen Schweineproduktion e.V. (ZDS, 2004); their definition is shown in Table 4. For association studies, the selected SNPs within BVES, CS, EGFR, ZDHHC5, SLC3A2, COQ9, AHNAK, UN, LYZ, VTN and KERA were genotyped using around 1,800 animals from 6 pig populations including PI(a), PIF1(a,b,c), DL(a) and DUPI. For the ZYX gene, the SNPs were genotyped in 870 animals from PI(b), DL(b), F1 and PIF1(b).

Table 4: Definitions of traits related to carcass and meat quality as analysed in this study

Traits	Definitions of traits
loin eye area (LEA) [cm^2]	Area of M. longissimus dorsi (Mld) at 13th/14th rib
fat area (FA) [cm^2]	Fat area on Mld at 13th/14th rib
meat to fat ratio (MFR)	Ratio of meat and fat area
shoulder fat depth (BF1) [cm]	Depth of fat and skin on muscle, mean of 3 measures at thickest point
fat depth at 10th rib (BF2) [cm]	Depth of fat and skin on muscle, mean of 3 measures at thinnest point
loin fat depth (BF3) [cm]	Depth of fat and skin on muscle, mean of 3 measures at thinnest point
average back fat (ABF) [cm]	Mean value of shoulder fat depth, back fat tenth rib and loin fat depth

Table 4: Definitions of traits related to carcass and meat quality as analysed in this study (continued)

Traits	Definitions of traits
meat color (OPTO)	Meat color 24 h p.m. in Mld at $13^{th}/14^{th}$ rib; OPTO star
pH1	pH value in Mld at $13^{th}/14^{th}$ rib 45 minute post mortem (p.m.)
pH24	pH value in Mld at $13^{th}/14^{th}$ rib 24 hour p.m.
conductivity1 (CON1)	Conductivity in Mld at $13^{th}/14^{th}$ rib 45 minute p.m.
conductivity24 (CON24)	Conductivity in Mld at $13^{th}/14^{th}$ rib 24 hour minute p.m.
shear force (SF) [N]	Shear force was measured by the Instron-4310 equipment
drip loss (DRIP) [%]	% of weight loss of Mld collected at 24 h p.m., held for 48 h at 4°C
cooking loss (COOK) [%]	% of weight loss of Mld incubated in water at 75°C for 50 minutes
thaw loss (THAW) [%]	% of weight loss of Mld frozen at −20°C

Table 5: Data collection pig populations and traits measured with mean and standard deviations

	DUPI (n=417)	PI(a) (n=259)	PIF1(a) (n=481)	PIF1(b) (n=331)	PIF1(c) (n=338)	DL(a) (n=290)	PI(b) (n=190)	F1 (n=188)	DL(b) (n=192)
No. of sire	5	16	10	57	116	39	43	27	31
No. of litters	44	64	232	114	141	283	99	108	104
No. of slaughter days	51	7	11	37	63	77	66	50	89
LEA	50.99 ± 5.51	59.30 ± 6.83	51.92 ± 6.00	54.40 ± 4.91	53.51 ± 4.99	43.02 ± 4.34	60.38 ± 5.46	43.29 ± 4.94	43.73 ± 5.15
FA	16.04 ± 3.15	-	-	14.34 ± 2.50	14.70 ± 3.06	20.71 ± 3.47	11.82 ± 2.14	21.45 ± 3.40	20.73 ± 3.32
MFR	0.32 ± 0.07	-	-	0.26 ± 0.05	0.28 ± 0.07	0.49 ± 0.11	0.20 ± 0.04	0.50 ± 0.11	0.48 ± 0.10
BF1	3.35 ± 0.44	-	-	2.98 ± 0.41	3.37 ± 0.44	3.71 ± 0.46	3.03 ± 0.45	3.76 ± 0.52	3.53 ± 0.46
BF2	1.64 ± 0.31	-	-	1.46 ± 0.27	1.91 ± 0.37	2.10 ± 0.36	1.47 ± 0.27	1.94 ± 0.37	1.87 ± 0.38
BF3	1.34 ± 0.34	-	-	1.04 ± 0.29	1.33 ± 0.37	1.86 ± 0.43	0.82 ± 0.26	1.77 ± 0.42	1.70 ± 0.41
ABF	2.11 ± 0.31	1.84 ± 0.31	2.61 ± 0.41	1.83 ± 0.27	2.20 ± 0.33	2.57 ± 0.36	1.77 ± 0.27	2.49 ± 0.38	2.37 ± 0.35
OPTO	68.57 ± 5.69	72.45 ± 6.54	70.39 ± 8.83	67.24 ± 5.97	68.03 ± 6.52	69.88 ± 5.82	62.45 ± 7.19	65.72 ± 6.08	67.68 ± 7.21
pH1	6.56 ± 0.21	6.48 ± 0.18	6.24 ± 0.26	6.48 ± 0.26	6.14 ± 0.33	6.32 ± 0.28	6.09 ± 0.38	6.47 ± 0.21	6.60 ± 0.18
pH24	5.51 ± 0.10	5.53 ± 0.13	5.57 ± 0.11	5.51 ± 0.09	5.48 ± 0.09	5.48 ± 0.11	5.49 ± 0.08	5.50 ± 0.08	5.52 ± 0.10
CON1	4.36 ± 0.62	4.53 ± 0.68	2.91 ± 0.60	4.29 ± 0.74	5.14 ± 1.75	4.31 ± 0.75	5.89 ± 3.37	4.25 ± 0.52	4.07 ± 0.54
CON24	2.82 ± 0.85	3.25 ± 0.84	3.45 ± 0.95	3.13 ± 1.14	5.45 ± 2.19	3.98 ± 1.71	4.85 ± 2.65	2.63 ± 0.78	2.52 ± 0.89

Table 5: Data collection pig populations and traits measured with mean and standard deviations (continued)

	DUPI	PI(a)	PIF1(a)	PIF1(b)	PIF1(c)	DL(a)	PI(b)	F1	DL(b)
SF	35.44 ± 6.98	38.14 ± 7.86	38.13 ± 7.28	41.83 ± 7.59	-	-	-	-	-
DRIP	2.10 ± 0.96	1.47 ± 0.76	1.94 ± 0.79	2.67 ± 1.42	5.51 ± 2.15	4.48 ± 2.04	-	-	-
THAW	8.09 ± 1.98	7.50 ± 2.61	9.08 ± 3.97	8.77 ± 1.89	-	-	-	-	-
COOK	24.97 ± 2.13	23.80 ± 2.16	25.39 ± 2.07	25.24 ± 1.85	-	-	-	-	-

3.1.2 Equipments

Vortex	G-560E	Scientific Industries Inc., USA
Centrifuge	5417C	Eppendorf-Netheler-Hinz GmbH, Hamburg
Centrifuge	Z320	Hermle-Labortechnik, Wehingen
Heater	FDB02AD	Techne (Cambridge) Ltd., England
UV transilluminator	TFML-40	LTF-Labortechnik GmbH & CO. KG, Wasserburg
Pyrosequencer	PSQ96MA	Biotage, Sweden
Vacuum prep tool	PyroMarkQ96	Biotage, Sweden
PCR thermocycler	HBPX 110	Thermo Electron GmbH, Ulm
Spectrophotometer	ND-1000	Nanodrop technologies, USA
Working chamber	HS12	Heraeus instruments, Hanau
Sequencer	ABI3130	Applied Biosystems, USA
Power supply	GFS200/400	Phamacia, Sweden
Electrophoresis box	-	Angewandte Gentechnologische Systeme GmbH, Heidelberg

3.1.3 Buffers, chemicals, reagents and kits

For PCR and sequencing

dNTPs	Promega, Mannheim
Buffer (10X)	Sigma-Aldrich, Taufkirchen
Oligonucleotide primer	Sigma-Aldrich, Taufkirchen
Taq polymerase	Sigma-Aldrich, Taufkirchen

Agarose	Sigma-Aldrich, Taufkirchen
TAE (50X)	Tris 2 M
	Acetic acid 57% (v/v)
	EDTA, pH8.0 0.05 M
PCR purification kit	QIAGEN, Hilden
For Pyrosequencing technique	
Binding buffer pH 7.6	10 mM Tris-HCl
	2M NaCl
	1mM EDTA
	0.1% Tween 20
Annealing buffer (1X), pH 7.6	20 mM Tris-Acetat
	2 mM MgAc2
Denaturation solution	0.2 M NaOH
Washing buffer, pH7.6	10 mM Tris-Acetat
Streptavidin sepharose	GE healthcare, München
Pyro Gold Q96 reagents	QIAGEN, Hilden
For single base extension technique	
Shrimp Alkaline Phosphatase (SAP)	Fermentas, St. Leon-Rot
Exonuclease I, *E.coli* (ExoI)	Fermentas, St. Leon-Rot
SNaPshot multiplex kit	Applied Biosystems, USA
Hi-Di Formamide	Applied Biosystems, USA
GeneScan 120 LIZ Size Standard	Applied Biosystems, USA

3.1.4 Software

Primer3 v. 0.4.0 (Rozen and Skaletsky, 2000)

Multalin (Corpet, 1988)

BioEdit v.7.0.9 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html)

BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi)

ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html)

PedCheck (O'Connell and Weeks, 1998)

CRI-MAP v. 2.4 (Green et al. 1990)

AutoDimer (Vallone and Butler, 2004)

PyroMark Assay Design Software 2.0 (QIAGEN GmbH, Hilden)

PSQ96MA 2.1.1 (Biotage, Sweden)

Gene Mapper v. 4.0 (Applied Biosystems, USA)

SAS v.9.1 (SAS Institute Inc., USA)

JMP genomics v. 3.1 (SAS Institute Inc., USA)

3.2 Methods

3.2.1 Screening for polymorphism sites

In silico analysis by comparing several sequences from the database revealed the possible targets for PCR amplification. The putative SNPs were confirmed by comparative sequencing of PCR fragments in a SNP discovery panel of unrelated animals including one animal each of the breeds German Landrace, German Large White, and Pietrain. The screening primers used in this study are shown in Table 6. The polymerase chain reactions were performed in a 25 µl volume containing 50 ng DNA of the respective panel sample, 1× PCR buffer (with 1.5 mM MgCl$_2$), 250 µM of each dNTP, 0.2 µM of each primer and 0.5 U of Taq DNA polymerase. The PCR procedures were performed under the following conditions: initial denaturing at 94 °C for 5 min followed by 40 cycles of 30 sec at 94 °C, 30 sec at 60 °C and 1 min at 72 °C and a final elongation of 8 min at 72 °C. The PCR products were purified using the PCR purification kit (QIAGEN) and comparatively sequenced in both directions using an ABI3130 sequencer.

3.2.2 Genotyping

SNPs were selected for genotyping based on suitability of the surrounding sequences to design primers for either pyrosequencing or single base extension assays, position in regions with potential function, exon-intron structure and preference for those causing amino acid exchange. For association studies, the selected SNPs within BVES, CS, EGFR, ZDHHC5, SLC3A2, COQ9, AHNAK, UN, LYZ, VTN and KERA were genotyped using

around 1,800 animals from 6 pig populations including PI(a), PIF1(a,b,c), DL(a) and DUPI. For the ZYX gene, the SNPs were genotyped in 870 animals from PI(b), DL(b), F1 and PIF1(b). Two genotyping techniques were used in this study (Table 7). The SNPs in SLC3A2, AHNAK, CS, VTN, LYZ, KERA and ZYX were genotyped by pyrosequencing technique. The target PCR products were prepared using the Vacuum Prep Tool. Therefore, 3 µl Streptavidin Sepharose beads were added to 37 µl Binding buffer (10 mM Tris-HCl pH 7.6, 2 M NaCl, 1 mM EDTA, 0.1% Tween 20) and mixed with 20 µl PCR product and 20 µl high purity water for 5 min at room temperature using a Vortex mixer. The beads containing the immobilised templates were captured onto the filter probes after applying the vacuum and then washed with 70% ethanol for 5 sec, denaturation solution (0.2 M NaOH) for 5 sec and washing buffer (10 mM Tris-Acetate pH 7.6) for 5 sec. The vacuum was switched off and the beads released into a PSQ 96 well plate containing 40 µl annealing buffer (20 mM Tris-Acetate, 2 mM MgAc2 pH 7.6) with 0.5 µM sequencing primer. The samples were heated to 80°C for 2 min and then allowed to cool down to room temperature. The sequencing reaction was performed using the Pyro Gold Reagent Kit in the PSQ96MA Pyrosequencing instrument according to the manufacturer's instructions.

On the other hand, the SNPs within BVES, EGFR, COQ9 and UN were genotyped by single base extension (SBE) technique. 5 µl target PCR product were purified by mixing them with 0.67 U Exonuclease I (ExoI) and 1.67 U shrimp alkaline phosphates (SAP) (Fermentas), then incubating them for 1 hour at 37°C and 15 min at 75°C. SBE was performed using the SNaPshot® multiplex kit (Applied Biosystems) in a volume of 5 µl (containing 0.02 µM of the synthethic clean DNA template, 0.2 µM primer mix, and 0.2 µM SNP primer extension premix) for 25 cycles (96°C 10 sec, 50°C 5 sec, 60°C 30 sec). The SBE products were purified again by adding 0.5 U SAP, and then incubating them for 1 hour at 37°C and 15 min at 75°C. 0.5 µl SBE products were added to 9.25 µl Hi-Di formamide and 0.25µl GeneScan-120 LIZ size standard (Applied Biosystems). The genotypes were determined using the ABI 3130 DNA Analyzer (Applied Biosystems).

Table 6: Screening primers used in this study

Gene symbol	Acc. No	Screening primers	Product
BVES	NM_001144112.1	Fwd. ATGAGACCACCTGCGAAAAC	852 bp
		Rvs. CCCCGAAGAAACTGGTGTAA	
CS	NM_214276.1	Fwd. CTGCCATGGCCTTACTCACT	648 bp
		Rvs. TTGCTGCAACACAAGGTAGC	
		Fwd. GCTCAGTGCAGCCATTACAG	674 bp
		Rvs. CCTGTTCCAGGAGGACATTG	
	CK464063.1	Fwd. ATGGCCACGCAGTACTAAGG	704 bp
		Rvs. GGGAAAGAGGAGCCAGATTC	
EGFR	NM_214007.1	Fwd GAATCGCAGGAGAAAACAGC	909 bp
		Rvs. CCGTAGCTCCAGACGTCACT	
		Fwd CGCAGCATGTCAAGATCACT	908 bp
		Rvs. CAGTCCTCCGTTGATGCAG	
		Fwd ACCCCCACTACCAGAACTCC	859 bp
		Rvs. TGGCTTATCCTCTTGCACCT	
ZDHHC5	CK464654.1	Fwd. CTTGGGAGACTCAGGCATTC	693 bp
		Rvs. CAGGGGTTAAGGAAGGGAAG	
SLC3A2	EU587016.1	Fwd. GAGACCTAGCGAGCCTGAAG	660 bp
		Rvs. AGAGCAGCAGCTGGTAGAGC	
		Fwd. GGCTTCTGACCTCCTCTGTG	528 bp
		Rvs. CCTCATAGGGCTCCAGGTTC	
COQ9	CN156459.1	Fwd. AGCCAAGTCTCTGGGTCTCTC	606 bp
		Rvs. TAGCTTACGTCCCTGCTTGC	
	DN104150.1	Fwd. ACTGCCAAGCAGGTGAAGTC	707 bp
		Rvs. TCCTGCAACATGTGCTAGTGT	
AHNAK	BX922331.2	Fwd. GGGGTGGATATCAACTTCCCTA	617 bp
		Rvs. GTCAGCTCCACCTCAGGAAG	
UN (unknown)	NW_001886512.1	Fwd. GCTGTTTCACGGCAAGAATC	385 bp
		Rvs. CGCACCTTTGTTGTTCTGAG	
LYZ	NM_214392.1	Fwd. GGTCTATGATCGGTGCGAGT	622 bp
		Rvs. GACCAACAATAATTCTCTTAGCAAACT	
		Fwd. TCCGAAGCAAGAGCATAAGG	621 bp
		Rvs. CTTTTTACAGCATGCATAAATTCAC	
VTN	NM_214104.1	Fwd. GTGTGACGAGCTGTGCTCTTAC	292 bp
		Rvs. TCCCACTGCACAGCTCTTCCTC	
KERA	XM_001927128.1	Fwd. TGGTATCTTTATCTTGAAAACAATCTG	309 bp
		Rvs. ATTGTGCTGCAGGTCAAGAAG	
ZYX	BP440079.1	Fwd. GCCCAAAGTGAATCCTTTCC	562 bp
		Rvs. GAAGTGCGTTTGGCTCTGA	
		Fwd. CCCAGGGATAAAGTGAGCAG	921 bp
	BW975440.1	Rvs. GTTGCACTTCTCCAGGGTGT	
		Fwd. CAACAGCTGATGCAGGACAT	676 bp
		Rvs. ACTGATGGGGAATGGATCTG	

Table 7: Details of genotyping primers, methods, and genotyped SNPs

Gene	Genotyping primers	Product	method	Polymorphism
LYZ	For. TGTTTGGCCAAGTGGGAAAG Rev. biotin-GATATGTGACAGGCATTAACTGC Seq. GAATATTTCAAATTAATAGC	152 bp	Pyroseq.	c.240A>C
	For. TGTGCAAAGAGGGTTGTCAG Rev. biotin-GTTAATTATATCACAAGAAAATTGGAAGG Seq. GGTTGTCAGAGATCCAC	87 bp	Pyroseq.	c.365A>T
VTN	For. GTGTGACGAGCTGTGCTCTTAC Rev. biotin-TCCCACTGCACAGCTCTTCCTC Seq. CTACCAGAGCTGCTGC	366 bp	Pyroseq.	c.154A>G c.156C>T
KERA	For. TGGTATCTTTATCTTGAAAACAATCTG Rev. biotin-ATTGTGCTGCAGGTCAAGAAG Seq. CATTCCTGAGAAGCCATT	309 bp	Pyroseq.	c.303C>T
UN (unknown)	For. GCTGTTTCACGGCAAGAATC Rev. CGCACCTTTGTTGTTCTGAG Seq. CGGATTGTAAGTGGATTCTCTTCTC	385 bp	SBE	g.1,022,434G >T
AHNAK	Fwd. GGGGTGGATATCAACTTCCCTA Rvs. biotin-GGCCTTGGAGCTCTTCAGGTC Seq1.CTAAAGCAGAGGCCAGC Seq2. TCAATTTTTCCAAACC	210 bp	Pyroseq.	c.12907A>G c.13014G>T
	Fwd. biotin-CACCGCTCAAATTCCTTCAG Rvs. GTCAGCTCCACCTCAGGAAG Seq. ACCAAAGGTACCAAATT	320 bp	Pyroseq.	c.13281A>G c.13290A>C>G c.13294C>T
ZYX	Fwd. CCCTGGGAGGTGCTTTCC Rvs. biotin-CCCTTGGGGTGTCCACTG Seq1. GGTGCTTTCCCTC	190 bp	Pyroseq.	c.279C>T
	Fwd. biotin-TGAGGTCCCCATACAGCTCC Rvs. GGCTTGGTATTGGACTTGGAAA Seq2. CTCCCAGGCCTCCAT Seq3. TGGAAATGAATGGCG	463 bp	Pyroseq.	c.399A>G c.522A>G
BVES	Fwd. ATGAGACCACCTGCGAAAAC Rvs. CCCCCATACAAAGTCCATCT Seq. [6T]GGGTTGGTTCTTCCAACTACTCT	205 bp	SBE	c.186G>T
COQ9	Fwd. CAGCATGTTTGGGAATGATG Rvs. CTACGCTTTTGAGCCTGCTT Seq.[12T]GATACTGCATTTTGTGACCCA	223 bp	SBE	c.453A>G
	Fwd. TTGGAAAACGCAACACTCAA Rvs. TCTGACCAGGTTGAGCACAC Seq. [17T]GAGAGGCCACGACAGGAACG	308 bp	SBE	+1247A>T
CS	Fwd. biotin-ACTTAAAAGACATTTTGGCTGACC Rvs. ATTCCTCGTTCGGTCTTAATTCTG Seq. CTGGCTTGCTCCTTAGGT	55 bp	Pyroseq.	c.120G>T
ZDHHC5	Fwd. ATGCCCCTCGTACTAGTTCCTCCT Rvs. biotin-CGTCGGGCTTGCCAAAAC Seq. GGGCAAGACTCCACT	102 bp	Pyroseq.	c.1803C>T
SLC3A2	Fwd. TGCCATGGGATGAATCCAG Rvs. biotin-AGCCAGGGTCTCCGCTCT Seq. GCACCTTAGGACCTGT	200 bp	Pyroseq.	c.1326A>G c.1336Indel [AGC]
EGFR	Fwd. ACCCCCACTACCAGAACTCC Rvs. TGGCTTATCCTCTTGCACCT Seq. [27T]GACCCTTACAGATGCCGTTTGA	859 bp	SBE	c.3543A>G

3.2.3 Genetic mapping

The regional assignments of all loci were performed in the DUPI population, the data were firstly checked for any genotyping errors by using Pedcheck (version 1.1) (O'Connell and Weeks, 1998), then using the CRI-MAP package (version 2.4). Two-point and multi-point procedures were used for linkage mapping (Green et al. 1990).

3.2.4 Association study

The association between genotypic and phenotypic variation was analyzed using a general mixed model (PROC Mixed, SAS v. 9.1; SAS Inc., Cary, NC, USA). The analyses were done for each gene separately within each of the pig populations. Apart from the fixed effect of genotype, the model included the fixed effect of sex for carcass traits for PI(a), PIF1(a) and DUPI and the random effect of sire (sire x dam in DUPI), the random effect of slaughter date for meat quality traits and slaughter weight as a covariate for carcass traits, respectively. The RYR1 genotype and the interaction between the genotype of candidate genes and RYR1 genotypes were included as a fixed effect in the PI(b) and PIF1(b,c) lines. If the candidate genes had more than one polymorphism (VTN, LYZ, AHNAK, SLC3A2, COQ9, ZYX), the haplotype phase between SNPs was inferred using the expectation-maximization algorithm (PROC Haplotype, SAS v. 9.1). The association analyses were done using the diplotypes, i.e. combinations of haplotypes, as fixed effects instead of genotype in the models described above. Only those animals whose haplotype pairs were assigned with the probability 1 were used. The genotype distribution in commercial pig populations were tested for Hardy-Weinberg equilibrium by a χ^2 test. Least square mean values for the genotypes and diplotypes were compared by t-test and p-values were adjusted by the Tukey-Kramer correction.

The model for association analysis was:

$$\mathrm{Y}_{ijklmno} = \mu + \mathrm{GENO}_j + \mathrm{SEX}_k + \mathrm{RYR1}_l + (\mathrm{GENO \; x \; RYR1})_{jk} + \mathrm{sire}_m + \mathrm{sladate}_n + \mathrm{SLAEW}_o + e_{ijklmno}$$

Where:

$\mathrm{Y}_{ijklmno}$ = the phenotype traits measured on the individual i (see table 4)

μ	= the overall mean of the trait
$GENO_j$	= the fixed effects of SNP genotype for each gene j (see table 7)
SEX_k	= the fixed effects of gender for carcass traits k (k=1; male, 2; female) (this parameter was excluded from the model for PI(b), PIF1(b), PIF1(c) and DL(b) because only male animal available)
$RYR1_l$	= the fixed effects of RYR1 genotype for PI(b), PIF1(b) and PIF1(c) (l=1; homozygous MHS susceptibility mutation, 2; MHS heterozygous carrier and 3; MHS free homozygous wild type) (this parameter was excluded from the model for PI(a), PIF1(a) and DUPI because only homozygous wild type animal available)
$(GENO \times RYR1)_{jl}$	= the interaction between the j-th SNP genotype and the l-th RYR1 genotype
$sire_m$	= the random effect of sire m (in DUPI, this parameter was replaced by family) (m = Id of sire in commercial lines or m = Id of family in DUPI)
$sladate_n$	= the random effect of slaughter date n for meat quality traits
$SLAEW_o$	= carcass weight o as covariance for carcass traits
$e_{ijklmno}$	= the residual error associated with the observation

Additive genetic effect of each locus was estimated as half of the difference between the homozygous groups: $a = 1/2(BB-AA)$, with A and B that indicate the first and the second allele of the analysed markers, respectively. The dominance effect was estimated as the difference between the heterozygous group and the average of the 2 homozygous groups in each locus: $d = AB-1/2(AA+BB)$. The estimates of effects were tested by t-test on significant deviation from zero. Correction for multiple testing, to allow correction for the fact that a large number of traits were analysed with a large number of SNP (2100 tests were performed in this study), and hence a high probability of false positive results, a pFDR correction was applied (JMP genomics v. 3.1) (Table 66). Principal component (PC) was analyzed using a factor analysis technique (PROC Factor, SAS v. 9.1). PC partitions the total variation into unrelated sets containing correlated fractional values (Table 67-75).

4. Results

4.1 Molecular characterization and detection of polymorphisms

4.1.1 The blood vessel epicardial substance (BVES)

The porcine BVES mRNA sequence (Acc. No: NM_001144112.1) consists of 2326 bp including 161 bp of 5' untranslated region (UTR), 984 bp of coding region and 1181 bp of 3'UTR. The porcine BVES protein consists of 327 amino acids which show 92% homology to the human (Acc. No: NP_009004.2), 91% to the cattle (Acc. No: XP_590718.3) and 87% to the mouse (Acc. No: NP_077247.1). The comparative analysis between porcine BVES mRNA sequence and porcine genomic DNA sequence (Acc. No: CU407220.2) revealed 8 exons. One silence SNP (c.186 G>T) was found in the coding region of exon2, and was selected for genotyping (Figure 4).
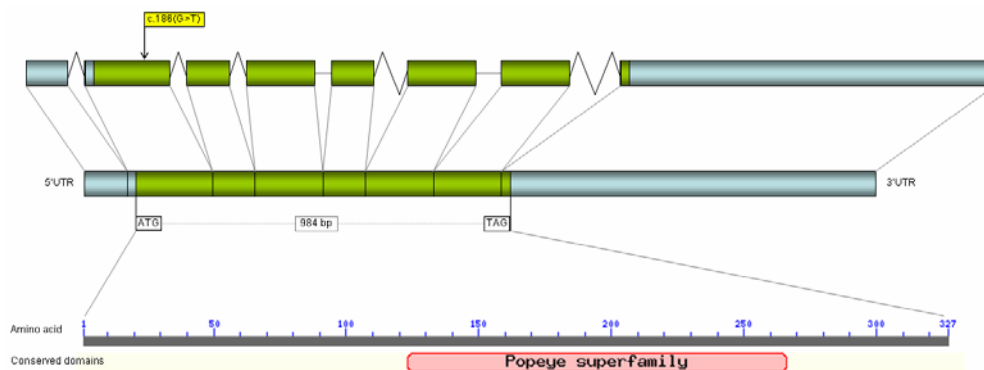
Figure 4: Structure of porcine BVES gene

4.1.2 The solute carrier family 3, member 2 (SLC3A2)

Two overlapping porcine expressed sequence tags (ESTs) (Acc. No: EU587016.1 and BP141705.1) provided the complete porcine SLC3A2 cDNA sequence which contains 1912 bp including 159 bp of 5'UTR, 1695 bp of coding region and 58 bp of 3'UTR. The porcine SLC3A2 protein consists of 564 amino acids which show 79% homology to the human (Acc. No: NP_001012682.1), 82% to the cattle (Acc. No: NP_001019659.2) and 73% to the mouse sequence (Acc. No: NP_001154885.1). The comparative analysis between the porcine SLC3A2 cDNA sequence and the porcine genomic DNA sequence

(Acc. No: FP340373.2) revealed 10 exons. Six polymorphism sites were detected within coding regions including three SNPs in exon7 (c.1103A>G, c.1113C>T and c.1119C>T), one SNP in exon8 (c.1200C>G) and one SNP (c.1326A>G) and one Indel polymorphism (c.1336indel[AGC]) in exon9. The SNP at position c.1103A>G causes an amino acid exchange from Arginine to Histidine (p.Arg368His) and the Indel polymorphism (c.1336indel[AGC]) leads to the addition or removal of one amino acid Serine (p.Ser446-). For this candidate gene, two polymorphisms (c.1326A>G and c.1336indel[AGC]) located in the conserved domains for trehalose synthase (TreS) (an enzyme involved in the glycogen metabolism) were selected for genotyping (Figure 5).
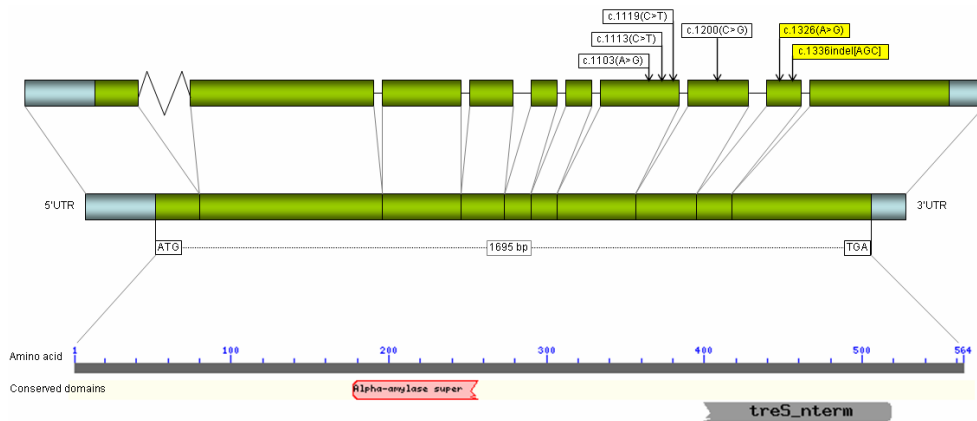
Figure 5: Structure of porcine SLC3A2 gene

4.1.3 The AHNAK

The structure of the porcine AHNAK gene can be deduced by comparison of the full-length human AHNAK mRNA sequence (Acc. No: NM_001620.1), porcine EST (Acc. No: BW975869.1) and the porcine genomic DNA sequence (FP102338.2). The comparative analysis revealed 5 exons covering 250 bp of 5'UTR, 13509 bp of coding sequence and 871 bp of 3'UTR. The porcine AHNAK protein consists of 4502 amino acids which show 63% homology to the human (Acc. No: NP_001611.1), 69% to the horse (Acc. No: XP_001916358.1) and 63% to the mouse (Acc. No: NP_033773.1). Five SNPs (c.12907A>G, c.13014G>T, c.13281A>G, c.13290A>C>G and c.13294C>T) were detected within the coding region of exon5. The SNP at position c.12907A>G effects an

amino acid exchange from Isoleucine to Valine (p.Ile4303Val). All Five SNPs were genotyped for further association analysis (Figure 6).
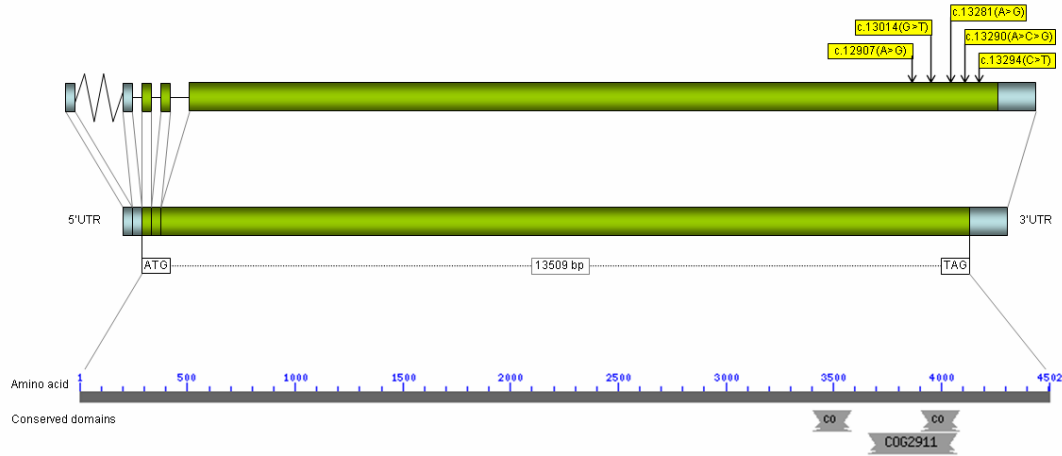
Figure 6: Structure of porcine AHNAK gene

4.1.4 The zinc finger, DHHC domain containing 5 (ZDHHC5)

The comparison of human ZDHHC5 mRNA sequence (Acc. No: NM_015457.2) and several porcine ESTs (Acc. No: DT320938.1, BX671032.2, gnl|ti|2132872638, gnl|ti|2132873247, gnl|ti|2132872202, CK464654.1) and the porcine genomic DNA sequence (CU914175.2) suggested the structure of porcine ZDHHC5 gene containing 12 exons, 252 bp of 5'UTR, 2151 bp of coding region and 333 bp of 3'UTR.
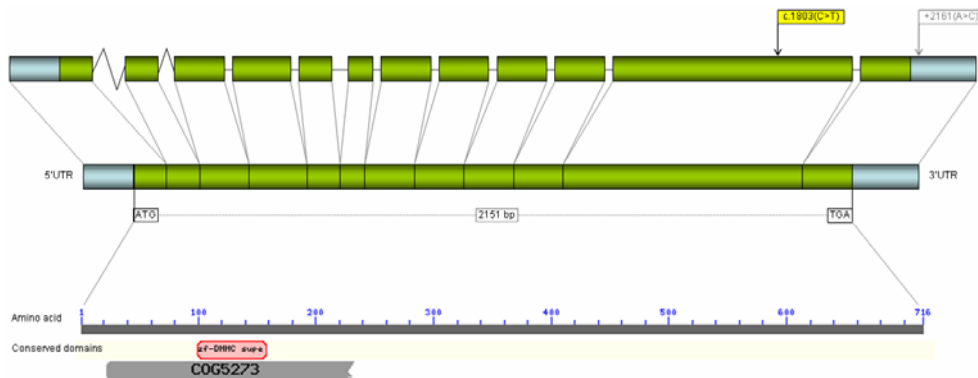
Figure 7: Structure of porcine ZDHHC5 gene

The porcine ZDHHC5 gene encodes 716 amino acids which show 97% similarity to the human (Acc. No: NP_056272.2), 95% to the cattle (Acc. No: XP_612592.4) and 97% to the mouse (Acc. No: NP_659136.1). Two SNPs were detected, one located in the coding area of exon11 (c.1803C>T) and another one located in the 3'UTR (+2161A>C). The silent SNP c.1803C>T was genotyped for association analysis (Figure 7).

4.1.5 The citrate synthase (CS)

The porcine CS mRNA (Acc. No: NM_214276.1) consists of 1437 nucleotides including 6 bp of 5' UTR, 1395 bp of coding region and 36 bp of 3'UTR. The porcine CS protein consists of 464 amino acids which show 96% homology to the human (Acc. No: NP_004068.2), 96% to the cattle (Acc. No: NP_001038186.1) and 95% to the mouse (Acc. No: NP_080720.1). The comparative analysis between porcine CS mRNA sequence and porcine genomic DNA sequence (Acc. No: CU498845.2) revealed 11 exons. Three SNPs were detected including one silent SNP (c.120G>T) located within the coding region of exon3 and two SNPs located in the 3'UTR (+1578A>G and +1622A>G).
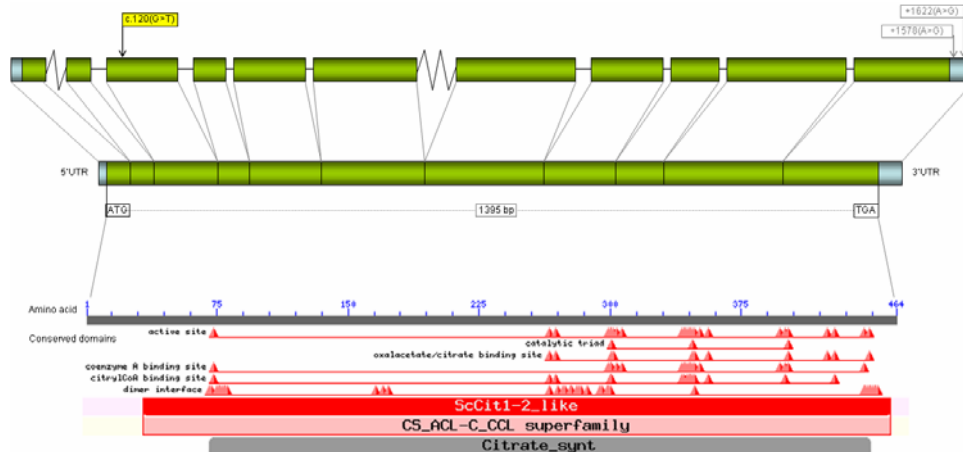
Figure 8: Structure of porcine CS gene

The coding area of porcine CS gene contains several conserved domains for several features related to the condensation of acetyl coenzyme A (AcCoA) with oxaloacetate (OAA) to form citrate and coenzyme A (CoA), the first step in the citric acid cycle (TCA

or Krebs cycle). Therefore, the SNP c.120G>T was genotyped for association analysis (Figure 8).

4.1.6 The Lysozyme (LYZ)

The porcine LYZ mRNA sequence (Acc. No: NM_214392.1) consists of 1239 bp including 34 bp of 5' untranslated region (UTR), 447 bp of coding region and 758 bp of 3'UTR. The coding region encodes 148 amino acids which show 71% homology to the human (Acc. No: NP_000230.1), 65% to the cattle (Acc. No: NP_001073808.1) and 72% to the mouse (Acc. No: NP_038618.1). The comparative analysis between porcine mRNA sequence and genomic DNA sequence (Acc. No: CU469188.2) revealed the structure of porcine LYZ gene that contains 4 exons. Screening for the SNP showed 4 SNPs within the coding region, including two SNPs in the exon2 (c.240A>C and c.255C>T) and another two SNPs in the exon3 (c.365A>T and c.370C>T). All SNPs located in the area of conserved domains such as a catalytic site, a catalytic cleft and a Ca^{2+}-binding site. Among these SNPs, a SNP c.365A>T causing an amino acid exchange from Glutamin to Leucine (p.Gln122Leu). For association analysis, two SNPs (c.240A>C and c.365A>T) were selected for genotyping (Figure 9).
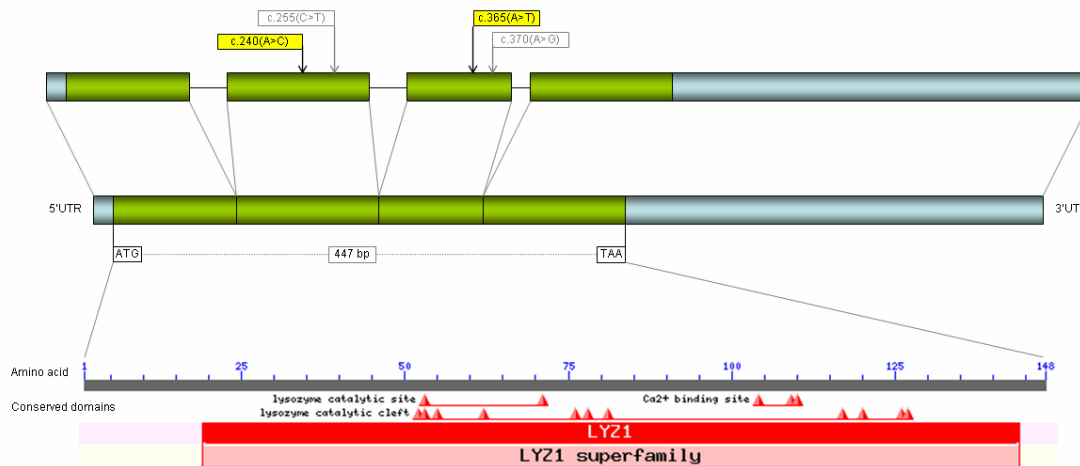
Figure 9: Structure of porcine LYZ gene

4.1.7 The Keratocan (KERA)

The porcine KERA mRNA sequence (Acc. No: XM_001927128.1) consists of 2027 bp including 34 bp of 5'UTR, 1092 bp of coding region and 900 bp of 3'UTR. The coding region encodes 363 amino acids which show 90% homology to the human (Acc. No: NP_008966.1), 91% to the cattle (Acc. No: NP_776335.1) and 86% to the mouse (Acc. No: NP_032464.1). The comparative analysis between porcine KERA mRNA sequence and porcine genomic DNA sequence (Acc. No: CU468030.2) revealed 2 exons. Screening for SNP showed three silent SNPs (c.303C>T, c.315A>C and c.528A>G) located in the coding region of exon1 and also located in the area of Leucine rich repeat (LRR) domains that involved in protein-protein interactions. For association analysis, a SNP c.303C>T was selected for genotyping (Figure 10).
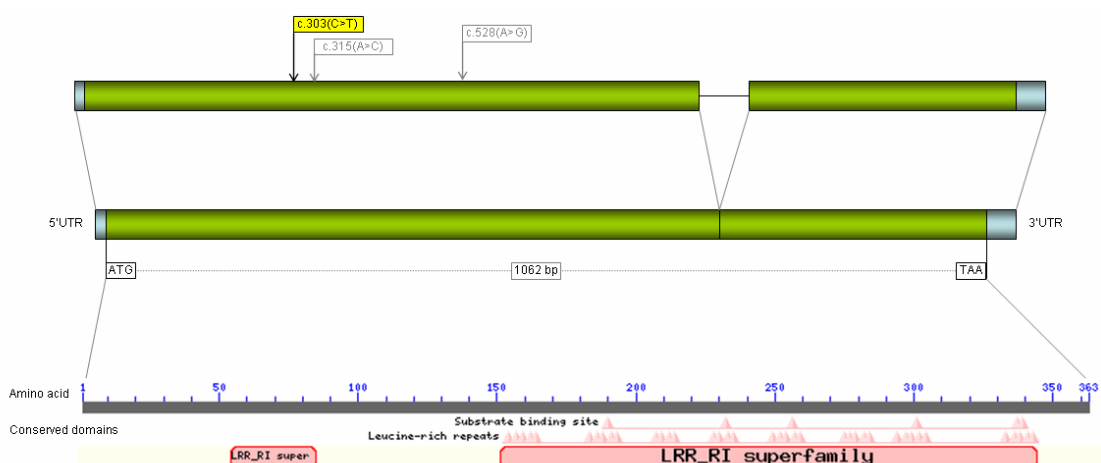
Figure 10: Structure of porcine KERA gene

4.1.8 The coenzyme Q9 homologue (COQ9)

Three overlapping of porcine ESTs (Acc. No: DT332099.1, CN156459.1 and DN104150.1) provided the complete porcine COQ9 cDNA sequence which contains of 1625 bp including 9 bp of 5'UTR, 954 bp of coding region and 662 bp of 3'UTR. The porcine COQ9 gene encodes 317 amino acids which show 92% similarity to the human (Acc. No: NP_064708.1), 87% to the cattle (Acc. No: NP_001039767.1) and 87% to the mouse (Acc. No: NP_080728.1). The comparison between porcine COQ9 cDNA sequence and porcine

genomic DNA sequence (Acc. No: FP326735.2) revealed 9 exons. Four SNPs were detected including two silent SNPs within coding region of exon4 (c.453A>G) and of exon6 (c.639G>T) and another two SNPs (+1247A>T and +1484C>T) in the 3'UTR. The SNPs in the coding region located in the conserved domain "diverge_rpsU" which is found in a number of Alphaproteobacteria and involved with the regulation of the initiation of protein translation. In this study, two SNPs (c.453A>G and (+1247A>T) were genotyped for association analysis (Figure 11).
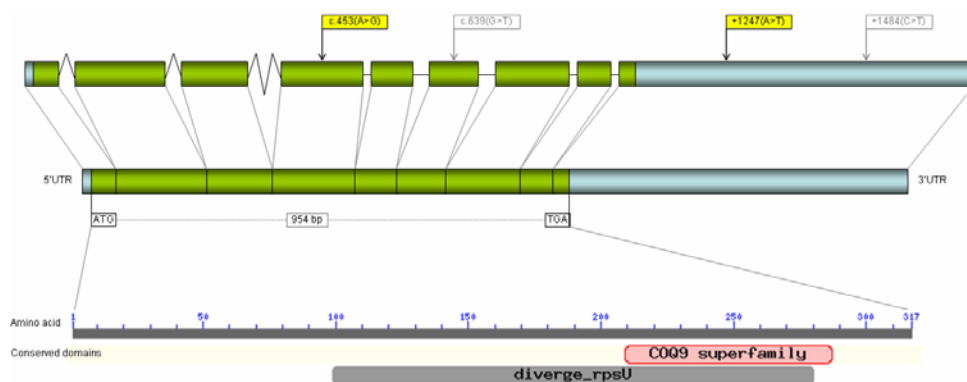
Figure 11: Structure of porcine COQ9 gene

4.1.9 Unknown (UN)

For unknown gene (non-annotated EST, Affymetrix probe set ID: Ssc.25503.1.S1_at), a SNP (g.1,022,434G>T) was detected at the position 1,022,434 of porcine genomic sequence (Sus scrofa chromosome 7 genomic contig, Acc. No: NW_001886512.1) which located in the area 20503 bp far from 5' flanking side of the putative pig RGMA sequence. This SNP was genotyped for association analysis.

4.1.10 The epidermal growth factor receptor (EGFR)

The porcine EGFR mRNA sequence (Acc. No: NM_214007.1) consists of 5038 bp including 126 bp of 5'UTR, 3630 bp of coding region and 1282 bp of 3'UTR. The coding region encodes 1209 amino acids which show 88% homology to the human (Acc. No: NP_005219.2), 92% to the cattle (Acc. No: XP_592211.3) and 87% to the rat (Acc. No: NP_113695.1). The structure of porcine EGFR gene was deducted by comparison of

porcine EGFR mRNA sequence, human EGFR mRNA sequence (Acc. No: NM_005228.3) and human genomic DNA sequence (Acc. No: AC006977.3, AC073324.6) revealed 28 exons. The coding area of porcine EGFR gene contains several conserved domains for several features involved in signaling pathways leading to a broad range of cellular responses including cell proliferation, differentiation, migration, growth inhibition, and apoptosis. Screening for SNP showed three SNPs including one silent SNP (c.3543A>G) in the coding region of exon28 and two SNPs (+3782A>G and +3802C>T) in the 3'UTR. For association analysis, a SNP c.3543A>G was selected for genotyping (Figure 12).
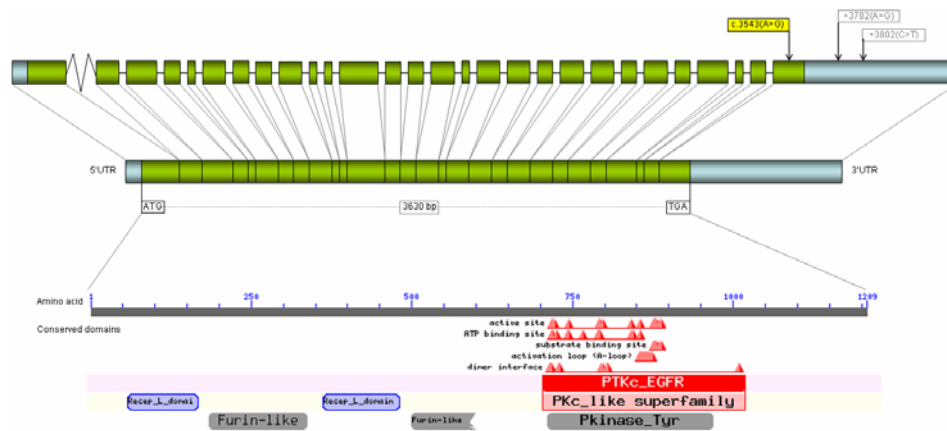
Figure 12: Structure of porcine EGFR gene

4.1.11 The vitronectin (VTN)

The porcine VTN mRNA sequence (Acc. No: NM_214104.1) consists of 1547 bp including 48 bp of 5' untranslated region (UTR), 1380 bp of coding region and 119 bp of 3'UTR. The coding region encodes 459 amino acids which show 71% homology to the human (Acc. No: NP_000629.3), 70% to the cattle (Acc. No: NP_001030222.1) and 69% to the mouse (Acc. No: NP_035837.1). The comparative analysis between porcine mRNA sequence and genomic DNA sequence (Acc. No: FP565711.1) revealed the structure of porcine VTN gene that contains 8 exons. Three SNPs (c.154A>G, c.156C>T and c.382C>T) were detected within the coding region of exon2. Among these SNPs, two SNPs (c.154A>G and c.156C>T) located in the area of Somatomedin B domain involved in the regulation of proteolysis. Especially, the SNP c.154A>G effects an amino acid

change from Threonine to Alanine (p.Thr52Ala). For association analysis, two SNPs (c.154A>G and c.156C>T) were selected for genotyping.
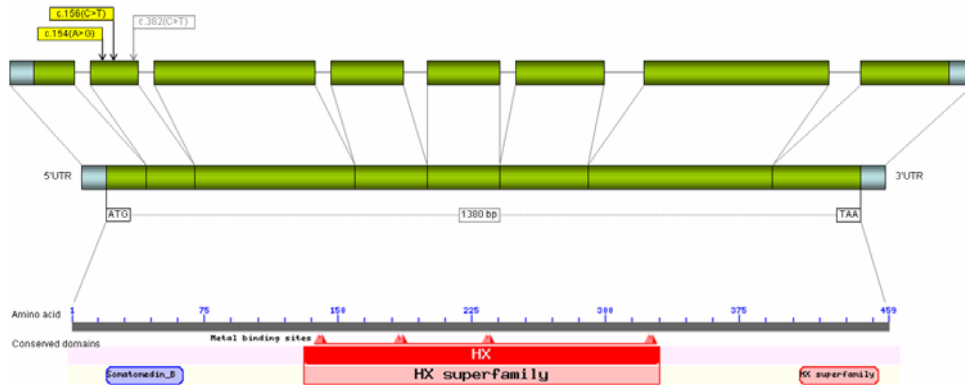
Figure 13: Structure of porcine VTN gene

4.1.12 The Zyxin (ZYX)

The overlapping of porcine ESTs (Acc. No: AJ680915.1, BP440079.1, EH008233.1 and BW975440.1) provided the full-length porcine ZYX cDNA sequence which contains of 1874 bp including 71 bp of 5'UTR, 1707 bp of coding region and 96 bp of 3'UTR. The porcine ZYX gene encodes 568 amino acids which showed similarity to 88% of human (Acc. No: NP_001010972.1), 90% of cattle (Acc. No: NP_001071569.1) and 84% of mouse (Acc. No: NP_035907.1).
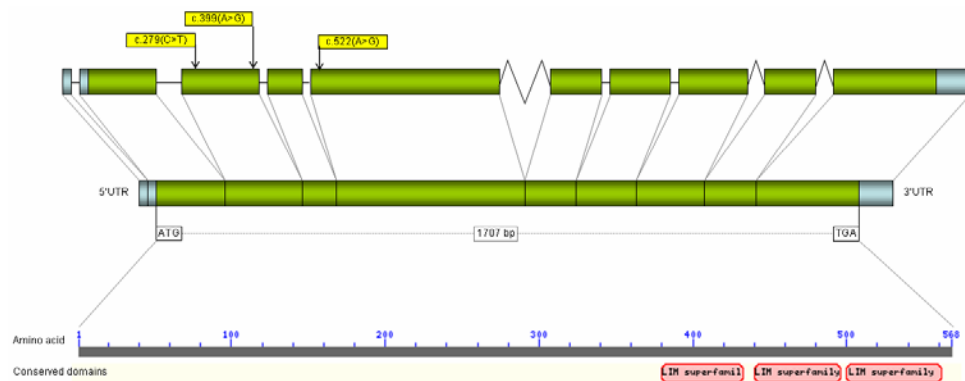
Figure 14: Structure of porcine ZYX gene

The comparison between porcine ZYX cDNA sequence and porcine genomic DNA sequence (Acc. No: CU929961.2) revealed 10 exons. At the 3' region of ZYX gene contains "LIM" domains involved in protein-protein interaction. Three silent SNPs were detected and genotyped including two SNPs in exon3 (c.279C>T and c.399A>G) and one SNP (c.639G>T) in exon5 (Figure 14).

Table 8: Detected SNPs, location and method used for genotyping

Acc. No.	Gene symbol	Confirmed SNP	Amino acid exchange
NM_001144112.1	BVES	c.186G>T (genotyped)	-
EU587016.1, BP141705.1	SLC3A2	c.1103A>G c.1113C>T c.1119C>T c.1200C>G c.1326A>G (genotyped) c.1336Indel[AGC] (genotyped)	Arginine→Histidine - - - - [Serine]
BX922331.2	AHNAK	c.12907A>G (genotyped) c.13014G>T (genotyped) c.13281A>G (genotyped) c.13290A>C>G (genotyped) c.13294C>T (genotyped)	Isoleucine→Valine - - - -
BX671032.2, CK464654.1	ZDHHC5	c.1803C>T (genotyped) +2161A>C	- -
NM_214276.1	CS	c.120G>T (genotyped) +1578A>G +1622A>G	- - -
NM_214392.1	LYZ	c.240A>C (genotyped) c.255C>T c.365A>T (genotyped) c.370A>G	- - Glutamine→Leucine -
XM_001927128.1	KERA	c.303C>T (genotyped) c.315A>C c.528A>G	- - -
CN156459.1, DN104150.1	COQ9	c.453A>G (genotyped) c.639G>T +1247A>T(genotyped) +1484C>T	- - - -
NW_001886512.1	UN	g.1,022,434G>T (genotyped)	-
NM_214007.1	EGFR	c.3543A>G (genotyped) +3782A>G +3802C>T	- - -
NM_214104.1	VTN	c.154A>G (genotyped) c.156C>T (genotyped) c.382C>T	Threonine→Alanine - -
BP440079.1, BW975440.1	ZYX	c.279C>T (genotyped) c.399A>G (genotyped) c.522A>G (genotyped)	- - -

The average length of a PCR product was 654 bp and the average SNP per base pair was 1:
361, two loci (BVES and UN) contained just one SNP whereas other loci contained two to
five SNPs. Most SNPs were detected in coding regions, and some SNPs (CS, EGFR,
COQ9 and ZDHHC5) were also detected in 3'UTR regions. The transitions detected in
SLC3A2, AHNAK, LYZ and VTN, resulted in amino acid substitutions. Total, twenty one
SNPs and one Indel were selected for genotyping; four of twelve genes were genotypes by
single base extension technique, the other eight by pyrosequencing technique (Table 8).

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

4.2.1 Association analysis of BVES

4.2.1.1 Genotype and allele frequencies of BVES c.186G>T

Genotype and allele frequencies of BVES c.186G>T are shown in Table 9. In PIF1(a,b)
and DL the genotype distribution was not in Hardy-Weinberg equilibrium since the
number of heterozygous animals was higher than expected (P<0.05). In PI, the 'G' allele
occurred with higher frequency (0.58), whereas in the other populations the 'G' allele
segregated with lower frequency (≤0.45).

Table 9: Genotype and allele frequencies of BVES c.186G>T in pigs

Populations	Genotype frequency			Allele frequency		HWE
	GG	GT	TT	G	T	
DUPI (n=287)	0.08	0.45	0.46	0.31	0.69	
PI (n=231)	0.36	0.43	0.21	0.58	0.42	NS
PIF1(a) (n=291)	0.16	0.57	0.27	0.45	0.55	*
PIF1(b) (n=305)	0.12	0.53	0.34	0.39	0.61	*
PIF1(c) (n=335)	0.12	0.52	0.36	0.38	0.62	NS
DL (n=268)	0.03	0.41	0.56	0.23	0.77	*

* P<0.05
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.1.1 The effects of BVES c.186G>T on phenotypic traits in pigs

The association analysis of c.186G>T for meat quality and carcass traits revealed
significant associations with DRIP and pH24 in PIF1(b) and DUPI respectively (Table 10).
Animals with the genotype 'GG' had higher DRIP than animals with the genotype 'TT'
(P<0.05) in PIF1(b). The heterozygous genotype 'GT' led to a higher BF3 than the

homozygous genotype 'TT' in PIF1(b). In DUPI, animals with the genotype 'GT' had lower pH24 than animals with the genotype 'TT' (P<0.01).

Table 10: Least square means (LSM) and standard errors (SE) for meat quality traits across BVES c.186G>T in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		GG	GT	TT	
pH24	DUPI	5.48^{ab} (0.02/19)	5.48^a (0.01/116)	5.52^b (0.01/118)	0.0075
BF3	PIF1(b)	0.98^{cd} (0.05/38)	1.09^c (0.03/162)	1.00^d (0.03/105)	0.0304
DRIP	PIF1(b)	3.07^c (0.24/38)	2.59^{cd} (0.16/161)	2.47^d (0.18/103)	0.0495

$^{c-d}$ P<0.05, $^{a-b}$ P<0.01
Within rows, values with the same letter are not significantly different

4.2.2 Association analysis of SLC3A2

4.2.2.1 Genotype and allele frequencies of SLC3A2 c.1326A>G

The allele and genotype frequencies of c.1326A>G are shown in Table 11. The presence of the allele 'A' was very high (≥0.71) across all populations. In all commercial pig populations, the genotype distribution was in Hardy-Weinberg equilibrium. In general, the frequency of the genotype 'GG' was very low (≤0.08) compared to the other genotypes 'AG' (0.08-0.48) and 'AA' (0.47-0.92).

Table 11: Genotype and allele frequencies of SLC3A2 c.1326A>G in pigs

Populations	Genotype frequency			Allele frequency		HWE
	AA	AG	GG	A	G	
DUPI (n=285)	0.47	0.48	0.05	0.71	0.29	
PI (n=221)	0.68	0.30	0.02	0.83	0.17	NS
PIF1(a) (n=282)	0.92	0.08	-	0.96	0.04	NS
PIF1(b) (n=307)	0.58	0.36	0.07	0.76	0.24	NS
PIF1(c) (n=303)	0.66	0.30	0.04	0.81	0.19	NS
DL (n=260)	0.52	0.40	0.08	0.72	0.28	NS

** P<0.01
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.2.2 The effects of SLC3A2 c.1326A>G on phenotypic traits in pigs

The study revealed an association of c.1209A>G with carcass traits (Table 12). Animals carrying the homozygous genotype 'AA' had lower MFR and BF3 than the heterozygous animals in PIF1(b) and DL (P<0.05) respectively.

Table 12: Least square means (LSM) and standard errors (SE) for carcass traits across SLC3A2 c.1326A>G in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		AA	AG	GG	
MFR	PIF1(b)	0.26^c (0.01/178)	0.28^d (0.01/109)	0.27^{cd} (0.01/20)	0.0352
BF3	DL	1.78^c (0.05/134)	1.94^d (0.05/105)	1.84^{cd} (0.10/21)	0.0226

$^{c-d}$ P<0.05, $^{a-b}$ P<0.01
Within rows, values with the same letter are not significantly different

4.2.2.3 Genotype and allele frequencies of SLC3A2 c.1336Indel[AGC]

For the Indel polymorphism ('D' representing the deletion of 'AGC' and 'I' representing the insertion of 'AGC'), the appearance of the heterozygous 'DI' was more frequent than expected in PIF1 (a,b,c). The range of frequency of 'D' was changing widely from 0.81 (in DL) to 0.34-0.35 (in DUPI and PI). In DUPI, PI and PIF1(a), the presence of the homozygous 'DD' was lowest (0.00-0.12), whereas in PIF1 (b,c) and DL the homozygous 'II' occurred with lowest frequency (0.02-0.20) (Table 13).

Table 13: Genotype and allele frequency of SLC3A2 c.1336Indel[AGC] in pigs

Populations	Genotype frequency			Allele frequency		HWE
	DD	DI	II	D	I	
DUPI (n=285)	0.07	0.53	0.40	0.34	0.66	
PI (n=221)	0.12	0.46	0.42	0.35	0.65	NS
PIF1(a) (n=282)	-	0.84	0.16	0.42	0.58	***
PIF1(b) (n=303)	0.26	0.58	0.16	0.55	0.45	**
PIF1(c) (n=303)	0.23	0.57	0.20	0.51	0.49	*
DL (n=260)	0.65	0.33	0.02	0.81	0.19	NS

* P<0.05, ** P<0.01, *** P<0.01
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.2.4 The effects of SLC3A2 c.1336Indel[AGC]on phenotypic traits in pigs

The analysis of c.1336Indel[AGC] revealed significant associations with various measures of carcass fatness traits in PI, PIF1(a,b,c) and DL (Table 14). In PI, animals with homozygous deletion 'DD' had higher ABF than heterozygous animals, whereas in PIF1(a,b) the homozygous insertion 'II' provided the lowest ABF (P<0.05). For traits related to fat content such as BF2 and FA, the deletion 'D' tends to be associated with high fat content in PIF1(b) (P<0.05); this observation was opposite compared to the DL line, in which the homozygous insertion 'II' provided highest BF1 (P<0.01). Moreover, the

analysis also revealed significant associations with various measures of meat quality traits in PIF1(a,b,c) and DL. Animals having homozygous 'II' offered highest COOK and SF in PIF1(a), while animals having homozygous 'DD' had highest THAW and lowest PH24 in PIF1(b) and highest CON1 in PIF1(c). In DL the deletion 'D' tends to be associated with low DRIP (P<0.05).

Table 14: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across SLC3A2 c.1336Indel[AGC] in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		DD	DI	II	
ABF	PI	1.87[c] (0.06/27)	1.75[d] (0.04/101)	1.82[cd] (0.04/93)	0.0402
ABF	PIF1(a)	2.67[c] (0.05/69)	2.65[c] (0.04/179)	2.49[d] (0.06/34)	0.0228
SF	PIF1(a)	37.22[a] (1.15/66)	38.50[c] (0.88/173)	42.14[bd] (1.42/31)	0.0061
COOK	PIF1(a)	25.20[c] (0.41/66)	25.56[cd] (0.33/173)	26.40[d] (0.48/31)	0.0443
ABF	PIF1(b)	1.90[c] (0.03/79)	1.83[cd] (0.02/175)	1.78[d] (0.04/49)	0.0458
BF2	PIF1(b)	1.54[c] (0.03/79)	1.45[cd] (0.02/175)	1.40[d] (0.04/49)	0.0169
FA	PIF1(b)	14.88[c] (0.31/79)	14.27[cd] (0.23/175)	13.78[d] (0.37/49)	0.0389
THAW	PIF1(b)	9.39[a] (0.25/76)	8.64[b] (0.20/164)	8.34[b] (0.30/48)	0.0016
pH1	PIF1(b)	6.55[c] (0.03/79)	6.45[d] (0.02/175)	6.51[cd] (0.04/49)	0.0220
pH24	PIF1(b)	5.49[c] (0.01/79)	5.51[cd] (0.01/175)	5.53[d] (0.01/49)	0.0422
CON1	PIF1(c)	5.63[c] (0.25/69)	5.04[d] (0.20/172)	5.29[cd] (0.26/62)	0.0310
BF1	DL	3.73[c] (0.04/168)	3.68[a] (0.05/86)	4.22[bd] (0.17/6)	0.0098
DRIP	DL	4.37[c] (0.24/167)	4.83[cd] (0.31/86)	6.44[d] (0.78/6)	0.0173

[c-d] P<0.05, [a-b] P<0.01
Within rows, values with the same letter are not significantly different

4.2.2.5 Diplotype and haplotype frequencies of the SLC3A2

In almost all populations, except PIF1 (a) and DL, the haplotype 'H2' was more frequent than the other two haplotypes ('H1' and 'H3'), which results in the diplotype 'H1/H2' becoming the major diplotype observed in most of the commercial pigs, whereas in DUPI and PI, the main diplotypes were 'H2/H2' or 'H2/H3' (Table 15).

Table 15: Diplotype and haplotype frequencies of SLC3A2 gene in pigs

Haplotype	DUPI (n=285)	PI (n=221)	PIF1(a) (n=264)	PIF1(b) (n=304)	PIF1(c) (n=303)	DL (n=260)
AD [H1]	0.05	0.18	0.52	0.31	0.32	0.53
AI [H2]	0.66	0.65	0.44	0.45	0.49	0.19
GD [H3]	0.29	0.17	0.04	0.24	0.19	0.28
Diplotype						
H1/H1	-	0.02	0.24	0.08	0.08	0.28
H1/H2	0.07	0.24	0.61	0.34	0.37	0.21
H1/H3	0.02	0.09	0.02	0.12	0.11	0.28
H2/H2	0.40	0.42	0.13	0.16	0.20	0.02
H2/H3	0.46	0.22	-	0.24	0.19	0.12
H3/H3	0.05	0.02	-	0.06	0.04	0.08

4.2.2.6 The effects of the SLC3A2 haplotype on phenotypic traits in pigs

The association analysis between haplotype and carcass and meat quality traits confirmed the results described in the previous sections, especially with the effect of Indel c.1336Indel[AGC]. According to the results, the diplotype 'H2/H2' is related with lower ABF and BF2 in PIF1(a,b); in contrast it is related with higher BF1 in DUPI. For meat quality traits, the diplotype 'H2/H2' is associated with lower THAW in PIF1(b). In PIF1(a), the diplotype 'H2/H2' is associated with higher SF. Moreover, the diplotype 'H1/H2' animals had lower pH1 than the diplotype 'H1/H1' animals in PIF1(b). In PIF1(c), the diplotype 'H1/H1' had higher CON1 than the diplotype 'H1/H2' and 'H2/H3' (Table 16).

Table 16: Least square means (LSM) and standard errors (SE) for carcass and meat quality traits across haplotypes of SLC3A2 in pigs

	BF1	ABF	SF	BF2	pH1	THAW	CON1
	DUPI	PIF1(a)	PIF1(a)	PIF1(b)	PIF1(b)	PIF1(b)	PIF1(c)
H1/H1	-	2.67^c (0.06/64)	37.27^a (1.21/61)	1.50^{cd} (0.06/24)	6.62^c (0.05/24)	9.59^{ac} (0.39/23)	6.27^c (0.37/24)
H1/H2	3.45^{cd} (0.12/21)	2.64^{cd} (0.04/161)	38.64^{abc} (0.94/155)	1.45^c (0.03/103)	6.45^d (0.03/103)	8.69^d (0.23/99)	5.13^d (0.23/113)
H1/H3	2.81^c (0.22/6)	2.73^{cd} (0.14/5)	36.10^{abcd} (3.10/5)	1.61^d (0.05/36)	6.50^{cd} (0.04/36)	9.30^c (0.35/35)	5.60^{cd} (0.32/33)
H2/H2	3.43^d (0.05/114)	2.49^d (0.06/34)	42.38^{bd} (1.45/31)	1.40^c (0.04/49)	6.51^{cd} (0.04/49)	8.33^{bd} (0.30/48)	5.28^{cd} (0.26/62)
H2/H3	3.29^{cd} (0.05/130)	-	-	1.45^{cd} (0.03/72)	6.49^{cd} (0.03/72)	8.57^d (0.26/65)	4.96^d (0.26/59)
H3/H3	3.40^{cd} (0.14/14)	-	-	1.47^{cd} (0.06/20)	6.55^{cd} (0.05/20)	9.28^a (0.43/19)	5.36^{cd} (0.53/12)
P-value	0.0291	0.0432	0.0102	0.0376	0.0287	0.0241	0.0156

$^{c-d}$ P<0.05, $^{a-b}$ P<0.01

Within columns, values with the same letter are not significantly different

4.2.3 Association analysis of AHNAK

4.2.3.1 Genotype and allele frequencies of AHNAK c.12907A>G

The distribution of AHNAK c.12907A>G is displayed in Table 17. There was a large range in the frequency of the allele 'A' from 0.07 to 0.41 when comparing PI to DUPI, whereas in PIF1 (a,b,c) and DL the range of the allele 'A' was between 0.12 and 0.15. In all commercial pig populations, the frequency of the genotype 'AA' was very low (0.00-0.02) compared to the other genotypes 'AG' (0.14-0.27) and 'GG' (0.72-0.86). In addition, a significant deviation from the Hardy-Weinberg equilibrium was found in PIF1(a) (P<0.05).

Table 17: Genotype and allele frequencies of AHNAK c.12907A>G in pigs

Populations	Genotype frequency			Allele frequency		HWE
	AA	AG	GG	A	G	
DUPI (n=406)	0.15	0.51	0.34	0.41	0.59	
PI (n=242)	-	0.14	0.86	0.07	0.93	NS
PIF1(a) (n=349)	-	0.24	0.76	0.12	0.88	*
PIF1(b) (n=303)	0.02	0.23	0.75	0.14	0.86	NS
PIF1(c) (n=331)	0.01	0.27	0.72	0.14	0.86	NS
DL (n=288)	0.02	0.26	0.72	0.15	0.85	NS

* P<0.05
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.3.2 The effects of AHNAK c.12907A>G on phenotypic traits in pigs

The results of the association analysis of AHNAK c.12907A>G are summarized in Table 18. Analysis of carcass traits revealed an association with BF2 in PIF1(c) and with ABF, BF1, BF3, MFR, FA and LEA in DUPI. Analysis of meat quality traits revealed an association with OPTO in DL and with THAW in DUPI. The homozygous genotype 'GG' animals had lower LEA than the heterozygous animals in PIF1(a) but the opposite effect was found with BF2 in PIF1(c). In DUPI, animals carrying the 'G' allele produced higher carcass fat contents, which was found to be most pronounced in the trait MFR; animals with genotype 'GG' had the highest MFR (P<0.001). For meat quality traits, the homozygous genotype 'GG' animals had higher OPTO and THAW than the heterozygous animals in DL and DUPI (P<0.05).

Table 18: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across AHNAK c.12907A>G in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		AA	AG	GG	
LEA	DUPI	50.82^{cd} (0.66/62)	51.05^{c} (0.40/208)	49.66^{d} (0.45/136)	0.0143
FA	DUPI	15.90^{c} (0.37/62)	16.12^{c} (0.22/208)	16.88^{d} (0.25/136)	0.0091
MFR	DUPI	0.32^{c} (0.01/62)	0.32^{ac} (0.01/208)	0.35^{bd} (0.01/136)	0.0005
BF1	DUPI	3.27^{c} (0.06/62)	3.39^{cd} (0.04/208)	3.47^{d} (0.04/136)	0.0135
BF3	DUPI	1.28^{c} (0.04/62)	1.35^{cd} (0.03/208)	1.42^{d} (0.03/136)	0.0122
ABF	DUPI	2.04^{a} (0.04/62)	2.13^{ab} (0.02/208)	2.19^{b} (0.03/136)	0.0048
THAW	DUPI	7.85^{cd} (0.33/62)	8.02^{c} (0.24/204)	8.51^{d} (0.26/132)	0.0424
LEA	PIF1(a)	-	51.77^{c} (0.77/72)	50.33^{d} (0.65/255)	0.0149
BF2	PIF1(c)	-	1.84^{c} (0.04/88)	1.94^{d} (0.03/239)	0.0488
OPTO	DL	-	68.34^{c} (0.71/71)	70.36^{d} (0.46/185)	0.0131

$^{c-d}$ P<0.05, $^{a-b}$ P<0.01
Within rows, values with the same letter are not significantly different

4.2.3.3 Genotype and allele frequencies of AHNAK c.13014G>T

In Table 19 the allele and genotype frequencies of AHNAK c.13014G>T are shown. AHNAK c.13014G>T occurred in all pig population with the frequency of 'G' varying from 0.11 in PI to 0.59 in DUPI. In all commercial pig populations, the genotype distributions seem to be in the same trend, where the genotype 'GG' occurred with lower frequency compared to the genotype 'GT' and 'TT' respectively. However, the lowest frequency of a genotype was observed for the genotype 'TT' in DUPI. Moreover, the numbers of heterozygous animals were more frequent than expected in DUPI, PI and PIF1(a).

Table 19: Genotype and allele frequencies of AHNAK c.13014G>T in pigs

Populations	Genotype frequency			Allele frequency		HWE
	GG	GT	TT	G	T	
DUPI (n=407)	0.31	0.57	0.12	0.59	0.41	
PI (n=242)	-	0.23	0.77	0.11	0.89	*
PIF1(a) (n=349)	-	0.29	0.71	0.14	0.86	**
PIF1(b) (n=303)	0.05	0.34	0.61	0.22	0.78	NS
PIF1(c) (n=331)	0.05	0.38	0.57	0.24	0.76	NS
DL (n=287)	0.09	0.39	0.51	0.29	0.71	NS

* P<0.05, ** P<0.01
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.3.4 The effects of AHNAK c.13014G>T on phenotypic traits in pigs

The analysis of AHNAK c.13014G>T revealed significant associations with carcass traits and/or meat quality traits in PI, PIF1(b,c) and DUPI (Table 20). Highly significant effects were found on ABF, BF1, MFR and FA in DUPI (P<0.001), where carriers of the 'T' allele produced higher fat contents. The association observed for BF3 and FA are consistent in that way in PI, PIF1(c) and DUPI. Moreover, the 'T' allele carriers also produced higher COOK, pH24 and DRIP in PI, PIF1(b) and DUPI respectively (P<0.05).

Table 20: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across AHNAK c.13014G>T in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		GG	GT	TT	
COOK	PI	-	23.33^c (0.33/55)	24.00^d (0.22/186)	0.0474
LEA	DUPI	50.88^c (0.51/125)	50.86^c (0.39/233)	48.82^d (0.69/49)	0.0210
FA	DUPI	15.87^a (0.28/125)	16.15^b (0.22/233)	17.78^b (0.38/49)	0.0001
MFR	DUPI	0.32^a (0.01/125)	0.32^a (0.01/233)	0.37^b (0.01/49)	<0.0001
BF1	DUPI	3.32^a (0.05/125)	3.38^a (0.04/233)	3.60^b (0.06/49)	0.0009
BF3	DUPI	1.31^a (0.03/125)	1.35^{ac} (0.03/233)	1.48^{bd} (0.04/49)	0.0050
ABF	DUPI	2.08^a (0.03/125)	2.13^a (0.02/233)	2.27^b (0.04/49)	0.0005
DRIP	DUPI	2.01^c (0.12/124)	2.11^{cd} (0.10/227)	2.48^d (0.16/48)	0.0393
BF3	PIF1(b)	0.91^c (0.08/14)	1.01^{cd} (0.03/104)	1.09^d (0.02/185)	0.0294
pH24	PIF1(b)	5.46^c (0.02/14)	5.50^{cd} (0.01/104)	5.51^d (0.01/185)	0.0424
FA	PIF1(c)	12.68^c (0.81/17)	14.82^d (0.31/125)	14.85^d (0.26/187)	0.0341

[c-d] P<0.05, [a-b] P<0.01
Within rows, values with the same letter are not significantly different

4.2.3.5 Genotype and allele frequencies of AHNAK c.13281A>G

Table 21 gives the allele and genotype frequencies of AHNAK c.13281A>G. The frequency of the allele 'A' varied from 0.15 in DL to 0.52 in PI. The 'AA' genotype segregation in DUPI, PIF1(a,b,c) and DL was low (0.01-0.12) compared to PI (0.25). In addition, a X^2 test showed significant disequilibria (P<0.05) in all PIF1 lines.

Table 21: Genotype and allele frequencies of AHNAK c.13281A>G in pigs

Populations	Genotype frequency			Allele frequency		HWE
	AA	AG	GG	A	G	
DUPI (n=408)	0.09	0.37	0.54	0.28	0.72	
PI (n=251)	0.25	0.54	0.21	0.52	0.48	NS
PIF1(a) (n=375)	0.03	0.53	0.44	0.29	0.71	***
PIF1(b) (n=315)	0.12	0.63	0.24	0.44	0.56	***
PIF1(c) (n=336)	0.07	0.58	0.36	0.35	0.65	***
DL (n=290)	0.01	0.28	0.71	0.15	0.85	NS

* P<0.05, ** P<0.01, *** P<0.001
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.3.6 The effects of AHNAK c.13281A>G on phenotypic traits in pigs

The effects of AHNAK c.13281A>G on carcass and meat quality traits are shown in Table 22. In DUPI, the effect of AHNAK c.13281A>G on ABF, BF1, BF3, FA indicated that the 'G' allele tends to associate with lower backfat thickness and also lower MFR. The homozygous genotype 'GG' was associated with high LEA in DL. For meat quality, the homozygous genotype 'AA' was associated with the lowest DRIP and the allele 'A' decreased pH1 in DUPI (P<0.05), whereas the heterozygous genotype 'AG' animals had lower DRIP and CON24 in DL (P<0.01). Moreover, the heterozygous animals also had higher OPTO in PIF1(b) and DL (P<0.05) when compared to the homozygous 'GG' animals.

Table 22: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across AHNAK c.13281A>G in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		AA	AG	GG	
FA	DUPI	17.51[a] (0.43/37)	16.44[ab] (0.25/152)	16.06[b] (0.24/219)	0.0134
MFR	DUPI	0.36[a] (0.01/37)	0.33[ab] (0.01/152)	0.32[b] (0.01/219)	0.0032
BF1	DUPI	3.56[c] (0.07/37)	3.43[cd] (0.04/152)	3.34[d] (0.04/219)	0.0187
BF3	DUPI	1.44[cd] (0.05/37)	1.41[c] (0.02/152)	1.32[d] (0.03/219)	0.0140
ABF	DUPI	2.23[c] (0.05/37)	2.17[cd] (0.03/152)	2.09[d] (0.03/219)	0.0140
pH1	DUPI	6.47[c] (0.03/37)	6.53[cd] (0.02/152)	6.57[d] (0.02/219)	0.0279
DRIP	DUPI	2.61[c] (0.42/36)	2.17[cd] (0.11/147)	2.04[d] (0.11/217)	0.0187
OPTO	PIF1(b)	66.73[cd] (1.00/38)	68.15[c] (0.50/200)	66.18[d] (0.74/76)	0.0375
LEA	DL	-	42.07[c] (0.63/73)	43.75[d] (0.49/182)	0.0100
OPTO	DL	-	71.18[c] (0.73/82)	69.41[d] (0.52/205)	0.0281
CON24	DL	-	3.66[c] (0.23/82)	4.14[d] (0.18/205)	0.0457
DRIP	DL	-	3.89[a] (0.27/82)	4.67[b] (0.21/204)	0.0051

[c-d] P<0.05, [a-b] P<0.01
Within rows, values with the same letter are not significantly different

4.2.3.7 Genotype and allele frequencies of AHNAK c.13290A>C>G

In Table 23 the frequencies of alleles and genotypes for the triallelic SNP c.13290A>C>G is shown. In commercial pig populations, the presence of the 'C' allele was very low (0.00-0.05) compared to that in DUPI (0.18). The 'G' allele was the major allele in all populations with frequencies between 0.53 and 0.77. The homozygous genotype 'GG' segregated with high frequencies (0.46-0.57) in DUPI, PI and PIF1(b,c), whereas in PIF1(a) and DL the major genotype was 'AG' with frequencies between 0.47 and 0.48. Moreover, the appearances of the genotypes 'AC' and 'CG' were very low in commercial pigs compared to those in DUPI. In addition, the rare genotype 'CC' was only found in DUPI with a very low frequency (0.01) and the frequency of the 'AC' genotype was also higher than expected.

Table 23: Genotype and allele frequencies of AHNAK c.13290A>C>G in pigs

Populations	Genotype frequency						Allele frequency			HWE
	AA	AC	CC	CG	GG	AG	A	C	G	
DUPI (n=405)	-	0.11	0.01	0.23	0.49	0.16	0.13	0.18	0.69	
PI (n=251)	0.04	-	-	-	0.57	0.39	0.23	-	0.77	NS
PIF1(a) (n=374)	0.12	0.03	-	0.01	0.36	0.48	0.37	0.02	0.61	NS
PIF1(b) (n=314)	0.09	-	-	0.03	0.46	0.42	0.3	0.01	0.68	NS
PIF1(c) (n=335)	0.08	0.02	-	0.02	0.47	0.41	0.3	0.02	0.69	NS
DL (n=290)	0.17	0.03	-	0.07	0.26	0.47	0.42	0.05	0.53	NS

*** P<0.001
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.3.8 The effects of AHNAK c.13290A>C>G on phenotypic traits in pigs

The association analysis of the triallelic SNP c.13290A>C>G revealed significant associations with various measures of carcass and meat quality traits (Table 24). Almost all significant differences were found between the heterozygous genotype 'AG' and the homozygous genotype 'GG' and/or the rare heterozygous genotypes 'AC' and 'CG'. The results indicate that the 'G' allele tended to associate with low backfat thickness. The genotype 'GG' tended to produce carcasses with lower ABF in PIF1(a,b) and also produce higher CON1 in DL. The rare genotype 'AC' tended to increase the pH in both stages (pH1 and pH24) in PIF1(a,c).

Table 24: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across AHNAK c.13290A>C>G in pigs

Traits	Populations	AA	AG	AC	CG	GG	P-value
		Least square means (LSM) (SE/n)					
ABF	PIF1(a)	2.59^{cd} (0.06/43)	2.68^{c} (0.04/180)	2.85^{c} (0.10/10)	2.77^{cd} (0.13/5)	2.59^{d} (0.04/136)	0.0137
pH24	PIF1(a)	5.60^{cd} (0.02/43)	5.57^{c} (0.02/179)	5.66^{d} (0.04/9)	5.53^{cd} (0.05/5)	5.57^{cd} (0.02/136)	0.0233
BF2	PIF1(b)	1.55^{cd} (0.05/29)	1.50^{c} (0.03/133)	-	1.48^{cd} (0.11/8)	1.41^{d} (0.02/143)	0.0338
BF3	PIF1(b)	1.13^{cd} (0.05/29)	1.08^{c} (0.03/133)	-	0.95^{cd} (0.11/8)	0.99^{d} (0.03/143)	0.0165
ABF	PIF1(b)	1.93^{c} (0.05/29)	1.86^{cd} (0.02/133)	-	1.79^{cd} (0.10/8)	1.78^{d} (0.02/143)	0.0159
THAW	PIF1(b)	9.86^{c} (0.39/26)	8.83^{d} (0.23/130)	-	9.72^{cd} (0.69/8)	8.83^{d} (0.23/132)	0.0239
pH1	PIF1(c)	6.18^{cd} (0.06/27)	6.12^{c} (0.04/137)	6.59^{c} (0.17/6)	5.94^{d} (0.14/6)	6.15^{cd} (0.03/158)	0.0388
LEA	DL	44.24^{cd} (0.73/44)	42.63^{c} (0.53/121)	42.81^{cd} (1.89/5)	45.10^{d} (1.00/20)	42.83^{cd} (0.62/67)	0.0457
CON1	DL	4.20^{c} (0.12/49)	4.28^{c} (0.10/135)	4.37^{cd} (0.24/9)	4.51^{cd} (0.16/21)	4.54^{d} (0.11/76)	0.0427

$^{c-d}$ P<0.05, $^{a-b}$ P<0.01
Within rows, values with the same letter are not significantly different

4.2.3.9 Genotype and allele frequencies of AHNAK c.13294C>T

The distribution of the 'C' allele varied from 0.58 in DL to 0.87 in DUPI. The homozygous genotype 'CC' was the major genotype found in DUPI, PI and PIF1(b,c) while in PIF1(a) and DL, the presence of heterozygous pigs was more frequent (0.49). However, low frequencies of homozygous 'TT' animals were observed across all pig populations (0.00-0.17) (Table 25).

Table 25: Genotype and allele frequency of AHNAK c.13294C>T in pigs

Populations	Genotype frequency			Allele frequency		HWE
	CC	CT	TT	C	T	
DUPI (n=405)	0.73	0.27	-	0.87	0.13	
PI (n=253)	0.57	0.39	0.04	0.76	0.24	NS
PIF1(a) (n=373)	0.40	0.49	0.10	0.65	0.35	NS
PIF1(b) (n=316)	0.48	0.42	0.09	0.70	0.30	NS
PIF1(c) (n=336)	0.49	0.43	0.08	0.71	0.29	NS
DL (n=290)	0.34	0.49	0.17	0.58	0.42	NS

NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.3.10 The effects of AHNAK c.13294C>T on phenotypic traits in pigs

Significant associations of AHNAK c.13294C>T are displayed in Table 26. The effects on ABF, BF2 and BF3 in PIF1(a,b) and MFR in DL indicated that the 'C' allele decreased fatness and increased leanness. For meat quality traits, the homozygous genotype 'CC' offered the highest CON1 when compared to other genotypes in DL (P<0.01) and tended

to produce carcasses with lower pH24 in PIF1(b). The genotype 'CC' animals had lower THAW than the heterozygous animals in PIF1(a) but this difference was not found in PIF1(b) where the homozygous genotype 'TT' showed the highest THAW.

Table 26: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across AHNAK c.13294C>T in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		CC	CT	TT	
ABF	PIF1(a)	2.60[c] (0.04/150)	2.69[d] (0.04/184)	2.61[cd] (0.06/39)	0.0192
THAW	PIF1(a)	8.52[c] (0.46/141)	9.68[d] (0.42/177)	8.70[cd] (0.75/38)	0.0470
BF2	PIF1(b)	1.42[c] (0.02/152)	1.50[d] (0.03/134)	1.55[cd] (0.05/29)	0.0168
BF3	PIF1(b)	0.99[c] (0.02/152)	1.08[d] (0.03/134)	1.13[cd] (0.06/29)	0.0086
ABF	PIF1(b)	1.79[c] (0.02/152)	1.86[d] (0.02/134)	1.93[d] (0.05/29)	0.0061
pH24	PIF1(b)	5.50[c] (0.009/152)	5.52[d] (0.010/134)	5.49[cd] (0.018/29)	0.0454
THAW	PIF1(b)	8.87[c] (0.23/141)	8.83[c] (0.23/131)	9.86[d] (0.38/26)	0.0195
LEA	DL	43.32[cd] (0.57/88)	42.51[c] (0.52/124)	44.32[d] (0.72/45)	0.0463
MFR	DL	0.48[cd] (0.01/88)	0.51[c] (0.01/124)	0.46[d] (0.02/45)	0.0263
CON1	DL	4.53[c] (0.10/98)	4.29[d] (0.10/142)	4.20[d] (0.12/50)	0.0095

[c-d] P<0.05, [a-b] P<0.01
Within rows, values with the same letter are not significantly different

4.2.3.11 Diplotype and haplotype frequencies of AHNAK

When combining five SNPs to construct a haplotype, six possible haplotypes were segregating in PIF1(b,c) and DL, being named H1 (AGGGC), H2 (GGGCC), H3 (GGGGC), H4 (GTAGC), H5 (GTGAT) and H6 (GTGGC). Five haplotypes were detected in PIF1(a) and PI (H1, H3, H4, H5 and H6). Four haplotypes were detected in DUPI (H1, H2, H4 and H5) (Table 27). The major diplotype in PI was 'H4/H4', whereas in all PIF1 lines the most frequent diplotype was 'H4/H5'. For DL and DUPI, the main diplotypes detected in this study were 'H5/H5' and 'H1/H4' respectively.

Table 27: Diplotype and haplotype frequencies of the AHNAK gene in pigs

Haplotype	PI (n=149)	PIF1(a) (n=278)	PIF1(b) (n=259)	PIF1(c) (n=315)	DL (n=255)	DUPI (n=390)
AGGGC[H1]	0.07	0.11	0.13	0.14	0.14	0.40
GGGCC[H2]	-	0.02	0.01	0.01	0.05	0.20
GGGGC[H3]	0.05	-	0.06	0.08	0.09	-
GTAGC[H4]	0.51	0.29	0.44	0.35	0.15	0.27
GTGAT[H5]	0.23	0.34	0.30	0.29	0.41	0.13
GTGGC[H6]	0.13	0.20	0.04	0.11	0.14	-
Diplotypes						
H1/H1	-	-	0.02	-	-	0.15
H1/H2	-	-	-	-	0.03	0.14
H1/H3	-	-	-	0.03	0.03	-
H1/H4	-	0.11	0.16	0.16	0.05	0.25
H1/H5	0.08	-	0.08	0.09	0.17	0.13
H1/H6	-	0.04	-	-	0.03	-
H2/H4	-	-	-	-	-	0.09
H2/H5	-	0.03	-	-	0.04	0.12
H3/H4	0.10	-	-	0.10	0.03	-
H3/H5	0.02	-	0.02		-	-
H4/H4	0.40	0.04	0.14	0.08	-	0.09
H4/H5	-	0.27	0.37	0.26	0.17	0.04
H4/H6	0.22	0.20	0.09	0.13	0.08	-
H5/H5	0.06	0.12	0.12	0.09	0.20	-
H5/H6	0.12	0.14	-	0.08	0.14	-
H6/H6	-	0.04	-	-	-	-

4.2.3.12 The effects of AHNAK haplotypes on phenotypic traits in pigs

Some traits were found to have a significant relation with the AHNAK haplotype (Table 28). In PIF1(a), pH24 was significantly associated with the AHNAK haplotype, in the form that the diplotype 'H1/H4' offered lower values than the diplotype 'H2/H5' (P<0.05). In DL, the animals carrying the diplotype 'H1/H6' had higher LEA than the 'H4/H5' animals (P<0.05). Moreover, in the DUPI population, the AHNAK haplotype affected LEA, in the way that the diplotype 'H1/H5' offered higher values than the diplotype 'H4/H5'. The animals bearing the diplotype 'H1/H1' had lower carcass fatness (BF1, BF3, ABF and FA) than the 'H4/H4' animals. Finally, different haplotypes were also in association with MFR, the animals with the diplotype 'H4/H5' had the highest MFR.

Table 28: Least square means (LSM) and standard errors (SE) for carcass and meat quality traits across haplotypes of AHNAK in pigs

	LEA DUPI	FA DUPI	MFR DUPI	BF1 DUPI	BF3 DUPI	ABF DUPI	pH24 PIF1(a)	LEA DL
H1/H1	50.41[cd] (0.70/55)	15.82[c] (0.39/55)	0.32[c] (0.01/55)	3.26[c] (0.07/55)	1.28[c] (0.05/55)	2.04[a] (0.04/55)	-	-
H1/H2	50.93[cd] 90.70/54	15.93[cd] (0.40/54)	0.32[c] (0.01/54)	3.36[cd] (0.07/54)	1.33[cd] (0.05/54)	2.11[ab] (0.04/54)	-	47.15[cd] (1.93/5)
H1/H3	-	-	-	-	-	-	-	43.81[cd] (1.49/8)
H1/H4	50.25[cd] (0.57/93)	16.08[c] (0.32/93)	0.32[c] (0.01/93)	3.40[cd] (0.05/93)	1.39[cd] (0.04/93)	2.14[ab] (0.03/93)	5.55[c] (0.03/30)	41.30[cd] (1.39/10)
H1/H5	52.10[c] (0.78/47)	16.08[cd] (0.44/47)	0.31[a] (0.01/47)	3.34[cd] (0.07/47)	1.32[cd] (0.05/47)	2.10[ab] (0.05/47)	-	43.85[cd] (0.81/37)
H1/H6	-	-	-	-	-	-	5.63[cd] (0.04/12)	46.62[c] (1.60/7)
H2/H4	49.15[cd] (0.87/32)	16.51[cd] (0.49/32)	0.34[abcd] (0.01/32)	3.40[cd] (0.08/32)	1.47[cd] (0.06/32)	2.18[ab] (0.05/32)	-	-
H2/H5	51.23[cd] (0.81/45)	16.09[cd] (0.46/45)	0.32[c] (0.01/45)	3.32[cd] (0.08/45)	1.29[cd] (0.05/45)	2.07[ab] (0.05/45)	5.67[d] (0.04/8)	43.39[cd] (2.08/4)
H3/H4	-	-	-	-	-	-	-	44.79[cd] (1.57/7)
H4/H4	49.39[cd] (0.83/32)	17.65[d] (0.47/32)	0.36[d] (0.01/32)	3.60[d] (0.08/32)	1.48[d] (0.05/32)	2.27[b] (0.05/32)	5.55[cd] (0.04/10)	41.86[cd] (1.71/6)
H4/H5	47.00[d] (1.45/15)	18.06[cd] (0.81/15)	0.39[bd] (0.02/15)	3.65[cd] (0.14/15)	1.53[cd] (0.09/15)	2.30[ab] (0.09/15)	5.57[cd] (0.02/73)	40.86[d] (0.81/34)
H4/H6	-	-	-	-	-	-	5.59[cd] (0.02/56)	42.61[cd] (1.11/16)
H5/H5	-	-	-	-	-	-	5.61[cd] (0.03/32)	44.13[cd] (0.73/44)
H5/H6	-	-	-	-	-	-	5.57[cd] (0.03/39)	43.70[cd] (0.88/28)
H6/H6	-	-	-	-	-	-	5.56[cd] (0.04/12)	-
P-value	0.0273	0.0193	0.0005	0.0237	0.0197	0.0090	0.0200	0.0110

[c-d] $P<0.05$, [a-b] $P<0.01$

Within columns, values with the same letter are not significantly different

4.2.4 Association analysis of ZDHHC5

4.2.4.1 Genotype and allele frequencies of ZDHHC5 c.1803C>T

In all pig populations, the appearance of the 'C' allele was very high (0.77-0.91). In PIF1 (b,c) and DL, the 'C' allele was distributed with the same frequency (0.91), whereas in PI and PIF1(a) the allele distributions were almost the same (0.86 vs. 0.87). In general, the frequency of the genotype 'TT' was very low compared to the other genotypes 'CT' and 'CC'. However, the deviation of the genotype distribution from Hardy-Weinberg

equilibrium was detected in PIF1(a), where the heterozygous genotype was more frequent than expected (Table 29).

Table 29: Genotype and allele frequency of ZDHHC5 c.1803C>T in pigs

Populations	Genotype frequency			Allele frequency		HWE
	CC	CT	TT	C	T	
DUPI (n=279)	0.54	0.46	-	0.77	0.23	
PI (n=248)	0.73	0.25	0.02	0.86	0.14	NS
PIF1(a) (n=264)	0.73	0.27	-	0.87	0.13	*
PIF1(b) (n=397)	0.83	0.17	-	0.91	0.09	NS
PIF1(c) (n=334)	0.82	0.18	-	0.91	0.09	NS
DL (n=277)	0.84	0.15	0.01	0.91	0.09	NS

* $P<0.05$
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.4.2 The effects of ZDHHC5 c.1803C>T on phenotypic traits in pigs

The results of the association analysis of ZDHHC5 c.1803C>T with carcass and meat quality traits are shown in Table 30. Because of the very low frequency of the genotype 'TT' in PI and DL, it was removed from the association analysis. For carcass traits, the homozygous genotype 'CC' was associated with lower MFR and FA in DUPI and higher BF1 in PIF1(b). For meat quality traits, the genotype 'CC' was associated with higher OPTO and COOK in PIF1(c) and PI. In DUPI, the genotype 'CC' was associated with lower COOK ($P<0.05$). The most significant association was found with DRIP in DL ($P<0.001$) where the homozygous genotype 'CC' animals had lower DRIP than the heterozygous animals.

Table 30: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across ZDHHC5 c.1803C>T in pigs

Traits	Populations	Least square means (LSM) (SE/n)		P-value
		CC	CT	
MFR	DUPI	0.32[a] (0.007/133)	0.34[b] (0.008/98)	0.0233
FA	DUPI	16.11[c] (0.28/133)	16.94[d] (0.31/98)	0.0233
COOK	DUPI	24.46[c] (0.26/133)	25.06[d] (0.27/98)	0.0337
COOK	PI	23.98[c] (0.23/180)	23.19[d] (0.32/63)	0.0121
BF1	PIF1(b)	3.01[c] (0.03/246)	2.87[d] (0.06/51)	0.0359
OPTO	PIF1(c)	68.23[c] (0.55/275)	66.25[d] (0.98/59)	0.0480
DRIP	DL	4.30[a] (0.20/231)	5.41[b] (0.33/42)	0.0004

[c-d] $P<0.05$, [a-b] $P<0.01$ Within rows, values with the same letter are not significantly different

4.2.5 Association analysis of CS

4.2.5.1 Genotype and allele frequencies of CS c.120G>T

Table 30 shows the genotype and allele frequencies of CS c.120G>T. The 'G' allele was prevalent (0.76-0.98) across all pig populations. The homozygous genotype 'GG' was the major genotype found in all populations, while the rare genotype 'TT' was detected only in PIF1(c) and DL. Moreover, there were disequilibria in the genotype distribution in DL (Table 31).

Table 31: Genotype and allele frequencies of CS c.120G>T in pigs

Populations	Genotype frequency			Allele frequency		HWE
	GG	GT	TT	G	T	
DUPI (n=277)	0.79	0.21	-	0.89	0.11	
PI (n=248)	0.96	0.04	-	0.98	0.02	NS
PIF1(a) (n=264)	0.86	0.14	-	0.93	0.07	NS
PIF1(b) (n=297)	0.89	0.11	-	0.94	0.06	NS
PIF1(c) (n=337)	0.74	0.25	0.02	0.86	0.14	NS
DL (n=277)	0.55	0.43	0.03	0.76	0.24	**

** P<0.01
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.5.2 The effects of CS c.120G>T on phenotypic traits in pigs

The association analysis of CS c.120G>T revealed significant associations with various carcass traits in PI, PIF1(a,b) and DL (Table 32). Only two genotypes 'GG' and 'GT' were used to analyse associations due to the very low frequency of the genotype 'TT', which was found only in PIF1(c) and DL. The homozygous genotype 'GG' was associated with lower carcass fatness in PIF1(b) and DL. In PI, the homozygous genotype 'GG' was associated with lower LEA. For meat quality traits, the genotype 'GG' was associated with lower DRIP in PIF1(a) and also related with lower CON1 and higher pH1 in PIF1(c).

Table 32: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across CS c.120G>T in pigs

| Traits | Populations | Least square means (LSM) (SE/n) | | P-value |
		GG	GT	
LEA	PI	58.00[c] (0.58/238)	61.67[d] (1.53/10)	0.0136
DRIP	PIF1(a)	1.94[a] (0.09/227)	2.39[b] (0.16/37)	0.0015
FA	PIF1(b)	14.18[c] (0.20/264)	15.21[d] (0.45/33)	0.0252
pH1	PIF1(c)	6.18[a] (0.03/248)	6.07[b] (0.04/83)	0.0081
CON1	PIF1(c)	5.07[c] (0.16/248)	5.55[d] (0.22/83)	0.0295
BF3	DL	1.79[a] (0.04/152)	1.93[b] (0.05/118)	0.0069
ABF	DL	2.52[c] (0.03/152)	2.62[d] (0.03/118)	0.0239

[c-d] P<0.05, [a-b] P<0.01

Within rows, values with the same letter are not significantly different

4.2.6 Association analysis of LYZ

4.2.6.1 Genotype and allele frequencies of LYZ c.240A>C

In Table 33 the frequencies of alleles and genotypes for c.240A>C is shown. In all populations, the 'A' allele occurred with low frequencies (0.17-0.34), resulting in the frequency of animals having the 'AA' genotype also being low (0.00-0.11). In general, the appearance of the homozygous genotype 'CC' was more frequent than that of the genotypes 'AC' and 'AA'.

Table 33: Genotype and allele frequencies of LYZ c.240A>C in pigs.

| Population | Genotype frequency | | | Allele frequency | | HWE |
	AA	AC	CC	A	C	
DUPI (n=404)	-	0.42	0.58	0.21	0.79	
PI (n=232)	0.04	0.27	0.69	0.17	0.83	NS
PIF1(a) (n=378)	0.03	0.29	0.68	0.17	0.83	NS
PIF1(b) (n=315)	0.07	0.37	0.57	0.25	0.75	NS
PIF1(c) (n=321)	0.06	0.38	0.56	0.25	0.75	NS
DL (n=252)	0.11	0.47	0.42	0.34	0.66	NS

NS = not significant

HWE = Hardy-Weinberg equilibrium

4.2.6.2 The effects of LYZ c.240A>C on phenotypic traits in pigs

For LYZ c.240A>C, the homozygous genotype 'AA' was associated with lower pH1 and higher OPTO in PIF1(a) and PIF1(b). The heterozygous 'AC' animals had lower DRIP and CON24 than the homozygous 'AA' animals in PI and the homozygous 'CC' animals in PIF1(a) respectively (Table 34).

Table 34: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across LYZ c.240A>C in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		AA	AC	CC	
DRIP	PI	2.13[a] (0.28/9)	1.32[b] (0.17/62)	1.53[ab] (0.15/161)	0.0030
pH1	PIF1(a)	6.03[ac] (0.08/12)	6.28[bcd] (0.03/108)	6.24[abd] (0.02/258)	0.0106
CON24	PIF1(a)	3.61[cd] (0.31/11)	3.23[c] (0.18/106)	3.53[d] (0.16/257)	0.0156
OPTO	PIF1(b)	71.23[ac] (1.29/22)	66.58[bcd] (0.64/115)	67.71[abd] (0.52/178)	0.0028

[c-d] P<0.05, [a-b] P<0.01
Within rows, values with the same letter are not significantly different

4.2.6.3 Genotype and allele frequencies of LYZ c.365A>T

In Table 35 the frequencies of alleles and genotypes for c.365A>T are shown. The 'A' allele occurred with high frequencies (0.59-0.98) across all pig populations. In general, the homozygous genotype 'AA' was the major genotype, while the minor genotype 'TT' was detected only in DUPI, PI and PIF1(b,c). In PIF1(a), the genotype distribution deviated from Hardy-Weinberg equilibrium.

Table 35: Genotypes and allele frequency of LYZ c.365A>T in pigs.

Population	Genotype frequency			Allele frequency		HWE
	AA	AT	TT	A	T	
DUPI (n=403)	0.66	0.33	0.02	0.82	0.18	
PI (n=230)	0.36	0.46	0.18	0.59	0.41	NS
PIF1(a) (n=378)	0.63	0.37	-	0.81	0.19	***
PIF1(b) (n=318)	0.73	0.26	0.02	0.86	0.14	NS
PIF1(c) (n=316)	0.59	0.34	0.07	0.76	0.24	NS
DL (n=261)	0.95	0.05	-	0.98	0.02	NS

*** P<0.001
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.6.4 The effects of LYZ c.365A>T on phenotypic traits in pigs

Significant associations of c.365A>T with ABF and LEA were found in PI and PIF1(c) respectively, where the homozygous genotype 'TT' animals had highest ABF and the 'T' allele tended to decrease LEA. For meat quality, the heterozygous genotype 'AT' tended to relate with higher CON1, whereas the homozygous genotype 'TT' pigs had highest CON24 in DUPI. Animals containing the genotype 'TT' had higher THAW than the

heterozygous animals in PIF1(b). Moreover, the 'T' carriers tended to produce carcasses with lower pH1 (Table 36).

Table 36: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across LYZ c.365A>T in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		AA	AT	TT	
CON24	DUPI	2.99[abc] (0.07/261)	2.85[acd] (0.09/131)	3.76[bd] (0.29/8)	0.0047
pH1	DUPI	6.56[c] (0.02/261)	6.52[cd] (0.02/131)	6.40[d] (0.07/8)	0.0472
ABF	PI	1.78[abc] (0.04/83)	1.77[acd] (0.03/106)	1.92[bd] (0.05/41)	0.0121
CON1	PI	4.42[c] (0.11/83)	4.65[d] (0.10/106)	4.43[cd] (0.13/41)	0.0444
LEA	PIF1(c)	53.85[c] (0.42/186)	53.01[cd] (0.53/106)	51.12[d] (1.11/22)	0.0494
THAW	PIF1(b)	8.93[cd] (0.20/221)	8.55[c] (0.26/76)	10.79[d] (0.81/5)	0.0153

[c-d] P<0.05, [a-b] P<0.01
Within rows, values with the same letter are not significantly different

4.2.6.5 Diplotype and haplotype frequencies of LYZ

In Table 37 the frequencies of diplotypes and haplotypes of LYZ is shown. Three haplotypes segregated across all pig populations, including AA (H1), CA (H2) and CT (H3). In general, the haplotype 'CA' occurred with high frequencies (0.42-0.63). Haplotype combination revealed six possible diplotypes in this study and there were some differences in the frequencies of diplotypes in each pig population. The main diplotype found in DUPI was 'H1/H2' whereas the main diplotype in PI was 'H2/H3'. For PIF1(a,b), the major diplotype was 'H2/H2'. In addition, in PIF1(c) and DL the most frequent diplotype was 'H1/H2'.

Table 37: Diplotype and haplotype frequencies of the LYZ gene in pigs

Haplotype	DUPI (n=285)	PI (n=221)	PIF1(a) (n=264)	PIF1(b) (n=304)	PIF1(c) (n=303)	DL (n=260)
AA[H1]	0.21	0.17	0.17	0.25	0.25	0.34
CA[H2]	0.61	0.42	0.64	0.60	0.51	0.63
CT[H3]	0.18	0.41	0.19	0.15	0.24	0.03
Diplotypes						
H1/H1	-	0.04	0.03	0.08	0.06	0.11
H1/H2	0.34	0.14	0.23	0.29	0.27	0.45
H1H3	0.08	0.11	0.06	-	0.12	0.02
H2/H2	0.31	0.18	0.37	0.44	0.27	0.39
H2/H3	0.25	0.34	0.31	0.17	0.21	0.03
H3/H3	0.02	0.18	-	0.02	0.07	-

4.2.6.6 The effects of the LYZ haplotype on phenotypic traits in pigs

The study revealed an association of the LYZ haplotype with CON24 in DUPI and PIF1(a). In DUPI, the diplotype 'H3/H3', which occurred with the lowest frequency, had the highest CON24, which was not seen in PIF1(a). In PIF1(a), the 'H2/H2' animals had higher CON24 than 'H1/H2' animals (P<0.05). LYZ haplotype effects on DRIP, pH1 and OPTO were found in PI, PIF1(a) and PIF1(b) respectively, where the 'H1/H1' animals provided high DRIP and OPTO values in PI and PIF1(b) respectively. In contrast, the 'H1/H1'animals had lower pH1 than the 'H1/H2' animals in PIF1(a). In DL(a), the diplotype 'H1/H3' had the lowest OPTO (Table 38).

Table 38: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across haplotypes of LYZ in pigs

	CON24 DUPI	DRIP PI	pH1 PIF1(a)	CON24 PIF1(a)	OPTO PIF1(b)	OPTO DL(a)
H1/H1	-	2.16[c] (0.29/9)	6.03[c] (0.08/12)	3.55[cd] (0.30/11)	71.27[ac] (1.25/22)	69.58[c] (1.18/27)
H1/H2	2.94[cd] (0.09/137)	1.45[cd] (0.19/32)	6.30[d] (0.04/83)	3.10[c] (0.18/81)	66.63[b] (0.71/80)	70.18[a] (0.62/113)
H1/H3	2.87[c] (0.15/33)	1.21[d] (0.20/26)	6.18[cd] (0.06/22)	3.46[cd] (0.24/18)	-	61.34[bd] (2.57/5)
H2/H2	3.05[cd] (0.09/124)	1.51[cd] (0.18/41)	6.24[cd] (0.03/136)	3.51[d] (0.16/124)	67.46[d] (0.59/121)	69.99[c] (0.65/99)
H2/H3	2.83[c] (0.09/98)	1.58[cd] (0.16/78)	6.25[cd] (0.03/114)	3.48[cd] (0.17/88)	67.99[cd] (0.86/47)	70.62[c] (2.04/8)
H3/H3	3.75[d] (0.30/8)	1.44[cd] (0.18/41)	-	-	70.93[cd] (2.53/5)	-
P-value	0.0185	0.0212	0.0137	0.0231	0.0101	0.0228

[c-d] P<0.05, [a-b] P<0.01
Within columns, values with the same letter are not significantly different

4.2.7 Association analysis of KERA

4.2.7.1 Genotype and allele frequencies of KERA c.303C>T

The distribution of the allele 'A' was 0.41, 0.31, 0.30, 0.28, 0.28 and 0.23 in DUPI, PI, PIF1(a), PIF1(b), PIF1(c) and DL respectively (Table 39). In general, low frequencies of the homozygous 'CC' animals were detected in all populations. However, a difference in the major genotype was found in some populations, in DUPI and PI the heterozygous 'CT' animals were more frequent than 'TT' animals, in contrast the homozygous 'TT' animals

were more frequent than heterozygous animals in other populations. Moreover, the allele distribution deviated from Hardy-Weinberg equilibrium in PI and PIF1(b).

Table 39: Genotype and allele frequencies of KERA c.303C>T in pigs

Populations	Genotype frequency			Allele frequency		HWE
	CC	CT	TT	C	T	
DUPI (n=413)	0.14	0.54	0.32	0.41	0.59	
PI (n=247)	0.07	0.49	0.44	0.31	0.69	*
PIF1(a) (n=421)	0.07	0.45	0.47	0.30	0.70	NS
PIF1(b) (n=329)	0.05	0.46	0.50	0.28	0.72	*
PIF1(c) (n=318)	0.08	0.41	0.52	0.28	0.72	NS
DL (n=268)	0.05	0.37	0.59	0.23	0.77	NS

* P<0.05
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.7.2 The effects of KERA c.303C>T on phenotypic traits in pigs

An effect of KERA c.303C>T on carcass traits was found with LEA in DL where the homozygous 'TT' animals had higher LEA than the heterozygous animals. For the analysis of meat quality, the significant effects were detected with pH in both stages in PI, there the 'C' carriers tended to produce meat with higher pH1 and the animals with genotype 'CC' had higher pH24 compared to the heterozygous animals (Table 40).

Table 40: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across KERA c.303C>T in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		CC	CT	TT	
pH1	PI	6.54[c] (0.05/17)	6.49[cd] (0.03/121)	6.44[d] (0.03/109)	0.0358
pH24	PI	5.60[c] (0.03/17)	5.52[d] (0.01/121)	5.53[cd] (0.01/109)	0.0273
LEA	DL	44.21[cd] (1.38/10)	44.02[c] (0.56/89)	42.51[d] (0.50/141)	0.0324

[c-d] P<0.05, [a-b] P<0.01
Within rows, values with the same letter are not significantly different

4.2.8 Association analysis of COQ9

4.2.8.1 Genotype and allele frequencies of COQ9 c.453A>G

Allele and genotype frequencies of COQ9 c.453A>G are displayed in Table 41. The distribution of the allele 'A' varied from 0.36 in PI to 0.55 in PIF1(b). In all populations, the frequencies of the heterozygous genotype 'AG' were higher than those of the

genotypes 'AA' and 'GG'. In DUPI and PIF1(b), the 'AA' genotype occurred with higher frequency than the 'GG' genotype. In contrast, in PI, PIF1(a,c) and DL, the frequency of the 'AA' genotype was lower than that of the 'GG' genotype. Moreover, significant disequilibria in allele distribution was found in PIF1(a).

Table 41: Genotype and allele frequencies of COQ9 c.453A>G in pigs

Populations	Genotype frequency			Allele frequency		HWE
	AA	AG	GG	A	G	
DUPI (n=287)	0.22	0.62	0.16	0.53	0.47	
PI (n=229)	0.11	0.51	0.38	0.36	0.64	NS
PIF1(a) (n=284)	0.13	0.54	0.33	0.40	0.60	*
PIF1(b) (n=305)	0.31	0.47	0.21	0.55	0.45	NS
PIF1(c) (n=333)	0.15	0.51	0.34	0.40	0.60	NS
DL (n=266)	0.22	0.50	0.28	0.47	0.53	NS

* $P < 0.05$
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.8.2 The effects of COQ9 c.453A>G on phenotypic traits in pigs

The effects of COQ9 c.453A>G on different phenotypes are shown in Table 42. In PIF1(b), pigs carrying the 'AA' genotype produced higher fatness trait values, including BF2, MFR and FA compared to those having the 'AG' or 'GG' genotype, which is the most pronounced of all effects that were found on FA ($P < 0.001$). In addition, the 'A' carriers had increased pH1 in DUPI; in PI the heterozygous animals tended to produce meat with higher SF than the homozygous 'GG' animals.

Table 42: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across COQ9 c.453A>G in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		AA	AG	GG	
pH1	DUPI	6.63[c] (0.04/45)	6.52[cd] (0.02/164)	6.49[d] (0.03/44)	0.0311
SF	PI	37.10[cd] (1.89/25)	40.23[c] (1.40/117)	37.91[d] (1.49/86)	0.0334
FA	PIF1(b)	15.16[a] (0.28/96)	14.07[b] (0.24/144)	13.84[b] (0.32/65)	0.0004
MFR	PIF1(b)	0.28[c] (0.01/96)	0.26[d] (0.01/144)	0.26[d] (0.01/65)	0.0305
BF2	PIF1(b)	1.53[c] (0.03/96)	1.45[d] (0.02/144)	1.44[d] (0.04/65)	0.0433

[c-d] $P < 0.05$, [a-b] $P < 0.01$
Within rows, values with the same letter are not significantly different

4.2.8.3 Genotype and allele frequencies of COQ9 +1247A>T

Table 43 gives the allele and genotype frequencies of COQ9 +1247A>T. The distribution of the allele 'A' varied from 0.45 in PIF1(b) to 0.64 in PI. In all populations, the frequencies of the heterozygous genotype 'AG' were higher than those of the 'AA' and 'TT' genotypes. In DUPI, PI and PIF1 (a,c), the genotype 'TT' animals were less frequent than the 'AA' animals. Moreover, significant disequilibria in allele distribution was found in PIF1(a).

Table 43: Genotype and allele frequencies of COQ9 +1247A>T in pigs.

Populations	Genotype frequency			Allele frequency		HWE
	AA	AT	TT	A	T	
DUPI (n=287)	0.29	0.66	0.05	0.62	0.38	
PI (n=229)	0.38	0.51	0.11	0.64	0.36	NS
PIF1(a) (n=284)	0.33	0.54	0.13	0.60	0.40	*
PIF1(b) (n=305)	0.21	0.47	0.31	0.45	0.55	NS
PIF1(c) (n=333)	0.30	0.52	0.17	0.56	0.44	NS
DL (n=266)	0.24	0.47	0.29	0.48	0.52	NS

* $P<0.05$
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.8.4 The effects of COQ9 +1247A>T on phenotypic traits in pigs

COQ9 +1247A>T significantly influenced FA ($P<0.001$) in PIF1(b); the 'TT' pigs offered higher FA and BF2 compared to those having the 'AT' or 'AA' genotype. In DUPI, the 'T' allele reduced backfat thickness (ABF and BF1). The results showed that BF1 is highly related to COQ9 +1247A>T ($P<0.001$). On the other hand, the effects of this SNP on MFR and LEA indicated that the 'T' allele also increased carcass leanness. For meat quality, COQ9 +1247A>T was associated with SF, OPTO and THAW in PI, DL and DUPI respectively. In the LD muscle, pigs carrying the 'AT' genotype tended to have higher SF value than pigs carrying the 'AA' genotype. In addition, higher values for OPTO were found in 'AT' animals compared to 'TT' animals. THAW of homozygous 'TT' pigs was higher than in 'AT' or 'AA' pigs (Table 44).

Table 44: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across COQ9 +1247A>T in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		AA	AT	TT	
LEA	DUPI	49.64^c (0.59/78)	50.79^{cd} (0.45/165)	54.04^d (1.61/10)	0.0218
MFR	DUPI	0.34^c (0.008/78)	0.33^c (0.006/165)	0.27^d (0.022/10)	0.0181
BF1	DUPI	3.49^{ac} (0.05/78)	3.34^{abd} (0.04/165)	2.96^{bc} (0.15/10)	0.0008
ABF	DUPI	2.19^{ac} (0.03/78)	2.10^{abd} (0.03/165)	1.88^{bcd} (0.10/10)	0.0043
THAW	DUPI	8.11^a (0.30/78)	8.06^a (0.24/165)	10.54^b (0.72/10)	0.0027
SF	PI	37.91^c (1.49/86)	40.23^d (1.40/117)	37.10^{cd} (1.89/25)	0.0334
FA	PIF1(b)	13.84^a (0.32/65)	14.07^a (0.24/144)	15.16^b (0.28/96)	0.0004
MFR	PIF1(b)	0.26^c (0.01/65)	0.26^c (0.01/144)	0.28^d (0.01/96)	0.0305
BF2	PIF1(b)	1.44^c (0.04/65)	1.45^d (0.02/144)	1.53^d (0.03/96)	0.0433
OPTO	DL	69.11^{cd} (0.75/65)	70.86^c (0.56/124)	68.71^d (0.70/77)	0.0226

$^{c-d}$ P<0.05, $^{a-b}$ P<0.01
Within rows, values with the same letter are not significantly different

4.2.8.5 Diplotype and haplotype frequencies of COQ9

Table 45 displays the distribution of the COQ9 haplotypes in different pig populations. Four haplotypes were observed in this study, including AA (H1), AT (H2), GA (H3) and GT (H4). Three haplotypes were found in DUPI, PIF1(c) and DL, whereas only two haplotypes were observed in PI and PIF1(a,b). In general, the base haplotypes in all populations were 'H3' and 'H2', but there were the additional haplotypes 'H1' in DUPI and 'H4' in PIF1(c) and DL.

Table 45: Diplotype and haplotype frequencies of the COQ9 gene in pigs

Haplotype	DUPI (n=138)	PI (n=229)	PIF1(a) (n=284)	PIF1(b) (n=305)	PIF1(c) (n=332)	DL (n=266)
AA [H1]	0.19	-	-	-	-	-
AT [H2]	0.36	0.36	0.40	0.55	0.40	0.47
GA[H3]	0.45	0.64	0.60	0.45	0.57	0.48
GT [H4]	-	-	-	-	0.03	0.05
Diplotypes						
H1/H1	0.06	-	-	-	-	-
H1/H2	0.29	-	-	-	-	-
H1/H3	0.32	-	-	-	-	-
H2/H2	0.11	0.11	0.13	0.31	0.15	0.22
H2/H3	-	0.51	0.54	0.47	0.49	0.43
H2/H4	-	-	-	-	0.02	0.07
H3/H3	0.22	0.38	0.33	0.21	0.30	0.24
H3/H4	-	-	-	-	0.04	0.03

4.2.8.6 The effects of the COQ9 haplotype on phenotypic traits in pigs

The association analysis of the COQ9 haplotypes revealed a significant association with BF1 in DUPI, SF in PI, BF2, FA and MFR in PIF1(b) and LEA and OPTO in DL. In PI, the meat of animals with the diplotype 'H2/H3' had the highest SF values. In DUPI, animals having the 'H1/H2' diplotype had higher BF1 than the 'H2/H2' animals. Moreover, animals having the 'H2/H2' diplotype had the highest values of BF2, FA and MFR in PIF1(b). In DL, animals having the 'H3/H4' diplotype had lowest LEA; in contrast, the 'H3/H4' diplotype offered the highest OPTO values (Table 46).

Table 46: Least square means (LSM) and standard errors (SE) for carcass traits across haplotypes of COQ9 in pigs

	BF1 DUPI	SF PI	BF2 PIF1(b)	FA PIF1(b)	MFR PIF1(b)	LEA DL	OPTO DL
H1/H1	3.45[cd] (0.17/8)	-	-	-	-	-	-
H1/H2	3.44[c] (0.10/40)	-	-	-	-	-	-
H1/H3	3.44[cd] (0.09/44)	-	-	-	-	-	-
H2/H2	2.98[d] (0.15/15)	37.10[cd] (1.89/25)	1.53[c] (0.03/96)	15.16[a] (0.28/96)	0.28[c] (0.01/96)	43.41[c] (0.66/51)	68.49[c] (0.80/59)
H2/H3	-	40.23[c] (1.40/117)	1.45[d] (0.02/144)	14.07[b] (0.23/144)	0.26[d] (0.01/144)	43.09[c] (0.54/104)	70.53[cd] (0.60/115)
H2/H4	-	-	-	-	-	42.30[abcd] (1.07/16)	69.68[cd] (1.41/18)
H3/H3	3.56[cd] (0.15/31)	37.91[d] (1.49/86)	1.44[cd] (0.04/65)	13.84[b] (0.32/65)	0.26[d] (0.01/65)	43.58[a] (0.66/58)	69.06[cd] (0.76/65)
H3/H4	-	-	-	-	-	38.04[bd] (1.60/6)	74.45[d] (1.95/9)
P-value	0.0354	0.0334	0.0433	0.0004	0.0305	0.0185	0.0209

[c-d] $P<0.05$, [a-b] $P<0.01$
Within columns, values with the same letter are not significantly different

4.2.9 Association analysis of UN

4.2.9.1 Genotype and allele frequencies of UN g.1,022,434G>T

The genotype and allele frequencies of UN g.1,022,434G>T are shown in Table 47. The major allele across all populations except DL was the 'G' allele, resulting in the frequencies of the homozygous genotype 'GG' and the heterozygous genotype 'GT' being higher than those of the homozygous genotype 'TT'; whereas in DL the homozygous

genotype 'GG' had a lower frequency than the other two genotypes. Moreover, a genotype distribution that fit into Hardy-Weinberg equilibrium was found only in PI.

Table 47: Genotype and allele frequencies of UN g.1,022,434G>T in pigs.

Populations	Genotype frequency			Allele frequency		HWE
	GG	GT	TT	G	T	
DUPI (n=285)	0.32	0.54	0.14	0.59	0.41	
PI (n=228)	0.80	0.20	-	0.90	0.10	NS
PIF1(a) (n=288)	0.49	0.50	0.01	0.74	0.26	***
PIF1(b) (n=305)	0.33	0.54	0.13	0.60	0.40	*
PIF1(c) (n=329)	0.39	0.56	0.05	0.67	0.33	***
DL (n=266)	0.17	0.56	0.27	0.45	0.55	*

* $P<0.05$, *** $P<0.001$
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.9.2 The effects of UN g.1,022,434G>T on phenotypic traits in pigs

Among parameters for carcass traits, UN g.1,022,434G>T was associated with several fatness traits in PIF1(b,c) and DUPI (Table 48). Most of the significant differences were found between the homozygous 'GG' and the heterozygous 'GT', whereas the homozygous 'TT' was not significantly different. In PIF1(c), pigs bearing the genotype 'GG' had higher backfat thickness traits (FA, BF1 and ABF) than the heterozygous pigs ($P<0.05$).

Table 48: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across UN g.1,022,434G>T in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		GG	GT	TT	
BF2	DUPI	1.57[c] (0.04/77)	1.67[d] (0.03/136)	1.57[cd] (0.06/39)	0.0203
BF3	DUPI	1.26[c] (0.04/77)	1.39[d] (0.03/136)	1.38[cd] (0.06/39)	0.0170
ABF	DUPI	2.05[c] (0.03/77)	2.15[d] (0.03/136)	2.14[cd] (0.05/39)	0.0324
DRIP	PI	1.47[c] (0.15/182)	1.73[d] (0.17/46)	-	0.0318
SF	PI	38.04[b] (1.12/181)	42.03[a] (1.49/46)	-	0.0012
FA	PIF1(c)	15.28[c] (0.30/129)	14.51[d] (0.26/183)	13.82[cd] (0.81/16)	0.0480
BF1	PIF1(c)	3.45[c] (0.04/129)	3.33[d] (0.03/184)	3.34[cd] (0.10/16)	0.0337
BF2	PIF1(c)	1.95[c] (0.03/129)	1.90[cd] (0.03/184)	1.70[d] (0.09/16)	0.0347
ABF	PIF1(c)	2.25[c] (0.03/129)	2.18[d] (0.02/184)	2.10[cd] (0.08/16)	0.0485
pH24	DL	5.45[c] (0.07/40)	5.50[d] (0.01/134)	5.47[cd] (0.02/62)	0.0214

[c-d] $P<0.05$, [a-b] $P<0.01$
Within rows, values with the same letter are not significantly different

In DUPI, pigs bearing the genotype 'GG' had lower backfat thickness traits (ABF, BF2 and BF3) than the heterozygous pigs (P<0.05). In addition, lower DRIP and SF were found in pigs with the 'GG' genotype compared to those with the 'GT' genotype in PI. In DL, the homozygous 'GG' animals had lower pH24 than the heterozygous animals (P<0.05).

4.2.10 Association analysis of EGFR

4.2.9.1 Genotype and allele frequencies of EGFR c.3543A>G

The allele and genotype frequencies of EGFR c.3543A>G are shown in Table 49. EGFR c.3543A>G occurred in all pig populations with frequencies varying from 0.33 in DL to 0.73 in PIF1(a). Moreover, the appearance of the heterozygous genotype was lower than expected in PI. Low frequencies of the homozygous 'GG' animals were observed in PI and PIF1(a,b,c) whereas in DUPI and DL, the homozygous genotype 'AA' occurred with low frequencies. From the three genotypes ('AA', 'AG' and 'GG'), the 'AA' genotype was predominant in PI and PIF1(a), whereas in PIF1(b,c) the major genotype was the heterozygous 'AG'.

Table 49: Genotype and allele frequencies of EGFR c.3543A>G in pigs.

Populations	Genotype frequency			Allele frequency		HWE
	AA	AG	GG	A	G	
DUPI (n=277)	0.18	0.58	0.23	0.48	0.52	
PI (n=215)	0.51	0.35	0.14	0.69	0.31	**
PIF1 (a) (n=279)	0.53	0.41	0.06	0.73	0.27	NS
PIF1 (b) (n=305)	0.28	0.49	0.23	0.52	0.48	NS
PIF1 (c) (n=290)	0.28	0.54	0.17	0.56	0.44	NS
DL (n=252)	0.12	0.44	0.45	0.33	0.67	NS

** P<0.001
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.10.2 The effects of EGFR c.3543A>G on phenotypic traits in pigs

The effects of the EGFR c.3543A>G on different phenotypes are shown in Table 50. In general, this SNP was related to various carcass fatness traits in DUPI. Results showed that the 'G' allele increased backfat thickness, especially in BF3 (P<0.01). Moreover, pigs carrying the 'AA' genotype appeared to have higher LEA compared to the 'AG' pigs (P<0.05). However, in PIF1(b) the heterozygous pigs had higher BF1 than the 'GG' pigs.

In addition, the meat of the animals having the 'AA' genotype had higher CON24 and lower OPTO values compared to the heterozygous animals in PIF1(a) and PI respectively, whereas in DUPI the meat of the heterozygous pigs appeared to have high THAW (P<0.05).

Table 50: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across EGFR c.3543A>G in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		AA	AG	GG	
LEA	DUPI	52.38^c (0.83/42)	49.96^d (0.49/142)	50.15^{cd} (0.69/59)	0.0321
BF3	DUPI	1.19^{ac} (0.06/42)	1.35^{abd} (0.03/142)	1.48^{cb} (0.05/59)	0.0011
ABF	DUPI	2.02^c (0.05/42)	2.12^{cd} (0.03/142)	2.19^d (0.04/59)	0.0403
THAW	DUPI	8.04^{cd} (0.41/42)	8.38^c (0.28/142)	7.59^d (0.35/59)	0.0461
OPTO	PI	71.35^c (0.99/110)	73.70^d (1.05/75)	71.37^{cd} (1.39/30)	0.0330
CON24	PIF1(a)	3.54^a (0.17/147)	3.20^b (0.18/111)	3.45^{ab} (0.26/17)	0.0138
BF1	PIF1(b)	2.99^{ab} (0.05/86)	3.07^a (0.04/148)	2.89^b (0.05/71)	0.0117
pH24	PIF1(b)	5.53^c (0.01/86)	5.50^d (0.01/148)	5.50^d (0.01/71)	0.0475

$^{c-d}$ P<0.05, $^{a-b}$ P<0.01
Within rows, values with the same letter are not significantly different

4.2.11 Association analysis of VTN

4.2.11.1 Genotype and allele frequencies of VTN c.154A>G

The major allele of VTN c.154A>G across all commercial pig populations was the allele 'A' whereas the 'A' allele was slightly lower than the 'G' allele in DUPI; therefore the homozygous genotype 'AA' or the heterozygous genotype 'AG' was more frequent than the homozygous genotype 'GG' in commercial populations, but in DUPI, the animals with the genotype 'AA' were least frequent. In addition, the genotype distribution deviated from Hardy-Weinberg equilibrium in PIF1(b) (Table 51).

Table 51: Genotype and allele frequencies of VTN c.154A>G in pigs.

Populations	Genotype frequency			Allele frequency		HWE
	AA	AG	GG	A	G	
DUPI (n=356)	0.19	0.53	0.28	0.46	0.54	
PI (n=222)	0.36	0.50	0.15	0.60	0.40	NS
PIF1 (a) (n=292)	0.50	0.38	0.12	0.69	0.31	NS
PIF1 (b) (n=291)	0.46	0.45	0.08	0.69	0.31	NS
PIF1 (c) (n=313)	0.42	0.50	0.09	0.67	0.33	*
DL (n=240)	0.57	0.37	0.07	0.75	0.25	NS

* P<0.05
NS = not significant   HWE = Hardy-Weinberg equilibrium

4.2.11.2 The effects of VTN c.154A>G on phenotypic traits in pigs

Table 52 represents the effects of VTN c.154A>G on the traits analyzed in PIF1(a,b,c) and DUPI. VTN c.154A>G was associated with ABF in DUPI; lower ABF was found in pigs with the 'AA' genotype and higher ABF was seen in 'GG' pigs (P<0.05). Moreover, several meat quality traits were found to be associated with this SNP by the change of the homozygous 'AA' to the heterozygous 'AG' genotype; the animals carrying the 'AA' genotype had lower muscle pH24 and COOK than the 'AG' animals in PIF1(a) and PIF1(b) respectively. In DUPI, the animals carrying the 'AA' genotype had highest OPTO and CON24. In addition, higher DRIP was found in 'AA' animals compared to 'GG' animals in PIF1(a) (P<0.05). Also, there seemed to be a tendency of low pH1 and CON1 values in heterozygous pigs compared to 'GG' and/or 'AA' pigs in PIF1(c) and DUPI.

Table 52: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across VTN c.154A>G in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		AA	AG	GG	
ABF	DUPI	2.06[c] (0.04/63)	2.14[cd] (0.03/149)	2.18[d] (0.04/84)	0.0499
CON1	DUPI	4.38[cd] (0.10/63)	4.23[c] (0.08/149)	4.43[d] (0.09/84)	0.0443
CON24	DUPI	3.08[c] (0.11/63)	2.77[d] (0.08/149)	2.86[cd] (0.10/84)	0.0219
OPTO	DUPI	70.55[c] (0.90/63)	68.27[d] (0.75/149)	68.20[d](0.87/84)	0.0143
pH24	PIF1(a)	5.57[a] (0.02/145)	5.61[b] (0.02/112)	5.60[ab] (0.03/35)	0.0097
DRIP	PIF1(a)	2.10[c] (0.12/145)	1.87[cd] (0.12/113)	1.73[d] (0.16/35)	0.0122
COOK	PIF1(b)	25.02[c] (0.22/133)	25.54[d] (0.22/136)	25.68[cd] (0.33/33)	0.0198
pH1	PIF1(c)	6.17[c] (0.03/128)	6.09[d] (0.03/152)	6.25[c] (0.07/26)	0.0240

[c-d] P<0.05, [a-b] P<0.01
Within rows, values with the same letter are not significantly different

4.2.11.3 Genotype and allele frequencies of VTN c.156C>T

Table 53 represents the genotype and allele frequencies of VTN c.156C>T. The data from all pig populations indicated that the major allele was the allele 'T', resulting in the heterozygous genotype 'CT' and the homozygous genotype 'TT' being more frequent than the homozygous genotype 'CC'; especially in DUPI this genotype was not detected. A deviation from Hardy-Weinberg equilibrium was observed in PIF1(c).

Table 53: Genotype and allele frequencies of VTN c.156C>T in pigs.

Populations	Genotype frequency			Allele frequency		HWE
	CC	CT	TT	C	T	
DUPI (n=356)	-	0.21	0.79	0.11	0.89	
PI (n=222)	0.15	0.50	0.36	0.40	0.60	NS
PIF1 (a) (n=292)	0.12	0.38	0.50	0.31	0.69	NS
PIF1 (b) (n=291)	0.08	0.45	0.46	0.31	0.69	NS
PIF1 (c) (n=308)	0.07	0.48	0.45	0.31	0.69	*
DL (n=240)	0.07	0.37	0.57	0.25	0.75	NS

* $P<0.05$
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.11.4 The effects of VTN c.156C>T on phenotypic traits in pigs

For VTN c.156C>T, only two genotypes ('CT' and 'TT') were detected in DUPI, whereas the 'CC' genotype was seen in the other populations. The different genotypes of this SNP had effects on several carcass and meat quality traits. In DUPI, the results showed that BF2 is dependent on the genotype, the 'TT' pigs producing higher carcass fatness and lower leanness. For meat quality traits, the 'TT' pigs also had lower pH24 and COOK compared to 'CT' pigs in PIF1(a) and PIF1(b). On the other hand, the 'T' carriers had increased pH1 and DRIP in DUPI and PIF1(a) respectively (Table 54).

Table 54: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across VTN c.156C>T in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		CC	CT	TT	
pH24	PIF1(a)	5.60[cd] (0.03/35)	5.61[c] (0.02/111)	5.57[d] (0.02/146)	0.0160
DRIP	PIF1(a)	1.73[c] (0.16/35)	1.88[cd] (0.12/112)	2.10[d] (0.12/146)	0.0160
COOK	PIF1(b)	25.72[cd] (0.39/24)	25.53[c] (0.23/138)	25.04[d] (0.22/140)	0.0325
MFR	DUPI	-	0.32[c] (0.010/66)	0.34[d] (0.006/230)	0.0394
BF2	DUPI	-	1.56[c] (0.05/66)	1.66[d] (0.03/230)	0.0456
pH1	DUPI	-	6.50[c] (0.03/66)	6.57[d] (0.02/230)	0.0271

[c-d] $P<0.05$, [a-b] $P<0.01$
Within rows, values with the same letter are not significantly different

4.2.11.5 Diplotype and haplotype frequencies of VTN

When combining two SNPs to construct a haplotype, three haplotypes were segregating in DUPI (b) and PIF1(c), whereas in the other populations only two haplotypes could be found (Table 55). The major haplotype across all populations was 'AT', being named 'H1',

whose frequencies varied from 0.45 in DUPI to 0.75 in DL. Five diplotypes were found in DUPI and PIF1(c) whereas the other populations contained only three diplotypes. In commercial pigs, the diplotypes 'H1/H1' or 'H1/H2' were the major diplotypes whereas in DUPI, the main diplotype was 'H1/H3'.

Table 55: Diplotype and haplotype frequencies of the VTN gene in pigs

Haplotype	DUPI (n=138)	PI (n=229)	PIF1(a) (n=284)	PIF1(b) (n=305)	PIF1(c) (n=332)	DL (n=266)
AT [H1]	0.45	0.60	0.69	0.69	0.66	0.75
GC [H2]	0.11	0.40	0.31	0.31	0.31	0.25
GT [H3]	0.44	-	-	-	0.03	-
Diplotypes						
H1/H1	0.19	0.36	0.50	0.46	0.42	0.57
H1/H2	0.12	0.49	0.38	0.45	0.45	0.37
H1/H3	0.41	0.15	0.12	0.08	0.04	0.07
H2/H2	-	-	-	-	0.07	-
H2/H3	0.09	-	-	-	0.02	-
H3/H3	0.19	-	-	-	-	-

4.2.11.4 The effects of VTN haplotypes on phenotypic traits in pigs

The effects of the VTN haplotype on the phenotype are shown in Table 56. The association between haplotype and phenotype traits confirmed the results described in the previous sections indicating that VTN has a significant relation with some traits. For example pH1, DRIP and COOK. In DUPI, the diplotype 'H1/H3' provided highest fatness (BF2 and FA) and also provided highest MFR and pH1. Moreover, the diplotype 'H1/H1'offered lowest pH24 and COOK in PIF1(a and b) but associated with highest DRIP in PIF1(a).

Table 56: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across haplotypes of VTN in pigs

	BF2 DUPI	MFR DUPI	FA DUPI	pH1 DUPI	pH24 PIF1(a)	DRIP PIF1(a)	COOK PIF1(b)
H1/H1	1.60[c] (0.04/68)	0.33[cd] (0.01/68)	16.27[cd] (0.35/68)	6.53[c] (0.03/68)	5.57[c] (0.02/145)	2.11[c] (0.12/145)	25.02[c] (0.22/133)
H1/H2	1.59[cd] (0.05/44)	0.31[c] (0.01/44)	15.79[c] (0.42/44)	6.50[c] (0.03/44)	5.61[d] (0.02/111)	1.88[cd] (0.12/112)	25.53[d] (0.23/129)
H1/H3	1.71[d] (0.03/146)	0.34[d] (0.01/146)	16.85[d] (0.27/146)	6.59[d] (0.02/146)	-	-	-
H2/H2	-	-	-	-	5.60[cd] (0.03/35)	1.73[d] (0.16/35)	25.71[cd] (0.39/24)
H2/H3	1.54[c] (0.06/32)	0.32[cd] (0.01/32)	15.75[c] (0.50/32)	6.51[c] (0.04/32)	-	-	-
H3/H3	1.66[cd] (0.04/66)	0.34[cd] (0.01/66)	16.79[cd] (0.35/66)	6.56[cd] (0.03/66)	-	-	-
P-value	0.0031	0.0294	0.0483	0.0331	0.0123	0.0134	0.0262

[c-d] P<0.05, [a-b] P<0.01
Within columns, values with the same letter are not significantly different

4.2.12 Association analysis of ZYX

4.2.12.1 Genotype and allele frequencies of ZYX c.279C>T

Allele and genotype frequencies of ZYX c.279C>T are shown in Table 57. In general, the 'C' allele was a major allele resulting in the homozygous genotype 'CC' being more frequent than the genotypes 'CT' and 'TT' respectively. However, in F1 the frequency of heterozygous animals was higher than that of the homozygous 'CC' animals and the genotype distribution was not in Hardy-Weinberg equilibrium because the number of heterozygous animal was higher than expected (P<0.05).

Table 57: Genotype and allele frequencies of ZYX c.279C>T in pigs.

Population	Genotype frequency			Allele frequency		HWE
	CC	CT	TT	C	T	
PIF1 (n=300)	0.68	0.29	0.03	0.83	0.17	NS
DL (n=192)	0.55	0.38	0.07	0.74	0.26	NS
F1 (n=188)	0.43	0.51	0.07	0.68	0.32	*
PI (n=190)	0.80	0.18	0.02	0.89	0.11	NS

* P<0.05
NS = not significant
HWE = Hardy-Weinberg equilibrium
4.2.12.2 The effects of ZYX c.279C>T on phenotypic traits in pigs

The association analysis of c. 279 C>T for carcass traits revealed significant associations with various measures of carcass fat as well as pH24 and OPTO in PI (Table 58). Significant differences were only found between the homozygous `CC´ and the heterozygous `CT´ genotypes, whereas the rare homozygous genotype `TT´ was not significantly different. The genotype `CC´ was associated with higher backfat thickness as well as higher pH24 and OPTO value in PI.

Table 58: Least square means (LSM) and standard errors (SE) for meat quality and carcass traits across ZYX c.279C>T in pigs

Traits	Population	Least square means (LSM) (SE/n)			P-value
		CC	CT	TT	
BF1	PI	3.11[c] (0.05/152)	2.86[d] (0.09/35)	2.92[cd] (0.25/3)	0.0265
BF2	PI	1.51[a] (0.03/152)	1.34[b] (0.05/35)	1.17[ab] (0.15/3)	0.0037
BF3	PI	0.88[c] (0.03/152)	0.73[d] (0.05/35)	0.81[cd] (0.14/3)	0.0158
ABF	PI	1.83[a] (0.03/152)	1.64[b] (0.05/35)	1.65[ab] (0.15/3)	0.0023
OPTO	PI	63.61[c] (0.86/152)	59.48[d] (1.45/35)	57.74[cd] (3.98/3)	0.0223
pH24	PI	5.51[c] (0.01/152)	5.46[d] (0.02/35)	5.42[cd] (0.05/3)	0.0194

[c-d] $P<0.05$, [a-b] $P<0.01$
Within rows, values with the same letter are not significantly different

4.2.12.3 Genotype and allele frequencies of ZYX c.399A>G

Table 59 represents the genotype and allele distribution of ZYX c.399A>G. The presence of the allele 'A' was higher than that of the allele 'G' across all populations varying from 0.78 in F1 to 0.93 in PI, resulting in the homozygous genotype 'AA' being more frequent than the genotypes 'AG' and 'GG'. However, a genotype distribution that fit Hardy-Weinberg equilibrium was detected only in PIF1.

Table 59: Genotype and allele frequencies of ZYX c.279C>T in pigs.

Population	Genotype frequency			Allele frequency		HWE
	AA	AG	GG	A	G	
PIF1 (n=300)	0.67	0.25	0.09	0.79	0.21	NS
DL (n=192)	0.87	0.04	0.09	0.89	0.11	***
F1 (n=188)	0.70	0.16	0.14	0.78	0.22	***
PI (n=190)	0.89	0.09	0.02	0.93	0.07	***

*** $P<0.001$
NS = not significant HWE = Hardy-Weinberg equilibrium

4.2.12.4 The effects of ZYX c.399A>G on phenotypic traits in pigs

Significant associations of the SNP c.399A>G are displayed in Table 60. The homozygous genotype of the minor allele `G´ is associated with significant higher least square means of fat measures (FA, BF3 and ABF) in DL.

Table 60: Least square means (LSM) and standard errors (SE) for carcass traits across ZYX c.399A>G in pigs

Traits	Population	Least square means (LSM) (SE/n)			P-value
		AA	AG	GG	
FA	DL	20.38^c (0.41/159)	21.91^{cd} (1.11/8)	22.43^d (0.82/16)	0.0259
BF3	DL	1.68^c (0.05/159)	1.85^{cd} (0.15/8)	1.95^d (0.11/16)	0.0294
ABF	DL	2.36^c (0.05/159)	2.55^{cd} (0.12/8)	2.55^d (0.09/16)	0.0328

$^{c-d}$ P<0.05, $^{a-b}$ P<0.01
Within rows, values with the same letter are not significantly different

4.2.12.5 Genotype and allele frequencies of ZYX c.522A>G

In Table 61 the frequencies of alleles and genotypes for ZYX c.522A>G are shown. In all populations, the 'A' allele occurred with low frequency (0.03-0.24), resulting in the frequency of animals having the 'AA' genotype also being low (0.00-0.11). In general, the appearance of the homozygous genotype 'GG' was more frequent than that of the heterozygous genotype 'AG' and the homozygous genotype 'AA'. Moreover, only in PIF1(b) and PI were the genotype distributions in Hardy-Weinberg equilibrium.

Table 61: Genotype and allele frequencies of ZYX c.522A>G in pigs.

Population	Genotype frequency			Allele frequency		HWE
	AA	AG	GG	A	G	
PIF1 (n=300)	0.08	0.34	0.59	0.24	0.76	NS
DL (n=192)	0.06	0.07	0.87	0.10	0.90	***
F1 (n=188)	0.11	0.21	0.68	0.21	0.79	***
PI (n=190)	-	0.07	0.93	0.03	0.97	NS

*** P<0.001
NS = not significant
HWE = Hardy-Weinberg equilibrium

4.2.12.6 The effects of ZYX c.522A>G on phenotypic traits in pigs

The effects of ZYX c.522A>G on carcass traits are displayed in Table 62. The results confirmed the effects from c.399A>G but this SNP shows a higher level of significance.

The homozygous genotype 'AA' is associated with significantly higher least square means of fat measures (FA, MFR, ABF and BF3) in DL.

Table 62: Least square means (LSM) and standard errors (SE) for carcass traits across ZYX c.522A>G in pigs

Traits	Populations	Least square means (LSM) (SE/n)			P-value
		AA	AG	GG	
FA	DL	23.71[a] (0.96/10)	19.36[b] (0.88/12)	20.41[b] (0.38/144)	0.0012
MFR	DL	0.58[a] (0.03/10)	0.43[b] (0.03/12)	0.48[b] (0.01/144)	0.0013
BF3	DL	2.22[a] (0.12/10)	1.56[b] (0.08/12)	1.69[b] (0.04/144)	0.0001
ABF	DL	2.73[a] (0.11/10)	2.28[b] (0.10/12)	2.36[b] (0.04/144)	0.0016

[c-d] $P<0.05$, [a-b] $P<0.01$
Within rows, values with the same letter are not significantly different

4.2.12.7 Diplotype and haplotype frequencies of ZYX

After combining three SNPs to construct a haplotype, two to four haplotypes were segregating across the populations. In detail, in DL, only two haplotypes segregated (H1 and H4), three haplotypes, including H1, H3 and H4 segregated in PI, whereas in F1 and PIF1 four haplotypes (H1, H2, H4 and H5) could be detected. Moreover, different diplotype frequencies were observed across all populations. In general, the diplotype 'H1/H1' was the major diplotype found in PIF1, DL and PI, whereas in F1 the diplotype 'H1/H4' was more frequent than the other diplotypes (Table 63).

Table 63: Diplotype and haplotype frequencies of ZYX in pigs.

Haplotype	PIF1 (n=300)	DL (n=192)	F1 (n=188)	PI (n=190)
CAG [H1]	0.66	0.76	0.55	0.82
CGA [H2]	0.16	-	0.10	-
CGG [H3]	-	-	-	0.07
TAG [H4]	0.13	0.24	0.23	0.11
TGA [H5]	0.05	-	0.12	-
Diplotypes				
H1/H1	0.61	0.52	0.29	0.71
H1/H3	-	-	-	0.10
H1/H4	0.29	0.42	0.54	0.19
H2/H2	0.04	-	-	-
H2/H5	0.06	-	0.12	-
H4/H4	-	0.06	0.05	-

4.2.12.8 The effects of the ZYX haplotype on phenotypic traits in pigs

The analysis of the ZYX haplotypes revealed some significant associations with carcass and meat quality traits that are displayed in Table 64. In PIF1, animals having the diplotypes 'H1/H1' or 'H1/H4' had lower pH24 than the 'H2/H2' animals, and the 'H1/H' animals also had lower DRIP than the 'H2/H5' animals. Moreover, lower fat measures (ABF and BF2) and were associated with the diplotype 'H1/H4' in PI. The diplotype 'H1/H4' provided highest LEA in PI.

Table 64: Least square means (LSM) and standard errors (SE) for carcass traits across haplotypes of ZYX in pigs

	pH24 PIF1	DRIP PIF1	LEA PI	ABF PI	BF2 PI
H1/H1	5.50^c (0.01/94)	2.47^c (0.19/94)	59.65^c (0.68/110)	1.81^c (0.04/110)	1.48^{cd} (0.04/110)
H1/H3	-	-	58.33^c (1.48/15)	1.82^{cd} (0.08/15)	1.60^c (0.08/15)
H1/H4	5.50^c (0.01/45)	2.60^{cd} (0.23/45)	63.38^d (1.18/30)	1.61^d (0.07/30)	1.33^d (0.07/30)
H2/H2	5.60^d (0.03/6)	2.37^{cd} (0.52/6)	-	-	-
H2/H5	5.51^{cd} (0.03/9)	3.84^d (0.45/9)	-	-	-
P-value	0.0191	0.0365	0.0097	0.0247	0.0326

$^{c-d}$ P<0.05, $^{a-b}$ P<0.01
Within columns, values with the same letter are not significantly different

4.3 Genetic mapping

Twenty eight full-sib families of the DUPI resource population were used for genetic mapping. Assignments of these twelve genes resulting from the two-point linkage analysis are shown in Table 65, together with the proximal and distal linked markers, LOD scores, and recombination fractions. BVES was assigned to SSC1, between the markers S0312 and SW2166. The result showed that three candidate genes, including SLC3A2, AHNAK and ZDHHC5, are located closely together on SSC2 in between the markers SW2623 and SW240. Another three candidate genes (CS, LYZ and KERA) were assigned to SSC5. COQ9 mapped to SSC6 between the markers S0035 and S0087. UN was assigned to SSC7 between the markers SW175 and S0115. In addition, EGFR is close to marker S0295. The assignment of VTN revealed its location on SSC12 in between the markers SW874 and SW605. Finally, ZYX was mapped to SSC18 between the markers SY4 and SW1808.

Table 65: Genetic mapping results

Gene	SSC.	Proximal linked loci	Rec. fraction	LOD	Kosambi cM	Distal linked loci	Rec. fraction	LOD	Kosambi cM
BVES	1	S0312	0.01	54.67	1.40	SW2166	0.21	7.88	20.9
SLC3A2	2	SW2623	0.11	11.30	12.4	AHNAK	0.02	19.32	2.1
AHNAK	2	SLC3A2	0.02	19.32	2.1	ZDHHC5	0.10	15.31	9.9
ZDHHC5	2	AHNAK	0.10	15.31	9.9	SW240	0.11	22.87	14.0
CS	5	SWR453	0.11	5.73	9.0	SW2425	0.08	9.01	6.0
LYZ	5	S0092	0.13	23.0	9.7	SW1134	0.05	7.09	4.6
KERA	5	SW1954	0.07	36.41	7.2	SW967	0.18	14.21	17.5
COQ9	6	S0035	0.21	4.00	31.8	S0087	0.19	6.25	27.5
UN	7	SW175	0.11	29.76	11.4	S0115	0.15	17.67	17.2
EGFR	9	S0295	0.38	0.26	43.6	-	-	-	-
VTN	12	SW874	0.25	3.71	28.7	SW605	0.17	4.49	17.9
ZYX	18	SY4	0.03	61.25	2.4	SW1808	0.15	7.42	8.5

5. Discussion

In this study, candidate genes were derived from their expression profiles determined in previous studies (Ponsuksili et al. 2008a,b); a shortlist of twelve genes was selected based on known function of the particular gene and/or its map position, giving preference to those genes located in QTL regions for meat quality traits. Up to date, many groups have used this strategy (functional genomic approach) for seeking candidate genes in different fields. For example, carcass and meat quality traits in pigs (Ponsuksili et al. 2005; Wimmers et al. 2007; Li et al. 2008), levels of androstenone in boars (Moe et al. 2008), immune responsiveness in pigs (Ponsuksili et al. 2008c), splay leg syndrome in piglets (Maak et al. 2009), anabolic agents responsiveness in heifers (Reiter et al. 2007) and nutrient transformation in cattle (Schwerin et al. 2006). Whereas other research groups have selected candidate genes based only on a physiologic basis and/or previously identified QTL regions, this approach may be difficult in the process of choosing specific genes from numbers of potential candidates for complex traits. In general, important biologic features of traits are directly reflected by transcript patterns, therefore the study of gene expression profiles can contribute to a better understanding of the molecular architecture and discover the detailed clues candidate genes provide for complex traits such as meat quality. Previously, expression profiles of the *M. longissimus dorsi* were compared between groups of animals exhibiting extreme differences in DRIP or pH24. The various techniques of expression profiling revealed a number of genes with phenotype-associated differential expression and provided functional categories of differentially regulated transcripts. 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 at high drip loss, the functional groups of oxidoreductase activity, lipid metabolism, and electron transport were identified. Therefore, six candidate genes (VTN, UN, LYZ, KERA, AHNAK and ZYX) were selected for further analysis in this study. The microarray expression levels of VTN and UN were down-regulated, LYZ, AHNAK and ZYX were up-regulated at high drip loss, whereas KERA was up-regulated at pH24 (Ponsuksili et al.

2008a). On the other hand, another six candidate genes (BVES, SLC3A2, ZDHHC5, CS, COQ9, EGFR) analysed in this study arise from their expression being correlated with water holding capacity (WHC). Previously, the expression analysis of *M. longissimus dorsi*-RNAs of 74 F2-animals of a DUPI resource population showed 1,279 transcripts whose expression data were correlated with 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. BVES, SLC3A2, CS, ZDHHC5 and COQ9 revealed negative correlation with drip loss (r = -0.81, -0.43, -0.38, -0.49 and -0.47 respectively; P≤0.001). EGFR and AHNAK showed positive correlation with drip loss (r = 0.66 and 0.53; P<0.0001) (Ponsuksili et al. 2008b). Thus these genes were used for further analyses, including identifying polymorphic sites, genotyping and mapping in order to evaluate their potential role as functional and/or positional candidate genes for carcass and meat quality, especially water holding capacity.

5.1 SNP detection

The polymorphism analysis of PCR fragments obtained from cDNA of twelve candidate genes, that were on average 654 bp in length, revealed an average of one polymorphic site per 361 bp. Almost all polymorphisms were situated in coding regions, some SNPs were also detected in the 3'UTRs of CS, EGFR, COQ9 and ZDHHC5. Fahrenkrug et al. (2002) detected one SNP per 184 bp in porcine ESTs, while Jungerius et al. (2003) found one SNP per 108 bp in coding and non-coding porcine genomic sequences. Moreover, Nonneman and Rohrer (2002) detected one SNP per 120 bp of non-coding sequence, while Sawera et al. (2000) detected one SNP per 220 bp in transcribed regions. The difference in numbers of the detected SNPs might be due to the differences in the panel of animals used to detect polymorphisms. In this study, a small number of commercially relevant breeds were used to detect SNPs including one animal each of the breeds Pietrain, German Large White and German Landrace. Most of the polymorphisms detected here were found to be segregating in the commercial populations. Twenty-two out of 38 polymorphisms were

selected for genotyping based on suitability of the surrounding sequences to design primers, position in regions with potential function, exon-intron structure and preference for those causing amino acid exchange.

5.2 Association analysis of candidate genes

5.2.1 The analysis of BVES

Blood vessel epicardial substance (BVES) is a membrane protein that is widely expressed throughout development and adulthood in several tissues including skeletal muscle. BVES is the prototypical member of the Popeye domain containing (popdc) gene family (Andrée et al. 2000; Smith and Bader, 2006). Structurally, it comprises a short intracellular N-terminus, three transmembrane helices that are necessary for membrane insertion and a long extracellular C-terminal domain. While BVES has a highly conserved primary amino acid sequence among different species, there are no studies identifying any protein domain linked to any molecular or cellular function. Functionally, BVES plays a role at cell junctions to establish and/or modulate cell adhesion or cell-cell interactions in epithelial cell types in a Ca^{2+}-independent manner (Wada et al. 2001; Osler et al. 2005; Lin et al. 2007). It has been known that a proteolytic degradation of several cell adhesion proteins takes place *post mortem* which is linked with the generation of drip channels (Huff-Lonergan and Lonergan, 2005). The present study revealed an association of BVES to BF3 and drip loss in PIF1(b), while an effect on pH24 was found in DUPI. A significant additive effect was observed for DRIP, while for BF3 a significant dominance effect was revealed. The location of BVES also supports these findings, since the gene is located on SSC1 (between marker S0312 and SW2166) in a region containing QTL for loin eye area and average backfat thickness in Berkshire x Yorkshire resource population (Thomsens et al. (2004). Moreover, QTL areas for carcass fatness traits (BF1, BF2, BF3 and ABF), meat color (OPTO), pH and conductivity (CON24) were detected in DUPI population (Liu et al. 2007) (Figure 15).
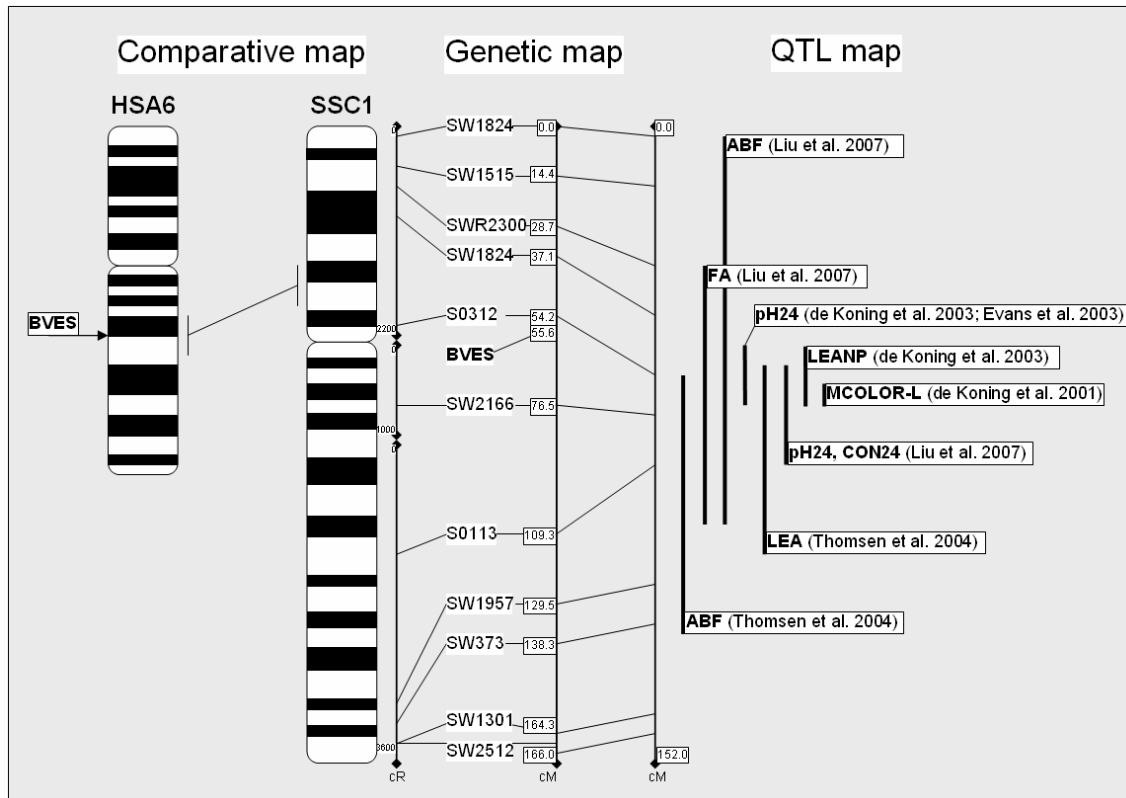
Figure 15: The location of BVES on SSC1

(Comparative map and RH map derived from Meyers et al. (2005): distance given in centi-Rays; cR, our own genetic map: distance between markers expressed in centi-Morgan; cM and QTL map based on pig qtl database: http://www.animalgenome.org/cgi-bin/QTLdb/SS/draw_chromap: distance given in centi-Morgan; cM)

5.2.2 The analysis of SLC3A2

The SLC3A2 (solute carrier family 3, member 2) gene is a member of the solute carrier family and encodes a cell surface transmembrane protein. Functionally, it plays a significant role in regulating integrin-mediated functions and regulates amino acid transport (Palacin and Kanai, 2004; Feral et al. 2005). SLC3A2 is reported as a *cis*-regulated functional positional candidate gene for drip loss (r = -0.43) (Ponsuksili et al. 2008b). In this study, two polymorphisms of SLC3A2 were studied. The first was a SNP at position 1326 (c.1326A>G) relative to the start codon, This SNP did not affect the protein sequence. However, effects on some carcass traits (MFR and BF3) were found in PIF1(b)

and DL. A significant dominance effect was found for BF3. Another polymorphism of SLC3A2 was the insertion/deletion of the three nucleotides "AGC" at position 1336 (c.1336Indel[AGC]), where the deletion resulted in one amino acid "Serine" being removed from the protein sequence at position 446 (p.Ser446-) in the area of the conserved domain for trehalose synthase (TreS). The SLC3A2 mutation c.1336Indel[AGC] may interfere with the glycogen metabolism, since the TreS plays a key role in the utilization of trehalose for the production of glycogen (Pan et al. 2008). The association study of c.1336Indel[AGC]) showed associations with several traits related to carcass fatness in PI, PIF1(a,b) and DL. The effect on ABF is consistent in several breeds, where the 'DD' genotype provide higher backfat (PI, PIF1(a,b)). A significant dominance effect was found for ABF in PI, while additive effects for ABF were found in PIF1(a,b), the 'D' was associated with an increase in ABF. For meat quality, effects on water binding properties (DRIP, THAW, COOK) were found in PIF1(a,b) and DL. The 'D' decreased COOK and DRIP in PIF1(a) and DL respectively, whereas in PIF1(b) it was associated with an increase in THAW. Effects on pH and CON1 were found in PIF1(b) and PIF1(c) respectively. Significant dominance effects were found for pH1 and CON1 in PIF1(b) PIF1(c) respectively, while significant additive effects were detected for SF and pH24 in PIF1(a) and PIF1(b) respectively. The association of the 'DD' genotype with both high backfat and high water holding capacity in this study is in agreement with the general overall findings by Huff-Lonergan et al. (2003), who reported a positive relationship between these two traits. Comparative mapping revealed that pig chromosome 2 (SSC2) (Figure 16) shares homology with human chromosome 11 (HSA11). Moreover, SLC3A2 was genetically assigned to SSC2 (in accordance with the localization of human SLC3A2 to HSA11q13), where a number of QTL areas were reported, e.g. for backfat thickness (DUPI population; Liu et al. 2007, 2008; Berkshire x Yorkshire population, Thomsen et al. 2004), loin eye area (Thomsen et al. 2004), and drip loss (Berkshire x Yorkshire, Malek et al. 2001; commercial population; van Wijk et al. 2006) and pH24 (Meishan x Pietrain population; Lee et al. 2003).

5.2.3 The analysis of AHNAK

AHNAK, a 700 kDa protein, is expressed in a variety of cells and has been implicated in different cell-type-specific functions (Gentil et al. 2001; Borgonovo et al. 2002; Haase et al. 2004). In muscle cells, AHNAK is localized at the sarcolemma membrane and T-tubules (Gentil et al. 2003). Recently, the carboxyl-terminal AHNAK domain was identified to link the Ca^{2+} channels to the actin-based cytoskeleton (Hohaus et al. 2002) and to exert a stabilizing effect on muscle contractility via its interaction with the actin of the thin filaments (Haase et al. 2004). Moreover, AHNAK is an activator for phospholipase C-γ (PLC-γ), an enzyme in cellular signal transduction involved in cell growth, proliferation and metabolism. (Lee et al. 2004). The activation of PLC-γ mediates several cellular responses, including cytoskeletal rearrangements that lead to protection of the plasma membrane in response to mechanical stress (Ryan et al. 2000; Ruwhof et al. 2001). AHNAK play a role in membrane repair process through interaction with partner proteins, including the annexin2/S100A10 complex and dysferlin (Huang et al. 2007) Therefore, AHNAK is an abundant muscular protein with possible important functions associated with structural support of the plasma membrane. The study in human muscle biopsies revealed that expression of AHNAK was associated with low maximal oxygen uptake (Vo_{2max}), increased with aging, and decreased with exercise training. AHNAK thus seems to reflect poor muscle fitness (Parikh et al. 2008). It has been known that a high proportion of type 1 fibers (slow-twitch muscle fibers) is associated with a high Vo_{2max} (Matolin et al. 1994). This opens up the interesting implication that AHNAK could also represent a link to micro-structure of muscle and finally meat quality. Microarray analysis and qPCR showed that AHNAK was up-regulated in the high drip loss group (Ponsuksili et al. 2008a). In this study, all five SNPs were related with carcass traits, especially LEA, ABF and BF3. FA, MFR, BF1 and ABF were highly significant with the SNP c.13014G>T (P<0.001). The SNP c.12907A>G was also highly associated with MFR (P<0.001). Moreover, almost all meat quality traits except SF were associated with AHNAK. In particular, the trait DRIP was associated with the SNPs c.13014G>T and c.13281A>G (P<0.05). The AHNAK effects on carcass and meat quality traits are mainly additive effect for example the 'G' allele of SNP c.13014G>T or the 'G' allele of c.13281A>G decreased both carcass fatness

and DRIP in DUPI. Interestingly, the association of 'GG' genotype of both SNPs c.13014G>T and c.13281A>G with both low DRIP and low ABF in DUPI population are in contrast with the general findings that reported a negative relationship between these two traits (Huff-Lonergan et al. 2003; Ponsuksili et al. 2009). The AHNAK haplotype showed associations mainly to carcass traits, the most pronounced of all effects was found on MFR (P<0.001). Moreover, AHNAK is located on SSC2 (Figure 16) in accordance with the localization of human AHNAK to HSA11q12 (Kudoh et al. 1995), in a QTL region for drip loss. The QTL for drip loss in this region was found in other studies (DUPI population; Liu et al. 2007, 2008, Berkshire x Yorkshire population; Malek et al. 2001, commercial population; van Wijk et al. 2006). The correlation between drip loss and AHNAK is high (r = 0.53; P<0.0001) and the eQTL for AHNAK indicates a *cis*-acting mode of regulation with genome-wide significance (LOD score = 6.4; F = 18.2) (Ponsuksili et al. 2008b). Moreover, QTL for loin eye area (Thomsen et al. 2004) and pH24 (Meishan x Pietrain population; Lee et al. 2003) were reported.

5.2.4 The analysis of ZDHHC5

The zinc finger, DHHC domain containing 5 (ZDHHC5) gene is a member of the ZDHHC gene family, at least 24 members have been identified in mammalian genome (Fukata et al. 2004), which encodes palmitoyl acyltransferase enzymes (PATs), the enzymes responsible for protein palmitoylation (Roth et al. 2002; Fukata et al. 2004). It has been known that protein function is affected by its expression level, localization, interaction with other proteins, and its posttranslational modifications. Many proteins can be modified by palmitolation on cysteine residues. Palmitoylation serves a number of important biological roles including affect on the localization, trafficking, degradation of a protein and activity of many signaling proteins (Draper and Smith, 2009; Leong et al. 2009), protein stability, as well as protein-protein and protein-lipid interaction (Dunphy and Linder, 1998; Resh, 1999; Putilina et al. 1999). The study revealed an association of ZDHHC5 c.1803C>T to COOK in PI and DUPI, BF1 in PIF1(b), OPTO in PIF1(c) and DRIP in DL. The other effects on MFR and FA were found in DUPI. In particular, the ZDHHC5 c.1803C>T was highly associated with drip loss (P = 0.0004), the homozygous genotype 'CC' animals had

lower DRIP than the heterozygous animals in DL. Previously, the negative correlation between drip loss and ZDHHC5 expression in DUPI was reported (r = -0.49) (Ponsuksili et al. 2008b). ZDHHC5 was mapped on SSC2 closed to AHNAK (Figure 16), coinciding with QTL areas for backfat thickness, loin eye area (DUPI population; Liu et al. 2007, 2008, Berkshire x Yorkshire; Thomsen et al. 2004), drip loss (Liu et al. 2007, 2008, Berkshire x Yorkshire population; Malek et al. 2001, commercial population; van Wijk et al. 2006) and pH24 (Meishan x Pietrain (MP) population; Lee et al. 2003).
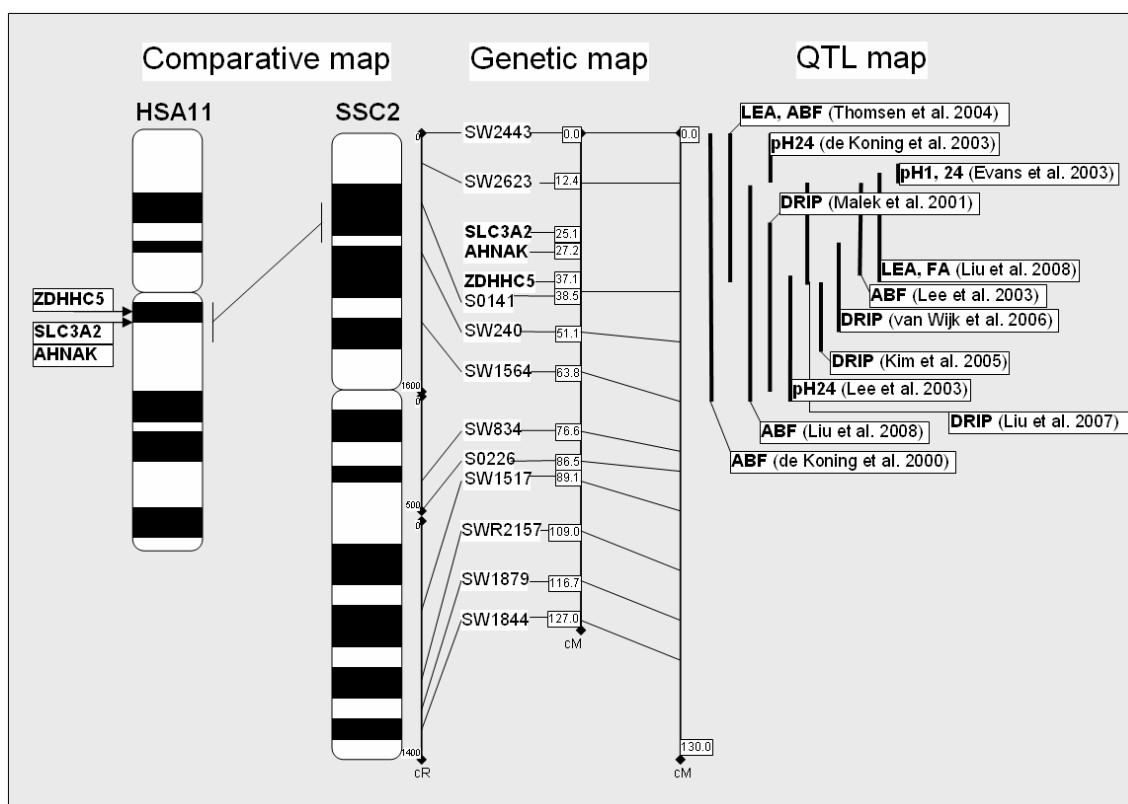
Figure 16: The location of SLC3A2, AHNAK and ZDHHC5 on SSC2

(Comparative map and RH map derived from Meyers et al. (2005): distance given in centi-Rays; cR, our own genetic map: distance between markers expressed in centi-Morgan; cM and QTL map based on pig qtl database: http://www.animalgenome.org/cgi-bin/QTLdb/SS/draw_chromap: distance given in centi-Morgan; cM)

5.2.5 The analysis of CS

Citrate synthase (CS) is a key regulator of aerobic energy production in cells; it is an enzyme in the mitochondrial matrix. Functionally, it catalyzes the condensation of Acetyl-CoA and oxaloacetate to citrate and CoA in the first step of the citric acid cycle. CS activity was measured to determine the relationship between fiber composition and muscle oxidative capacity (Chaudhary et al. 1992; Delp and Duan, 1996; Papinaho et al. 1996; Kohn et al. 2005). Mattson et al. (2002) showed that activity of CS in hamster skeletal muscle was strongly correlated with muscle fiber types in the rank order of type IIA ($r = 0.68$; P<0.001), type I ($r = 0.44$; P<0.018) and type IIB ($r = -0.55$; P = 0.002), similar to the rat (Delp and Duan, 1996). Henckel et al. (1997) reported that the activity of CS was positively correlated with lean meat content, muscle capillarity and heme pigment in *M. longissimus dorsi* of Danish Landrace and Yorkshire pigs. Moreover, a study in bulls revealed that CS activity in "red" muscle (supraspinatus) was higher than "white" or "mix" muscles (semitendinosus and longissimus dorsi) (Vestergaard et al. 2000). A study in turkeys and pigs showed that slow-growing animals had higher CS activity than fast-growing animal as well as "red" muscle had higher CS activity than "white" muscle. Also the "white" muscle showed a rapid pH decline shortly after slaughter (Oksbjerg et al. 2000; Werner et al. 2005). Recently, a study in bulls from three breeds (Belgian Blue, Limousin and Aberdeen Angus) revealed that the bulls with the highest drip loss (Belgian Blue bulls), also had the lowest CS activity (Cuvelier et al. 2006). Strong CS activity might help to maintain the quality of meat in terms of water-holding capacity indirectly by maintaining the amount of ATP in muscle during the early postmortem stage and lowering the rate of pH decline (Huff-Lonergan and Lonergan 2005). Therefore, the gene encoding the CS protein may take part in controlling meat quality. Previously, the study of gene expression profiles in pig muscle revealed that CS had negative correlation with drip loss ($r = -0.38$; P≤0.001) (Ponsuksili et al. 2008b), indicates that pig's muscle with high aerobic metabolism (or high CS expression) relates to low drip loss. In this study, we observed associations of CS with LEA in PI, DRIP in PIF1(a), FA in PIF1(b), pH1 and CON1 in PIF1(c), BF3 and ABF in DL. The relationship of the CS polymorphism to loin eye area or fat area traits in pigs is not unexpected, base on the reported role of CS in muscle fiber

composition. Under physiological conditions, the number of capillaries per fiber is positively correlated to fiber diameter, indicating a proliferation of capillaries to compensate for fiber hypertrophy (Wang et al. 1993). Positive correlation between the activity of citrate synthase and the number of capillaries was found by Henckel et al. (1997). Moreover, the CS gene was mapped to pig chromosome 5p12-p13 (Chaudhary et al. 1992) in the QTL regions (Figure 17) for pH24 (commercial population; Harmegnies et al. 2006), drip loss (Berkshire x Yorkshire population; Thomsen et al. 2004) and backfat thickness (Meishan x Large White; Milan et al. 2002)

5.2.6 The analysis of LYZ

Lysozyme (LYZ) was mapped to pig chromosome 5p11 (Chaudhary et al. 1997) which has been confirmed by our genetic mapping result (Figure 17). The location of LYZ coincides with QTL regions for QTL for pH24, meat color (commercial population; Harmegnies et al. 2006) and drip loss (Berkshire x Yorkshire population; Thomsen et al. 2004). A QTL for conductivity at 24 hour p.m. is also located downstream nearby this area (Meishan x Pietrian population; Lee et al. 2003). LYZ belongs to a family of enzymes which damage bacterial cell walls by catalyzing the hydrolysis of the β-(1,4)-glycosidic linkage between n-acetylglucosamine and the muramic acid of the peptidoglycan layer. LYZ displays antimicrobial, antitumoral and immunomodulatory properties, which have been extensively studied (Ibrahim et al. 2001; Gorbenko et al. 2007). However, the function of LYZ relating to meat quality is poorly understood. As previously published, the expression level of LYZ in *M. longissimus dorsi* was up-regulated in the high drip loss group (fold change 1.70; P = 0.04) (Ponsuksili et al. 2008a). The study of the cellular model for induction of drip loss in meat by Lambert et al. (2001) showed that anoxia/ischemia, which represents the situation in muscle cells upon slaughter, may lead to drip loss. Anoxia/ischemia is characterized by an increased concentration of cellular free Ca^{2+}, cell swelling and an elevated production of reactive oxygen species (ROS), which affects the muscular content of osmolytes and cell water. Sanoudou et al. (2004) reported that antioxidant enzymes play key roles in the cell, protecting it against ROS. The study of transcriptional profiles of postmortem human skeleton muscle revealed that antioxidant

enzymes were up-regulated (Sanoudou et al. 2004). Liu et al. (2006a,b) found that LYZ suppresses ROS generation and oxidant stress response gene transcription and provides protection against acute and chronic oxidant injury. Therefore, it can be reasoned that LYZ up-regulation in the high drip loss group occurs due to the cellular response to high oxidative stress. In this study, we detected a SNP (c.365A>T) causing an amino acid exchange from Glutamin to Leucine (p.Gln122Leu), located in a conserved domain of LYZ which includes several features such as a catalytic site, a catalytic cleft and a Ca^{2+}-binding site (Oasba and Kumar, 1997; Permyakov and Berliner, 2000). For the association study of LYZ, two SNPs were genotyped (c.240A>C and c.365A>T). The SNP c.240A>C was associated with meat quality traits only, including OPTO, pH1, CON24 and DRIP (P<0.05). Significant additive and dominance effects were observed for DRIP in PI, pH1 in PIF1(a) and OPTO in PIF1(b), while for CON24 in PIF1(a) only significant dominance effect was revealed. The SNP c.365A>T was associated with LEA, ABF, pH1, CON(1,24), THAW and DRIP (P<0.05). Significant additive and dominance effects were found for CON24 in DUPI, ABF in PI, THAW in PIF1(b), while only significant effects were detected for pH1 in DUPI and LEA in PIF1(c), and only a significant dominance effect was detected for CON1 in PI. The haplotype of LYZ showed associations with the same traits that were effected by the SNP c.240A>C. Interestingly, LYZ showed a consistent association with CON24 in DUPI and PIF1(a). LYZ is probably involved in releasing the charge of liquid contents of muscle cells during the postmortem protein degradation, since there is evidence for the disruption of the membrane integrity due to the insertion of LYZ into the lipid bilayer which induces the release of the aqueous contents (Posse et al. 1994).

5.2.7 The analysis of KERA

Keratocan (KERA) is a keratan sulfate proteoglycan of the extracellular matrix (ECM). The gene KERA, which encodes the core protein keratocan, is a class II member of the small leucine rich repeat proteoglycan (SLRP) gene family (Iozzo, 1998). It is known that SLRP modulate tissue organization, cellular proliferation, matrix adhesion, growth factor and cytokine responses, and sterically protect the surface of collagen type I and II fibrils from proteolysis (Melrose et al. 2008). The ECM is a major determinant in 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 (Velleman, 2000). The gene expression level of KERA was up-regulated at pH24 (Ponsuksili et al. 2008a). In this study, three associations were found, including LEA and pH of both stages (P<0.05). A significant additive effect was detected for pH1, while for pH24 significant additive and dominance effect were detected in PI. Furthermore, the position of KERA was located on SSC5 (Figure 17) in the QTL region for backfat thickness and pH24 (Berkshire x Yorkshire population; Malek et al. 2001).

Figure 17: The location of CS, LYZ and KERA on SSC5

(Comparative map and RH map derived from Meyers et al. (2005): distance given in centi-Rays; cR, our own genetic map: distance between markers expressed in centi-Morgan; cM and QTL map based on pig qtl database: http://www.animalgenome.org/cgi-bin/QTLdb/SS/draw_chromap: distance given in centi-Morgan; cM)

5.2.8 The analysis of COQ9

The COQ9 (coenzyme Q9 homologue) gene encodes an enzyme that is required for the biosynthesis of coenzyme Q (Johnson et al. 2005), which is well defined as a crucial component of the oxidative phosphorylation process in mitochondria which converts the energy in carbohydrates and fatty acids into ATP (Crane 2001). In the early postmortem stage, the supply of oxygen in muscle is depleted thus, a shift from aerobic to anaerobic metabolism and an increase in the accumulation of lactic acid leads to a pH decline thereby influencing the water holding capacity in muscle (Huff-Lonergan and Lonergan 2005). On the other hand, strong coenzyme Q activation might help to maintain the ATP content in muscle, thus decreasing the rate of the pH decline. Beside its roll in the energy metabolism, the reduced form of coenzyme Q can protect cells from oxidative stress, by exerting its antioxidant function either directly on superoxide radicals or indirectly on lipid radicals (Lenaz et al. 1998; Kucharská et al. 2004). Thus it may relate to meat quality due to the high levels of antioxidants in the meat influencing the activity of μ-calpain which affects proteolysis and early postmortem shear force (Huff-Lonergan and Lonergan 2005). The COQ9 gene was mapped to the human chromosome 16q13 which comparative mapping locates on SSC6 (Figure 18) in QTL areas of carcass fatness traits (Meishan x Yorkshire population; Paszek et al. 2001, Meishan x Pietrain population; Yue et al. 2003), loin eye area, fat area (DUPI population; Liu et al. 2007, 2008), conductivity at 24 hour p.m. (Duroc x Berlin miniature pig population; Wimmers et al. 2006), pH1 (White Duroc x Chinese Erhualian population; Duan et al. 2009), pH24 (commercial population; Kim et al. 2005) and drip loss (commercial population; de Koning et al. 2001, Hampshire x Landrace; Markljung et al. 2008, DUPI population; Liu et al. 2008). Previously, COQ9 was also reported as a *cis*-regulated functional positional candidate gene for drip loss (r = -0.47) (Ponsuksili et al. 2008b). The polymorphisms c.453A>G and +1247A>T had the same allelic frequencies in PI, PIF1(a) and PIF1(b), a feature that suggests that they present high linkage disequilibria in each of the three breeds, thus c.453A>G and +1247A>T showed the same effect on SF in PI and on BF2, FA and MFR in PIF1(b). Another effect of c.453A>G was found on pH1 in DUPI, while the +1247A>T was associated with OPTO in DL and ABF, BF1, FA, MFR, LEA and THAW in DUPI. Considering these results, the

COQ9 effects on carcass traits are mainly additive effect for example the 'A' allele of SNP +1247A>T increased carcass fatness (ABF and BF1 in DUPI, FA in PIF1(b)). Some significant dominance effects were found for SF, THAW and OPTO. The association analysis between haplotypes and phenotype traits confirmed the results from the single SNP and provided a new significant association with LEA in DL.
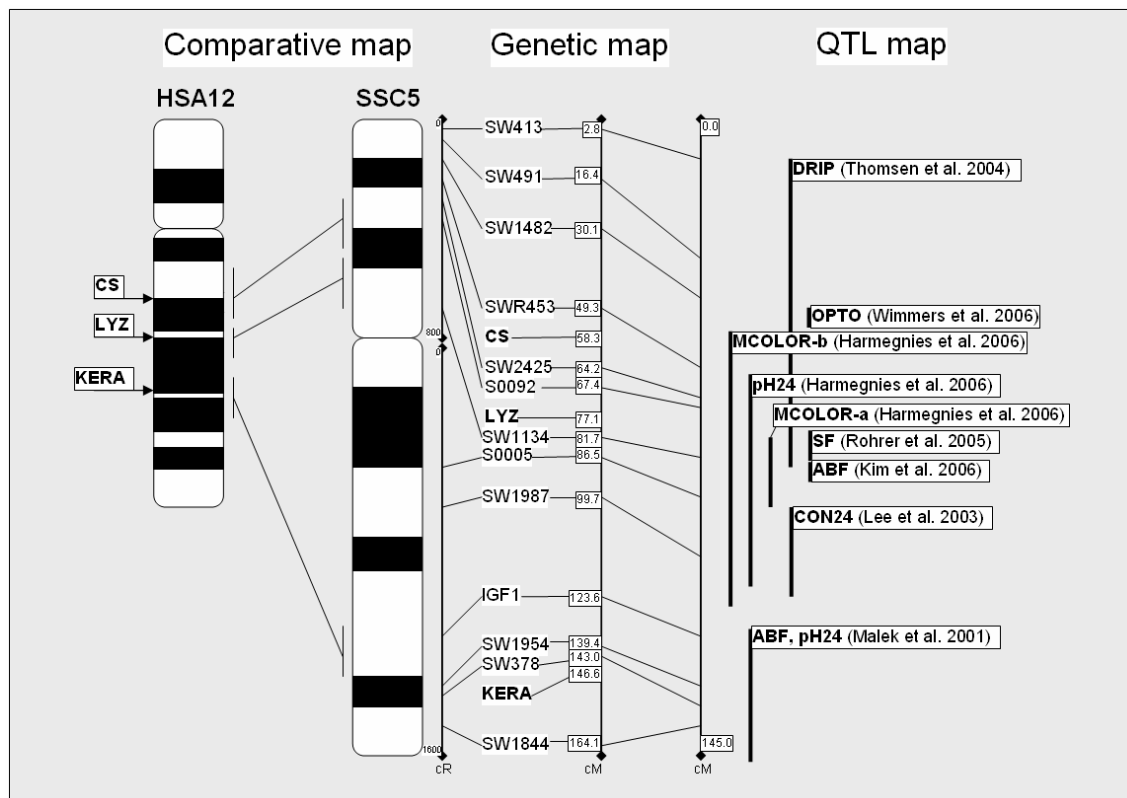

Figure 18: The location of COQ9 on SSC6

(Comparative map and RH map derived from Meyers et al. (2005): distance given in centi-Rays; cR, our own genetic map: distance between markers expressed in centi-Morgan; cM and QTL map based on pig qtl database: http://www.animalgenome.org/cgi-bin/QTLdb/SS/draw_chromap: distance given in centi-Morgan; cM)

5.2.9 The analysis of UN

Unknown locus (UN) (a non-annotated EST; Affymetrix probe set ID: Ssc.25503.1.S1_at) was not in a coding area of any known gene (no significant similarity found by using ORF

and BLASX), with a SNP (g.1,022,434G>T   Acc. No: NW_001886512.1) located 20 kb from the 5' flanking side of the putative pig RGMA sequence. The expression level of UN was down-regulate in high drip loss group and showed high negative correlation with drip loss (r = -0.58) (Ponsuksili et al. 2008a,b). Moreover, the genetic mapping result revealed the location of UN on SSC7 (Figure 19) in QTL areas for backfat thickness, cooking loss (DUPI population; Liu et al. 2007), loin eye area (DUPI population; Edwards et al. 2008) shear force (commercial population; Harmegnies et al. 2006) and pH24 (Meishan x Pietrain population; Yue et al. 2003, Large White x Meishan population; Su et al. 2004), therefore this locus was considered a positional candidate gene. Here, effects of this locus on pH, DRIP and SF were found. A significant additive effect was detected for BF2 in PIF1(c), the 'G' allele increased BF2. Significant dominance effects were detected for BF2 and pH24 in DUPI and DL respectively.

Figure 19: The location of UN on SSC7

(Our own genetic map: distance between markers expressed in centi-Morgan; cM and QTL map based on pig qtl database: http://www.animalgenome.org/cgi-bin/QTLdb/SS/draw_chromap: distance given in centi-Morgan; cM)

5.2.10 The analysis of EGFR

The epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein that constitutes one of four members of the erbB family of tyrosine kinase receptors. Binding of EGFR to its cognate ligands leads to autophosphorylation of receptor tyrosine kinases and subsequent activation of signal transduction pathways that are involved in regulating cellular proliferation, differentiation, and survival (Herbst, 2004). Ligand-independent phosphorylation of receptors can be induced directly by exposure to hydrogen peroxide (H_2O_2, a generation of reactive oxygen species; ROS) and also by different oxidative stress-inducing agents (Rosette and Karin, 1996; Finkel, 1998). Meves et al. (2001) reported that EGFR phosphorylation responses to oxidative stress which linked to intracellular H_2O_2 levels. The most compelling evidence in favour of a role of the cytoskeleton in EGF-induced signal transduction has been obtained by the finding that the EGFR itself is an actin-binding protein (den Hartigh et al. 1992). It was demonstrated that a member of the small leucine rich repeat proteoglycan (SLRP) family (the family of keratocan), decorin is specifically interacts with the EGF receptor (EGFR) and causes a sustained activation of the EGFR (Iozzo, 1999). An early signal generated by the activation of EGFR upon ligand binding is a transient increase in the cytosolic concentration of free calcium ion ($[Ca^{2+}]_{cyt}$) (Villalobo et al. 2000). Entry of extracellular Ca^{2+}, and Ca^{2+} release from intracellular stores, both appear to contribute to the generation of the EGF-mediated $[Ca^{2+}]_{cyt}$ spike (Hughes et al. 1991; Peppelenbosch et al. 1992; Schalkwijk et al. 1995). Early post mortem higher Ca^{2+} concentration causes rapid contraction, an increase in the rate of muscle metabolism, and accelerated pH decline with resulting higher drip (Huff-Lonergan and Lonergan, 2005). Previously, EGFR was one of the gene sets associated with calcium signaling pathways that showed a high positive correlation with drip loss (r = 0.67) (Ponsuksili et al. 2008b). In this study, EGFR showed significant associations with OPTO in PI, CON24 in PIF1(a), BF1 and pH24 in PIF1(b), while in DUPI it was associated with ABF, BF3, MFR, LEA and THAW. Significant additive effects were detected for LEA, ABF, BF3, MFR in DUPI and pH24 in PIF1(b), while significant dominance effects were detected for LEA, THAW in DUPI, OPTO in PI and BF1 in PIF1(b). Comparative and genetic mapping revealed the location of EGFR on SSC9

(Figure 20) linked closely to a QTL area for backfat thickness (Yorkshire population; Kim et al. 2006). A QTL for loin eye area was also located nearby upstream of the EGFR position (DUPI population; Liu et al. 2007).
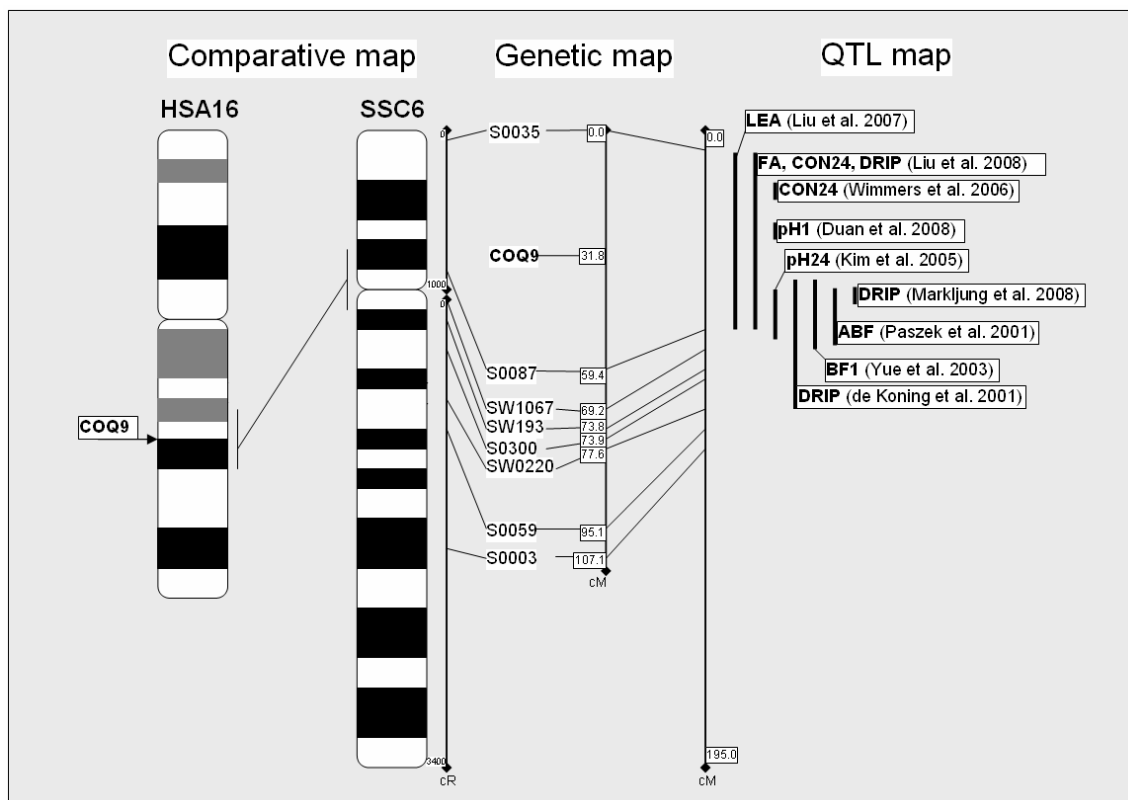

Figure 20: The location of EGFR on SSC9
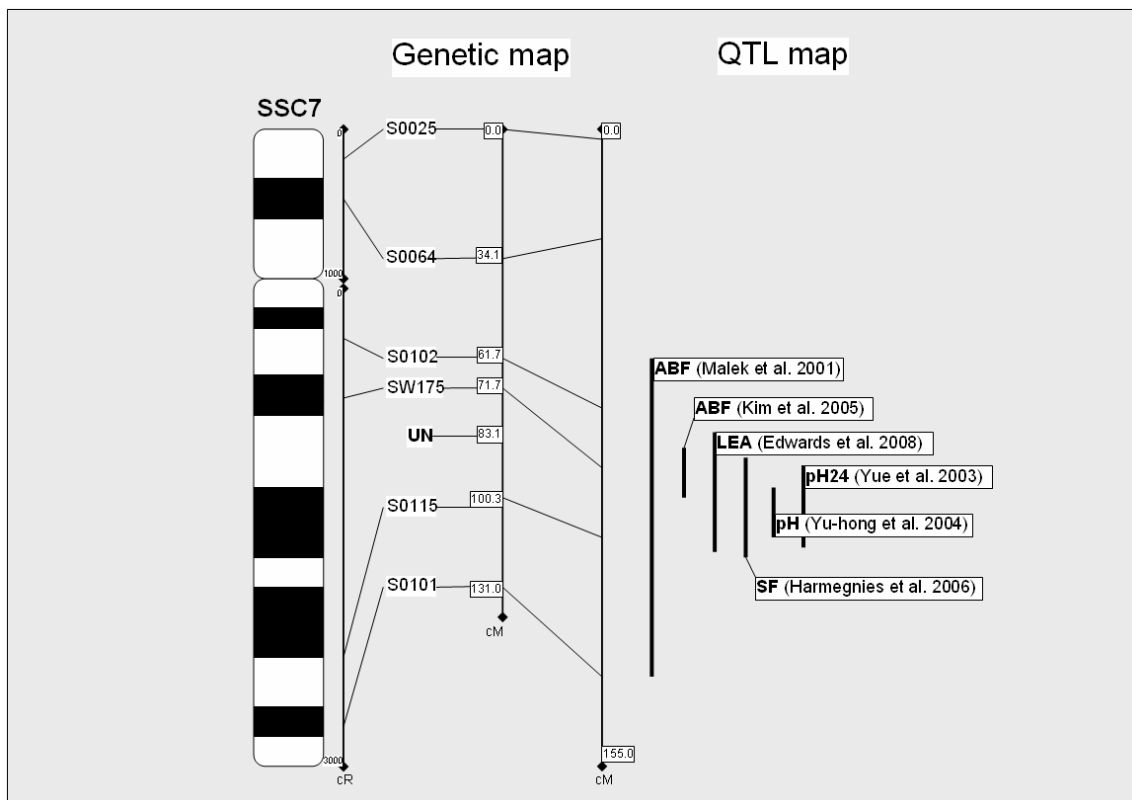
(Comparative map and RH map derived from Meyers et al. (2005): distance given in centi-Rays; cR, our own genetic map: distance between markers expressed in centi-Morgan; cM and QTL map based on pig qtl database: http://www.animalgenome.org/cgi-bin/QTLdb/SS/draw_chromap: distance given in centi-Morgan; cM)

5.2.11 The analysis of VTN

Vitronectin (VTN) is a multifunctional glycoprotein found in plasma and the extracellular matrix. It is a component of the urokinase system (Kricker et al. 2003; Lynn et al. 2005). Functionally, VTN promotes cell adhesion and spreading and modulation of cell morphology, inhibits the membrane-damaging effect of the terminal cytolytic complement pathway, and binds to several serpin serine protease inhibitors (Kjaergaard et al. 2007;

Schar et al 2008). Furthermore, it was demonstrated that insulin-like growth factor-binding protein-4 (IGFBP-4) proteolytic degradation regulated by vitronectin (Mazerbourg et al. 2000). The study in human revealed that complexes comprising IGF and IGF-binding proteins bound to the extracellularmatrix (ECM) protein vitronectin significantly enhance cellular functions relevant to wound repair (Hyde et al. 2004; Upton et al. 2008). In this study, we detected a SNP in the somatomedin B (SMB) domain of VTN, which binds to the urokinase receptor on the cell surface, promoting cell adhesion (Blasi, 1997; Chapman and Wei, 2001). The somatomedin B domain of vitronectin binds to plasminogen activator inhibitor-1 (PAI-1), and stabilizes it. Thus vitronectin serves to regulate proteolysis initiated by plasminogen activation (Zhou et al. 2003). This SNP (c.154A>G) that effects an amino acid exchange from a polar amino acid to a non-polar amino acid (p.Thr52Ala) might alter the function of this domain. Here, effects on pH and DRIP were observed that may be due to interference with the cell adhesion. For SNP c.154A>G, significant additive effects were detected for ABF, OPTO in DUPI, DRIP in PIF1(a) and COOK in PIF1(b), while significant dominance effects were detected for CON1 and CON24 in DUPI. For SNP c.156C>T, a significant additive effect was found for DRIP in PIF1(a). The mapping result revealed the position of VTN on SSC12 (Figure 21) in the QTL regions of carcass and meat quality traits i.e. meat color (Bergshire x Yorkshire population; Malek et al. 2001, commercial population; Harmegnies et al. 2006), diameter of muscle fiber (Duroc x Berlin miniature pig population; Wimmers et al. 2006), backfat thickness (Bergshire x Yorkshire population; Thomsen et al. 2004), loin eye area (Duroc x Berlin miniature pig population; Ponsuksili et al. 2005) and pH24 (Duroc x Pietrain population; Edwards et al. 2008). According to the PigQTL database, no QTL for drip loss was detected in this area.
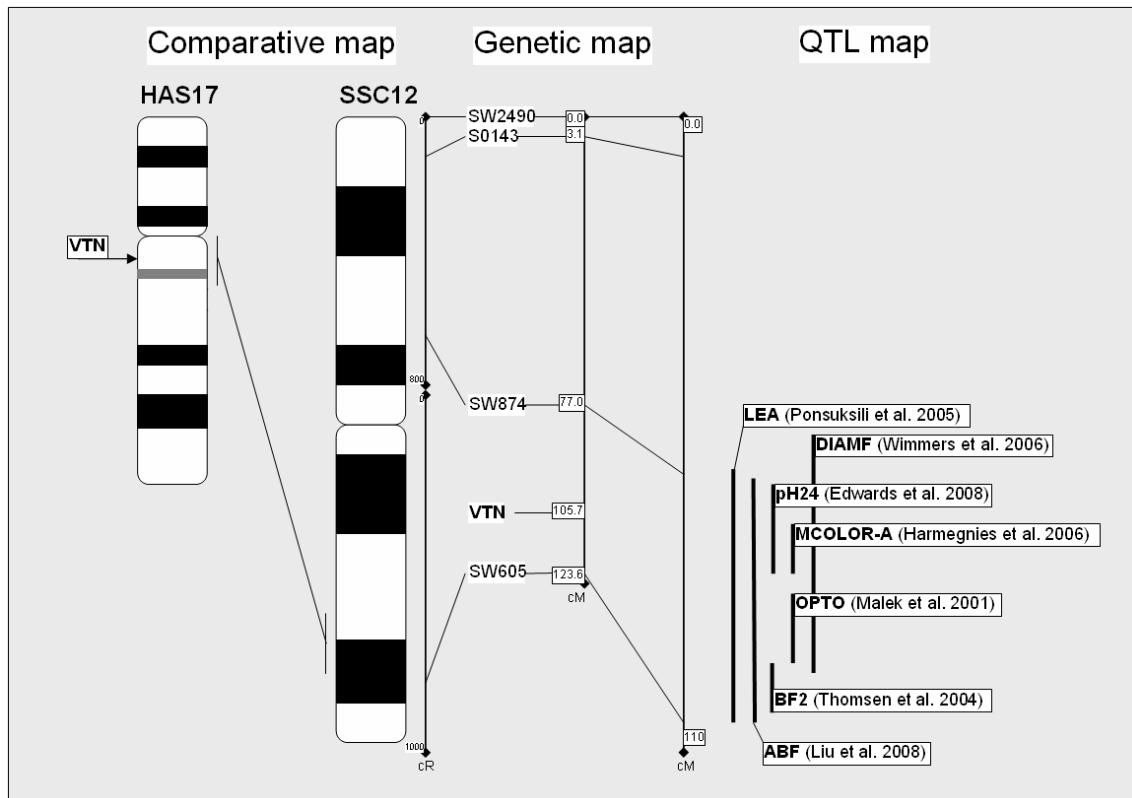
Figure 21: The location of VTN on SSC12

(Comparative map and RH map derived from Meyers et al. (2005): distance given in centi-Rays; cR, our own genetic map: distance between markers expressed in centi-Morgan; cM and QTL map based on pig qtl database: http://www.animalgenome.org/cgi-bin/QTLdb/SS/draw_chromap: distance given in centi-Morgan; cM)

5.2.12 The analysis of ZYX

Zyxin (ZYX) is one of the proteins in focal adhesions along the actin fibers and interacts with the actin cross linking protein α-actinin. ZYX has been postulated to play a role in actin organization, signal transduction (Macalma et al. 1996; Nix et al. 2001), cellular response to mechanical stress (Yoshigi et al. 2005) and cell-cell adhesion (Hansen and Beckerle, 2006). Structurally, ZYX has a N-terminal domain which interacts with SH3 domains of proteins involved in signal transduction, and a C-terminal LIM-domain comprising three copies of a cysteine- and histidine-rich motif known to mediate protein-protein and/or protein-DNA interactions involved in the regulation of cell proliferation and

differentiation (Hoffman et al. 2003). Due to the involvement of ZYX in cell structure and cell interconnection, ZYX is a candidate gene for carcass and meat quality traits in pigs. Three SNPs (c.279C>T, c.399A>G and c.522A>G) of ZYX were analysed. The SNP c.279C>T showed significant associations with carcass fatness traits (BF1, BF2, BF3 and ABF), OPTO and pH24 in PI, whereas the other two SNPs were associated with BF3 and ABF in DL. For the SNP c.279C>T, a significant additive effect was found for BF2, the 'C' allele increased BF2. The 'G' allele of SNP c.399A>G and 'A' allele of SNP c.522A>G increased carcass fatness (ABF and BF3). Furthermore, the ZYX haplotype showed significant associations with ABF and BF2 in PI, which corresponds to the results obtained for the SNP c.279A>C and also revealed new significant associations with DRIP and pH24 in PIF1(b) (P<0.05). The result of the genetic mapping showed that the ZYX gene is located on SSC18 (Figure 22) in the area of several QTL affecting carcass and meat quality traits such as average backfat (Berkshire x Yorkshire population; Malek et al. 2001), drip loss (commercial population; de Koning et al. 2001) and cooking loss (DUPI population; Liu et al. 2007) as well muscle fiber diameter (Duroc x Berlin miniature pig population; Wimmers et al. 2006). Moreover, several other candidate genes were also reported in this region including CAPZA2 (capping protein muscle Z-line, alpha 2), IFRD1 (Interferon-related developmental regulator 1), IGFBP3 (insulin-like growth factor binding protein 3) PGAM2 (phosphoglycerate mutase 2) and PRKAG2 (protein kinase, AMP-activated, gamma 2 non-catalytic subunit) (Jennen et al. 2007).
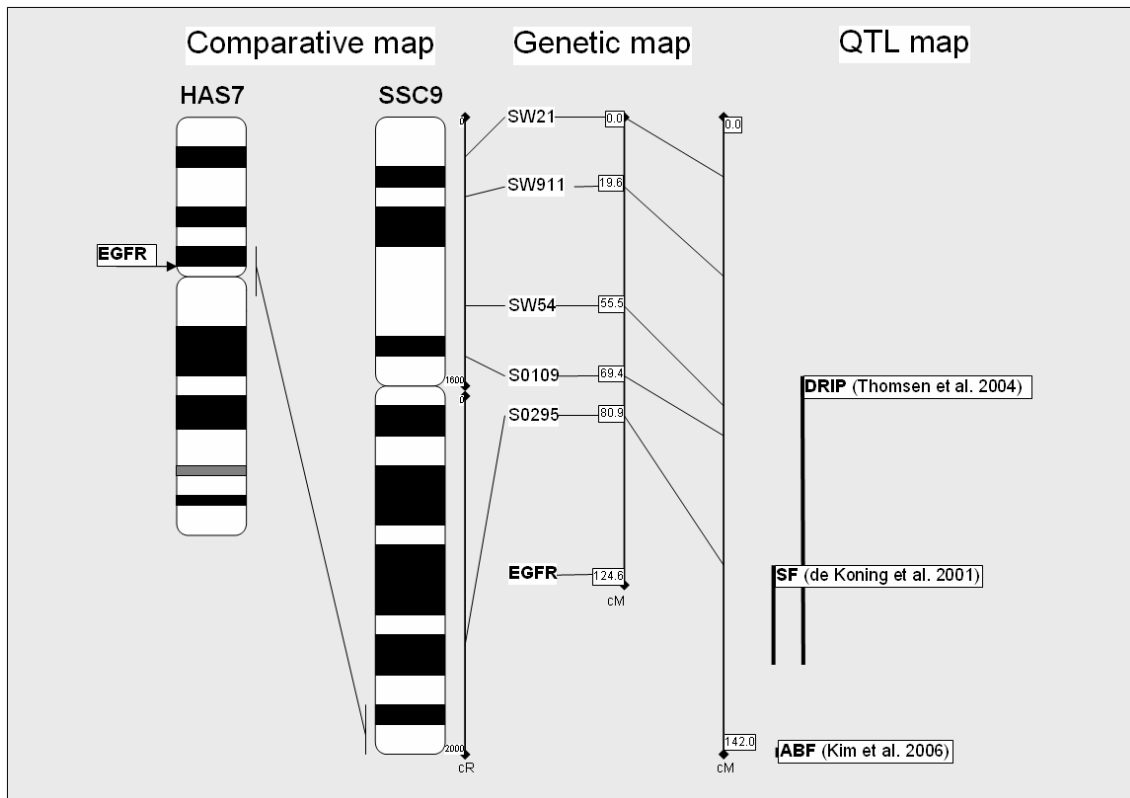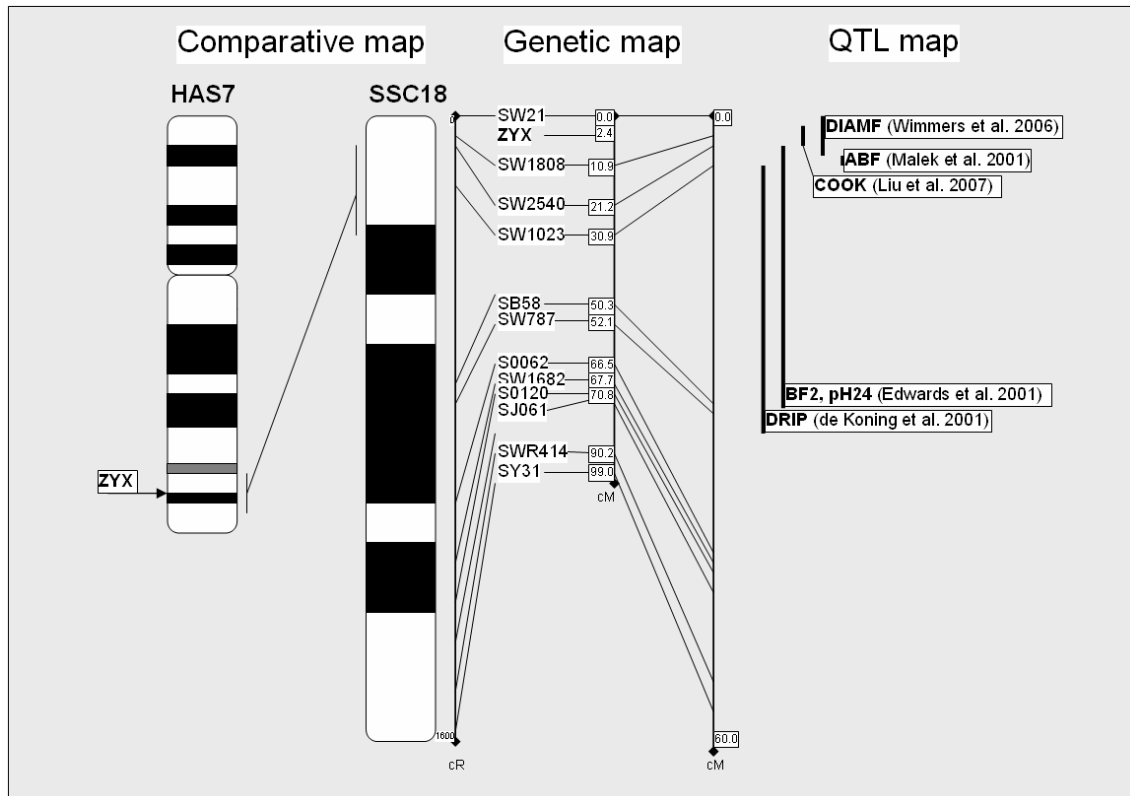
Figure 22: The location of ZYX on SSC18

(Comparative map and RH map derived from Meyers et al. (2005): distance given in centi-Rays; cR, our own genetic map: distance between markers expressed in centi-Morgan; cM and QTL map based on pig qtl database: http://www.animalgenome.org/cgi-bin/QTLdb/SS/draw_chromap: distance given in centi-Morgan; cM)

5.3 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 (Lee et al. 2000; Estévez et al. 2004; 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). This is not surprising as 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.

Positively correlated transcripts were enriched in functional categories and pathways like extracellular matrix receptor interaction and calcium signaling. Transcripts with negative correlations dominantly represented biochemical processes including oxidative phosphorylation, mitochondrial pathways, as well as transporter activity (Figure 23). Many studies have shown that the degradation of the cytoskeleton and other structural proteins play an important role in drip loss at the postmortem stage (Melody et al. 2004; Lonergan and Lonergan, 2005; Zhang et al. 2006; Scheffler and Gerrard, 2007). 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).
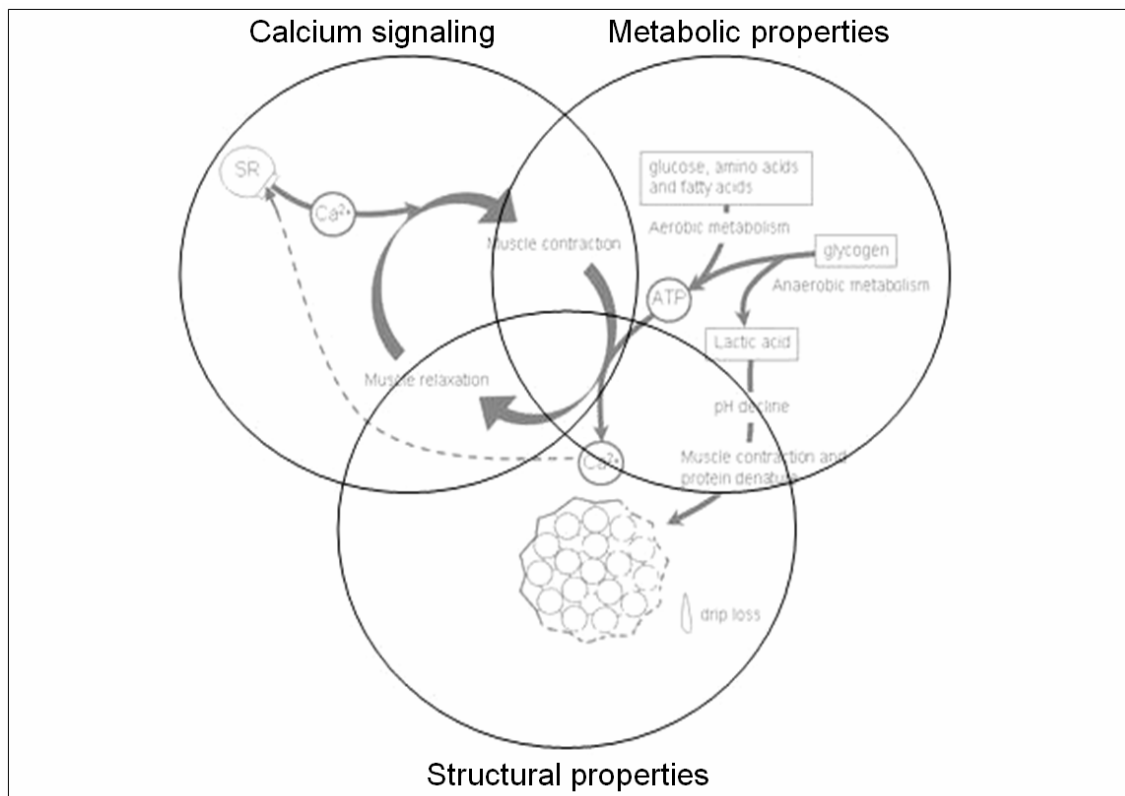
Figure 23: Functional pathways related to meat quality in terms of drip loss

The hypothesis is that if the genes in the extracellular matrix receptor interaction pathway promote muscle proteins that can better withstand degradation during the postmortem stage and the genes in the calcium signaling and/or the oxidative phosphorylation pathway maintain the ATP levels in muscle *post mortem* or reduce the rate of the pH decline, it might help to improve the meat quality, especially the water holding capacity.

In the present study, the candidate genes can be separated into two groups. The first group, LYZ, AHNAK, ZYX and EGFR, was reported as genes up-regulated or positively correlated with drip loss. In this group, only LYZ, AHNAK and ZYX were found to be significantly associated with drip loss in at least one pig population (P<0.05). The second group, genes down-regulated or negatively correlated with drip loss consists of VTN, UN, BVES, SLC3A2, CS, ZDHHC5 and COQ9. KERA, a gene up-regulated at high pH24 can be assigned to the same group of genes negatively correlated with drip loss, since it is known that drip loss is negatively correlated with pH. Six of these eight genes were significantly associated with drip loss in at least one pig population, including VTN, UN, BVES, SLC3A2, CS (P<0.05) and ZDHHC5 (P<0.001). In total, nine out of twelve genes were associated with drip loss in this study. Some genes expression levels showed correlation with drip loss 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 genes effects may be strongly dependent on the environmental effects that can mask an association. In general, heritability estimates for drip loss are quite low, varying from 0.08-0.30 depending on the method of drip measurement or the breed (Sellier, 1998; Sonesson et al. 1998; van Wijk et al. 2005; Suzuki et al. 2005; Hermesch et al. 2000).

Moreover, individual candidate genes can be assigned to 3 main groups (calcium signaling, metabolic properties and structural properties) according to their functions as described in the previous section. The 'calcium signaling' group includes EGFR and AHNAK. The 'metabolic properties' group includes CS and COQ9 which are both involved in the oxidative metabolism. The 'structural properties' group includes BVES, SLC3A2, KERA and ZYX. The rest of the candidate genes were assigned to a 'other properties' group. Some candidate genes may have special functions such as antioxidant (LYZ and COQ9) or cell membrane repair (AHNAK). 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). 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 (Figure 24). Muscle structural and metabolic properties expressed during life affect meat quality at post mortem.
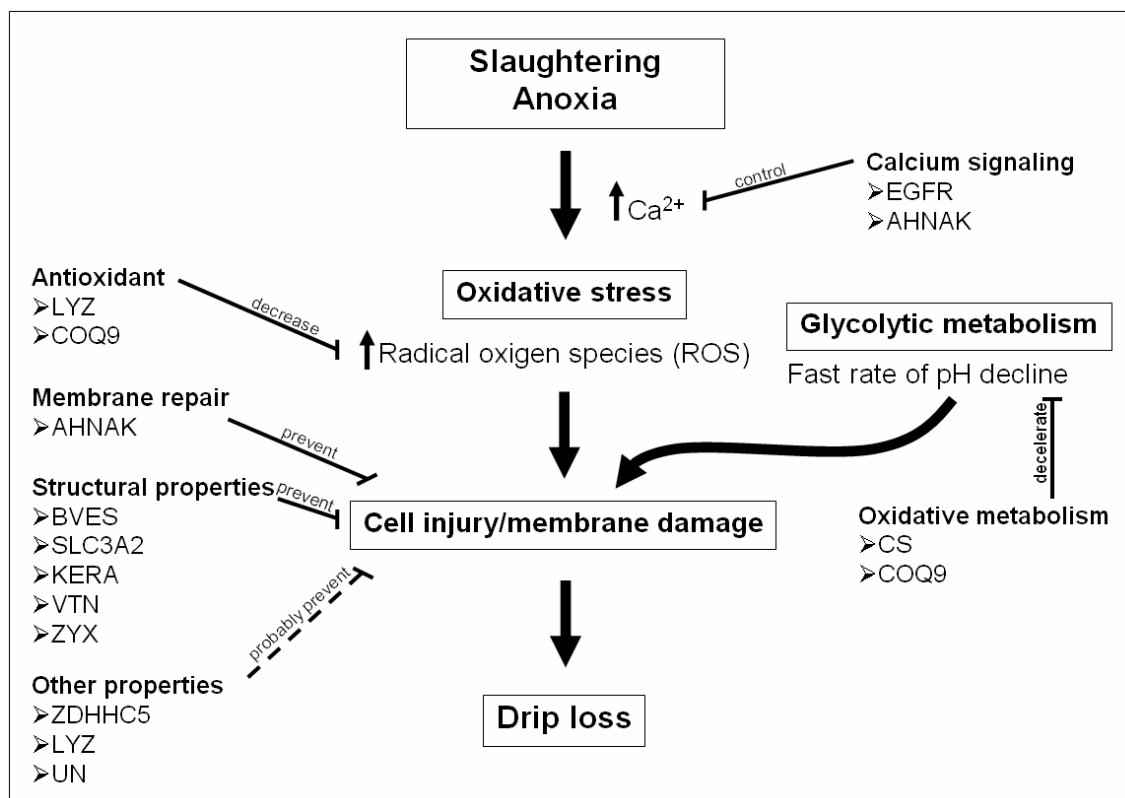
Figure 24: Hypothetical model for candidate genes in drip loss[1] formation

[1]Drip loss is usually expressed as a percent, gives an indication of water loss (difference in weight between 0 and 72 hours post mortem) (Honikel, 1998)

Although, most of our twelve candidate gene were selected based on their function that may related to meat quality, also many significant associations with carcass 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; Suzuki et al. 2005; Kušec et al.

2003; Ponsuksili et al. 2009); drip loss for example had strong positive correlations with loin eye area and conductivity at 24 hour p.m. and strong negative correlations with average backfat thickness, fat area, meat color (OPTO) and pH at 24 hour p.m. (Table 1) (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. The deviations from HWE that were observed for most genes in some crossbred populations are likely because of differences of the allele frequencies in the parental lines; deviations from HWE observed in purebreds is potentially due to selection. This may indicate that selection of different pig breeds took place due to different strategies (Goliásová and Wolf, 2004; Otto et al. 2007). In general, the Pietrain is very popular as a terminal sire, because it is renowned for its very high yield of lean meat and it is well known that German Landrace is a good maternal line (Mörlein et al. 2007). 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). Heritabilities of meat quality traits are relative low. Borchers et al. (2007) reported heritability of drip loss equal to 0.14 in Pietrain pigs after the MHS gene was corrected. Heritability of 0.37 for initial pH value in Pietrain pig was reported (Knapp et al. 1997). Suzuki et al. (2005) reported heritability for drip loss, cooking loss and pH in Duroc pigs were 0.14, 0.09 and 0.07 respectively. Shear force is a high heritable trait ($h^2 = 0.54$) in commercial pigs (Lindholm-Perry et al. 2009). In this study, q-values obtained for all 194 significant associations at p-value less than 0.05 varied from 0.06 to 0.41. This should be taken into account when interpreting the results. But the q-values derived from the FDR analysis provide a conservative estimate of the proportion of results that are falsely positive. Ideally,

these q-values should be small. However, in this study, some of the analyzed carcass and meat quality traits are correlated, especially carcass fatness traits (BF1, BF2, BF3, ABF, FA, MFR) are stronly correlated with each other. Therefore when SNP-traits association analyses are performed, the SNPs often showed significant associations with related traits. That means that correcting for the number of tests made (2100) is very stringent, because trait correlation is not taken into account. In this case, principal component (PC) analysis was suggested to reduce a whole set of correlated variables of carcass and meat quality to uncorrelated linear functions of the original variables without a significant loss of information (Karlson, 1992). The results of the PC analysis in this study are presented in Table 67-75 for carcass and meat quality parameters. In fact already ≤ 8 PC explain about 90% of the total variation. That indicates that a correlation for 2100 tests is too stringent. Principal component analyses suggest a reduction of number of test to 1280 corresponding to higher nominal values for significance thresholds. Assuming a q-value of 0.25 as a threshold, 26 significant associations still remained at p-value less than 0.01. Considering these results found that AHNAK was highly associated with MFR (SNP c.12907A>G, c.13014G>T and haplotype; P<0.001) and carcass fatness (ABF, FA, BF1) (SNP c.13014G>T; P<0.001), ZDHHC5 c.1803C>T was highly associated with DRIP (P<0.001), COQ9 was highly associated with FA (SNP c. 453A>G, +1247A>T and haplotype) and BF1 (SNP +1247A>T), and ZYX was associated with BF3 (SNP c.522A>G; P<0.001). Moreover, these candidate genes show mainly additive effect therefore, they 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 close to them and carcass and meat quality traits. The 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 for carcass and meat quality.

5.4 Future prospect

One of the main limitations to the dissection of economically important traits in livestock species has been the lack of a sufficient number of genetic markers for the development of

high-density and high-throughput assays for association studies. The genetic regulation of quantitative traits is complex and the identification of the genes that underlie genetic variation requires large numbers of genetic markers, such as microsatellites or SNPs. To date, many QTL (quantitative trait loci) have been localized to large chromosomal regions in several species of domestic animals, including the pig (Hu and Reecy, 2007; Rothschild et al. 2007). The need for more genetic markers is also supported by the extent of linkage disequilibrium (LD) in the pig genome, which has been estimated to extend from as little as 40-60 kb up to 400 kb in the commonly used commercial pig breeds, such as Duroc, Landrace and Large White (Jungerius et al. 2005; Amaral et al. 2008). It has been predicted that a marker density of 5-10 markers per cM (centiMorgan) will be needed to conduct whole genome association studies in the pig (Amaral et al. 2008; Du et al. 2007). In recent years, new sequencing technologies have emerged which offer great promise for marker discovery due to their ability to efficiently generate large amounts of sequence data, both in terms of time and cost. They are usually referred to as "second generation" or "next generation" sequencing technologies and include the Illumina Genome Analyzer (previously Solexa) and Roche's 454 FLX system, these instruments have been widely used for genome sequencing and re-sequencing and SNP discovery (Morozova and Marra, 2008). The pig SNP chip includes already validated SNPs as well as SNPs identified de novo. The high density 60K SNP chip will be an extremely valuable tool for the pig genomics community for a variety of applications including QTL and LD mapping, association studies and genomic selection (Morozova and Marra, 2008; Ramos et al. 2008) 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. Twelve genes, *BVES*, *SLC3A2*, *AHNAK*, *ZDHHC5*, *CS*, *LYZ*, *KERA*, *COQ9*, *UN* (a non-annotated EST), *EGFR*, *VTN* and *ZYX* 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, *in silico* analysis was performed which then suggested the target areas for amplification. Thirty seven SNPs and one Indel polymorphism were confirmed by sequence alignment using a panel of unrelated animals (one each of Pietrain, German Large White and German Landrace). Twenty-two out of these thirty-eight polymorphisms were selected randomly for genotyping. For the association studies, the SNPs of the first eleven genes were genotyped in ca. 1,800 animals from 6 pig populations including commercial herds of Pietrain (PI(a/b)), Pietrain x (German Large White x German Landrace) (PIF1(a/b/c)), and German Landrace (DL(a/b)) and one experimental F_2-population Duroc x Pietrain (DUPI). For ZYX, the SNPs were genotyped in 870 animals from 4 pig populations including PI, DL, F1 and PIF1. The assignments of all loci were performed in the DUPI population. The genetic mapping established the location of *BVES* on SSC1, of *SLC3A2*, *AHNAK* and *ZDHHC5* on SSC2, of *CS*, *LYZ* and *KERA* on SSC5, of *COQ9* on SSC6, of *UN* on SSC7, of *EGFR* on SSC9, of *VTN* on SSC12 and of *ZYX* on SSC18 respectively, coinciding with QTL regions for carcass and meat quality traits. Sixteen phenotypic traits including seven carcass traits (LEA, FA, MFR, BF1, BF2, BF3 and ABF) and nine meat quality traits (OPTO, pH1, pH24, CON1, CON24, SF, DRIP, COOK and THAW) were used to determine the association with candidate genes. All genes showed at least three associations at P<0.05. In particular, nine genes (*BVES*, *SLC3A2*, *AHNAK*, *ZDHHC5*, *CS*, *LYZ*, *UN*, *VTN* and *ZYX*) were associated with the trait DRIP.

In detail, the association analysis of *BVES* showed effects on BF3, DRIP (P<0.05) and pH24 (P<0.01). For *SLC3A2*, two polymorphisms were genotyped. The SNP c.1326A>G had effects on MFR and BF3 (P<0.05), whereas the Indel c.1336Indel[AGC] was associated with FA, BF1, BF2, ABF, pH1, CON1, SF, DRIP, COOK and THAW (P<0.05).

When combining the two polymorphisms to construct a haplotype, some traits (BF1, BF2, ABF, pH1, SF and THAW) were found to have significant relations with a certain genotype. For *AHNAK*, five SNPs were genotyped, including c.12907A>G, c.13014G>T, c.13281A>G, c.13290A>C>G and c.13294C>T (Acc. No: BX922331.2). In general, all five SNPs were related with carcass traits, especially LEA, ABF and BF3. FA, MFR, BF1 and ABF were highly significantly affected by the SNP c.13014G>T (P<0.001). The SNP c.12907A>G was also highly associated with MFR (P<0.001). Moreover, almost all meat quality traits except SF were associated with *AHNAK*. In particular, the trait DRIP was associated with the SNPs c.13014G>T and c.13281A>G (P<0.05). The *AHNAK* haplotype showed associations mainly with carcass traits, the most pronounced of all effects was found on MFR (P<0.001). The analysis of *ZDHHC5* revealed significant associations with FA, MFR, BF1, OPTO and COOK (P<0.05) and exhibited a highly significant association with DRIP (P<0.001). For *CS*, beside the effects on DRIP, associations with LEA, FA, BF3, ABF, pH1 and CON1 were also detected (P<0.05). For *LYZ*, two SNP were genotyped (c.240A>C and c.365A>T). The SNP c.240A>C was associated only with meat quality traits, including OPTO, pH1, CON24 and DRIP (P<0.05), whereas the SNP c.365A>T was associated with LEA, ABF, pH1, CON, THAW and DRIP (P<0.05). The haplotype of *LYZ* showed associations with the same traits that were affected by the SNP c.240A>C. For *KERA*, three associations were found, including LEA and the pH of both stages (P<0.05). The analysis of two SNPs (c.453A>G and +1247A>T) in *COQ9* revealed significant associations with various measures of carcass and meat quality traits. The SNP c.453A>G was associated with FA, MFR, BF2, pH1 and SF, whereas the SNP +1247A>T was associated with nearly all carcass traits and OPTO, SF and THAW. The haplotype effects of *COQ9* were nearly the same that were found in the SNP +1247A>T. Interestingly, FA was highly influenced by both of the SNP and the haplotype of *COQ9* (P<0.001), BF1 was highly associated with the SNP +1247A>T (P<0.001), whereas OPTO was highly related with the *COQ9* haplotype (P<0.001). The analysis of *UN* showed significant associations with carcass fatness traits and pH24, SF and DRIP (P<0.05). For *EGFR*, several significant associations were detected, including LEA, BF1, BF3, ABF, OPTO, pH24, CON24 and THAW (P<0.05). In *VTN*, two SNPs (c.154A>G and c.156C>T)

were studied. The effects of the SNP c.154A>G were detected on ABF and meat quality traits such as OPTO, pH, CON, DRIP and COOK (P<0.05), whereas the SNP c.156C>T had effects on MFR, BF2, pH, DRIP and COOK (P<0.05). The association analysis between the *VTN* haplotype and phenotypic traits confirmed the effect of the SNP c.156C>T and also showed a new significant association with FA (P<0.05). Finally, three SNPs (c.279C>T, c.399A>G and c.522A>G) of *ZYX* were analysed. The SNP c.279C>T showed significant associations with carcass fatness traits (BF1, BF2, BF3 and ABF), OPTO and pH24, whereas the other two SNPs were associated with FA, BF3 and ABF. Furthermore, the *ZYX* haplotype showed significant associations with ABF, BF2 and pH24 which corresponds to the results obtained for the SNP c.279A>C and also revealed a new significant association with DRIP (P<0.05) and LEA (P<0.01).

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, twelve candidate genes were investigated, the polymorphisms were detected and also the regional assignments were performed. The study used knowledge about their physiological roles to support their putative involvement in the genetic regulation of carcass and meat quality traits. The association study of twelve candidate genes revealed statistic evidence for a link between the genetic variation at these loci or close to them and carcass and meat quality traits.

Zusammenfassung

Die vorliegende Arbeit analysiert Kandidatengene, die aufgrund ihrer differentiellen Expression und/oder ihrer von dem Merkmal Wasserbindungsvermögen abhängigen Expression ausgewählt wurden. Zwölf Gene, *BVES*, *SLC3A2*, *AHNAK*, *ZDHHC5*, *CS*, *LYZ*, *KERA*, *COQ9*, *UN* (ein unannotiertes EST), *EGFR*, *VTN* und *ZYX* wurden basierend auf ihrer bekannten Funktion und/oder ihrer Zuordnung zu QTL-Regionen für Schlachtkörper- und Fleischqualitätsmerkmale ausgewählt. Zur Identifikation von Polymorphismen wurde eine *in silico*-Analyse durchgeführt, die Zielgebiete für die Amplifikation nahelegte. 37 SNPs und ein Indel-Polymorphismus wurden durch das Sequenzalignment einer Auswahl nicht-verwandter Tiere (jeweils ein Pietrain, Deutsches Edelschwein und Deutsche Landrasse) bestätigt. Von 38 Polymorphismen wurden 22 zufällig für die Genotypisierung ausgewählt. Für die Assoziationsstudien wurden die SNPs der ersten elf Gene in ca. 1.800 Tieren aus sechs Schweinepopulationen genotypisiert. Dazu gehörten kommerzielle Herden der Rassen Pietrain (PI(a/b)), Deutsche Landrasse (DL(a/b)) und der Drei-Rassen-Kreuzung Pietrain x (Deutsches Edelschwein x Deutsche Landrasse) (PIF1(a/b/c)) sowie eine experimentelle F2-Population aus Duroc x Pietrain (DUPI). Für *ZYX* wurden die SNPs in 870 Tieren aus vier Schweinepopulationen genotypisiert; dazu gehörten PI, DL, F1 und PIF1. Die Zuordnungen aller Loci wurden in der DUPI-Population durchgeführt. Die genetische Kartierung zeigte, dass *BVES* auf SSC1, *SLC3A2*, *AHNAK* und *ZDHHC5* auf SSC2, *CS*, *LYZ* und *KERA* auf SSC5, *COQ9* auf SSC6, *UN* auf SSC7, *EGFR* auf SSC9, *VTN* auf SSC12 und *ZYX* auf SSC18 lokalisiert sind, jeweils in QTL-Regionen für Schlachtkörper- und Fleischqualitätsmerkmale. Sechzehn phänotypische Merkmale, darunter sieben Schlachtkörpermerkmale (LEA, FA, MFR, BF1, BF2, BF3 und ABF) und neun Fleischqualitätsmerkmale (OPTO, pH1, pH24, CON1, CON24, SF, DRIP, COOK und THAW) wurden mit den Kandidatengenen assoziiert. Alle Gene zeigten mindestens drei Assoziationen mit P<0,05. Insbesondere waren neun Gene (*BVES*, *SLC3A2*, *AHNAK*, *ZDHHC5*, *CS*, *LYZ*, *UN*, *VTN* und *ZYX*) mit dem Merkmal DRIP assoziiert.

Die Assoziationsanalyse von *BVES* zeigte im Einzelnen einen Effekt auf BF3, DRIP (P<0,05) und pH24 (P<0,01). Für *SLC3A2* wurden zwei Polymorphismen genotypisiert.

Der SNP c.1326A>G hatte einen Effekt auf MFR und BF3 (P<0,05), während der Indel c.1336Indel[AGC] mit FA, BF1, BF2, ABF, pH1, CON1, SF, DRIP, COOK und THAW (P<0,05) assoziiert war. Aus den beiden Polymorphismen wurde ein Haplotyp konstruiert und einige Merkmale (BF1, BF2, ABF, pH1, SF und THAW) zeigten signifikante Zusammenhänge mit einem bestimmten Genotyp. Für *AHNAK* wurden fünf SNPs genotypisiert; darunter waren c.12907A>G, c.13014G>T, c.13281A>G, c.13290A>C>G und c.13294C>T (Acc. No: BX922331.2). Generell standen alle fünf SNPs mit Schlachtkörpermerkmalen in Verbindung, vor allem LEA, ABF und BF3. FA, MFR, BF1 und ABF waren hoch signifikant mit dem SNP c.13014G>T (P<0,001). Der SNP c.12907A>G war außerdem stark assoziiert mit MFR (P<0,001). Ferner waren alle Fleischqualitätsmerkmale außer SF mit *AHNAK* assoziiert. Vor allem das Merkmal DRIP zeigte eine starke Assoziation mit dem SNP c.13014G>T und c.13281A>G (P<0,05). Der *AHNAK*-Haplotyp zeigte hauptsächlich Assoziationen zu Schlachtkörpermerkmalen. Der deutlichste Effekt wurde auf MFR (P<0,001) gefunden. Die Analyse von *ZDHHC5* ließ siginifikante Assoziationen mit FA, MFR, BF1, OPTO und COOK (P<0,05) erkennen und zeigte eine hoch signifikante Assoziation mit DRIP (P<0,001). Für *CS* wurden, neben dem Effekt auf DRIP, weitere Assoziationen mit LEA, FA, BF3, ABF pH1 und CON1 entdeckt (P<0,05). Für *LYZ* wurden zwei SNPs genotypisiert (c.240A>C und c.365A>T). Der SNP c.240A>C war nur mit Fleischqualitätsmerkmalen assoziiert, darunter OPTO, pH1, CON24 und DRIP (P>0,05), während der SNP c.365A>T mit LEA, ABF, pH1, CON, THAW und DRIP (P>0,05) assoziiert war. Der Haplotyp von *LYZ* zeigte Assoziationen mit denselben Merkmalen, die von dem SNP c.240A>C beeinflusst wurden. Für *KERA* wurden drei Assoziationen gefunden (LEA und beide pH-Werten (P<0,05)). Die Analyse von zwei SNPs (c.453A>G und +1247A>T) in *COQ9* ließ signifikante Assoziationen mit verschiedenen Messgrößen für Schlachtkörper- und Fleischqualitätsmerkmale erkennen. Der SNP c.453A>G war mit FA, MFR, BF2, pH1 und SF assoziiert, während der SNP +1247A>T mit nahezu allen Schlachtkörpermerkmalen und OPTO, SF und THAW assoziiert war. Die Haplotypeneffekte von *COQ9* waren nahezu identisch mit den Effekten, die im SNP +1247A>T gefunden wurden. Interessanterweise wurde FA durch beide SNPs und den Haplotyp von *COQ9* stark beeinflusst (P<0,001). BF1 war stark assoziiert mit dem

SNP +1247A>T (P<0,001), während OPTO stark vom *COQ9*-Haplotyp abhängig war (P<0,001). Die Analyse von *UN* zeigte signifikante Assoziationen mit Schlachtkörper-Fettgehaltsmerkmalen und pH24, SF und DRIP (P<0,05). Für *EGFR* wurden verschiedene signifikante Assoziationen gefunden, darunter LEA, BF1, BF3, ABF, OPTO, pH24, CON24 und THAW (P<0,05). In *VTN* wurden zwei SNPs (c.154A>G und c.156C>T) untersucht. Effekte des SNPs c.154A>G konnten auf ABF und Fleischqualitätsmerkmale wie OPTO, pH, CON, DRIP und COOK festgestellt werden (P<0,05), während der SNP c.156C>T Effekte auf MFR, BF2, pH, DRIP und COOK hatte (P<0,05). Die Assoziationsanalyse zwischen dem *VTN*-Haplotyp und den Phänotyp-Merkmalen bestätigte die Effekte des SNPs c.156C>T und zeigte außerdem eine neue signifikante Assoziation mit FA (P<0,05). Schließlich wurden drei SNPs von *ZYX* analysiert (c.279C>T, c.399A>G and c.522A>G). Der SNP c.279C>T zeigte signifikante Assoziationen mit Schlachtkörper-Fettgehaltsmerkmalen (BF1, BF2, BF3 und ABF), OPTO und pH24, während die beiden anderen SNPs mit FA, BF3 und ABF assoziiert waren. Weiterhin wies der *ZYX*-Haplotyp signifikante Assoziationen mit ABF, BF2 und pH24 auf, was mit den Ergebnissen für den SNP c.279C>T korrespondiert und es zeigte sich außerdem eine neue signifikante Assoziation mit DRIP (P<0,05) und LEA (P<0,01).

Die individuellen Kandidatengene zeigten verschiedene Effekte auf bestimmte Merkmale, die, soweit aus der Literatur bekannt, miteinander korreliert sind. Allerdings zeigte keines der Gene signifikante Assoziationen mit einem bestimmten Merkmal in allen Populationen. Dies könnte an den rassenspezifischen Effekten liegen, die zu dem extremen Muskelphänotyp der Schweinerassen beitragen, an einem unvollständigen Kopplungsungleichgewicht mit kausalen Mutationen und/oder an Effekten im Kontext von DNA-Variationen an anderen interagierenden Loci. Dafür spricht, dass die analysierten Merkmale quantitative Merkmale sind, die von verschiedenen Loci kontrolliert werden.

Fazit: Es wurden zwölf Kandidatengene untersucht, Polymorphismen ermittelt und regionale Zuordnungen durchgeführt. Mit dem Wissen um ihre physiologische Rolle unterstützt diese Studie die putative Beteiligung der Kandidatengene an der genetischen Regulation von Schlachtkörper- und Fleischqualitätsmerkmalen. Die Assoziationsstudien der zwölf Kandidatengene zeigten statistische Beweise für einen Zusammenhang von

genetischer Variation an oder in der Nähe dieser Loci mit Schlachtkörper- und Fleischqualitätsmerkmalen.

7. References

Amaral AJ, Megens HJ, Crooijmans RP, Heuven HC, Groenen MA (2008): Linkage disequilibrium decay and haplotype block structure in the pig. Genetics. 179, 569-579

Andrée B, Hillemann T, Kessler-Icekson G, Schmitt-John T, Jockusch H, Arnold HH, Brand T (2000): Isolation and characterization of the novel popeye gene family expressed in skeletal muscle and heart. Dev. Biol. 223, 371-382

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

Bee G, Anderson AL, Lonergan SM, Huff-Lonergan E (2007): Rate and extent of pH decline affect proteolysis of cytoskeletal proteins and water-holding capacity in pork. Meat Sci. 76, 359-365

Blasi F (1997): uPA, uPAR, PAI-1: Key intersection of proteolytic, adhesive and chemotactic highways? Immunol. Today. 18, 415-417

Blomberg le A, Garrett WM, Guillomot M, Miles JR, Sonstegard TS, Van Tassell CP, Zuelke KA (2006): Transcriptome profiling of the tubular porcine conceptus identifies the differential regulation of growth and developmentally associated genes. Mol. Reprod. Dev. 73, 1491-1502

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

Borgonovo B, Cocucci E, Racchetti G, Podini P, Bachi A, Meldolesi J (2002): Regulated exocytosis: a novel, widely expressed system. Nat. Cell Biol. 4, 955-962

Bowker BC, Grant AL, Forrest JC Gerrard DE (2000): Muscle metabolism and PSE pork. J. Anim. Sci. 79, 1-8

Cagnazzo M, te Pas MF, Priem J, de Wit AA, Pool MH, Davoli R, Russo V (2006): Comparison of prenatal muscle tissue expression profiles of two pig breeds differing in muscle characteristics. J. Anim. Sci. 84, 1-10

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

Carr TR, Kauffmann RG, Meeker DL, Meisinger DL (1997): Factors reducing pork value pork industry handbook, PIH-135. West Lafayette, IN: Purdue University Cooperative Extension Service, pp. 1-4

Chapman HA, Wei Y (2001): Protease crosstalk with integrins: the urokinase receptor paradigm. Thromb. Haemost. 86, 124-129

Chaudhary R, Chowdhary BP, Harbitz I, Gustavsson I, Evans CT (1992): Localization of the citrate synthase (CS) gene to the p12-p13 bands of chromosome 5 in pigs by in situ hybridization. Hereditas. 117, 39-43

Chaudhary R, Wintero AK, Fredholm M, Chowdhary BP (1997): FISH mapping of seven cDNA sequences in the pig. Chromosome Res. 5, 545-549

Chen H, Li C, Fang M, Zhu M, Li X, Zhou R, Li K, Zhao S (2009): Understanding Haemophilus parasuis infection in porcine spleen through a transcriptomics approach. BMC Genomics 10, 64

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

Ciesĺak D, Kapelański W, Blicharski T, Pierzchala M (2000): Restriction fragment length polymorphisms in myogenin and myf3 genes and their influence on lean meat content in pigs. J. Anim. Breed Genet. 117, 43-55

Ciobanu DC, Bastiaansen J, Malek M, Helm J, Woollard J, Plastow GS, Rothschild MF (2001): Evidence for new alleles in the protein kinase AMP-activated, subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. Genetics 159, 1151-1162

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

Corpet F (1988): Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res. 16, 10881-10890

Crane FL (2001): Biochemical functions of coenzyme Q10. J. Am. Coll. Nutr. 20, 591-598

Crosier AE, Farin CE, Rodriguez KF, Blodin P, Alexander JE, Farin PW (2002): Development of skeletal muscle and expression of candidate genes in bovine fetuses from embryos produced in vivo or in vitro. Biol. Reprod. 67, 401-408

Cuvelier C, Clinquart A, Hocquette JF, Cabaraux JF, Dufrasne I, Istasse L, Hornick JL (2006): Comparison of composition and quality traits of meat from young finishing bulls from Belgian Blue, Limousin and Aberdeen Angus breeds. Meat Sci. 74, 522-531

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

Dekkers JCM (2004): Commercial application of marker- and gene-assited selection in livestock: Strategies and lessons. J. Anim. Sci. (E. Suppl.), E313-E328

Delp MD, Duan C (1996): Composition and size of type I, IIA, IID/X and IIB fibers and citrate synthase activity of rat skeletal muscle. J. Appl. Physiol. 80, 261-270

den Hartigh JC, van Bergen en Henegouwen PMP, Verkleij AJ, Boonstra J (1992): The EGF-receptor is an actin-binding protein. J. Cell Biol. 119, 349-355

Draper JM, Smith CD (2009): Palmitoyl acyltransferase assays and inhibitors (Review). Mol. Membr. Biol. 26, 5-13

Du FX, Clutter AC, Lohuis MM (2007): Characterizing linkage disequilibrium in pig populations. Int. J. Biol. Sci. 3, 166-178

Duan YY, Ma JW, Yuan F, Huang LB, Yang KX, Xie JP, Wu GZ, Huang LS (2009): Genome-wide identification of quantitative trait loci for pork temperature, pH decline, and glycolytic potential in a large-scale White Duroc x Chinese Erhualian resource population. J. Anim. Sci. 87, 9-16

Dunphy JT, Linder ME (1998): Signalling functions of protein palmitoylation. Biochim. Biophys. Acta 1436, 245-261

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

Essen-Gustavsson B, Karlstrom K, Lundstrom K (1992): Muscle fibre characteristics and metabolic response at slaughter in pigs of different halothane genotypes and their relation to meat quality. Meat Sci. 31, 1-11

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

Evans GJ, Giuffra E, Sanchez A, Kerje S, Davalos G, Vidal O, Illán S, Noguera JL, Varona L, Velander I, Southwood O I, de Koning DJ, Haley CS, Plastow GS, Andersson L (2003): Identification of quantitative trait loci for production traits in commercial pig populations. Genetics. 164, 621-627

Fahrenkrug SC, Freking BA, Smith TP, Rohrer GA, Keele JW (2002): Single nucleotide polymorphism (SNP) discovery in porcine expressed genes. Anim. Genet. 33, 186-95

Feral CC, Nishiya N, Fenczik CA, Stuhlmann H, Slepak M, Ginsberg MH (2005): CD98hc (SLC3A2) mediates integrin signaling. PNAS. 102, 355-360

Fernandez X. Neyraud E, Astruc T, Sante V (2002): Effects of halothane genotype and pre-slaughter treatment on pig meat quality. part 1. post mortem metabolism, meat quality indicators and sensory traits of m. longissimus lumborum, Meat Sci. 62, 429-437

Finkel T (1998): Oxygen radicals and signaling. Curr. Opin. Cell. Biol. 10, 248-253

Fontanesi L, Davoli R, Nanni Costa L, Beretti F, Scotti E, Tazzoli M, Tassone F, Colombo M, Buttazzoni L, Russo V (2008): Investigation of candidate genes for glycolytic potential of porcine skeletal muscle: Association with meat quality and production traits in Italian Large White pigs. Meat Sci. 80, 780-787

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

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

Fukata M, Fukata Y, Adesnik H, Nicoll RA, Bredt DS (2004): Identification of PSD-95 palmitoylating enzymes. Neuron. 44, 987-996

Gentil BJ, Delphin C, Mbele GO, Deloulme JC, Ferro M, Garin J, Baudier J (2001): The giant protein AHNAK is a specific target for the calcium- and zinc-binding S100B protein: potential implications for Ca^{2+} homeostasis regulation by S100B. J. Biol. Chem. 276, 23253-23261

Gentil BJ, Delphin C, Benaud C, Baudier J (2003): Expression of the giant protein AHNAK (desmoyokin) in muscle and lining epithelial cells. J. Histochem. Cytochem. 51, 339-348

Goliásová E, Wolf J (2004): Impact of the ESR gene on litter size and production traits in Czech Large White pigs. Anim. Genet. 35, 293-297

Gorbenko GP, Ioffe VM, Kinnunen PKJ (2007): Binding of lysozyme to phospholipid bilayers: evidence for protein aggregation upon membrane association. Biophys. J. 93, 140-153

Green P, Falls K, Crooks S (1990): Documentation for CRI-MAP, Version 2.4. http://www.animalgenome.org/~hu/CRIMAPwkshp/crimap-doc.html.

Green JA, Kim JG, Whitworth KM, Agca C, Prather RS (2006): The use of microarrays to define functionally-related genes that are differentially expressed in the cycling pig uterus. Soc. Reprod. Fertil. Suppl. 62, 163-176

Haase H, Pagel I, Khalina Y, Zacharzowsky U, Person V, Lutsch G, Petzhold D, Kott M, Schaper J, Morano I (2004): Thecarboxyl-terminal AHNAK domain induces actin bundling andstabilizes muscle contraction. FASEB. J. 18, 839-841

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

Hansen MDH, Beckerle MC (2006): Opposing roles of Zyxin/LPP ACTA repeats and the LIM domain region in cell-cell adhesion. J. Biol. Chem. 281, 16178-16188

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

Hausman GJ, Poulos SP, Richardson RL, Barb CR, Andacht T, Kirk HC, Mynatt RL (2006): Secreted proteins and genes in fetal and neonatal pig adipose tissue and stromal-vascular cells. J. Anim. Sci. 84, 1666-1681

Henckel P, Oksbjerg N, Erlandsen E, Barton-Gade P, Bejerholm C (1997): Histo- and biochemical characteristics of the Longissimus Dorsi muscle in pigs and their relationships to performance and meat quality. Meat Sci. 47, 311-321

Herbst RS (2004): Review of epidermal growth factor receptor biology. Int. J. Radiat. Oncol. Biol. Phys. 59, 21-26

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

Hoffman LM, Nix DA, Benson B, Boot-Hanford R, Gustafsson E, Jamora C, Menzies AS, Goh KL, Jensen CC, Gertler FB, Fuchs E, Fässler R, Beckerle MC (2003): Targeted disruption of the murine zyxin gene. Mol. Cell. Biol. 23, 70-79

Hohaus A, Person V, Behlke J, Schaper J, Morano I, Haase H (2002): The carboxyl-terminal region of AHNAK provides a link between cardiac L-type Ca^{2+} channels and the actin-based cytoskeleton. FASEB. J. 16, 1205-1216

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

Hu ZL, Reecy JM (2007): Animal QTLdb: beyond a repository. A public platform for QTL comparisons and integration with diverse types of structural genomic information. Mamm. Genom 18, 1-4

Huang Y, Laval SH, van Remoortere A, Baudier J, Benaud C, Anderson LV, Straub V, Deelder A, Frants RR, den Dunnen JT, Bushby K, van der Maarel SM (2007): AHNAK, a novel component of the dysferlin protein complex, redistributes to the cytoplasm with dysferlin during skeletal muscle regeneration. FASEB J. 21, 732-742

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, Baas TJ, Malek M, Dekkers JCM, Prusa K, Rothschild MF (2003): Correlations among selected pork quality traits. J. Anim. Sci. 80, 617-627

Hughes AR, Bird GS, Obie JF, Thastrup O, Putney JW (1991): Role of inositol (1,4,5) trisphosphate in epidermal growth factor-induced Ca2+ signaling in A431 cells. Mol. Pharmacol. 40, 254-262

Hyde C, Hollier B, Anderson A, Harkin D, Upton Z (2004): Insulin-like growth factors (IGF) and IGF-binding proteins bound to vitronectin enhance keratinocyte protein synthesis and migration. J. Invest. Dermatol. 122, 1198-206

Ibrahim HR, Thomas U, Pellegrini A (2001): A helix-loop-helix peptide at the upper lip of the active site cleft of lysozyme confers potent antimicrobial activity with membrane permeabilization action. J. Biol. Chem. 276, 43767-43774

Iozzo RV (1998): Matrix proteoglycans: from molecular design to cellular function. Annu. Rev. Biochem. 67, 609-652

Iozzo RV (1999): The biology of the small leucine-rich proteoglycans. Functional network of interactive proteins. J. Biol. Chem. 274, 18843-18846

Jennen DGJ, Brings AD, Liu G, Jüngst 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, 2-11

Johnson A, Gin P, Marbois BN, Hsieh EJ, Wu M, Barros MH, Clarke CF, Tzagoloff A (2005): COQ9, a new gene required for the biosynthesis of coenzyme Q in Saccharomyces cerevisiae. J. Biol. Chem. 280, 31397-31404

Jungerius BJ, Rattink AP, Crooijmans RP, van der Poel JJ, van Oost BA, te Pas MF, Groenen MA (2003): Development of a single nucleotide polymorphism map of porcine chromosome 2. Anim. Genet. 34, 429-437

Jungerius BJ, Gu J, Crooijmans RPMA, Poel JJ, Groenen MA, van Oost BA, te Pas MF (2005): Estimation of the extent of linkage disequilibrium in seven regions of the porcine genome. Anim. Biotechnol. 16, 41-54

Kallweit E, Henning M, Baulain U (2007): Recent market requirement for pork quality in germany. Poljoprivreda 13, 25-29

Karlsson A (1992): The use of principal component analysis (PCA) for evaluating results from pig meat quality measurements. Meat Sci. 31, 423-433

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

Kim JJ, Zhao H, Thomsen H, Rothschild MF, Dekkers JCM (2005): Combined line-cross and half-sib QTL analysis of crosses between outbred lines. Genet. Res. 85, 235-248

Kim CW, Hong YH, Yun SI, Lee SR, Kim YH, Kim MS, Chung KH, Jung WY, Kwon EJ, Hwang SS, Park DH, Cho KK, Lee JG, Kim BW, Kim JW, Kang YS, Yeo JS, Chang KT (2006): Use of microsatellite markers to detect quantitative trait loci in Yorkshire pigs. J. Reprod. Dev. 52, 229-237

Kjaergaard M, Gårdsvoll H, Hirschberg D, Nielbo S, Mayasundari A, Peterson CB, Jansson A, Jørgensen TJD, Poulsen FM, Ploug M (2007): Solution structure of recombinant somatomedin B domain from vitronectin produced in Pichia pastoris. Protein Sci. 16, 1934-1945

Knapp P, Willam A, Solkner J (1997): Genetic parameters for leanmeat content and meat quality traits in different pig breeds. Lives. Prod. Sci., 52, 69-73

Kohn TA, Kritzinger B, Hoffman LC, Myburgh KH (2005): Characteristics of impala (*Aepyceros melampus*) skeletal muscles. Meat Sci. 69, 277-282

Kricker JA, Towne CL, Firth SM, Herington AC, Upton Z (2003): Structural and functional evidence for the interaction of insulin-like growth factors (IGFs) and IGF binding proteins with vitronectin. Endocrinology. 144, 2807-2815

Kucharská J, Ulicná O, Gvozdjáková A, Sumbalová Z, Vancová O, Bozek P, Nakano M, Greksák M (2004): Regeneration of coenzyme Q9 redox state and inhibition of oxidative stress by Rooibos tea (Aspalathus linearis) administration in carbon tetrachloride liver damage. Physiol. Res. 53, 515-521

Kudoh J, Wang Y, Minoshima S, Hashimoto T, Amagai M, Nishikawa T, Shtivelman E, Bishop JM, Shimizu N (1995): Localization of the human AHNAK/desmoyokin

gene (AHNAK) to chromosome band 11q12 by somatic cell hybrid analysis and fluorescence in situ hybridization. Cytogenet. Cell Genet. 70, 218-220

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, Ørtenblad N (2001): Cellular model for induction of drip loss in meat. J. Agric. Food Chem. 49, 4876-4883

Le Roy P, Naveau J, Elsen JM, Sellier P (1990): Evidence for a new major gene influencing meat quality in pigs. Genet. Res. 55, 33-44

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

Lee SS, Chen Y, Moran C, Stratil A, Reinder G, Bartenschlager H, Moser G, Geldermann H (2003): Linkage and QTL mapping for Sus scrofa chromosome 5. J. Anim. Breed. Genet. 120, 38-44

Lee IH, You JO, Ha KS, Bae DS, Suh PG, Rhee SG, Bae YS (2004): AHNAK-mediated activation of phospholipase C-gamma1 through protein kinase C. J. Biol. Chem. 279, 26645-26653

Lenaz G, Bovina C, D'Aurelio M, Fato R, Formiggini G, Genova ML, Giuliano G (1998): Role of mitochondria in oxidative stress and aging. Biochim. Biophys. Acta. 1366, 53-67

Lengerken GV, Maak S, Wicke M (2002): Muscle metabolism and meat quality of pigs and poultry. Veterinarija ir zootechnika. 20, 82-86

Leong WF, Zhou T, Lim GL, Li B (2009): Protein palmitoylation regulates osteoblast differentiation through BMP-induced osterix. PLoS One. 4, e4135

Li M, Zhu L, Li X, Shuai S, Teng X, Xiao H, Li Q, Chen L, Guo Y, Wang J (2008): Expression profiling analysis for genes related to meat quality and carcass traits during postnatal development of backfat in two pig breeds. Sci. China C Life Sci. 51, 718-733

Lin S, Zhao D, Bownes M (2007): Blood vessel/epicardial substance (bves) expression, essential for embryonic development, is down regulated by Grk/EFGR signaling Int. J. Dev. Biol. 51, 37-44

Lindholm-Perry AK, Rohrer GA, Holl JW, Shackelford SD, Wheeler TL (2009): Relationships among calpastatin single nucleotide polymorphisms, calpastatin expression and tenderness in pork longissimus. Anim. Genet. 40, 713-721

Liu H, Zheng F, Li Z, Uribarri J, Ren B, Hutter R, Tunstead JR, Badimon J, Striker GE, Vlassara H (2006a): Reduced acute vascular injury and atherosclerosis in hyperlipidemic mice transgenic for Lysozyme. Am. J. Pathol. 169, 303-313

Liu H, Zheng F, Cao Q, Ren B, Zhu L, Striker G, Vlassara H (2006b): Amelioration of oxidant stress by the defensin lysozyme. Am. J. Physiol. Endocrinol. Metab. 290, E824-E832

Liu M, Peng J, Xu D, Zheng R, Li F, Li J, Zuo B, Lei M, Xiong Y, Deng C, Jiang S (2007): Associations of MFY5 gene polymorphisms with meat quality traits in different domestic pig (Sus scrofa) populations. Genet. Mol. Biol. 30, 370-374

Liu M, Peng J, Xu DQ, Zheng R, Li FE, Li JL, Zuo B, Lei MG, Xiong YZ, Deng CY, Jiang SW (2008): Association of MYF5 and MYOD1 gene polymorphisms and meat quality traits in Large White x Meishan F2 pig populations. Biochem Genet. 46, 720-732

Liu G, Kim JJ, Jonas E, Wimmers K, Ponsuksili S, Murani E, Phatsara C, Tholen E, Juengst H, Tesfaye D, Chen JL, Schellander K (2008): Combined line-cross and half-sib QTL analysis in Duroc-Pietrain population. Mamm. Genome. 19, 429-438

Liu G, Jennen DGJ, Tholen E, Jüngst H, Kleinwächter T, Hölker M, Tesfaye D, Ün C, Schreinemachers HJ, Murani E, Ponsuksili S, Kim JJ, Schellander K, Wimmers K (2007): A genome scan reveals QTL for growth, fatness, leanness and meat quality in a Duroc-Pietrain resource population. Anim. Genet. 38, 241-252

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

Lynn GW, Heller WT, Mayasundari A, Minor KH, Peterson CB (2005): A model for the three-dimensional structure of human plasma vitronectin from small-angle scattering measurements. Biochem. 44, 565-574

Maak S, Boettcher D, Tetens J, Wensch-Dorendorf M, Nürnberg G, Wimmers K, Swalve HH, Thaller G (2009): Identification of candidate genes for congenital splay leg in piglets by alternative analysis of DNA microarray data. Int. J. Biol. Sci. 5, 331-337

Macalma T, Otte J, Hensler ME, Bockholt SM, Louis HA, Kalff-Suskei M, Grzeschiki K, Ahe DVD, Beckerle MC (1996): Molecular characterization of human zyxin. J. Biol. Chem. 271, 31470-31478

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

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

Matolin S, Vaverka F, Lunak J, Novak J, Horak V, Krejci P (1994): Histochemical and functional parameters in Nordic combination athletes. Physiol. Res. 43, 243-251

Mattson JP, Miller TA, Poole DC, Delp MD (2002): Fiber composition and oxidative capacity of hamster skeletal muscle. J. Histochem. Cytochem. 50, 1685-1692

Mazerbourg S, Zapf J, Bar RS, Brigstock DR, Monget P (2000): Insulin-like growth factor (IGF)-binding protein-4 proteolytic degradation in bovine, equine, and porcine preovulatory follicles: regulation by IGFs and heparin-binding domain-containing peptides. Biol. Reprod. 63, 390-400

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

Melrose J, Fuller ES, Roughley PJ, Smith MM, Kerr B, Hughes CE, Caterson B, Little CB (2008): Fragmentation of decorin, biglycan, lumican and keratocan is elevated in degenerate human meniscus, knee and hip articular cartilages compared with age-matched macroscopically normal and control tissues. Arthritis. Res Ther. 10: R79

Meves A, Stock SN, Beyerle A, Pittelkow MR, Peus D (2001): H_2O_2 mediates oxidative stress-induced epidermal growth factor receptor phosphorylation. Toxicology Letters 122, 205-214

Meyers SN, Rogatcheva MB, Larkin DM, Yerle M, Milan D, Hawken RJ, Schook LB, Beever JE (2005): Piggy-BACing the human genome II. A high-resolution, physically anchored, comparative map of the porcine autosomes. Genomics 86, 739-752

Milan D, Woloszyn N, Yerle M, Le Roy P, Bonnet M, Riquet J, Lahbib-Mansais Y, Caritez JC, Robic A, Sellier P, Elsen JM, Gellin J (1996): Accurate mapping of the "acid meat" RN gene on genetic and physical maps of pig chromosome 15. Mamm. Genome. 7, 47-51

Milan D, Jeon JT, Looft C, Amarger V, Robic A, Thelander M, Rogel-Gaillard C, Paul S, Iannuccelli N, Rask L, Ronne H, Lundström K, Reinsch N, Gellin J, Kalm E, Roy PL, Chardon P, Andersson L (2000): A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. Science. 288, 1248-1251

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

Moe M, Lien S, Bendixen C, Hedegaard J, Hornshøj H, Berget I, Meuwissen TH, Grindflek E (2008): Gene expression profiles in liver of pigs with extreme high and low levels of androstenone. BMC Vet. Res. 4, 29

Monin, G, Sellier P (1985): Pork of low technological quality with a normal rate of muscle pH fall in the immediate post-mortem period: The case of the Hampshire breed. Meat Sci.13, 49-63

Mörlein D, Link G, Murani E, Wimmers K, Wicke M (2007): Evaluation of three pig crossbreed types with respect to strategies to improve the meat quality: MHS

genotype rather than crossbreed type influences drip loss. Arch. Tierz. Dummerstorf 50, 605-618

Morozova O, Marra MA (2008): Applications of next-generation sequencing technologies in functional genomics. Genomics 92, 255-264

Nix DA, Fradelizi J, Bockholt S, Menichi B, Louvard D, Friederich E, Beckerle MC (2001): Targeting of zyxin to sites of actin membrane interaction and to the nucleus. J. Biol. Chem. 276, 34759-34767

Nobis W, Ren X, Suchyta SP, Suchyta TR, Zanella AJ, Coussens PM (2003): Development of a porcine brain cDNA library, EST database, and microarray resource. Physiol. Genomics 16, 153-159

Nonneman DJ, Rohrer GA (2002): Comparative mapping of a region on chromosome 10 containing QTL for reproduction in swine. Amin. Genet. 34, 42-46

Oasba PK, Kumar S (1997): Molecular divergence of lysozymes and alpha-lactalbumin. Crit. Rev. Biochem. Mol. Biol. 32, 255-306

O'Connell JR, Weeks DE (1998): PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am. J. Hum. Genet. 63, 259-266

Oksbjerg N, Petersen JS, Sørensen IL, Henckel P, Vestergaard M, Ertbjerg P, Møller AJ, Bejerholm C, Støier S (2000): Long-term changes in performance and meat quality of danish landrace pigs: A study on a current compared with an unimproved genotype. Anim. Sci. 71, 81-92

Osler ME, Chang MS, Bader DM (2005): Bves modulates epithelial integrity through an interaction at the tight junction. J. Cell Sci. 118, 4667-4678

Otto G, Roehe R, Looft H, Thoelking L, Knap PW, Rothschild MF, Plastow GS, Kalm E (2007): Associations of DNA markers with meat quality traits in pigs with emphasis on drip loss. Meat Sci. 75, 185-195

Palacin M, Kanai Y (2004): The ancillary proteins of HATs: SLC3 family of amino acid transporters. Eur. J. Physiol. 447, 490-494

Pan YT, Carroll JD, Asano N, Pastuszak I, Edavana VK, Elbein AD (2008): Trehalose synthase converts glycogen to trehalose. FEBS. J. 275, 3408-3420

Papinaho PA, Ruusunen MH, Suuronen T (1996): Relationship between muscle biochemical and meat quality properties of early deboned broiler breasts. J. Appl. Poultry Res. 5:126-133

Parikh H, Nilsson E, Ling C, Poulsen P, Almgren P, Nittby H, Eriksson KF, Vaag A, Groop LC (2008): Molecular correlates for maximal oxygen uptake and type 1 fibers. Am. J. Physiol. Endocrinol. Metab. 294, E1152-1159

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. Biotech. 12, 155-165

Peppelenbosch MP, Tertoolen LG, der Hertog J, de Laat SW (1992): Epidermal growth factor activates calcium channels by phospholipase A2/5-lipoxygenase-mediated leukotriene C4 production. Cell. 69, 295-303

Permyakov EA, Berliner LJ (2000): alpha-Lactalbumin: structure and function. FEBS. Lett. 473, 269-274

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

Ponsuksili S, Jonas E, Murani E, Phatsara C, Srikanchai T, Walz, C, Schwerin M, Schellander K, Wimmers K (2008b): 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, Wimmers K (2008c): Porcine genome-wide gene expression in response to tetanus toxoid vaccine. Dev. Biol. (Basel) 132, 185-95

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 (Online version) DOI 10.1007/s10142-009-0131-1

Posse E, De Arcuri BF, Morero RD (1994): Lysozyme interactions with phospholipid vesicles: relationships with fusion and release of aqueous content. Biochim. Biophys. Acta. 1193, 101-106

Putilina T, Wong P, Gentleman S (1999): The DHHC domain: a new highly conserved cysteine-rich motif. Mol Cell Biochem. 195, 219-226

Ramos PL, Crooijmans RPMA, Amaral AJ, bendixen J, Hedegaard J, Rohrer G, Smith C, Tassel J van, Taylor M, Groenen MAM (2008): High throughput SNP discovery and validation in the pig: towards the development of a high density swine SNP chip. Abstract in scientific journal or proceedings In: Pig Genome II, Ljubljana, Slovenia, 4 - 5 June, 2008

Reecy JM, Spurlock DM, Stahl CH (2006): Gene expression profiling: insights into skeletal muscle growth and development. J. Anim. Sci. 84, E150-154

Reiter M, Walf VM, Christians A, Pfaffl MW, Meyer HH (2007): Modification of mRNA expression after treatment with anabolic agents and the usefulness for gene expression-biomarkers. Anal. Chim. Acta. 586, 73-81

Resh MD (1999): Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. Biochim. Biophys. Acta 1451, 1-16

Rosenvolt K, Andersen HA (2003): Factors of significance for pork quality - a review. Meat Sci. 64, 219-237

Rosette C, Karin M (1996): Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. Science 274, 1194-1197

Roth AF, Feng Y, Chen L, Davis NG (2002): The yeast DHHC cysteine-rich domain protein Akr1p is a palmitoyl transferase. J. Cell Biol. 159, 23-28

Rothschild MF, Plastow GS (1999): Advances in pig genomics and industry applications. AgBiotechNet. 1, 1-7

Rothschild MF, Hu ZL, Jiang Z (2007): Advances in QTL mapping in pigs. Int. J. Biol. Sci. 3, 192-197

Rozen S, Skaletsky HJ (2000): Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ, pp 365-386

Ruwhof C, van Wamel JT, Noordzij LA, Aydin S, Harper JC, van der Laarse A (2001): Mechanical stress stimulates phospholipase C activity and intracellular calcium ion levels in neonatal rat cardiomyocytes. Cell Calcium. 29, 73-83

Ryan MJ, Gross KW, Hajduczok G (2000): Calcium-dependent activation of phospholipase C by mechanical distension in rennin-expressing As 4.1 cells. Am. J. Physiol. 279, E823-829

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

Sawera M, Cirera S, Jørgensen CB, Gorodkin J, Fredholm M (2006): Linkage mapping of gene-associated SNPs to pig chromosome 11. Anim. Genet. 37, 199-204

Schalkwijk CG, Spaargaren M, Defize LH, Verkleij AJ, Bosch H, Boonstra J (1995): Epidermal growth factor (EGF) induces serine phosphorylation-dependent activation and calcium-dependent translocation of the cytosolic phospholipase A2. Eur. J. Biochem. 231, 593-601

Schar CR, Blouse GE, Minor KH, Peterson CB (2008): A deletion mutant of vitronectin lacking the somatomedin B domain exhibits residual plasminogen activator inhibitor-1-binding activity. J. Biol. Chem. 283, 10297-10309

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

Schwerin M, Czernek-Schäfer 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, S19-34

Schwerin M, Kuehn C, Wimmers S, Walz C, Goldammer T (2006): Trait-associated expressed hepatic and intestine genes in cattle of different metabolic type--putative functional candidates for nutrient utilization. J. Anim. Breed. Genet. 123, 307-314

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

Sieczkowska H, Koćwin-Podsiadła M, Zybert A, E. Krzęcio E, Antosik K, Kamiński S, Wójcik E (2009): The association between polymorphism of PKM2 gene and glycolytic potential and pork meat quality. Meat Sci. 84, 180-185

Smith TK, Bader DM (2006): Characterization of Bves expression during mouse development using newly generated immunoreagents. Dev. Dyn. 235, 1701-1708

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

Stetzer AJ, McKeith FK (2003): Benchmaking value in the pork supply chain: quantitative strartegies and opportunities to improve quality. Savoy, IL: American Meat Science Association

Su YH, Xiong YZ, Jiang SW, Zhang Q, Lei MG, Zheng R, Deng CY (2004): Mapping quantitative trait loci for meat quality trait in a Large White x Meishan cross. Yi. Chuan. Xue. Bao. 31, 132-136

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

Te Pas MF, De Wit AA, Priem J, Cagnazzo M, Davoli R, Russo V, Pool MH (2005): Transcriptome expression profiles in prenatal pigs in relation to myogenesis. J. Muscle Res. Cell Motil. 26, 157-165

Te Pas MFW, Soumillion A (2001): The use of physiologic and functional genomic information of the regulation of the determination of skeleton muscle mass in livestock breeding strategies to enhance meat production. Curr. Genom. 2, 285-304

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

Tor M, Estany J, Villalba D, Cubiló D, Tibau J, Soler J, Sanchez A, Noguera JL (2001): A withinbreed comparison of RYR1 pig genotypes for performance, feeding behaviour, and carcass, meat and fat quality traits. J. Anim. Breed. Genet. 118, 417-427

Tuggle CK, Wang Y, Couture O (2007): Advances in swine transcriptomics. Int. J. Biol. Sci. 3, 132-152

Upton Z, Cuttle L, Noble A, Kempf M, Topping G, Malda J, Xie Y, Mill J, Harkin DG, Kravchuk O, Leavesley DI, Kimble RM (2008): Vitronectin: growth factor complexes hold potential as a wound therapy approach. J. Invest. Dermatol. 128, 1535-1544

Vallone PM, Butler JM (2004): AutoDimer: a screening tool for primer-dimer and hairpin structures. Biotechniques. 37, 226-231

van der Steen HAM, Prall GFW, Plastow GS (2005): Application of genomics to the pork industry. J. Anim. Sci. 83, E1-E8

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 MAM, 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

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

Verner J, Humpolíček P, Knoll A (2007): Impact of MYOD family genes on pork traits in Large White and Landrace pigs. J. Breed Genet. 124, 81-85

Vestergaard M, Therkildsen M, Henckel P, Jensen LR, Andersen HR, Sejrsen K (2000): Influence of feeding intensity, grazing and finishing feeding on meat and eating quality of young bulls and the relationship between muscle fibre characteristics, fibre fragmentation and meat tenderness. Meat Sci. 54, 187-195

Villalobo A, Ruano MJ, Palomo-Jiménez PI, Li H, Martín-Nieto J (2000): The molecular basis of calcium action in biology and medicine. In Calcium. Edited by: Pochet R, Donato R, Haiech J, Heizmann C, Gerke V. Boston: Kluwer Academic 287-303

Wada AM, Reese DE, Bader DM (2001): Bves: prototype of new class of cell adhesion molecules expressed during coronary artery development. Dev. 128, 2085-2093

Wang N, Hikida RS, Staron RS, Simoneau JA (1993): Muscle fibre types of women after resistance training - quantitative ultrastructure and enzyme activity. Pfügers Archiv. 424, 494-502

Weiler U, Appell HJ, Kremser M, Hofäcker S, Claus R (1995): Consequences of selection on muscle composition. a comparative study on gracilis muscle in wild and domestic pigs. Anat. Histol. Embryol. 24, 77-80

Werner G, Opalka JR, Gellerich FN, Wicke M (2005): The influence of mitochondrial function on meat quality in turkey and swine. Arch. Tierz. Dummerstorf. 48S, 106-114

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, Te Pas MFW, Chang KC, Davoli R, Merks JWM, Henne H, Muraniova M, da Costa N, Harlizius B, Schellander K, Böll I, Braglia S, de Wit AAC, Cagnazzo M, Fontanesi L, Prins D, Ponsuksili S (2007): Associations of functional candidate genes derived from gene-expression profiles of prenatal porcine muscle tissue with meat quality and muscle deposition. Anim. Genet. 38, 474-484

Yoshigi M, Hoffman LM, Jensen CC, Yost HJ, Beckerle MC (2005): Mechanical force mobilizes zyxin from focal adhesions to actin filaments and regulates cytoskeletal reinforcement. J. Cell Biol. 171, 209-215

Yue G, Schröffel JRJ, Moser G, Bartenschlager H, Reiner G, Geldermann H (2003): Linkage and QTL mapping for sus scrofa chromosome 12. J. Anim. Breed. Genet. 120, 95-102

ZDS, Zentral Verband der Deutschen Schweineprodukion e.V. (2004): Richtlinie fuer die Stationsprurefung auf Mastleistung, Schlachtkoerperwert und Fleischbeschaffenheit beim Schwein., Ausschuss fuer Leistungsprüfung und Zuchtwertschaetzung, Bonn, Germany

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

Zhao SH, Recknor J, Lunney JK, Nettleton D, Kuhar D, Orley S, Tuggle CK. (2005): Validation of a first-generation long-oligonucleotide microarray for transcriptional profiling in the pig. Genomics 86, 618-625

Zhou A, Huntington JA, Pannu NS, Carrell RW, Read RJ (2003): How vitronectin binds PAI-1 to modulate fibrinolysis and cell migration. Nat. Struct. Biol. 10, 541-54

Zhou QY, Fang MD, Huang TH, Li CC, Yu M, Zhao SH. (2009): Detection of differentially expressed genes between Erhualian and Large White placentas on day 75 and 90 of gestation. BMC Genomics 10, 337

Zuo B, Yang H, Lei MG, Li FE, Deng CY, Jiang SW, Xiong YZ (2007): Association of the polymorphism in GYS1 and ACOX1 genes with meat quality traits in pigs. Animal 1, 1243-1248

8. Appendix

Table 66: P-value, Q-value, additive and dominance effects of individual SNP

Polymorphisms	Traits	Populations	P-value	Q-value	a (± se)	d (± se)
BVES c.186G>T	pH24	DUPI	0.0075	0.3435	0.02(0.01)	-0.02(0.01)
BVES c.186G>T	BF3	PIF1(b)	0.0304	0.3622	0.01(0.03)	0.09(0.04)*
BVES c.186G>T	DRIP	PIF1(b)	0.0495	0.4111	-0.30(0.12)*	-0.18(0.16)
SLC3A2 c.1326A>G	MFR	PIF1(b)	0.0352	0.3698	0.005(0.01)	0.01(0.01)
SLC3A2 c.1326A>G	BF3	DL(a)	0.0226	0.3435	0.03(0.05)	0.13(0.07)*
SLC3A2 c.1336Indel[AGC]	ABF	PI(a)	0.0402	0.3914	-0.02(0.03)	-0.09(0.04)*
SLC3A2 c.1336Indel[AGC]	ABF	PIF1(a)	0.0228	0.3435	-0.09(0.03)*	0.07(0.04)
SLC3A2 c.1336Indel[AGC]	SF	PIF1(a)	0.0061	0.3070	2.46(0.77)**	-1.18(0.92)
SLC3A2 c.1336Indel[AGC]	COOK	PIF1(a)	0.0443	0.4052	0.60(0.24)*	-0.24(0.28)
SLC3A2 c.1336Indel[AGC]	ABF	PIF1(b)	0.0458	0.4068	-0.06(0.02)*	-0.01(0.03)
SLC3A2 c.1336Indel[AGC]	BF2	PIF1(b)	0.0169	0.3435	-0.07(0.03)**	-0.02(0.03)
SLC3A2 c.1336Indel[AGC]	FA	PIF1(b)	0.0389	0.3892	-0.56(0.22)*	-0.06(0.28)
SLC3A2 c.1336Indel[AGC]	THAW	PIF1(b)	0.0016	0.1399	-0.53(0.16)**	-0.21(0.21)
SLC3A2 c.1336Indel[AGC]	pH1	PIF1(b)	0.0220	0.3435	-0.02(0.02)	-0.07(0.03)*
SLC3A2 c.1336Indel[AGC]	pH24	PIF1(b)	0.0422	0.4018	0.02(0.01)*	0.01(0.01)
SLC3A2 c.1336Indel[AGC]	CON1	PIF1(c)	0.0310	0.3622	-0.17 (0.15)	0.42(0.18)*
SLC3A2 c.1336Indel[AGC]	BF1	DL(a)	0.0098	0.3435	0.24(0.09)**	-0.29(0.10)**
SLC3A2 c.1336Indel[AGC]	DRIP	DL(a)	0.0173	0.3435	1.04(0.39)**	-0.57(0.42)
SLC3A2 haplotype	BF1	DUPI	0.0291	0.3622	Non-est	Non-est
SLC3A2 haplotype	ABF	PIF1(a)	0.0432	0.4018	Non-est	Non-est
SLC3A2 haplotype	SF	PIF1(a)	0.0102	0.3435	Non-est	Non-est
SLC3A2 haplotype	BF2	PIF1(b)	0.0376	0.3808	Non-est	Non-est
SLC3A2 haplotype	pH1	PIF1(b)	0.0287	0.3622	Non-est	Non-est
SLC3A2 haplotype	THAW	PIF1(b)	0.0241	0.3435	Non-est	Non-est
SLC3A2 haplotype	CON1	PIF1(c)	0.0156	0.3435	Non-est	Non-est
AHNAK c.12907A>G	LEA	DUPI	0.0143	0.3435	-0.58(0.37)	0.82(0.44)
AHNAK c.12907A>G	FA	DUPI	0.0091	0.3435	0.49(0.20)*	-0.27(0.25)
AHNAK c.12907A>G	MFR	DUPI	0.0005	0.0830	0.01(0.01)**	-0.01(0.01)
AHNAK c.12907A>G	BF1	DUPI	0.0135	0.3435	0.10(0.03)**	0.02(0.04)
AHNAK c.12907A>G	BF3	DUPI	0.0122	0.3435	0.07(0.02)**	-0.002(0.03)
AHNAK c.12907A>G	ABF	DUPI	0.0048	0.2733	0.07(0.02)**	0.02(0.03)
AHNAK c.12907A>G	THAW	DUPI	0.0424	0.4018	0.33(0.16)*	-0.16(0.19)
AHNAK c.12907A>G	LEA	PIF1(a)	0.0149	0.3435	Non-est	Non-est
AHNAK c.12907A>G	BF2	PIF1(c)	0.0488	0.4094	Non-est	Non-est
AHNAK c.12907A>G	OPTO	DL(a)	0.0131	0.3435	Non-est	Non-est
AHNAK c.13014G>T	COOK	PI(a)	0.0474	0.4088	Non-est	Non-est
AHNAK c.13014G>T	LEA	DUPI	0.0210	0.3435	-1.03(0.41)*	1.01(0.48)*
AHNAK c.13014G>T	FA	DUPI	0.0001	0.0554	0.95(0.23)***	-0.68(0.26)*
AHNAK c.13014G>T	MFR	DUPI	<0.0001	0.0554	0.03(0.01)***	-0.02(0.01)**
AHNAK c.13014G>T	BF1	DUPI	0.0009	0.1246	0.14(0.04)***	-0.08(0.04)
AHNAK c.13014G>T	BF3	DUPI	0.0050	0.2733	0.09(0.03)**	-0.04(0.03)
AHNAK c.13014G>T	ABF	DUPI	0.0005	0.083	0.10(0.02)***	-0.05(0.03)
AHNAK c.13014G>T	DRIP	DUPI	0.0393	0.3895	0.24(0.09)*	-0.13(0.11)
AHNAK c.13014G>T	BF3	PIF1(b)	0.0294	0.3622	0.09(0.04)*	0.02(0.05)

* P<0.05, ** P<0.01, *** P<0.001

Table 66: P-value, Q-value, additive and dominance effects of individual SNP (continued)

Polymorphisms	Traits	Populations	P-value	Q-value	a (± se)	d (± se)
AHNAK c.13014G>T	pH24	PIF1(b)	0.0424	0.4018	0.03(0.01)*	0.01(0.01)
AHNAK c.13014G>T	FA	PIF1(c)	0.0341	0.3631	1.08(0.42)*	1.05(0.50)*
AHNAK c.13281A>G	FA	DUPI	0.0134	0.3435	-0.73(0.25)**	-0.34(0.30)
AHNAK c.13281A>G	MFR	DUPI	0.0032	0.2126	-0.02(0.01)**	-0.005(0.01)
AHNAK c.13281A>G	BF1	DUPI	0.0187	0.3435	-0.11(0.04)**	-0.02(0.05)
AHNAK c.13281A>G	BF3	DUPI	0.0140	0.3435	-0.06(0.03)*	0.03(0.03)
AHNAK c.13281A>G	ABF	DUPI	0.0140	0.3435	-0.07(0.03)**	0.004(0.03)
AHNAK c.13281A>G	pH1	DUPI	0.0279	0.3622	0.05(0.02)**	0.01(0.02)
AHNAK c.13281A>G	DRIP	DUPI	0.0187	0.3435	-0.29(0.10)**	-0.15(0.12)
AHNAK c.13281A>G	OPTO	PIF1(b)	0.0375	0.3808	-0.28(0.60)	1.7(0.73)*
AHNAK c.13281A>G	LEA	DL(a)	0.0100	0.3435	Non-est	Non-est
AHNAK c.13281A>G	OPTO	DL(a)	0.0281	0.3622	Non-est	Non-est
AHNAK c.13281A>G	CON24	DL(a)	0.0457	0.4068	Non-est	Non-est
AHNAK c.13281A>G	DRIP	DL(a)	0.0051	0.2733	Non-est	Non-est
AHNAK c.13290A>C>G	ABF	PIF1(a)	0.0137	0.3435	Non-est	Non-est
AHNAK c.13290A>C>G	pH24	PIF1(a)	0.0233	0.3435	Non-est	Non-est
AHNAK c.13290A>C>G	BF2	PIF1(b)	0.0338	0.3622	Non-est	Non-est
AHNAK c.13290A>C>G	BF3	PIF1(b)	0.0165	0.3435	Non-est	Non-est
AHNAK c.13290A>C>G	ABF	PIF1(b)	0.0159	0.3435	Non-est	Non-est
AHNAK c.13290A>C>G	THAW	PIF1(b)	0.0239	0.3435	Non-est	Non-est
AHNAK c.13290A>C>G	pH1	PIF1(c)	0.0388	0.3892	Non-est	Non-est
AHNAK c.13290A>C>G	LEA	DL(a)	0.0457	0.4068	Non-est	Non-est
AHNAK c.13290A>C>G	CON1	DL(a)	0.0427	0.4018	Non-est	Non-est
AHNAK c.13294C>T	ABF	PIF1(a)	0.0192	0.3435	0.002(0.03)	0.09(0.04)*
AHNAK c.13294C>T	THAW	PIF1(a)	0.0470	0.4088	0.09(0.39)	1.07(0.51)*
AHNAK c.13294C>T	BF2	PIF1(b)	0.0168	0.3435	0.06(0.03)*	0.01(0.04)
AHNAK c.13294C>T	BF3	PIF1(b)	0.0086	0.3435	0.07(0.03)*	0.02(0.04)
AHNAK c.13294C>T	ABF	PIF1(b)	0.0061	0.3070	0.07(0.03)**	0.003(0.03)
AHNAK c.13294C>T	pH24	PIF1(b)	0.0454	0.4068	-0.003(0.01)	0.03(0.01)*
AHNAK c.13294C>T	THAW	PIF1(b)	0.0195	0.3435	0.50(0.19)**	-0.53(0.24)*
AHNAK c.13294C>T	LEA	DL(a)	0.0463	0.4069	0.50(0.41)	-1.31(0.54)*
AHNAK c.13294C>T	MFR	DL(a)	0.0263	0.3578	-0.01(0.01)	0.04(0.01)**
AHNAK c.13294C>T	CON1	DL(a)	0.0095	0.3435	-0.16(0.06)**	-0.08(0.08)
AHNAK haolotype	LEA	DUPI	0.0273	0.3599	Non-est	Non-est
AHNAK haolotype	FA	DUPI	0.0193	0.3435	Non-est	Non-est
AHNAK haolotype	MFR	DUPI	0.0005	0.0830	Non-est	Non-est
AHNAK haolotype	BF1	DUPI	0.0237	0.3435	Non-est	Non-est
AHNAK haolotype	BF3	DUPI	0.0197	0.3435	Non-est	Non-est
AHNAK haolotype	ABF	DUPI	0.0090	0.3435	Non-est	Non-est
AHNAK haolotype	pH24	PIF1(a)	0.0200	0.3435	Non-est	Non-est
AHNAK haolotype	LEA	DL(a)	0.0110	0.3435	Non-est	Non-est
AHNAK haolotype	CON1	DL(a)	0.0242	0.3435	Non-est	Non-est
AHNAK haolotype	OPTO	DL(a)	0.0097	0.3435	Non-est	Non-est
ZDHHC5 c.1803C>T	MFR	DUPI	0.0233	0.3435	Non-est	Non-est
ZDHHC5 c.1803C>T	FA	DUPI	0.0233	0.3435	Non-est	Non-est
ZDHHC5 c.1803C>T	COOK	DUPI	0.0337	0.3622	Non-est	Non-est
ZDHHC5 c.1803C>T	COOK	PI(a)	0.0121	0.3435	Non-est	Non-est

* P<0.05, ** P<0.01, *** P<0.001

Table 66: P-value, Q-value, additive and dominance effects of individual SNP (continued)

Polymorphisms	Traits	Populations	P-value	Q-value	a (± se)	d (± se)
ZDHHC5 c.1803C>T	BF1	PIF1(b)	0.0359	0.3704	Non-est	Non-est
ZDHHC5 c.1803C>T	OPTO	PIF1(c)	0.0480	0.4089	Non-est	Non-est
ZDHHC5 c.1803C>T	DRIP	DL(a)	0.0004	0.0830	Non-est	Non-est
CS c.120G>T	LEA	PI(a)	0.0136	0.3435	Non-est	Non-est
CS c.120G>T	DRIP	PIF1(a)	0.0015	0.1399	Non-est	Non-est
CS c.120G>T	FA	PIF1(b)	0.0252	0.3517	Non-est	Non-est
CS c.120G>T	pH1	PIF1(c)	0.0081	0.3435	Non-est	Non-est
CS c.120G>T	CON1	PIF1(c)	0.0295	0.3622	Non-est	Non-est
CS c.120G>T	BF3	DL(a)	0.0069	0.3371	Non-est	Non-est
CS c.120G>T	ABF	DL(a)	0.0239	0.3435	Non-est	Non-est
LYZ c.240A>C	DRIP	PI(a)	0.0030	0.2126	-0.30(0.13)*	-0.51(0.15)***
LYZ c.240A>C	pH1	PIF1(a)	0.0106	0.3435	0.11(0.04)**	0.14(0.05)**
LYZ c.240A>C	CON24	PIF1(a)	0.0156	0.3435	-0.04(0.14)	-0.34(0.16)*
LYZ c.240A>C	OPTO	PIF1(b)	0.0028	0.2114	-1.76(0.67)**	-2.89(0.86)***
LYZ c.365A>T	CON24	DUPI	0.0047	0.2733	0.39(0.15)*	-0.52(0.16)**
LYZ c.365A>T	pH1	DUPI	0.0472	0.4088	-0.08(0.04)*	0.04(0.04)
LYZ c.365A>T	ABF	PI(a)	0.0121	0.3435	0.07(0.03)*	-0.08(0.03)*
LYZ c.365A>T	CON1	PI(a)	0.0444	0.4052	0.004(0.07)	0.22(0.09)*
LYZ c.365A>T	LEA	PIF1(c)	0.0494	0.4111	-1.36(0.59)*	0.52(0.76)
LYZ c.365A>T	THAW	PIF1(b)	0.0153	0.3435	0.93(0.40)*	-1.31(0.45)**
LYZ haplotype	CON24	DUPI	0.0185	0.3435	Non-est	Non-est
LYZ haplotype	DRIP	PI(a)	0.0212	0.3435	Non-est	Non-est
LYZ haplotype	pH1	PIF1(a)	0.0137	0.3435	Non-est	Non-est
LYZ haplotype	CON24	PIF1(a)	0.0231	0.3435	Non-est	Non-est
LYZ haplotype	OPTO	PIF1(b)	0.0101	0.3435	Non-est	Non-est
LYZ haplotype	OPTO	DL(a)	0.0228	0.3435	Non-est	Non-est
KERA c.303C>T	pH1	PI(a)	0.0358	0.3704	-0.05(0.02)*	-0.003(0.03)
KERA c.303C>T	pH24	PI(a)	0.0273	0.3599	-0.04(0.02)*	-0.05(0.02)*
KERA c.303C>T	LEA	DL(a)	0.0324	0.3622	-0.85(0.70)	0.66(0.81)
COQ9 c.453A>G	pH1	DUPI	0.0311	0.3622	-0.07(0.03)*	-0.04(0.03)
COQ9 c.453A>G	SF	PI(a)	0.0334	0.3622	0.41(0.86)	2.73(1.06)*
COQ9 c.453A>G	FA	PIF1(b)	0.0004	0.083	-0.66(0.19)***	-0.43(0.26)
COQ9 c.453A>G	MFR	PIF1(b)	0.0305	0.3622	-0.01(0.00)*	-0.01(0.01)
COQ9 c.453A>G	BF2	PIF1(b)	0.0433	0.4018	-0.05(0.02)*	-0.04(0.03)
COQ9 +1247A>T	LEA	DUPI	0.0218	0.3435	2.2(0.86)*	-1.05(0.90)
COQ9 +1247A>T	MFR	DUPI	0.0181	0.3435	-0.03(0.01)**	0.02(0.01)
COQ9 +1247A>T	BF1	DUPI	0.0008	0.1208	-0.27(0.08)***	0.11(0.08)
COQ9 +1247A>T	ABF	DUPI	0.0043	0.2645	-0.15(0.05)**	0.06(0.05)
COQ9 +1247A>T	THAW	DUPI	0.0027	0.2114	1.21(0.37)**	-1.27(0.39)**
COQ9 +1247A>T	SF	PI(a)	0.0334	0.3622	-0.41(0.86)	2.73(1.06)*
COQ9 +1247A>T	FA	PIF1(b)	0.0004	0.083	-0.66(0.19)***	-0.43(0.26)
COQ9 +1247A>T	MFR	PIF1(b)	0.0305	0.3622	0.01(0.00)*	-0.01(0.01)
COQ9 +1247A>T	BF2	PIF1(b)	0.0433	0.4018	0.05(0.02)*	-0.04(0.03)
COQ9 +1247A>T	OPTO	DL(a)	0.0226	0.3435	-0.20(0.49)	1.95(0.71)**
COQ9 haplotype	BF1	DUPI	0.0354	0.3698	Non-est	Non-est
COQ9 haplotype	SF	PI(a)	0.0334	0.3622	Non-est	Non-est
COQ9 haplotype	BF2	PIF1(b)	0.0433	0.4018	Non-est	Non-est

* P<0.05, ** P<0.01, *** P<0.001

Table 66: P-value, Q-value, additive and dominance effects of individual SNP (continued)

Polymorphisms	Traits	Populations	P-value	Q-value	a (± se)	d (± se)
COQ9 haplotype	FA	PIF1(b)	0.0004	0.0830	Non-est	Non-est
COQ9 haplotype	MFR	PIF1(b)	0.0305	0.3622	Non-est	Non-est
COQ9 haplotype	LEA	DL(a)	0.0185	0.3435	Non-est	Non-est
COQ9 haplotype	OPTO	DL(a)	0.0209	0.3435	Non-est	Non-est
UN g.1,022,434G>T	BF2	DUPI	0.0203	0.3435	0.003(0.03)	0.10(0.04)**
UN g.1,022,434G>T	BF3	DUPI	0.0170	0.3435	0.06(0.03)	0.07(0.04)
UN g.1,022,434G>T	ABF	DUPI	0.0324	0.3622	0.05(0.03)	0.05(0.03)
UN g.1,022,434G>T	DRIP	PI(a)	0.0318	0.3622	Non-est	Non-est
UN g.1,022,434G>T	SF	PI(a)	0.0012	0.1329	Non-est	Non-est
UN g.1,022,434G>T	FA	PIF1(c)	0.0480	0.4089	-0.73(0.43)	-0.04(0.48)
UN g.1,022,434G>T	BF1	PIF1(c)	0.0337	0.3622	-0.05(0.05)	-0.07(0.06)
UN g.1,022,434G>T	BF2	PIF1(c)	0.0347	0.3671	-0.12(0.05)*	0.07(0.06)
UN g.1,022,434G>T	ABF	PIF1(c)	0.0485	0.4089	-0.07(0.04)	0.01(0.05)
UN g.1,022,434G>T	pH24	DL(a)	0.0214	0.3435	0.004(0.01)	0.04(0.01)**
EGFR c.3543A>G	LEA	DUPI	0.0321	0.3622	-1.12(0.56)*	-1.3(0.62)*
EGFR c.3543A>G	BF3	DUPI	0.0011	0.1329	0.14(0.04)***	0.01(0.04)
EGFR c.3543A>G	ABF	DUPI	0.0403	0.3914	0.09(0.03)*	0.02(0.04)
EGFR c.3543A>G	THAW	DUPI	0.0461	0.4069	-0.23(0.24)	0.56(0.27)*
EGFR c.3543A>G	OPTO	PI(a)	0.0330	0.3622	0.01(0.67)	2.34(0.95)*
EGFR c.3543A>G	CON24	PIF1(a)	0.0138	0.3435	-0.05(0.12)	-0.29(0.15)
EGFR c.3543A>G	BF1	PIF1(b)	0.0117	0.3435	-0.05(0.03)	0.13(0.05)**
EGFR c.3543A>G	pH24	PIF1(b)	0.0475	0.4088	-0.02(0.01)*	-0.01(0.01)
VTN c.154A>G	ABF	DUPI	0.0499	0.4123	0.06(0.02)*	0.02(0.03)
VTN c.154A>G	CON1	DUPI	0.0443	0.4052	0.03(0.06)	-0.18(0.07)*
VTN c.154A>G	CON24	DUPI	0.0219	0.3435	-0.10(0.07)	-0.21(0.09)*
VTN c.154A>G	OPTO	DUPI	0.0143	0.3435	-1.18(0.48)*	-1.11(0.62)
VTN c.154A>G	pH24	PIF1(a)	0.0097	0.3435	0.02(0.01)	0.02(0.01)
VTN c.154A>G	DRIP	PIF1(a)	0.0122	0.3435	-0.19(0.07)*	-0.04(0.1)
VTN c.154A>G	COOK	PIF1(b)	0.0198	0.3435	0.33(0.16)*	0.19(0.21)
VTN c.154A>G	pH1	PIF1(c)	0.0240	0.3435	0.02(0.05)	-0.09(0.06)
VTN c.156C>T	pH24	PIF1(a)	0.0160	0.3435	-0.02(0.01)	0.02(0.01)
VTN c.156C>T	DRIP	PIF1(a)	0.0160	0.3435	0.18(0.07)*	-0.04(0.10)
VTN c.156C>T	COOK	PIF1(b)	0.0325	0.3622	-0.34(0.19)	0.15(0.24)
VTN c.156C>T	MFR	DUPI	0.0456	0.4068	Non-est	Non-est
VTN c.156C>T	BF2	DUPI	0.0394	0.3895	Non-est	Non-est
VTN c.156C>T	pH1	DUPI	0.0271	0.3599	Non-est	Non-est
VTN haplotype	BF2	DUPI	0.0031	0.2126	Non-est	Non-est
VTN haplotype	MFR	DUPI	0.0294	0.3622	Non-est	Non-est
VTN haplotype	FA	DUPI	0.0483	0.4089	Non-est	Non-est
VTN haplotype	pH1	DUPI	0.0331	0.3622	Non-est	Non-est
VTN haplotype	pH24	PIF1(a)	0.0123	0.3435	Non-est	Non-est
VTN haplotype	DRIP	PIF1(a)	0.0134	0.3435	Non-est	Non-est
VTN haplotype	COOK	PIF1(b)	0.0262	0.3578	Non-est	Non-est
ZYX c.279C>T	BF1	PI(b)	0.0265	0.3578	-0.09(0.13)	-0.15(0.15)
ZYX c.279C>T	BF2	PI(b)	0.0037	0.2364	-0.17(0.08)*	0.002(0.09)
ZYX c.279C>T	BF3	PI(b)	0.0158	0.3435	-0.03(0.07)	-0.12(0.08)
ZYX c.279C>T	ABF	PI(b)	0.0023	0.1910	-0.09(0.07)	-0.10(0.08)

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 66: P-value, Q-value, additive and dominance effects of individual SNP (continued)

Polymorphisms	Traits	Populations	P-value	Q-value	a (± se)	d (± se)
ZYX c.279C>T	OPTO	PI(b)	0.0223	0.3435	-2.94(2.03)	-1.19(2.31)
ZYX c.279C>T	pH24	PI(b)	0.0194	0.3435	-0.05(0.02)	-0.002(0.03)
ZYX c.399A>G	BF3	DL(b)	0.0259	0.3578	0.14(0.05)*	0.04(0.15)
ZYX c.399A>G	ABF	DL(b)	0.0328	0.3622	0.10(0.04)*	0.10(0.12)
ZYX c.399A>G	FA	DL(b)	0.0259	0.3578	1.03(0.41)*	0.50(1.14)
ZYX c.522A>G	BF3	DL(b)	0.0001	0.0554	-0.27(0.06)***	-0.40(0.13)**
ZYX c.522A>G	ABF	DL(b)	0.0016	0.1399	-0.19(0.05)***	-0.27(0.11)*
ZYX c.522A>G	FA	DL(b)	0.0012	0.1329	-1.65(0.48)***	-2.70(0.95)**
ZYX c.522A>G	MFR	DL(b)	0.0013	0.1350	-0.05(0.02)**	-0.10(0.03)**
ZYX haplotype	pH24	PIF1(b)	0.0191	0.3435	Non-est	Non-est
ZYX haplotype	DRIP	PIF1(b)	0.0365	0.3742	Non-est	Non-est
ZYX haplotype	LEA	PI(b)	0.0097	0.3435	Non-est	Non-est
ZYX haplotype	ABF	PI(b)	0.0247	0.3477	Non-est	Non-est
ZYX haplotype	BF2	PI(b)	0.0326	0.3622	Non-est	Non-est

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 67: Proportion of variance explained by each principle component (PC) and standardized loadings of the first ten PCs in DUPI pigs

Principle component (PC)	1	2	3	4	5	6	7	8	9	10
Total variance (%)	31.34	12.20	11.01	7.36	6.72	5.85	5.33	4.01	3.61	3.34
Cumulative (%)	31.34	43.55	54.55	61.91	68.63	74.48	79.82	83.83	87.44	90.78
LEA	-0.25	-0.03	0.34	-0.55	0.62	-0.13	0.28	-0.05	-0.10	-0.05
FA	0.89	0.09	0.11	-0.08	0.04	-0.03	0.06	0.04	-0.11	-0.04
MFR	0.89	0.10	-0.06	0.19	-0.24	0.03	-0.09	0.05	-0.06	-0.01
BF1	0.81	0.13	0.11	-0.10	0.08	0.09	-0.02	0.03	0.06	-0.25
BF2	0.75	0.21	0.01	-0.16	0.19	0.15	-0.04	-0.13	-0.05	0.27
BF3	0.86	0.19	-0.08	-0.01	0.07	-0.02	-0.02	0.07	0.06	0.07
ABF	0.94	0.20	0.03	-0.10	0.13	0.09	-0.03	0.00	0.03	0.00
OPTO	0.13	-0.42	0.62	0.35	0.14	0.08	-0.07	0.05	0.32	0.31
pH1	0.27	-0.73	-0.19	-0.03	0.23	0.23	0.19	0.14	0.23	-0.01
pH24	0.17	-0.26	0.69	0.06	-0.18	-0.19	0.07	0.46	-0.25	-0.16
CON1	-0.06	0.47	0.17	0.09	-0.32	0.15	0.77	-0.01	0.08	0.13
CON24	-0.26	0.56	0.50	0.16	0.18	-0.23	-0.23	-0.07	-0.04	0.21
SF	-0.27	0.14	0.51	0.15	0.05	0.67	-0.11	-0.23	-0.05	-0.30
DRIP	-0.31	0.66	-0.13	0.06	0.22	-0.05	-0.06	0.36	0.43	-0.21
THAW	-0.16	0.12	-0.39	0.55	0.49	0.23	0.11	0.25	-0.35	0.09
COOK	-0.39	0.14	-0.02	-0.55	-0.22	0.45	-0.22	0.36	-0.06	0.26

Table 68: Proportion of variance explained by each principle component (PC) and standardized loadings of the first ten PCs in PI(a) pigs

Principle component (PC)	1	2	3	4	5	6	7	8	9	10
Total variance (%)	23.57	17.50	12.50	9.87	7.93	6.91	6.41	5.24	4.16	4.06
Cumulative (%)	23.57	41.07	53.57	63.44	71.37	78.28	84.69	89.93	94.09	98.15
LEA	0.11	-0.45	0.09	0.69	0.29	0.32	-0.18	-0.19	0.08	0.20
ABF	-0.22	-0.64	0.19	0.29	0.25	-0.32	0.23	0.42	-0.01	-0.17
OPTO	0.86	0.18	0.04	-0.04	0.13	-0.07	0.09	0.25	0.01	0.17
pH1	0.20	-0.30	-0.69	-0.10	-0.03	0.52	0.03	0.27	-0.19	-0.11
pH24	0.85	0.17	0.09	-0.08	0.05	0.04	-0.04	0.19	0.31	0.11
CON1	0.00	0.18	0.66	0.21	-0.46	0.40	0.32	0.10	-0.04	-0.07
CON24	0.17	0.67	0.20	0.09	0.53	0.15	-0.08	-0.04	-0.10	-0.38
SF	-0.06	0.63	-0.37	0.43	0.04	-0.17	0.36	0.00	-0.24	0.23
DRIP	-0.62	0.29	0.29	-0.11	0.09	0.10	-0.42	0.38	-0.16	0.27
THAW	-0.59	0.01	0.03	-0.38	0.41	0.25	0.43	-0.03	0.23	0.17
COOK	-0.50	0.44	-0.39	0.35	-0.18	0.01	-0.10	0.19	0.41	-0.13

Table 69: Proportion of variance explained by each principle component (PC) and standardized loadings of the first ten PCs in PIF1(a) pigs

Principle component (PC)	1	2	3	4	5	6	7	8	9	10
Total variance (%)	22.30	16.60	12.53	9.88	8.77	7.03	5.88	5.73	4.50	3.70
Cumulative (%)	22.30	38.90	51.43	61.31	70.08	77.11	82.99	88.71	93.21	96.91
LEA	0.08	-0.01	-0.26	-0.79	0.44	0.19	0.03	0.15	0.18	0.08
ABF	-0.06	-0.65	0.34	0.27	-0.07	0.41	0.21	0.32	0.19	0.17
OPTO	-0.54	0.40	0.42	-0.09	0.08	-0.39	-0.05	0.25	-0.08	0.36
pH1	-0.77	0.11	-0.13	0.04	-0.04	-0.23	0.27	-0.09	0.41	-0.21
pH24	-0.53	0.30	0.44	0.12	0.23	0.31	-0.39	-0.13	0.22	-0.06
CON1	0.69	0.31	0.44	0.00	0.10	0.08	-0.15	-0.13	0.05	-0.09
CON24	0.49	0.25	0.58	-0.08	0.14	-0.09	0.50	-0.12	0.06	-0.03
SF	-0.26	0.72	-0.06	0.04	-0.06	0.33	0.18	0.39	-0.23	-0.24
DRIP	0.68	0.24	-0.14	0.16	-0.14	-0.26	-0.20	0.42	0.35	-0.05
THAW	0.06	-0.10	-0.23	0.51	0.80	-0.12	0.06	0.08	-0.08	-0.03
COOK	0.18	0.64	-0.46	0.26	-0.08	0.26	0.14	-0.22	0.15	0.35

Table 70: Proportion of variance explained by each principle component (PC) and standardized loadings of the first ten PCs in PIF1(b) pigs

Principle component (PC)	1	2	3	4	5	6	7	8	9	10
Total variance (%)	26.19	16.09	12.34	8.11	6.91	5.72	4.39	4.13	3.52	3.13
Cumulative (%)	26.19	42.28	54.62	62.73	69.63	75.35	79.74	83.87	87.39	90.51
LEA	-0.20	0.25	0.11	0.61	-0.61	-0.07	-0.09	0.31	-0.04	0.10
FA	0.83	0.13	-0.02	-0.14	-0.08	-0.17	-0.14	0.46	0.02	-0.02
MFR	0.82	-0.01	-0.07	-0.41	0.22	-0.11	-0.08	0.27	0.04	-0.07
BF1	0.74	0.21	0.08	0.29	-0.02	0.07	-0.04	-0.27	0.23	-0.30
BF2	0.72	0.25	-0.02	0.33	-0.03	0.07	-0.04	-0.02	-0.01	0.07
BF3	0.77	0.07	-0.05	0.00	0.10	-0.05	0.24	-0.13	-0.27	0.41
ABF	0.91	0.21	0.01	0.26	0.03	0.04	0.05	-0.20	0.02	0.01
OPTO	0.13	-0.48	0.69	0.08	0.14	0.18	-0.02	0.17	-0.06	0.07
pH1	0.11	-0.75	-0.29	0.11	-0.07	0.03	0.21	0.04	0.25	0.27
pH24	0.18	-0.22	0.80	-0.07	-0.08	0.11	0.15	0.02	-0.30	-0.18
CON1	-0.17	0.59	0.32	-0.04	0.13	0.12	0.52	0.22	0.38	0.01
CON24	-0.19	0.71	0.34	-0.20	-0.11	0.19	0.02	-0.12	-0.06	0.18
SF	-0.18	-0.06	0.61	0.24	0.43	-0.23	-0.38	-0.06	0.25	0.22
DRIP	-0.19	0.77	-0.10	-0.21	0.08	-0.12	-0.20	0.01	-0.06	0.12
THAW	-0.11	0.12	-0.36	0.29	0.42	0.70	-0.14	0.21	-0.10	0.00
COOK	-0.32	0.17	-0.15	0.47	0.49	-0.45	0.26	0.11	-0.23	-0.13

Table 71: Proportion of variance explained by each principle component (PC) and standardized loadings of the first ten PCs in PIF1(c) pigs

Principle component (PC)	1	2	3	4	5	6	7	8	9	10
Total variance (%)	33.76	26.93	8.08	6.60	5.80	4.40	4.28	3.50	3.04	1.87
Cumulative (%)	33.76	60.69	68.77	75.38	81.17	85.57	89.85	93.35	96.39	98.27
LEA	-0.50	0.11	0.74	0.07	-0.20	0.32	0.00	0.20	0.04	-0.02
FA	0.84	0.00	-0.13	-0.16	-0.32	0.34	-0.04	0.16	0.08	-0.01
MFR	0.87	-0.03	-0.37	-0.16	-0.19	0.18	-0.03	0.04	0.06	0.01
BF1	0.77	-0.03	0.18	0.22	0.13	-0.29	-0.33	0.30	0.15	0.03
BF2	0.73	0.17	0.35	-0.11	0.15	0.06	0.24	-0.37	0.28	-0.01
BF3	0.82	0.08	0.18	0.07	0.00	-0.01	0.16	-0.02	-0.50	-0.03
ABF	0.93	0.09	0.29	0.08	0.12	-0.12	0.00	-0.01	-0.02	0.00
OPTO	0.06	-0.66	-0.13	0.06	0.61	0.28	0.19	0.19	0.04	0.03
pH1	0.04	-0.88	0.14	-0.10	-0.16	-0.06	0.05	-0.03	-0.04	0.41
pH24	0.11	-0.53	-0.10	0.75	-0.14	0.19	-0.14	-0.24	0.01	-0.03
CON1	0.01	0.72	-0.16	0.36	-0.19	-0.15	0.43	0.16	0.13	0.12
CON24	-0.03	0.86	-0.08	0.15	0.21	0.23	0.00	0.09	-0.08	0.13
DRIP	-0.03	0.84	-0.02	-0.04	0.15	0.14	-0.34	-0.19	-0.03	0.20

Table 72: Proportion of variance explained by each principle component (PC) and standardized loadings of the first ten PCs in DL(a) pigs

Principle component (PC)	1	2	3	4	5	6	7	8	9	10
Total variance (%)	33.98	22.98	9.00	7.43	7.32	4.56	3.95	3.04	2.86	2.39
Cumulative (%)	33.98	56.97	65.97	73.40	80.72	85.28	89.23	92.27	95.13	97.52
LEA	-0.58	-0.05	0.11	0.52	0.56	0.16	-0.05	0.09	0.20	-0.01
FA	0.69	0.28	0.03	0.57	-0.22	0.07	-0.18	0.01	0.14	0.04
MFR	0.83	0.22	-0.02	0.20	-0.45	-0.03	-0.10	-0.04	0.01	0.03
BF1	0.66	0.24	0.04	-0.36	0.40	-0.17	-0.37	-0.07	0.10	0.10
BF2	0.72	0.26	-0.02	0.18	0.31	0.29	0.07	-0.08	-0.43	-0.05
BF3	0.79	0.23	-0.06	-0.12	0.05	0.03	0.41	0.21	0.26	-0.07
ABF	0.87	0.27	0.00	-0.18	0.29	0.04	0.04	0.06	0.05	-0.01
OPTO	0.37	-0.43	0.56	0.23	0.13	-0.41	0.26	-0.23	-0.03	0.07
pH1	0.26	-0.79	-0.08	-0.10	-0.03	0.34	0.08	-0.06	0.06	0.41
pH24	0.24	-0.53	0.67	-0.11	-0.09	0.10	-0.24	0.29	-0.05	-0.06
CON1	-0.27	0.57	0.52	-0.22	-0.12	0.37	0.05	-0.32	0.15	-0.09
CON24	-0.37	0.72	0.33	-0.03	-0.07	-0.02	0.15	0.29	-0.14	0.21
DRIP	-0.31	0.83	0.01	0.05	0.04	-0.13	-0.05	-0.05	0.01	0.26

Table 73: Proportion of variance explained by each principle component (PC) and standardized loadings of the first ten PCs in DL(b) pigs

Principle component (PC)	1	2	3	4	5	6	7	8	9	10
Total variance (%)	38.29	13.87	12.40	9.57	7.19	5.61	3.79	3.36	3.00	2.74
Cumulative (%)	38.29	52.15	64.55	74.13	81.32	86.93	90.72	94.09	97.08	99.82
LEA	-0.35	-0.28	0.54	0.56	0.34	0.03	0.24	0.10	0.01	0.08
FA	0.84	-0.20	0.12	-0.04	0.06	0.22	0.36	0.06	-0.20	-0.01
MFR	0.86	-0.03	-0.21	-0.35	-0.13	0.15	0.16	0.02	-0.16	-0.06
BF1	0.79	0.08	0.24	0.02	0.19	-0.21	0.04	-0.47	0.09	-0.03
BF2	0.76	-0.09	0.25	0.23	0.09	-0.15	-0.36	0.23	-0.21	-0.19
BF3	0.84	-0.07	0.06	-0.05	-0.16	0.06	-0.01	0.24	0.37	0.24
ABF	0.95	-0.02	0.22	0.07	0.05	-0.12	-0.11	-0.03	0.11	0.00
OPTO	0.18	0.81	-0.20	0.30	0.06	0.06	0.18	0.12	0.18	-0.31
pH1	0.26	-0.17	-0.62	0.21	0.49	0.43	-0.19	-0.08	0.04	0.06
pH24	0.16	0.88	0.10	0.13	0.03	0.03	-0.06	-0.01	-0.23	0.34
CON1	-0.20	0.19	0.17	-0.68	0.62	-0.14	0.02	0.16	0.04	-0.01
CON24	-0.22	0.17	0.72	-0.21	-0.12	0.56	-0.16	-0.09	0.06	-0.11

Table 74: Proportion of variance explained by each principle component (PC) and standardized loadings of the first ten PCs in PI(b) pigs

Principle component (PC)	1	2	3	4	5	6	7	8	9	10
Total variance (%)	37.61	20.75	10.64	8.97	6.65	4.82	4.39	2.92	2.00	1.09
Cumulative (%)	37.61	58.36	69.00	77.97	84.62	89.44	93.83	96.76	98.76	99.85
LEA	-0.25	0.05	0.76	-0.28	0.50	0.01	0.16	0.06	0.01	-0.01
FA	0.81	0.14	-0.16	0.11	0.47	-0.14	0.11	0.18	-0.06	0.00
MFR	0.82	0.12	-0.44	0.21	0.20	-0.12	0.02	0.11	-0.05	0.02
BF1	0.66	0.43	0.10	-0.18	-0.34	0.11	0.45	0.00	-0.10	-0.03
BF2	0.70	0.23	0.24	-0.27	-0.15	0.12	-0.46	0.28	0.05	0.02
BF3	0.82	0.14	0.06	-0.12	0.17	-0.18	-0.15	-0.43	0.15	-0.01
ABF	0.86	0.37	0.15	-0.23	-0.19	0.05	0.04	-0.06	0.00	0.00
OPTO	0.35	-0.39	0.47	0.44	-0.29	-0.43	0.09	0.11	0.13	0.04
pH1	0.47	-0.74	-0.07	-0.05	0.07	0.35	0.15	0.00	0.18	0.21
pH24	0.32	0.21	0.36	0.73	0.06	0.38	-0.10	-0.11	-0.10	-0.03
CON1	-0.36	0.80	-0.16	0.18	0.05	0.10	0.12	0.11	0.37	-0.06
CON24	-0.45	0.82	0.07	0.06	0.00	-0.14	-0.04	-0.05	-0.08	0.28

Table 75: Proportion of variance explained by each principle component (PC) and standardized loadings of the first ten PCs in F1 pigs

Principle component (PC)	1	2	3	4	5	6	7	8	9	10
Total variance (%)	42.30	13.54	11.33	8.61	6.62	4.78	4.46	3.57	2.48	2.21
Cumulative (%)	42.30	55.84	67.17	75.78	82.40	87.18	91.64	95.21	97.69	99.90
LEA	-0.60	0.03	0.10	0.69	0.21	-0.18	-0.09	0.19	0.16	0.05
FA	0.86	-0.01	-0.08	0.13	-0.06	-0.22	0.23	0.25	0.21	-0.11
MFR	0.90	-0.02	-0.12	-0.25	-0.17	-0.08	0.21	0.09	0.08	-0.10
BF1	0.82	0.02	-0.01	0.08	0.24	0.17	-0.33	-0.22	0.20	-0.19
BF2	0.78	0.06	-0.12	0.42	0.03	-0.11	0.02	0.03	-0.41	-0.09
BF3	0.89	0.06	-0.06	0.01	0.02	0.09	0.01	0.02	0.05	0.44
ABF	0.95	0.05	-0.07	0.18	0.13	0.08	-0.15	-0.09	-0.02	0.05
OPTO	0.30	-0.13	0.80	0.00	-0.14	0.35	-0.10	0.32	-0.05	-0.04
pH1	-0.05	-0.79	0.01	0.25	0.23	0.27	0.40	-0.16	0.02	-0.01
pH24	0.29	-0.07	0.80	-0.08	0.10	-0.42	0.07	-0.27	0.00	0.04
CON1	-0.06	0.68	0.10	-0.21	0.63	0.12	0.24	0.10	-0.04	-0.03
CON24	-0.07	0.71	0.16	0.38	-0.40	0.21	0.24	-0.23	0.07	-0.02