RENATA HERNANDES PITA

CANDIDATE GENES FOR MEAT QUALITY AND GROWTH IN SWINE

A Dissertation submitted to the Animal Science Graduate Program of the Federal University of Viçosa in partial fulfillment of the requirements of the degree *Doctor Scientiae*.

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RESUMO

PITA, Renata Hernandes, D.S., Universidade Federal de Viçosa, Julho 2004. **Genes candidatos para qualidade da carne e crescimento em suínos**. Orientadora: Simone Eliza Facioni Guimarães. Conselheiros: Paulo Sávio Lopes e Robledo de Almeida Torres.

A carne suína é uma das mais importantes fontes de proteína no mundo, sendo assim, um aumento da qualidade e da taxa de crescimento desses animais é um fator extremamente importante para a produção de suínos. Assim, o objetivo desse estudo foi identificar novos genes candidatos relacionados com a qualidade da carne, composição corporal e crescimento em suínos. Para esse estudo foi estabelecido uma família base compreendida de 3 gerações de animais oriundos do cruzamento entre as linhas Berkshire e Yorkshire. Para genes relacionados com qualidade da carne foram considerados: Proliferador de peroxissoma ativado pelo receptor alfa (*Peroxisome proliferator – Activated receptor alpha* -PPARA) e Peptídeo tirosina tirosina (*Peptideo tyrosina tyrosina* -PYY), devido ao seu grande papel em humanos e camundongos. Para crescimento foram estudados mais 3 genes baseados na sua localização em regiões de QTL (*quantitative trait loci*) no cromossomo 5 de suíno e também pelo seu papel fisiológico em humanos e

camundongos: Dominíos CASP2 e RIPKI contendo adaptador com domínio da morte (*CASP2 e RIPKI domain containing adaptor with death domain* - CRADD), suppressor da sinilazição 2 da citoquinas (*suppressor of cytokine signaling 2* - SOCS2) e receptor viral de semaforina codificada *(viral encoded semaphrin receptor* - PLXNC1). Polimorfismos foram identificados e usados para mapear esses três genes no cromossomo suíno. O PPARA, CRADD, SOCS2 e PLXNC1 foram mapeados no cromossomo 5 de suínos e o PYY foi mapeado no cromossomo 12. Análises de associações foram feitas na população de estudo Berkshire e Yorkshire e observou-se efeitos significativos do genótipo PPARA-*BsrG*I para peso ao nascimento (p<0,05), perda de água por gotejamento (p<0,05), e área de olho de lombo (p<0,1), com os animais com genótipos 11 apresentando maior peso ao nascimento, menor perda de água por gotejamento e maior área de olho de lombo. Em relação aos genótipos do PYY-*Hinf*I, este apresentaram efeitos significativos para mamoreio (p<0,01), espessura de toucinho no lombo (p<0,05) e capacidade de retenção de água (p<0,05), espessura de toucinho média (p<0,1), firmeza (p<0,1), e potencial glicolítico médio (p<0,1), sendo que os animais 22 apresentaram maior marmoreio, maior espessura de toucinho no lombo e espessura de toucinho média e também apresentaram maior firmeza e maior potencia glicolítico, já os animais 11 apresentaram menor capacidade de retenção de água com animais. Para os genes relacionados com crescimento foram feitas duas análises estatísticas. Uma análise considerando os genes individualmente no modelo e outra com os três genes simultaneamente no modelo. A análise com os três genes revelou que houve efeito significativo do genótipo CRADD-*Ava*I sobre o lipídeo total (p<0,001), ganho diário médio durante o teste (p<0,1), marmoreio (p<0,1) e resistência média

da carne ($p<0,1$), sendo que os animais 11 apresentaram menor teor de lipídios e menor marmoreio e os animais 22 apresentaram menor ganho diário e maior resistência da carne. Análise considerando os três genes no modelo mostrou que o SOCS2-*SacII* apresentaram efeitos significativos sobre peso aos 16 dias de teste ($p<0,05$), glicogênio médio ($p<0,05$) e ganho diário médio ao desmame ($p<0,05$), e peso da carcaça e cor ($p<0,1$), onde o genótipo 22 apresentou maior peso aos 16 dias, menor teor de glicogênio médio, maior ganho diário ao desmame e menores valores de peso da carcaça e cor. Finalmente, os genótipos do PLXNC1-*Msc*I apresentaram efeitos significativos sobre o ganho médio diário no teste ($p<0,01$) e lombo hormel Minolta ($p<0,01$) e área de olho de lombo e pH do pernil ($p<0,1$), sendo que os animais com o genótipo 12 apresentando menor ganho diário médio, e os animais 11 apresentando uma cor mais forte, e os animais 22 apresentando os maiores valores de área de olho de lombo e pH do pernil. O uso destes genes na seleção assistida por marcadores podem resultar em melhoras substanciais para os programas de melhoramento genético de suínos.

ABSTRACT

PITA, Renata Hernandes, D.S., Universidade Federal de Viçosa, July 2004. **Candidate genes for meat quality and growth in swine**. Adviser: Simone Eliza Facioni Guimarães. Committee members: Paulo Sávio Lopes and Robledo de Almeida Torres.

Pork is one of the most important animal protein sources in the world and the increment of meat quality and growth rate have become important factors for pig producers. The objective of this study was to find positional candidate genes related with meat quality, body composition and growth in the pig. A three generation Berkshire and Yorkshire resource family was used to find and study genes with relevant phenotypes for meat quality and growth. Two candidate genes were considered for meat quality: the Peroxisome proliferator – Activated receptor alpha (PPARA) and Peptide YY or peptide tyrosine tyrosine (PYY). Also, it was investigated three more genes based on their positional locations in QTL (quantitative traits loci) regions on pig chromosome 5 and their importance in high growth. These genes were: CASP2 and RIPKI domain containing adaptor with death domain (CRADD), suppressor of cytokine signaling 2 (SOCS2) and viral encoded semaphorin receptor (PLXNC1). Single nucleotide polymorphisms were identified and used to map these genes to pig chromosomes. The

PPARA, CRADD, SOCS2 and PLXNC1 genes were mapped on swine chromosome 5 and PYY gene was mapped on chromosome 12. Association analyses on the Berkshire and Yorkshire population revealed significant effects of PPARA- *Bsr*GI genotypes for birth weight and average drip loss (p<0.05), and loin eye area (p<0.1). Animals 11 had higher birth weight and loin eye area, and smaller drip loss. The PYY- *Hinf* I genotypes showed significant effects on marbling (p<0.01), lumbar back fat and water holding capacity (p<0.05) and average back fat, firmness and average glycolytic potential (p<0.1). Animals 22 had more marbling, lumbar back fat, average back fat, firmness and average glycolytic potential, while animals 11 had smaller water holding capacity. For the high growth genes two analyses were made. One analysis was made with individual genes and another with all three genes in the model. The analysis with all three genes showed that there were significant effects of CRADD-*AvaI* genotypes on total lipid (p<0.001), and average daily gain on test, marbling and average instron force (p<0.1). Animals 11 had smaller total lipid and marbling, while animals 22 had smaller average daily on test and average instron force. Association analysis with SOCS2- *SacII* genotypes showed significant effects on 16 day weight, average daily gain to weaning and average glycolytic (p<0.05 level), and carcass weight and color (p<0.1). Animals 22 had higher 16 day weight, average daily gain to weaning, and lower average glycolytic, carcass weight and color. The PLXNC1- *MscI* genotypes showed significant effects on average daily gain on test and hormel loin Minolta (p<0.01), and loin eye area and ham pH (p<0.1). Animals 12 had smaller average daily gain, animals 11 higher color (hormel loin Minolta), and animals 22 with higher loin eye area and ham pH. In conclusion, the use of these genes in marker-assisted selection may result in substantial improvements in selection programs.

1. INTRODUCTION

Conventional selection methods using phenotypic information to assist in the selection of animals with favorable alleles have been practiced for millennia. The response to direct selection for many traits such as reproductive traits have been slow because heritabilitity of many traits is low or the trait is measured after slaughter or they are very expensive to measure phenotypically, such as meat quality characteristics, reproductive traits and diseases traits. (De Vries and Plastow, 1998). Recently, advances in molecular genetic engineering have promised to revolutionize agricultural practices (Lande and Thompson, 1990) .The molecular biology should be integrated with conventional selection methods to obtain the maximum improvement in the economic value of domesticated populations.

The development of molecular biology techniques and the application of these techniques to farm animals have progressed rapidly and have opened new vistas for investigators wishing to identify genes that control quantitative traits (quantitative trait loci - QTL). These include the Halothane (HAL) or stress gene (Christian 1972; Fujii et al., 1991), the Rendement Napole (RN) or acid meat gene (Monin and Sellier, 1985; Ciobanu et al., 2001), the heart-fatty acid binding protein (H-FABP) gene, the fatty acid binding protein (A-FABP) gene (Gerbens et al., 1998), Estrogen receptor (ER) gene (Rothschild et al., 1997) and many others.

Traits such as feed intake, growth, body composition and meat quality are quantitative traits and each can be affected by a large number of genes. However, some of these genes might have a large effect. Such genes, if found, are called major genes. Most of these quantitative traits are also influenced by many other

genes that have smaller individual effects but large aggregate effects on the trait (Kennedy et al., 1992).

The development and application of molecular biology techniques to farm animals have progressed rapidly allowing us to identify chromosomal regions and genes responsible for differences in pork production. Known functional roles of genes and its genomic locations in species that are rich in genome information, such as human and mouse, can be used to find positional candidate genes (Lander et al., 2001. Therefore, specific genes identified in the human genome in regions that are syntenic with a specific region in the pig, as identified using bi-directional chromosomal painting (Goureau et al., 1996), could be a source of positional candidate genes in the pig for traits of interest.

The candidate gene approach has proven extremely powerful for studying the genetic architecture of complex traits. Quantitative trait locus (QTL) mapping is frequently used to identify genomic regions associated with a phenotypic trait of interest. These regions are generally large and may have thousands of putative genes. By definition, all genes in the QTL region are candidate loci for the trait and fine mapping in the region of QTL can reduce the number of candidate genes.

The pig industry is very interested in both the selective breeding of pigs and the use of gene technology because it can play an important role in enhancing pork quality (De Vries et al., 2000).

2. REVIEW OF LITERATURE

2.1. Pig gene mapping

Mapping genes and construction of gene maps are fundamental approaches to achieve the comprehensive understanding of an organism and also, to detect genes or regions with major influences on production traits.

The gene mapping efforts have focused basically on two types of maps, genetic linkage and physical maps. The genetic linkage map defines the genetic distance between two genes or markers as the frequency of recombination caused by crossing over during meiosis. The physical map is to assign genes or markers to specific chromosomal regions regardless of the rate of recombination.

The physical gene map is useful to characterize specific chromosome regions of interest and to facilitate further positional cloning. A somatic cell hybrid panel (Yerle et al., 1996) is an important tool to map the gene. In the pig, the method retains the fragments of all pig chromosomes in the 27 hybrid lines and the porcine somatic cells were fused with rodent tumor cells and only hybrids cells could be positively selected. During subsequent cell divisions a total of 27 somatic cell hybrids were obtained and characterized cytogenetically. Over 1000 genes and markers have been mapped using the somatic hybrid panel.

Genetic maps are primarily developed as tools for the localization and characterization of genes controlling important phenotypic traits. The major impetus for gene mapping research in livestock species is to identify genes underlying economic trait loci (Johansson, et al., 1995).

Another important tool of pig gene mapping is comparative mapping using the well-developed human and mouse gene maps. Comparative gene mapping is

based on the observation that genes that are closely linked in one species tend to be closely linked in other species if the species have evolved from a common source. The completed human sequence will be compared with non-human sequences and those comparisons will give scientists new insights into evolutionary, biochemical, genetic, metabolic, or physiological pathways (Tilghman, 1996).

Species that are closely related usually accumulate fewer rearrangements and therefore have many long conserved segments. The evidence of conserved segments for mammals is considerable, therefore many human and mouse genes have been mapped with some segments showing strong conservation (Nadeau and Sankoff, 1998).

Comparative mapping between humans and other organisms has revealed that synteny relationships are often conserved across a wide range of species. Therefore, groups of genes that are tightly linked in humans have a high probability of also being linked in other species (Rettenberger et al., 1995). The relationship between position and function of genes in one species can also explain the potential function of the gene in other species because the evolutionary processes might have conserved the clustered multigene families in the genome and the conserved clusters might be homologous to those in other organisms (Clark, 1999).

Recent genome analyses on more amenable organisms such as the mouse, *Drosophila*, *Caenorhabditis elegan*s (worm), *Saccharomyces cerevisiae* and *Escherichia coli* make it possible to advance from identification of new genes to systematic studies of their functions. Comparisons between human and porcine chromosome revealed extensive conservation, but with gene order rearrangements (Johansson et al 1995; Rettenberger et al., 1995; Goureau et al., 1996; Larsen et

al., 1999). These results contributed to the specification of borders of homologous segments in each species and have allowed the orientation of these segments.

To explore the molecular genetics of growth or reproduction it will be necessary to identify and isolate the candidate genes involved in specific trait development (Rettenberger, et al., 1995).

2.2. Candidate gene approach

This approach assumes that candidate genes represent a proportion of the QTL that determine a particular trait. The following are steps involved in a candidate gene analysis (Rothschild and Soller, 1997):

1) Choosing the candidate gene. In this case three sources of information can be used: Physiological approach, mutational approach and positional approach. The physiological approach involves prior knowledge of the biochemistry and / or physiology of a trait to draw up a list of genes. The mutational approach looks for mutations in genes from other species, and the positional approach combines information about a gene's chromosomal location with QTL information for easier identification of a potential causative gene. Also, comparative mapping can be used to utilize information of known genes in another species to map a gene in the species of interest.

The QTL analyses have increased the number of new positional candidate genes. These QTL analyses involve employing a genomic scan where generally F2 or backcross families are used and genotypes are obtained for many markers. Several experiments have been completed and are beginning to produce interesting and useful results (Rothschild and Plastow, 1999). The QTL scans have identified several chromosomes that are now targets for further confirmation of the

chromosomal region, advanced fine mapping of QTL, and positional comparative candidate gene analysis.

2) Designing primer sequences to amplify the gene. The primers can be made from existing databases or using sequence similarity between species.

3) Uncovering polymorphisms in the gene. Several technologies exist to uncover the polymorphisms in candidate genes. The PCR-RFLP test allows amplification and analysis of large number of individuals.

4) Analysis of associations between traits of interest and genotype for the candidate gene.

Several candidate gene analyses have been successfully conducted. For example, in pigs from PIC (Pig Improvement Company), there has been an increase in the rate of genetic response by up to 30% by incorporating the ESR genotype in selection indices for dam lines in nucleus herds. Also, PIC reduced mortalities of between 4-16 per 1000 head to near zero and improved meat quality in commercial products when they removal the Halothane stress gene (Rothschild and Plastow, 1999).

2.3. Candidate genes

2.3.1. Meat quality

Meat quality is generally defined as darker color, more water holding capacity, and better texture, which describes meat that is free of the PSE syndrome (pale, soft, and exudative). However, meat quality cannot be defined using only one parameter such as appearance (Wariss and Brown, 1987). The quality of meat can be affected by five aspects hygienic, compositional, nutritional, sensorial and

technological i.e. the suitability to processing, storage and distribution (Nardone and Valfrè, 1999).

Pig meat quality is more important than for others animals because of its use in processed or technological products. The main characteristics of technological quality are the emulsifying capacity, from which depends on the stability of fat and meat emulsions; the preservation length, related to post-mortem pH, shear force and micro-organisms development; and water retention (Nardone and Valfrè, 1999). Molecular biology will allow to improve technological quality of meat, as well as chemical and organoleptic characteristics. Particularly, it may be possible to identify unfavorable alleles and then remove them or increase the favorable ones with selection.

2.3.1.1. Peroxisome proliferator – Activated receptor alpha (PPARA)

Perixisome proliferator activated receptors (PPARs) define a subfamily of nuclear hormone receptors, which regulate the transcription of genes involved in metabolism of lipids. They are nuclear hormone receptors that mediate the effects of fatty acids and their derivatives at the transcriptional level. To date, three mammalian PPAR subtypes have been isolated; termed PPARA (PPAR alpha), PPARD (PPAR delta) and PPARG (PPAR gamma). PPARs play important roles in lipid metabolism, which make them interesting as candidate genes for intramuscular fat (IMF) and other fat-related meat quality traits (Bocher et al., 2002).

Peroxisome proliferator – Activated receptor alpha (PPARA) is a nuclear hormone receptor member of the superfamily of nuclear receptors activated by endogenous and xenobiotic ligands (Mangelsdorf et al., 1995). PPARA plays a key

role in regulating the catabolic pathway of lipids in response to a variety of compounds named peroxisome proliferators (PPs). Its expression, in mouse, is mainly detected in tissues exhibiting high rates of β-oxidation, i.e. in liver, kidney, heart and skeletal muscle where it promotes cellular uptake, activation and oxidation of fatty acids through activation of target gene expression. In contrast to rodents, in which PPARA is highly expressed in liver, in pig PPARA is highly expressed in adipose tissue and to a much lesser extent in liver and skeletal muscle (Ding et al., 2000).

The physiological response to peroxisome proliferators is species-dependent and in rats and mice this involves an increase in size and number of hepatic peroxisomes (peroxisome proliferation) that after sustained exposure leads to tumor growth in the liver. Importantly, humans treated with the fibrate drug, whose administration reduces the serum level of triglycerides and cholesterol, appear refractory to these pleitropic responses of PPs (Sundvold et al., 2001).

PPARA is involved in fatty acid oxidation by up-regulating the expression of the acyl-Coa oxidase and carnitine palmitoyltransferase enzymes (Ding et al., 2000). Recently, a missense mutation has been identified in the human exon 5 of PPARA gene resulting in the substitution of a leucine for a valine at codon 162. This mutation was associated with reduced adiposity and may be involved in the pathogenesis of obesity (Bossé et al., 2003).

Michalik et al. (2001) showed that α, β and γ isotypes of peroxisome proliferator-activated receptor are expressed in the mouse epidermis during fetal development and that they disappear progressively from the interfollicular epithelium after birth. Interestingly, PPAR α and β expression is reactivated in the adult epidermis after various stimuli, resulting in keratinocyte proliferation and

differentiation such as tetradecanoylphorbol acetate topical application, hair plucking, or skin wound healing. PPARA is mainly involved in the early inflammation phase of the healing.

2.3.1.2. Peptide YY

The pancreatic polypeptide (PP) family consists of three peptides: pancreatic polypeptide, peptide tyrosine tyrosine (PYY), and neuropeptide tyrosine (NPY) and they are found both in the central nervous system and in peripheral tissue (Lundberg et al., 1982). PYY and pancreatic polypeptide are mainly found in gastrointestinal mucosa and pancreas and are involved in the control of digestive functions, while NPY is the most abundant neuropeptide present in the nervous systems. Many nutrients, hormones, growth factors, and neurotransmitters have been demonstrated to stimulate PYY release from mucosal endocrine cells in the distal intestine. Some studies have shown that bile acid, but not nutrients, plays a crucial role in the regulation of PYY secretion (Onaga et al., 2002).

The receptors belong to the large family of G protein-coupled receptors and are denoted as Y receptores subtypes (Y1, Y2, Y4, Y5, and Y6). This receptors bind all three peptides, but with different affinities (Balasubramaniam, 1997).

PYY has two molecular forms. The major molecular known as PYY_{1-36}, consists of 36 amino acids. The secondary, truncated, molecular form is PYY_{3-36}, in this form lacks the amino terminal and have reduced affinity to Y1 receptors. PYY is predominantly present in the endocrine cells of intestine, pancreas, small quantities has been reported to be present in the brain, and in some peripheral neurons in the gastrointestinal tract (Balasubramaniam, 1997).

2.3.2. Region of high growth

The study of mammalian growth-control genes is essential for elucidating the mechanism of growth at the tissue, organ, or whole-body level and the identification of quantitative trait loci (QTL) involved in the control of growth in the swine is of obvious importance. In mouse, the high growth (hg) mutation is a 460 Kb deletion of chromosome 10 which causes a 30-50% increase in growth in the homozygous animal without becoming obese (Bradford and Famula, 1984). In high-growth mice was observed that they have increased levels of plasma insulin-like growth factor I (IGF-I) and decreased levels of plasma and pituitary growth hormone (GH), suggesting that the causal mutation influences growth through deregulating the GH/IGF1 system (Horvat and Medrano, 2001). Animals with extreme body growth are often accompanied by poor reproductive performance. Cargill et al. (1999) suggest that the hypothalamic-pituitary unit of the high growth female provides an inadequate signal to the ovaries to maintain pregnancy and the luteal failure may be due to insufficient prolactin.

Studies with the mouse have shown that three genes, CASP2 and RIPKI domain containing adaptor with death domain (CRADD), suppressor of cytokine signaling-2, also known as Cish2 (SOCS2) and viral encoded semaphorin receptor (Vespr or Plexin C1 or PLXNC1), have been identified within this region of high growth (Wong et al., 2002). In the chicken, the CRADD gene was mapped on chromosome 1 in a potential growth QTL region and showed conserved synteny with regions of human chromosome 12 and mouse chromosome 10 (Smith et al., 2000).

Recently, the high growth mouse deletion was determined with 486,178 bp, with one breakpoint at 633 bp downstream of SOCS2 exon 2, and the other 8589

bp upstream to PLXNC1 exon 3, resulting in the fusion of the two genes, SOCS2 exons 1-2 and PLXNC1 exons 3-20, and deletion of all CRADD gene in the high growth genome (Wong et al., 2002).

2.3.2.1. CRADD gene

CRADD is an adapter molecule that contains an aminoterminal CARD (Caspase Recruitment Domain) region and a carboxy-terminal “death domain”. It mediates the action of cysteine proteases involved in the apoptosis pathway (Duan and Dixit, 1997). The human death adaptor molecule CRADD shares a very high homology with the mouse CRADD gene. Therefore, possibly the increase in cell number observed in high growth is the result of alterations in the apoptosis pathway (Horvat and Medrano, 1998).

Also, CRADD interact with caspase-2 and the caspase-2 knockout mouse has an attenuated female germ cell apoptosis resulting in a significant increase in the number of primordial follicles in the postnatal ovary, which could be related to increase of ovulation rate in high growth females (Dilts et al., 1991). Overexpression of CRADD cDNA in 3T3L1 cell inhibited differentiation in mouse, suggesting that CRADD plays a role in controlling differentiation of mouse preadipocytes and, perhaps, in other cell types, in addition to its established role in apoptosis (Felmer et al., 2003).

2.3.2.2. SOCS2 gene

SOCS2 is a member of the suppressor of cytokine signaling family, a group of related proteins implicated in the down regulation of cytokine action through inhibition of the Janus kinase (JAK) signal transducers and activators of

transcription (STAT) signal-transduction pathway (Greenhalgh et al., 2002). The suppressors of cytokine signaling (SOCS) proteins are a family of eight SH2 domain-containing proteins, comprising cytokine-inducible SH2 domain-containing protein (CIS) and SOCS-1-7. SOCS proteins operate as part of a classical negative feedback loop, in which activation of cytokine signaling leads to their expression. (Greenhalgh et al., 2002).

SOCS2 deficient animals exhibit accelerated post-natal growth resulting in a 30-50% increase in body weight by 12 weeks of age, significant increase in bone and body lengths, thickening of the skin due to collagen deposition, and increases in internal organ size due apparently from elevated cell numbers rather than increase cell size. SOCS2 is an essential down regulator of growth hormone (GH) signalling *in vivo*, and its absence leads to increase growth through increase production of IGF-1 (Metcalf et al., 2000) and SOCS2 regulates neuronal differentiation by inhibiting growth hormone signaling (Turnley et al., 2002).

Greenhalgh et al. (2002) produced mice that transgenically overexpress SOCS2 and find that this mouse did not have repressed growth and actually, significantly increase in a number of growth parameters. Also, they found that SOCS2 interacts with endogenous GH receptors in primary cells, and using synthesized peptides they observed that SOCS2 shows one site of interaction to Tyrisine 595 (Tyr^{595}) of the GH receptor.

2.3.2.3. PLXNC1 gene

The mouse PLXNC1 protein is a member of the semaphorin family, which is a large family of secreted and transmembrane proteins best known for their involvement in neural development and also associated with the immune system

(Comeau et al., 1998). The extracellular domains of Plexin contain approximately 500 amino acid semaphorin domains. Semaphorins are implicated in cardiac and skeletal development, in the immune response, in the regulation of angiogenesis and in tumor growth and metastasis (Tamagnone et al., 1999).

The Plexins subfamily including four genes Plexin-A, -B, -C, and –D. The Plexin –A subfamily includes Plexin-A1, Plexin-A2, Plexin-A3 and Plexin-A4, whose genes are located on human chromosome 3, 1, X and 7, respectively. The Plexin-B subfamily includes Plexin –B1 (located on human chromosome 3), Plexin-B2 (human chromosome 22) and Plexin-B3 located on human chromosome X. The Plexin-C subfamily is defined by Plexin-C1 and the Plexin-D subfamily contains Plexin-D1 (located in human chromosome 3).

These three genes in the high growth region are conserved between human chromosome 12 and mouse chromosome 10 (Wong et al., 2002). In humans, this high-growth region corresponds to an interval of 100 to 103 cM from the top on genetic map of human chromosome 12 and from 12q21.33-q23.1 on a cytogenetic map (Horvat and Medrano, 1995). These identified chromosomal regions and genes that regulate lean growth in pigs are of obvious importance to the pig industry.

3. OBJECTIVES

The objectives of this study were 1) to investigate the location of high growth genes and meat quality genes in the pig genome and 2) to evaluate their association with a variety of growth and meat related phenotypes in pigs.

4. MATERIALS AND METHODS

4.1. Animals

The experiment began in 1996 and animals F2 were born and collected in 1997 to 1999. The group of animals used in this study was a three-generation family of pigs generated by an intercross between two purebred Berkshire boars and nine purebred Yorkshire sows that were used to produce nine F1 litters. The boars were chosen from commercial boar studs and were mated artificially to sows from the Iowa State University Swine Breeding Farm. From the resulting F1 litters, 8 boars and 26 gilts were selected to produce the 525 F2 animals used in this study. A total of 65 matings were made to produce four sets of F2 offspring. The pigs were weighed at weekly intervals and sent to a commercial facility to be slaughtered when they reached approximately 110 kg. These breeds were chosen for their divergence in growth and body composition phenotypes. Details of the rearing and management procedures are described in Malek et al. (2001a, b).

4.2. Management

The F1 animals used for breeding were put in outside lots with shelter and gilts were bred at 8 - 9 months of age, and sows were bred after weaning their respective litters during the course of the experiment. The females farrowed in rooms that contained 12 farrowing crates and they were fed a 15% protein lactation diet *ad libitum*. The F2 pigs were weaned at 16 - 21 days of age and the feed was made available at 10 - 14 days of age. Litters were kept together during the growing and finishing phases. At weaning, males were castrated and the pigs were moved to a nursery, where they received a 21% protein complete feed for 5 - 7

days and then a 20% protein complete feed for 3 weeks. This was changed to an 18% protein ration for another 2 - 3 weeks. When the pigs left the nursery, they were placed in pens that allowed for an average of eight sq. ft. per pig. The diet was changed to an 18.8% protein diet until the pig's weight reached 34 kg on a pen average. At that time, the diet was changed to a 17.5% protein diet until pigs reached 72 kg, and then to a 16% protein diet until the pigs went to market. All diets were fortified with vitamins and minerals for the age of the pig. Water was provided *ad libitum*. The slaughter point was determined by weighing pigs at weekly intervals when they approached 110 kg.

4.3. Traits measured

For the research described in this thesis, body composition and meat quality were evaluated on the basis of 39 traits (Malek et al., 2001 a, b).

The traits measured on the live animal were: birth weight, 16 day weight, average daily gain from birth to weaning, and average daily gain on test from weaning to slaughter. After slaughter and chilling, carcass traits were evaluated at the plant by trained personnel according to National Pork Producers Council guidelines (NPPC, 1991). Traits recorded were carcass weight, carcass length, tenth rib back fat, lumbar back fat, last rib back fat, average back fat, and loin eye area.

Subjective traits evaluated after slaughter and chilling were: marbling, firmness, and color. Marbling, firmness and color were scored on a scale from 1 to 5, with higher values indicating greater marbling, greater firmness, and darker color.

Color also was evaluated objectively with a Minolta chromometer and a Hunter lab scan. Minolta and Hunter L values measure light reflectance of the muscle. Lower values indicate darker color, which is desirable, and higher values indicate paler, lighter colored meat. Color was measured at two locations: 1) at the Hormel slaughter plant in Austin, Minnesota at 24 hrs after slaughter and 2) at the Iowa Sate University Meat Laboratory in Ames 48 hrs after slaughter. All measurements were taken by trained personnel following the guidelines of the National Pork Producers Council (NPPC, 1991).

Other objective measurements were muscle pH, drip loss, water holding capacity, gycolytic potential, total lipid and muscle fiber type composition. Muscle pH was measured in the longissimus dorsi and the semimembranosus muscles at 24 hours after slaughter, using a glass penetration pH electrode.

Drip loss measures the amount of moisture (purge) lost from the product over a period of time. Drip loss was measured on a size-standardized sample from the longissimus dorsi (3 cm in diameter and 2.5 cm thick) (Honikel et al., 1986; Kauffman et al., 1986a) that was collected at 48 hours postmortem. The sample was weighed, suspended in a plastic bag, held at 4°C for 72 hours, and re-weighed at the end of the holding time. Drip loss was calculated as the percentage of product weight that was lost over the 72 hour storage period. This was done with duplicate samples and the average value was used for analysis.

Water holding capacity is a complementary measure of the ability of meat to retain water. Water holding capacity was measured using the filter paper press method (Kauffman et al., 1986 b), which evaluates the amount of moisture lost from the surface of the loin shortly after cutting. A pre-weighed piece of filter paper, which was exposed to the atmosphere for 10 minutes, was placed on a fresh cut of

the loin muscle 48 hours postmortem for three seconds to allow it to absorb surface moisture, and then re-weighed. The difference in weight was used as the measure of water holding capacity (Kauffman et al., 1986 a), with a lower value indicating that less moisture was lost from the tissue, which is more desirable.

To evaluate the sensory characteristics of the meat (juiciness, tenderness, chewiness, pork flavor, and off-flavor) was used a 10-point category scale. The scale was anchored on the left end with a term representing a low degree of juiciness, tenderness, chewiness, flavor, and off-flavor intensity. On the right end of the scale was a term representing a high degree of each characteristic. Any flavor that was not associated with normal pork flavor was considered as an off-flavor. The values for each pork chop were averaged across the three panelists.

For these sensory characteristics of the meat were taken a vacuum packaged boneless chops from the longissimus dorsi of each animal in 48 hours after slaughter and stored for 10 days at 4°C. Following the storage period, chops were broiled to 71°C in an electric oven broiler (Amana Model ARE 60) that had been preheated to 210°C. The temperature of each chop was monitored in the center of the chop using thermocouples (Chromega/Alomega) attached to an Omega digital thermometer (Model DSS-650, Omega Engineering).

Cooking loss was calculated from weights taken before and after broiling and was expressed as a percentage. Instrumental measurement of tenderness (Average Instron force) of the broiled chops was evaluated using a circular five-pointed star-probe (9 mm in diameter with 6 mm between each point) attached to an Instron Universal Testing Machine (Model 1122). A 100 kg load cell was used with a crosshead speed of 200 mm/min. The star-probe attachment was used to determine the amount of force needed to puncture and compress the chop to 80%

of the sample height. Each chop was punctured 3 times and the average was recorded.

Sensory evaluation of the broiled chops was done using three highly-trained professional sensory panelists. Panelists were seated in individual booths with red lighting overhead to mask any differences in product color. Cubes, 1.3 cm in size, were removed from the center of the broiled loin chops, placed in preheated, individually-coded glass petri dishes and served to each panelist. Room temperature deionized, distilled water and unsalted crackers were used to cleanse the palates of the panelists between samples.

At 48 hours postmortem, a sub-sample of the loin was frozen and sent to the University of Illinois, where glycogen, free glucose, glucose-6-P, and lactate content were measured in μMol/g (Monin and Sellier, 1985). Postmortem metabolism of elevated glycogen stores results in increased production of lactate, which is a pH lowering by-product of muscle metabolism. Glycolytic potential is a measure of glycogen stores and was calculated as follows: glycolytic potential = 2 x ([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate] (Monin and Sellier, 1985; Maribo et al., 1999). Glycolytic potential is expressed in μMol lactate equivalents per gram muscle wet weight. In addition to glycolytic potential and lactate concentration, residual glycogen concentration was used as a trait of interest in this study.

Total lipid in the longissimus dorsi was measured as described by Bligh and Dyer (1959) and expressed as a percentage of tissue weight. Total lipids were then dissolved in isopropanol and assayed for concentration of total cholesterol using an enzymatic procedure (Sigma Cholesterol Kit No. 352, Sigma Chemical Co., St. Louis, MO). Cholesterol was reported in mg per 100 g of tissue.

Muscle fiber type composition was evaluated in 48-hour postmortem samples from the longissimus dorsi by separation of myosin isoforms on high porosity SDS-PAGE gels. The procedure used was as described by Talmadge and Roy (1993) but with modifications as described by Huff-Lonergan et al., (2002). Results were expressed as the ratio of the density of the II a b and of myosin to the density of the IIb band within a sample. Porcine diaphragm muscle (extracted as described in Huff-Lonergan et al., 2002) was used as a standard on each gel to aid in identifying the myosin isoforms. Diaphragm muscle contains primarily type IIa, IIx, and type I associated myosin isoforms (Talmadge and Roy, 1993).

See Table 1 and 2 in Appendix for more details of the traits.

4.4. Candidate genes, primer sequences, PCR conditions and polymorphisms

In the first part, there were investigated two genes that were selected based principally on biological roles of the genes and probable locations in QTL regions on pig chromosomes (Malek et al., 2001a,b). They are very interesting genes to increase the meat quality. These genes were the Peroxisome proliferator – Activated receptor alpha (PPARA or PPARα), and Peptide YY or peptide tyrosine tyrosine (PYY). Also, there were investigated three more genes based on their locations in QTL regions on pig chromosome 5. The genes were the CASP2 and RIPKI domain containing adaptor with death domain (CRADD), suppressor of cytokine signaling 2 (SOCS2), also known as Cish2 and viral encoded semaphorin receptor (Vespr or Plexin C1 or PLXNC1). These genes have been identified within this region of high growth in the mouse (Wong et al., 2002) and they are potentially very interesting candidate genes in swine.

The gene nomenclature and the gene symbol used in this work follow the rules developed by the HUGO / GDB nomenclature committee (http://www.gene.ucl.ac.uk/nomenclature).

PPARA primers were designed in exon 4 (PP1F) and exon 5 (PP1R) from a published porcine partial cDNA sequence (GenBank accession no. AF228696).

For the PYY gene, a consensus primer (PY1F and PY1R) was designed in conserved regions among the human, mouse and bovine PYY gene sequences (GenBank accession nos. NM_004160, NM145435, and L37369, respectively).

For the CRADD and PLXNC1 genes primers were designed from swine ETSs (Expressed sequence tags) from Iowa State university database. For SOCS2, a complete cDNA sequence of porcine SOCS2 (Genbank accession AY312266) was used to design primers. Primer sequences, fragment sizes and accession numbers are given in Table 1.

Table 1. PCR primers, fragment size (bp) and amplification conditions

Gene		Primer sequences (5' → 3')	Fragment size (bp)	Annealing temperatures	Extension time 72 °C for
PPARA	PP1F	TCTCCAGCCTCCAGCCCCTC	~ 1,700	45 s at 60 °C	30 s
	PP1R	CACAGGCTTCATACGCAGGA			
	PP2F	CATTCGGCTAAAGCTGGTCT	~ 317	45 s at 57 °C	30 s
	PP2R	TGACTAGTTCTAATTATTCCGAGGATCTGCTGTAC			
PYY	PY1F	AACCGCTACTACGCCTCCCTG	~900	30 s at 58 °C	1 min
	PY1R	ACCACACACAGCCCTCCAGCC			
	PY2F	GAG AGC TGG AAG AAT AGA AGC	~175	30s at 58 °C	30 s
	PY2R	ACC ACA GCC CTC CAG CC			
CRADD	CR1F	TTCCCAGCACTCCCTTTTTAG	~ 635	30 s at 58 °C	45 s
	CR1R	ACCCCATCACGGCAGAAA			
SOCS2	SO1F	ACCCAACCCTCCACTTTCTC	~ 897	30 s at 58 °C	45 s
	SO1R	GCAACCCTCCTCCTTTCC			
PLXNC1	PL1F	GCACAAGTTCAAAGTAAAAGAAATG	~ 1,366	30 s at 55 °C	30 s
	PL1R	GGGCATCCAAAAAGTCAAAA			

The PCR reactions were performed using 12.5 ng of porcine DNA, 1x PCR buffer, 1.25 mM $MgCl_2$ (PP1F and PP1R), 1.0 mM $MgCl_2$ (PP2F and PP2R), 0.75 mM $MgCl_2$ (PY1F and PY1R), 1.5 mM $MgCl_2$ (CRADD and PLXNC1) and 1.0 mM $MgCl_2$ (SOCS2), 0.125 mM dNTPs, 0,3 μM of each primer, and 0.5 U Taq DNA polymerase (Promega, Madison, WI, USA) in a 10 μL final volume. The PCR profile included 4 min at 94 °C; 35 cycles of 45 s at 94 °C. Annealing temperatures (TA) and extension time specific for each primer in Table 1, and 45 s at 72 °C (CRADD and SOCS2) or 30 s (PLXNC1); and a final 7 min extension at 72 °C in a PTC200 (MJ Research, Inc., Watertown, MA, USA). Primer sequences, fragment sizes, annealing temperatures and extension time are given in Table 1.

The PCR products from several individuals from several commercial breeds of pigs were directly sequenced. The sequences were analyzed with Sequencher software (Gene Codes Corporation, version 4.0.5, Ann Arbor, MI) to identify polymorphisms. Sequences were compared with human sequence for similarity.

The PCR-restriction fragment length polymorphism (RFLP) tests were used to genotype the identified polymorphisms in the candidate genes. After digesting with specific restriction enzymes that recognize polymorphic sites, the digested PCR products were separated on 3-4% agarose gels and stained with ethidium bromide. Locations of polymorphisms, restriction enzymes, and the digestion patterns are listed in Table 2.

Table 2. Primer, Polymorphism locations, restriction enzymes, size of the allelic polymorphisms.

Primer	Polymorphism locations	Restriction enzymes	Size of the allelic polymorphisms
PP2F and PP2R	Intron 4	*Bsr*GI	317-bp (allele 1), or 286 and 31-bp (allele 2)
PY2F and PY2R	3' UTR	*Hinf* I	175bp (allele 1), or 105 and 70 bp (allele 2)
CR1F and CR1R	5'UTR	*Ava*I	635, 470 and 165
SO1F and SO1R	intron 2	*SacII*	439, 262 (all genotypes) and 196 (only 12 and 22)
PL1F and PL1R	intron 26	*MscI*	1158 (11 and 12 genotypes), 617 and 541 (12 and 22 genotypes) and 65(all genotypes)

4.5. Linkage mapping and statistical analysis

The candidate genes were mapped to the Berkshire x Yorkshire family linkage map using the CRIMAP (version 2.4) mapping program (Green et al., 1990).

An analysis of variance procedure was used to test associations of candidate gene polymorphisms with meat quality and body composition traits using the SAS general linear model computer program (SAS/STAT, 1990). The traits were tested using a mixed-model, which included the fixed effects of sex, slaughter date, genotype and the random effect of dam. Sire was not used as it was confounded with slaughter date.

Live weight at slaughter was added in the model as a covariate for average backfat, tenth rib backfat, lumbar backfat, last rib backfat, loin eye area, carcass weight, carcass length, total lipid, marbling, and cholesterol.

For the traits average glycogen, average glycolytic potential and average lactate the Rendiment of Napole (RN) gene was added to the model as a fixed effect.

Similar analyses were also run with the CRADD, SOCS2 and PLNXC1 candidate genes included in the model. The traits were tested using a mixed-model included the fixed effects of sex, slaughter date, candidate genes and random effect of dam. Live weight at slaughter was added in the model as a covariate and RN was included as a fixed effect for the same traits as in individual analysis.

5. RESULTS

5.1. Identification of polymorphisms and linkage mapping

5.1.1. Meat quality genes

The 1,700-bp product from PP1F and PP1R was sequenced and the sequence revealed 85% and 87% exonic nucleotide identity to the corresponding human and mouse sequences of PPARA gene, respectively. Sequence analysis of the PCR products from several individual pigs of different breeds detected an intronic nucleotide substitution. A restriction enzyme that would recognize this substitution was not found. Thus, a restriction site was created using a mismatch primer that created a recognition site for the enzyme *Bsr*GI (PP2F and PP2R). The PCR fragment from these primers was 317 bp and spanned intron 4 and exon 5. The porcine PPARA genomic sequence has been submitted to GenBank (accession no.AY344366).

The PPARA gene was significantly linked with several markers on porcine chromosome 5 (SSC5). For SSC5, no QTL was detected near the PPARA locus in the Berkshire and Yorkshire crossed family. Two-point linkage analysis determined that the most closely linked markers (recombination fraction and LOD score) were ACR (0.06, 21.77) and SW413 (0.10, 15.02). The best map order for PPARA produced by multipoint linkage analysis with other linked markers (with distances in centimorgans listed between markers) was:

PPARA - 6.3 – ACR – 2.3 – SW413 – 27.3 – SW1482 – 36.8 – SW2 – 24.9 – SW904 – 13.1 – IGF1 – 7.0 – SW995 – 10.2 – SW1954 – 3.2 – SW378.

The porcine sequence from PY1F and PY1R was identified as the porcine PYY gene spanning exons 2 and the 3' UTR and showed 86% and 76% exonic identity to the corresponding human and bovine PYY sequences, respectively. Sequence analysis of the PCR products from several individual pigs of different breeds detected a 3' UTR nucleotide substitution, which is a recognition site for the enzyme *Hinf* I. The porcine PYY sequence has been submitted to GenBank (accession no. AY344365). Using this sequence, pig specific primers, PY02F and PY02R, were designed to amplify a 175 bp product.

The porcine PYY gene was assigned to chromosome 12 (P=1.00) and the p11- (2/3 p13) region (P= 0.89) by PCR analysis of a pig-rodent somatic cell hybrid panel (Yerle et al., 1996).

Two-point and multipoint linkage analyses were performed using CRIMAP 2.4 against other genotypes in the Iowa State Berkshire x Yorkshire resource population. Most significant linkage between the PYY gene and other markers were obtained with microsatellites S0229 (recombination fraction = 0.19 and LOD = 41.17) and SW874 (recombination fraction = 0.13 and LOD = 59.93) on chromosome 12. The best map order for the PYY gene produced by multipoint linkage analyses with other linked markers was (with distances between markers in centimorgans): S0229 – 20.9 – PYY- 14.6 - SW874 – 12.3 – S0090 – 14.7 – S0147 – 22.9 – SWC23 – 12.2 - SW2180. In Figure 1 is shown the QTL analyses and the comparative mapping between human chromosome 17 and swine 12.

5.1.2. High growth genes

The CRADD primers amplified a fragment of approximately 635 bp spanning exon 3 and 5'UTR. After sequencing this product showed 88% similarity to the

previously submitted mRNA of the human CRADD gene. Sequencing PCR products of several individual pigs of different breeds showed one nucleotide substitution in the 5’ UTR situated within an *Ava*I restriction enzyme recognition site.

The SOCS2 primers amplified a fragment of approximately 897 bp, which spanned exon 2 and exon 3, and this product after sequencing showed 88% of similarity to the previously submitted mRNA of the human SOCS2 gene. Sequencing PCR products of several individual pigs of different breeds showed one nucleotide substitution in the intron 2 situated within a *SacII* restriction enzyme recognition site.

For PLXNC1 the primers amplified a fragment of approximately 1,366 bp and spanned exon 26 and exon 27. The PCR product was sequenced and the sequence showed 91% of similarity to the previously submitted mRNA of the human Plexin gene. Comparing several sequences of different pig breeds revealed one nucleotide substitution situated within a *MscI* restriction enzyme recognition site in the intron 26 of this gene.

A single nucleotide polymorphism (SNP) was discovered for each gene and confirmed by enzyme for all of the genes. The enzyme, the fragments sizes are presented in Table 2.

The CRADD, SOCS2, and PLXNC-1 candidate genes were significantly linked with several markers on porcine chromosome 5 (SSC5) at a general position that contains several QTL for growth and body composition traits (Figure 1). This QTL region is homologous to parts of human chromosomes 12. Two-point linkage analysis determined that CRADD, SOCS2 and PLXNC1 are very close

and there are not recombination fraction between CRADD, SOCS2 and the marker SW1954 (Figure 1).

The best map order for the high growth genes produced by multipoint linkage analyses with other linked markers were (with distances between markers in centimorgans): PPARA – 6.3 – ACR- 2.3 – SW413 – 27.3 – SW1482 – 32.0 – GPR49 – 4.8 – SW2 – 24.9 – SW904 – 13.1 –IGF1 – 7.1 – SW995 – 4.0 – PLXNC1 – 7.1 – SW1954 – 0.0 – CRADD – 0.0 – SOCS2 – 3.5 – SW378 – 0.6 – ATP2B1 – 8.5 – DUSP6.

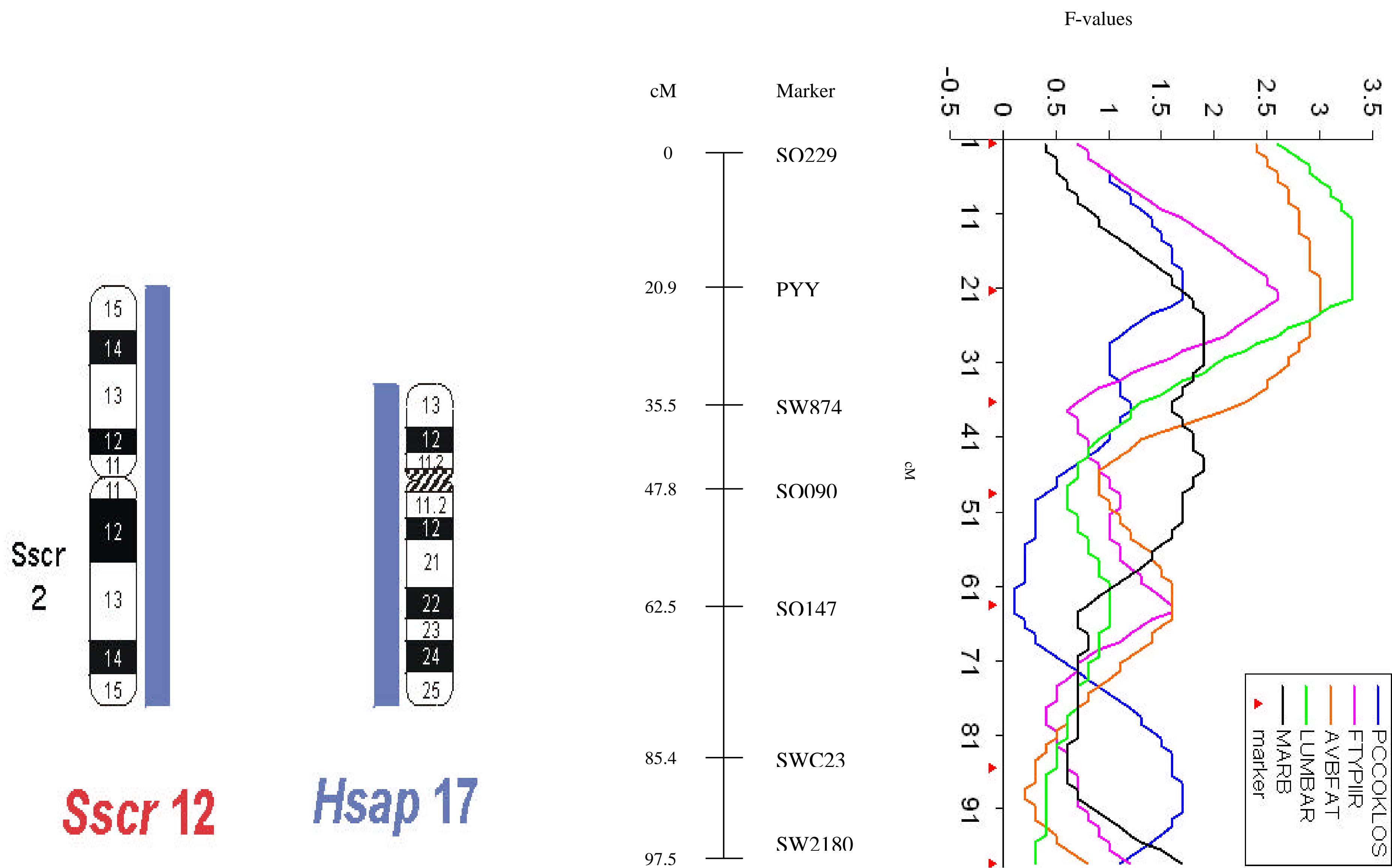

Figure 1. Comparative physical and linkage map of pig chromosome12 with human chromosome 17 and QTL analyses on pig chromosome 12. The shared regions summarize previous results of chromosomal painting.

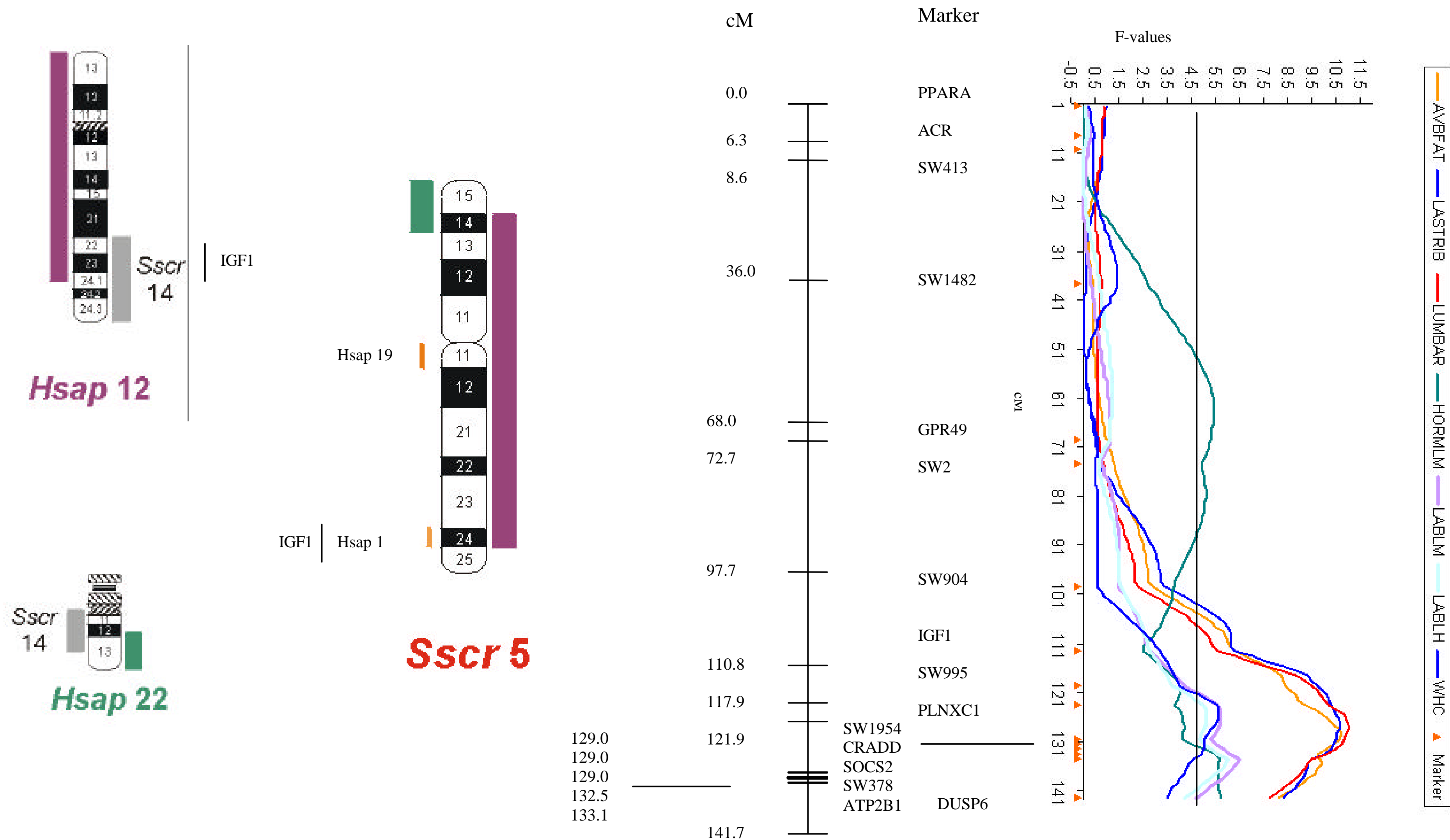

Figure 2. Comparative physical and linkage map of pig chromosome 5 with human chromosomes 12 and 22 and QTL analyses on pig chromosome 5. The shaded regions summarize previous results of chromosomal painting.

5.2. Berkshire x Yorkshire F2 association study

5.2.1. Meat quality genes

Frequencies of Allele 1 in the Berkshire and Yorkshire grandparents were 0.25 and 0.22 for PPARA, and 1.00 and 0.33 for PYY. The frequency of allele 1 in the total F2 Berkshire x Yorkshire population was 0.17 for PPARA, and 0.73 for PYY.

Some significant associations were found between the genotypes of candidate genes and meat quality phenotypes (Table 3 and 4).

There were significant effects of PPARA genotypes on birth weight and average drip loss ($p<0.05$), and loin eye area ($p<0.1$). Animals 11 had higher birth weight and loin eye area, and smaller drip loss. Also, a low significance was observed for cholesterol, hormel loin Hunter, average lactate, and average glycolytic potential ($p<0.20$). Animals 22 had higher cholesterol level and hormel loin Hunter while genotypes 11 had the lowest amount of average glycolytic potential and average lactate.

The PYY genotypes showed significant effects on marbling ($p<0.01$), lumbar back fat, water holding capacity ($p<0.05$), and average back fat, firmness, and average glycolytic potential ($p<0.1$). Animals 22 had more marbling, lumbar back fat, average back fat, firmness, and average glycolytic potential, while genotypes 11 had the smallest amount of water holding capacity. The total lipid, fiber type II ratio, ham Hunter, ham Minolta, flavor score and average glycogen, and average lactate were nearly significant ($p<0.2$). Animals 22 had higher total lipid, fiber type II ratio, average glycogen, and average glycolytic potential, and smaller ham Hunter and ham Minolta, while genotypes 11 had the smallest flavor score.

Table 3 - Association results of PPARA gene with phenotypes in a Berkshire x Yorkshire pig population. The number of animals is in parenthesis.

TRAIT	PPARA Genotype			
	11	**12**	**22**	**Pr > F**
Average back fat (cm) (AVBFAT)	3.19±0.20 (10)	3.30±0.06 (156)	3.25±0.05 (339)	0.6796
Carcass weight (kg) (CARCWT)	87.15±0.88 (10)	87.39±0.23 (156)	87.05±0.18 (339)	0.3734
Last rib back fat (cm) (LASTRIB)	3.27±0.21 (10)	3.17±0.06 (156)	3.13±0.05 (339)	0.6853
Loin eye area (cm^2) (LEA)	**37.24±1.79 (10)**	**36.66±0.65 (156)**	**35.61±0.57 (339)**	**0.0959**
Carcass Length (cm)(LENGTH)	83.72±0.79 (10)	84.06±0.20 (156)	84.19±0.15 (339)	0.7226
Lumbar back fat (cm) (LUMBAR)	3.42±0.25 (10)	3.59±0.07 (156)	3.52±0.06 (339)	0.5780
Tenth rib back fat (cm) (TENTHRIB)	2.93±0.24 (10)	3.11±0.07 (156)	3.10±3.10 (339)	0.7722
Average daily gain to weaning (kg) (ADGWT)	0.20±0.02 (10)	0.23±0.01 (156)	0.24±0.01 (338)	0.4287
Average daily gain on test (kg) (AGDTEST)	0.66±0.02 (10)	0.68±0.01 (156)	0.69±0.01 (338)	0.2033
Birth weight (kg) (BIRTHWT)	**1.65±0.11 (10)**	**1.48±0.04 (156)**	**1.55±0.04 (338)**	**0.0416**
16 day weight (kg) (SIXTHEWT)	4.47±0.46 (10)	4.87±0.16 (156)	4.98±0.14 (338)	0.4524
Cholesterol (mg/100g) (CHOLES)	**53.91±2.71 (10)**	**58.16±0.68 (156)**	**58.90±0.52 (339)**	**0.1353**
Marbling (MARB)	3.37±0.25 (10)	3.67±0.07 (156)	3.68±0.06 (339)	0.4670
Total lipid (%) (TOTLIPPR)	3.30±0.44 (10)	3.11±0.16 (156)	3.09±0.14 (339)	0.8797
Average drip loss (%) (AVDRIPPR)	**4.18±0.70 (10)**	**6.01±0.18 (156)**	**5.68±0.14 (338)**	**0.0161**
Color (COLOR)	3.49±0.18 (10)	3.27±0.04 (156)	3.23±0.03 (338)	0.3165
Firmness (FIRM)	3.56±0.23 (10)	3.44±0.05 (153)	3.36±0.04 (334)	0.3534
Fiber type I (FTYPI)	0.11±0.05 (10)	0.07±0.01 (153)	0.08±0.01 (334)	0.8046
Fiber type II ratio (FTYPIIR)	0.57±0.30 (10)	1.03±0.07(153)	1.03±0.05 (334)	0.3184
Ham Hunter (HAMH)	41.08±0.22 (10)	41.24±0.29 (156)	41.74±0.21 (338)	0.2991
Ham Minolta (HAMM)	16.87±1.03 (10)	17.15±0.24 (156)	17.55±0.18 (338)	0.3211
Ham pH (HAMPH)	5.88±0.07 (10)	5.89±0.01 (156)	5.89±0.01 (338)	0.9722
Hormel loin Hunter (HORMLH)	**42.15±1.81 (10)**	**44.39±0.61(156)**	**45.03±0.53 (338)**	**0.1622**
Hormel loin Minolta (HORMLM)	20.96±1.65 (10)	21.65±0.46 (156)	21.02±0.37 (338)	0.3850
Hormel loin pH (HORMLPH)	5.78±0.06 (10)	5.77±0.01 (156)	5.76±0.01 (338)	0.8519
Lab Loin Hunter (LABLH)	44.96±1.26 (10)	46.93±0.33 (156)	46.98±0.25 (338)	0.2763
Lab loin Minolta (LABLM)	20.23±1.20 (10)	22.10±0.31(156)	22.05±0.23 (338)	0.3049
Lab loin pH (LABLPH)	5.89±0.06 (10)	5.82±0.01 (156)	5.81±0.01 (338)	0.5422
Water holding capacity (g) (WHC)	0.16±0.04 (10)	0.20±0.01 (156)	0.19±0.01 (338)	0.6141
Average Instron force (kg) (AVINSFOR)	4.49±0.30 (10)	4.38±0.08 (153)	4.51±0.06 (334)	0.3077
Chew score (CHEWSCR)	2.74±0.35 (10)	2.52±0.09 (153)	2.41±0.06 (334)	0.3958
Flavor score (FLAVSCR)	2.45±0.60 (10)	2.70±0.14 (153)	2.89±0.10 (334)	0.4407
Juiciness score (JUICSCR)	6.15±0.53 (10)	6.03±0.12 (153)	6.04±0.08 (334)	0.9728
Off Flavor score (OFFLAVSC)	1.72±0.70 (10)	1.48±0.17 (153)	1.46±0.12 (334)	0.9343
Percent cooking loss (%) (PCCOKLOS)	18.02±1.55 (10)	18.72±0.35 (153)	18.70±0.24 (334)	0.9047
Tenderness score (TENDSCR)	7.55±0.46 (10)	7.78±0.11 (153)	7.85±0.08 (334)	0.7122
Average glycogen (μmol/g) (AVGGG)	7.64±1.19 (10)	8.99±0.27 (155)	8.75±0.19 (335)	0.4569
Average glycolytic potential (μmol/g) (AVGP)	98.60±6.12 (10)	106.75±1.51 (155)	104.23±1.10 (335)	0.1772
Average lactate (mmol/g) (AVLAC)	**83.22±4.76 (10)**	**88.89±1.16 (155)**	**86.68±0.84 (335)**	**0.1528**

Table 4 - Association results of PYY gene with phenotypes in a Berkshire x Yorkshire pig population. The number of animals is in parenthesis.

TRAIT	PYY Genotype			
	11	12	22	Pr > F
Average back fat (cm) (AVBFAT)	**3.23±0.05 (254)**	**3.30±0.06 (206)**	**3.41±0.09 (43)**	**0.0878**
Carcass weight (kg) (CARCWT)	87.10±0.19 (254)	87.27±0.20 (206)	87.18±0.38 (43)	0.7381
Last rib back fat (cm) (LASTRIB)	3.12±0.05 (254)	3.18±0.05 (206)	3.24±0.09 (43)	0.2848
Loin eye area (cm^2) (LEA)	36.00±0.59 (254)	36.01±0.62 (206)	35.25±0.89 (43)	0.5868
Carcass Length (cm) (LENGTH)	84.13±0.16 (254)	84.20±0.17 (206)	84.01±0.34 (43)	0.8427
Lumbar back fat (cm) (LUMBAR)	**3.49±0.06 (254)**	**3.60±0.07 (206)**	**3.77±0.11 (43)**	**0.0181**
Tenth rib back fat (cm) (TENTHRIB)	3.07±0.07 (254)	3.13±0.07 (206)	3.22±0.11 (43)	0.3392
Average daily gain to weaning (kg) (ADGWT)	0.24±0.01 (254)	0.23±0.01 (205)	0.22±0.01 (43)	0.4086
Average daily gain on test (kg) (AGDTEST)	0.69±0.01 (254)	0.69±0.01 (205)	0.68±0.01 (43)	0.2756
Birth weight (kg) (BIRTHWT)	1.54±0.04 (254)	1.54±0.04 (205)	1.48±0.05 (43)	0.3849
16 day weight (kg) (SIXTHEWT)	5.00±0.14 (254)	4.90±0.14 (205)	4.70±0.22 (43)	0.2939
Cholesterol (mg/100g) (CHOLES)	58.97±0.53 (254)	58.32±0.59 (206)	58.07±1.17 (43)	0.5366
Marbling (MARB)	**3.70±0.07 (254)**	**3.61±0.07 (206)**	**3.96±0.12 (43)**	**0.0064**
Total lipid (%) (TOTLIPPR)	**3.12±0.15 (254)**	**3.02±0.16 (206)**	**3.41±0.23 (43)**	**0.1239**
Average drip loss (%) (AVDRIPPR)	5.74±0.15 (254)	5.83±0.16 (205)	5.84±0.31 (43)	0.8359
Color (COLOR)	3.23±0.03 (254)	3.28±0.04 (205)	3.28±0.08 (43)	0.4528
Firmness (FIRM)	**3.41±0.05 (254)**	**3.33±0.05 (203)**	**3.55±0.10 (43)**	**0.0728**
Fiber type I (FTYPI)	0.082±0.01 (250)	0.08±0.01 (203)	0.08±0.02 (42)	0.9842
Fiber type II ratio (FTYPIIR)	**1.05±0.06 (250)**	**0.97±0.06 (203)**	**1.20±0.13 (42)**	**0.1872**
Ham Hunter (HAMH)	**41.85±0.23 (254)**	**41.39±0.26 (205)**	**40.90±0.52 (43)**	**0.1172**
Ham Minolta (HAMM)	17.62±0.20 (254)	17.28±0.22 (205)	16.88±0.44 (43)	0.1695
Ham pH (HAMPH)	5.89±0.01 (254)	5.882±0.01 (205)	5.93±0.03 (43)	0.4454
Hormel loin Hunter (HORMLH)	44.98±0.55 (254)	44.77±0.57 (205)	43.82±0.87 (43)	0.3298
Hormel loin Minolta (HORMLM)	21.30±0.39 (254)	21.032±0.42 (205)	21.712±0.74 (43)	0.5780
Hormel loin pH (HORMLPH)	5.77±0.01 (254)	5.75±0.01 (205)	5.79±0.03 (43)	0.3680
Lab Loin Hunter (LABLH)	46.92±0.27 (254)	46.94±0.29 (205)	47.30±0.55 (43)	0.7894
Lab loin Minolta (LABLM)	21.99±0.25 (254)	22.07±0.27 (205)	22.54±0.52 (43)	0.5945
Lab loin pH (LABLPH)	5.82±0.01(254)	5.82±0.01 (205)	5.82±0.03 (43)	0.9477
Water holding capacity (g) (WHC)	**0.19±0.01 (254)**	**0.22±0.01 (204)**	**0.21±0.02 (43)**	**0.0160**
Average Instron force (kg) (AVINSFOR)	4.50±0.07 (250)	4.45±0.07 (203)	4.44±0.14 (42)	0.7612
Chew score (CHEWSCR)	2.44±0.07 (250)	2.46±0.08 (203)	2.54±0.15 (42)	0.8126
Flavor score (FLAVSCR)	2.94±0.11 (250)	2.66±0.13 (203)	2.81±0.26 (42)	0.1787
Juiciness score (JUICSCR)	6.03±0.09 (250)	6.10±0.106 (203)	5.93±0.22 (42)	0.7380
Off Flavor score (OFFLAVSC)	1.57±0.13 (250)	1.32±0.15 (203)	1.55±0.30 (42)	0.3204
Percent cooking loss (%) (PCCOKLOS)	19.01±0.27 (250)	18.40±0.31 (203)	18.21±0.66 (42)	0.2239
Tenderness score (TENDSCR)	7.83±0.09 (250)	7.84±0.10 (203)	7.72±0.20 (42)	0.8332
Average glycogen (mmol/g) (AVGGG)	**8.63±0.21** (253)	**8.89±0.24 (204)**	**9.75±0.52 (41)**	**0.1146**
Average glycolytic potential (mmol/g) (AVGP)	**103.77±1.20 (253)**	**105.94±1.35 (204)**	**109.07±2.67 (41)**	**0.0985**
Average lactate (µmol/g) (AVLAC)	86.50±0.94 (253)	88.13±1.04 (204)	89.65±2.08 (41)	0.1967

5.2.2. High growth genes

Frequencies of allele 1 in the Berkshire and Yorkshire grandparents were 0.75 and 0.67 for CRADD, 0.75 and 0.33 for S0CS2, and 1.00 and 0.78 for PLXNC1. The frequency of allele 1 in the total F2 Berkshire x Yorkshire population was 0.75 for CRADD, 0.58 for S0CS2, and 0.89 for PLXNC1.

Many significant associations were found between the genotype of candidate genes and meat quality phenotypes in the Berkshire x Yorkshire family (Table 5, 6 and 7).

There were significant effects of CRADD genotypes on total lipid (p<0.001), average daily gain on test and fiber type I (p<0.05), and lumbar back fat, flavor score, and marbling (p<0.10). Animals 11 had smaller total lipid, fiber type I, lumbar back fat, and marbling. Animals with genotypes 22 had smaller average daily gain on test and flavor score. The average back fat, last rib back fat, average instron force, and off flavor score were also nearly significant (p<0.20). Animals 11 had smaller average back fat and last rib back fat, and genotypes 22 had highest average instron force, and off flavor score.

Association analyses with SOCS2 genotypes showed significant effects on 16 day test (p<0.01), average daily gain to weaning, fiber type II ratio (p<0.05) and average glycogen (p<0.1). Animals 22 had higher 16 day weight and daily gain to weaning, and smaller fiber type II and average glycogen. The average back fat and carcass weight showed nearly significant effects (p<0.20). Animals 22 had smaller average back fat and carcass weight.

The PLXNC1 genotypes showed significant effects on average daily gain on test and hormel loin Minolta (p<0.01), and loin eye area (p<0.05). Animals 22 had higher daily gain on test and loin eye area, and smaller index of Hormel loin

Minolta. Also, nearly significant effects was found for ham pH and hormel loin Hunter ($p<0.20$). Animals 22 had higher amount of hormel loin Hunter and smaller ham pH.

An analysis including all three genes as fixed effects (Table 8, 9 and 10) revealed significant effects for CRADD genotypes on total lipid ($p<0.001$), average daily gain on test, marbling and average instron force ($p<0.1$). Animals 11 had smaller total lipid and marbling, while animals with genotypes 22 had smaller average daily on test and average instron force. Also nearly significant effects for color, flavor score and off flavor score ($p<0.20$). Animals 22 had higher score of color and off flavor score, and smaller flavor score.

The SOCS2 genotypes showed significant effects on 16 day test, average daily gain to weaning and average glycogen ($p<0.05$), color and carcass weight ($p<0.1$). Animals 22 had higher 16 day weight, average daily gain to weaning, and lower average glycolytic, carcass weight and color. Also, nearly significant effects for fiber type II ratio, chew score and tenderness score ($p<0.20$). Animals 11 had higher chew score and fiber type II ratio and smaller tenderness score.

For PLXNC1 genotypes revealed significant effects on average daily gain on test and hormel loin Minolta ($p<0.01$), loin eye area and ham pH ($p<0.1$) with animals 12 showing smaller average daily gain, animals 11 higher color (hormel loin Minolta), and animals 22 with higher loin eye area and ham pH. Also, hormel loin Hunter and chew score ($p<0.2$). Animals 22 had higher hormel loin Hunter and smaller chew score.

Table 5 - Association results of CRADD gene with phenotypes in a Berkshire x Yorkshire pig population. The number of animals is in parenthesis.

TRAIT	**CRADD Genotype**			
	11	**12**	**22**	**Pr > F**
Average back fat (cm) (AVBFAT)	**3.22±0.05 (270)**	**3.34±0.06 (228)**	**3.37±0.22 (7)**	**0.1840**
Carcass weight (kg) (CARCWT)	87.06±0.20 (270)	87.32±0.22 (228)	87.12±0.93 (7)	0.6107
Last rib back fat (cm) (LASTRIB)	**3.11±0.05 (270)**	**3.20±0.05 (228)**	**3.47±0.22 (7)**	**0.1801**
Loin eye area (cm^2) (LEA)	35.86±0.62 (270)	36.14±0.65 (228)	33.22±1.96 (7)	0.2903
Carcass Length (cm) (LENGTH)	84.21±0.17 (270)	84.09±0.18 (228)	83.79±0.82 (7)	0.7952
Lumbar back fat (cm) (LUMBAR)	**3.47±0.06 (270)**	**3.66±0.07 (228)**	**3.55±0.27 (7)**	**0.0635**
Tenth rib back fat (cm) (TENTHRIB)	3.06±0.07 (270)	3.17±0.07 (228)	3.09±0.26 (7)	0.3986
Average daily gain to weaning (kg) (ADGWT)	0.24±0.01(271)	0.23±0.01 (228)	0.24±0.03 (7)	0.9869
Average daily gain on test (kg) (AGDTEST)	**0.68±0.01 (271)**	**0.70±0.01 (228)**	**0.670±0.03 (7)**	**0.0318**
Birth weight (kg) (BIRTHWT)	1.55±0.03 (271)	1.51±0.04 (228)	1.53±0.12 (7)	0.6373
16 day weight (kg) (SIXTHEWT)	4.97±0.15 (271)	4.93±0.16 (228)	4.97±0.50 (7)	0.9677
Cholesterol (mg/100g) (CHOLES)	58.26±0.56 (270)	59.20±0.62 (228)	57.00±2.81 (7)	0.3861
Marbling (MARB)	**3.60±0.07 (270)**	**3.79±0.07 (228)**	**3.86±0.27 (7)**	**0.0707**
Total lipid (%) (TOTLIPPR)	**2.84±0.16 (270)**	**3.45±0.17 (228)**	**3.28±0.49 (7)**	**0.0006**
Average drip loss (%) (AVDRIPPR)	5.79±0.15 (271)	5.78±0.17 (228)	5.70±0.74 (7)	0.9937
Color (COLOR)	3.27±0.03 (271)	3.22±0.04 (228)	3.51±0.19 (7)	0.2270
Firmness (FIRM)	3.37±0.05 (271)	3.42±0.05 (227)	3.29±0.24 (7)	0.6523
Fiber type I (FTYPI)	**0.07±0.01 (264)**	**0.10±0.01 (227)**	**0.16±0.05 (7)**	**0.0275**
Fiber type II ratio (FTYPIIR)	0.99±0.06 (264)	1.08±0.06 (227)	0.95±0.31 (7)	0.6062
Ham Hunter (HAMH)	41.58±0.23 (271)	41.65±0.26 (228)	40.05±1.2 (7)	0.4516
Ham Minolta (HAMM)	17.43±0.20 (271)	17.46±0.22 (228)	16.07±1.1 (7)	0.4273
Ham pH (HAMPH)	5.90±0.01 (271)	5.89±0.01 (228)	5.85±0.07 (7)	0.7798
Hormel loin Hunter (HORMLH)	44.74±0.59 (271)	44.92±0.63 (228)	43.16±1.99 (7)	0.6454
Hormel loin Minolta (HORMLM)	21.08±0.42 (271)	21.42±0.46 (228)	19.93±1.77 (7)	0.5933
Hormel loin pH (HORMLPH)	5.78±0.01 (271)	5.75±0.01 (228)	5.74±0.06 (7)	0.2804
Lab Loin Hunter (LABLH)	46.77±0.28 (271)	47.21±0.31 (228)	45.78±1.33 (7)	0.3287
Lab loin Minolta (LABLM)	21.87±0.27 (271)	22.31±0.30 (228)	20.86±1.27 (7)	0.2950
Lab loin pH (LABLPH)	5.83±0.01 (271)	5.81±0.01 (228)	5.76±0.06 (7)	0.3630
Water holding capacity (g) (WHC)	0.20±0.01 (271)	0.20±0.01 (228)	0.14±0.05 (7)	0.4313
Average Instron force (kg) (AVINSFOR)	**4.47±0.07 (251)**	**4.47±0.07 (214)**	**5.16±0.34 (6)**	**0.1280**
Chew score (CHEWSCR)	2.45±0.07 (270)	2.43±0.08 (218)	2.86±0.37 (7)	0.5185
Flavor score (FLAVSCR)	**2.68±0.11 (270)**	**3.04±0.13** (218)	**2.50±0.62 (7)**	**0.0698**
Juiciness score (JUICSCR)	6.02±0.09 (270)	6.08±0.11 (218)	6.08±0.53 (7)	0.9183
Off Flavor score (OFFLAVSC)	**1.61±0.14 (270)**	**1.28±0.16** (218)	**2.17±0.73 (7)**	**0.1513**
Percent cooking loss (%) (PCCOKLOS)	18.75±0.26 (270)	18.66±0.31 (218)	19.09±1.56 (7)	0.9475
Tenderness score (TENDSCR)	7.85±0.09 (270)	7.82±0.10 (218)	7.39±0.47 (7)	0.6417
Average glycogen (μmol/g) (AVGGG)	8.85±0.21 (268)	8.77±0.24 (225)	8.05±1.39 (7)	0.8320
Average glycolytic potential (μmol/g) (AVGP)	104.94±1.26 (268)	105.00±1.42 (225)	102.13±7.27 (7)	0.9254
Average lactate (μmol/g) (AVLAC)	87.17±0.96 (268)	87.54±1.09 (225)	86.27±5.64(7)	0.9447

Table 6 - Association results of SOCS2 gene with phenotypes in a Berkshire x Yorkshire pig population. The number of animals is in parenthesis.

	SOCS2 genotype			
TRAIT	**11**	**12**	**22**	**Pr > F**
Average back fat (cm) (AVBFAT)	**3.31±0.06 (161)**	**3.28±0.06 (255)**	**3.16±0.08 (10)**	**0.1930**
Carcass weight (kg) (CARCWT)	**86.99±0.23 (161)**	**87.35±0.19 (255)**	**86.89±0.29 (10)**	**0.1533**
Last rib back fat (cm) (LASTRIB)	3.19±0.06 (161)	3.16±0.05 (255)	3.05±0.07 (10)	0.2663
Loin eye area (cm^2) (LEA)	35.67±0.64 (161)	35.90±0.59 (255)	36.59±0.73 (10)	0.4089
Carcass Length (cm) (LENGTH)	84.26±0.20 (161)	84.10±0.16 (255)	84.17±0.25 (10)	0.7458
Lumbar back fat (cm) (LUMBAR)	3.60±0.08 (161)	3.54±0.07 (255)	3.43±0.09 (10)	0.2309
Tenth rib back fat (cm) (TENTHRIB)	3.13±0.08 (161)	3.12±0.07 (255)	2.99±0.09 (10)	0.3000
Average daily gain to weaning (kg) (ADGWT)	**0.22±0.01 (162)**	**0.24±0.01 (255)**	**0.25±0.01 (10)**	**0.0109**
Average daily gain on test (kg) (AGDTEST)	0.69±0.01 (162)	0.68±0.01 (255)	0.69±0.01 (10)	0.2562
Birth weight (kg) (BIRTHWT)	1.51±0.04 (162)	1.55±0.04 (255)	1.56±0.05 (10)	0.3419
16 day weight (kg) (SIXTHEWT)	**4.69±0.16 (162)**	**4.99±0.15 (255)**	**5.26±0.19 (10)**	**0.0084**
Cholesterol (mg/100g) (CHOLES)	58.87±0.69 (161)	58.47±0.56 (255)	58.53±0.87 (10)	0.8660
Marbling (MARB)	3.67±0.08 (161)	3.68±0.07 (255)	3.70±0.09 (10)	0.9572
Total lipid (%) (TOTLIPPR)	3.08±0.16 (161)	3.10±0.15 (255)	3.10±0.18 (10)	0.9958
Average drip loss (%) (AVDRIPPR)	5.83±0.19 (162)	5.87±0.15 (255)	5.51±0.23 (10)	0.3434
Color (COLOR)	3.30±0.04 (162)	3.25±0.03 (255)	3.19±0.06 (10)	0.3059
Firmness (FIRM)	3.40±0.06 (160)	3.40±0.05 (255)	3.36±0.07 (10)	0.8514
Fiber type I (FTYPI)	0.09±0.01 (160)	0.08±0.01 (250)	0.07±0.02 (9)	0.5785
Fiber type II ratio (FTYPIIR)	**1.14±0.07 (160)**	**1.04±0.06 (250)**	**0.84± 0.10 (9)**	**0.0405**
Ham Hunter (HAMH)	41.57±0.30 (162)	41.63±0.24 (255)	41.51±0.38 (10)	0.9545
Ham Minolta (HAMM)	17.39±0.25 (162)	17.48± 0.20 (255)	17.31±0.32 (10)	0.8671
Ham pH (HAMPH)	5.90±0.02 (162)	5.88±0.01 (255)	5.92±0.02 (10)	0.3829
Hormel loin Hunter (HORMLH)	44.78±0.62 (162)	44.86±0.57 (255)	44.74±0.72 (10)	0.9752
Hormel loin Minolta (HORMLM)	21.24±0.47 (162)	21.32± 0.40 (255)	21.12±0.57 (10)	0.9371
Hormel loin pH (HORMLPH)	5.76±0.02 (162)	5.76±0.01 (255)	5.79±0.02 (10)	0.4443
Lab Loin Hunter (LABLH)	46.86±0.34 (162)	47.04±0.28 (255)	46.91±0.42 (10)	0.8702
Lab loin Minolta (LABLM)	21.99±0.32 (162)	22.18±0.26 (255)	21.86±0.40 (10)	0.6934
Lab loin pH (LABLPH)	5.82±0.02 (162)	5.82±0.01 (255)	5.83±0.02 (10)	0.9330
Water holding capacity (g) (WHC)	0.20±0.01 (162)	0.21±0.01 (255)	0.19±0.01 (10)	0.7403
Average Instron force (kg) (AVINSFOR)	4.46±0.08 (150)	4.49±0.07 (255)	4.48±0.10 (9)	0.9345
Chew score (CHEWSCR)	2.53±0.09 (154)	2.46±0.08 (253)	2.34±0.11 (10)	0.3977
Flavor score (FLAVSCR)	2.94±0.15 (154)	2.74±0.11 (253)	2.76±0.19 (10)	0.4750
Juiciness score (JUICSCR)	6.03±0.13 (154)	6.01±0.09 (253)	6.14±0.16 (10)	0.7979
Off Flavor score (OFFLAVSC)	1.47±0.18 (154)	1.55±0.14 (253)	1.35±0.22 (10)	0.6772
Percent cooking loss (%) (PCCOKLOS)	18.81±0.36 (154)	18.78±0.28 (253)	18.34±0.46 (10)	0.6762
Tenderness score (TENDSCR)	7.72±0.12 (154)	7.81±0.09 (253)	8.01±0.15 (10)	0.2810
Average glycogen (mmol/g) (AVGGG)	**8.67±0.28 (161)**	**9.13±0.21 (251)**	**8.21±0.36 (10)**	**0.0564**
Average glycolytic potential (μmol/g) (AVGP)	105.70±1.58 (161)	105.21±1.27 (251)	103.57±1.99 (10)	0.6676
Average lactate (μmol/g) (AVLAC)	88.32±1.23 (161)	86.90±0.99 (251)	87.22±1.55 (10)	0.5769

Table 7 - Association results of PLXNC1 gene with phenotypes in a Berkshire x Yorkshire pig population. The number of animals is in parenthesis.

	PLXNC1 genotype			
TRAIT	**11**	**12**	**22**	**Pr > F**
Average back fat (cm) (AVBFAT)	3.29±0.05 (396)	3.20±0.07 (95)	3.14±0.19 (9)	0.3669
Carcass weight (kg) (CARCWT)	87.13±0.17 (396)	87.38±0.28 (95)	86.97±0.79 (9)	0.6619
Last rib back fat (cm) (LASTRIB)	3.17±0.05 (396)	3.06±0.07 (95)	3.17±0.19 (9)	0.2600
Loin eye area (cm^2) (LEA)	**35.70±0.55 (396)**	**36.81±0.71 (95)**	**39.40±1.61 (9)**	**0.0246**
Carcass Length (cm) (LENGTH)	84.20±0.15 (396)	84.01±0.25(95)	84.13±0.71(9)	0.7783
Lumbar back fat (cm) (LUMBAR)	3.57±0.06 (396)	3.49±0.09 (95)	3.38±0.23 (9)	0.4992
Tenth rib back fat (cm) (TENTHRIB)	3.13±0.06 (396)	3.04±0.09(95)	2.86±0.22 (9)	0.3465
Average daily gain to weaning (kg) (ADGWT)	0.23±0.01 (398)	0.24±0.01 (94)	0.24±0.02 (9)	0.5893
Average daily gain on test (kg) (AGDTEST)	**0.69±0.01 (398)**	**0.66±0.01 (94)**	**0.70±0.02 (9)**	**0.0018**
Birth weight (kg) (BIRTHWT)	1.54±0.04 (398)	1.53±0.05 (94)	1.58±0.10 (9)	0.8845
16 day weight (kg) (SIXTHEWT)	4.91±0.14 (398)	5.06±0.18 (94)	4.98±0.43 (9)	0.6188
Cholesterol (mg/100g) (CHOLES)	58.77±0.48 (396)	58.38±0.83 (95)	58.69±2.40 (9)	0.9068
Marbling (MARB)	3.70±0.07 (396)	3.62±0.09 (95)	3.64±0.24 (9)	0.6440
Total lipid (%) (TOTLIPPR)	3.07±0.15 (396)	3.24±0.19 (95)	3.24±0.42 (9)	0.5139
Average drip loss (%) (AVDRIPPR)	5.77±0.14 (398)	5.77±0.22 (94)	6.29±0.63 (9)	0.7125
Color (COLOR)	3.26±0.03 (398)	3.19±0.05 (94)	3.36±0.16 (9)	0.3686
Firmness (FIRM)	3.39±0.04 (398)	3.38±0.07 (94)	3.58±0.21 (9)	0.6286
Fiber type I (FTYPI)	0.08±0.01 (390)	0.07±0.02 (94)	0.10±0.05 (9)	0.6875
Fiber type II ratio (FTYPIIR)	1.05±0.05 (390)	0.94±0.09 (94)	0.91±0.27 (9)	0.5267
Ham Hunter (HAMH)	41.71± 0.21(398)	41.31±0.37(94)	40.23±1.10 (9)	0.3132
Ham Minolta (HAMM)	17.52±0.18 (398)	17.18±0.31 (94)	16.37±0.93 (9)	0.3427
Ham pH (HAMPH)	**5.90±0.01 (398)**	**5.86±0.02 (94)**	**5.85±0.07 (9)**	**0.1958**
Hormel loin Hunter (HORMLH)	**44.84±0.55 (398)**	**44.51±0.71 (94)**	**47.63±1.66 (9)**	**0.1432**
Hormel loin Minolta (HORMLM)	**21.36±0.38 (398)**	**21.06± 0.56 (94)**	**16.14±1.49 (9)**	**0.0023**
Hormel loin pH (HORMLPH)	5.76±0.01 (398)	5.76±0.02 (94)	5.81±0.06 (9)	0.6762
Lab Loin Hunter (LABLH)	46.98±0.25 (398)	47.01±0.40 (94)	45.71±1.14 (9)	0.5170
Lab loin Minolta (LABLM)	22.08±0.24 (398)	22.11±0.38 (94)	20.92±1.09 (9)	0.5523
Lab loin pH (LABLPH)	5.82±0.013 (398)	5.80±0.02 (94)	5.81±0.06 (9)	0.6365
Water holding capacity (g) (WHC)	0.20±0.01 (398)	0.19±0.01 (94)	0.22±0.04 (9)	0.6767
Average Instron force (kg) (AVINSFOR)	4.46±0.06 (374)	4.58±0.10 (82)	4.38±0.27 (9)	0.4660
Chew score (CHEWSCR)	2.42±0.07 (386)	2.58±0.11 (95)	2.22±0.32 (9)	0.2864
Flavor score (FLAVSCR)	2.89±0.11 (386)	2.67±0.18 (95)	2.20±0.55 (9)	0.3136
Juiciness score (JUICSCR)	6.06±0.09 (386)	5.89±0.16 (95)	6.69±0.48 (9)	0.2142
Off Flavor score (OFFLAVSC)	1.44±0.13 (386)	1.53±0.21 (95)	2.00±0.63 (9)	0.6641
Percent cooking loss (%) (PCCOKLOS)	18.79±0.24 (386)	18.64±0.45 (95)	18.14±1.39 (9)	0.8798
Tenderness score (TENDSCR)	7.85±0.08 (386)	7.72±0.14 (95)	8.12±0.41 (9)	0.4942
Average glycogen (μmol/g) (AVGGG)	8.76±0.19 (391)	8.98±0.35 (95)	9.23±1.07 (9)	0.8076
Average glycolytic potential (μmol/g) (AVGP)	104.60±1.12 (391)	105.93±1.89 (95)	111.81±5.55 (9)	0.4015
Average lactate (μmol/g) (AVLAC)	87.11±0.85 (391)	87.89±1.47 (95)	93.02±4.32 (9)	0.3893

Table 8 - Association results of CRADD gene with phenotypes in a Berkshire x Yorkshire pig including the three genes in the model.

TRAIT	**CRADD Genotype**			
	11	**12**	**22**	**Pr > F**
Average back fat (cm) (AVBFAT)	3.18 ±0.08	3.28 ± 0.09	3.31 ± 0.24	0.3827
Carcass weight (kg) (CARCWT)	86.99 ± 0.33	87.43 ± 0.36	87.39 ± 1.03	0.3196
Last rib back fat (cm) (LASTRIB)	3.12 ± 0.08	3.18 ± 0.09	3.50 ± 0.25	0.2721
Loin eye area (cm^2) (LEA)	37.08 ± 0.80	37.49 ± 0.85	35.45 ± 2.12	0.5197
Carcass Length (cm) (LENGTH)	84.20 ± 0.29	83.95 ± 0.32	84.41 ± 0.90	0.5560
Lumbar back fat (cm) (LUMBAR)	3.43 ± 0.10	3.59 ± 0.11	3.57 ± 0.30	0.2035
Tenth rib back fat (cm) (TENTHRIB)	2.98 ± 0.10	3.09 ± 0.11	2.88 ± 0.29	0.4188
Average daily gain to weaning (kg) (ADGWT)	0.23 ± 0.01	0.24 ± 0.01	0.25 ± 0.03	0.5592
Average daily gain on test (kg) (AGDTEST)	**0.68 ± 0.01**	**0.70 ± 0.01**	**0.67 ± 0.02**	**0.0846**
Birth weight (kg) (BIRTHWT)	1.56 ± 0.05	1.53 ± 0.06	1.56 ± 0.14	0.7579
16 day weight (kg) (SIXTHEWT)	4.88 ± 0.21	5.03 ± 0.22	5.10 ± 0.56	0.6625
Cholesterol (mg/100g) (CHOLES)	58.26 ± 0.99	59.11 ± 1.09	56.79 ± 3.13	0.5190
Marbling (MARB)	**3.56 ± 0.10**	**3.78 ± 0.11**	**3.66 ± 0.31**	**0.0537**
Total lipid (%) (TOTLIPPR)	**2.93 ± 0.20**	**3.56 ± 0.22**	**3.31 ± 0.53**	**0.0009**
Average drip loss (%) (AVDRIPPR)	5.98 ± 0.27	5.98 ± 0.30	6.00 ± 0.83	0.9998
Color (COLOR)	**3.33 ± 0.07**	**3.22 ± 0.07**	**3.46 ± 0.21**	**0.1301**
Firmness (FIRM)	3.45 ± 0.09	3.47 ± 0.09	3.39 ± 0.27	0.9242
Fiber type I (FTYPI)	0.08 ± 0.02	0.10 ± 0.02	0.13 ± 0.06	0.2628
Fiber type II ratio (FTYPIIR)	0.99 ± 0.11	1.02 ± 0.12	1.05 ± 0.35	0.9395
Ham Hunter (HAMH)	41.01 ± 0.44	41.06 ± 0.49	39.78 ± 1.41	0.6437
Ham Minolta (HAMM)	16.98 ± 0.37	16.99 ± 0.41	15.87 ± 1.18	0.6165
Ham pH (HAMPH)	5.87 ± 0.03	5.85 ± 0.03	5.82 ± 0.09	0.5358
Hormel loin Hunter (HORMLH)	45.58 ± 0.81	45.96 ± 0.87	44.21 ± 2.19	0.6354
Hormel loin Minolta (HORMLM)	19.36 ± 0.65	19.72 ± 0.71	18.20 ± 1.95	0.6362
Hormel loin pH (HORMLPH)	5.79 ± 0.02	5.76 ± 0.02	5.77 ± 0.07	0.3190
Lab Loin Hunter (LABLH)	46.32 ± 0.49	46.90 ± 0.53	45.25 ± 1.50	0.2632
Lab loin Minolta (LABLM)	21.49 ± 0.46	22.03 ± 0.51	20.41 ± 1.43	0.2597
Lab loin pH (LABLPH)	5.82 ± 0.02	5.79 ± 0.03	5.74 ± 0.07	0.2095
Water holding capacity (g) (WHC)	0.20 ± 0.02	0.21 ± 0.02	0.13 ± 0.05	0.3689
Average Instron force (kg) (AVINSFOR)	**4.46 ± 0.12**	**4.50 ± 0.13**	**5.27 ± 0.39**	**0.0974**
Chew score (CHEWSCR)	2.47 ± 0.13	2.39 ± 0.15	3.06 ± 0.42	0.2355
Flavor score (FLAVSCR)	**2.51 ± 0.21**	**2.80 ± 0.24**	**1.69 ± 0.70**	**0.1150**
Juiciness score (JUICSCR)	6.15 ± 0.19	6.24 ± 0.21	6.02 ± 0.62	0.8238
Off Flavor score (OFFLAVSC)	**1.86 ± 0.26**	**1.47 ± 0.29**	**2.66 ± 0.81**	**0.1035**
Percent cooking loss (%) (PCCOKLOS)	18.66 ± 0.53	18.41 ± 0.61	19.04 ± 1.77	0.8349
Tenderness score (TENDSCR)	7.82 ± 0.17	7.86 ± 0.19	7.06 ± 0.54	0.3081
Average glycogen (μmol/g) (AVGGG)	8.97 ± 0.41	9.08 ± 0.47	8.06 ± 1.60	0.7875
Average glycolytic potential (μmol/g) (AVGP)	107.73 ± 2.27	107.84 ± 2.53	108.25 ± 8.36	0.9970
Average lactate (μmol/g) (AVLAC)	89.61 ± 1.77	89.66 ± 1.97	92.24 ± 6.53	0.9181

Table 9 - Association results of SOCS2 gene with phenotypes in a Berkshire x Yorkshire pig including the three genes in the model.

	SOCS2 genotype			
TRAIT	**11**	**12**	**22**	**Pr > F**
Average back fat (cm) (AVBFAT)	3.29 ± 0.12	3.29 ± 0.12	3.20 ± 0.12	0.4667
Carcass weight (kg) (CARCWT)	**87.15 ± 0.52**	**87.59 ± 0.47**	**87.08 ± 0.48**	**0.0969**
Last rib back fat (cm) (LASTRIB)	3.30 ± 0.12	3.30 ± 0.12	3.20 ± 0.12	0.4328
Loin eye area (cm^2) (LEA)	36.48 ± 1.11	36.70 ± 1.04	36.83 ± 1.06	0.8962
Carcass Length (cm) (LENGTH)	84.30 ± 0.46	84.07 ± 0.42	84.19 ± 0.43	0.6261
Lumbar back fat (cm) (LUMBAR)	3.56 ± 0.15	3.55 ± 0.14	3.47 ± 0.14	0.6403
Tenth rib back fat (cm) (TENTHRIB)	2.99 ± 0.15	3.02 ± 0.14	2.94 ± 0.14	0.5624
Average daily gain to weaning (kg) (ADGWT)	**0.22 ± 0.02**	**0.24 ± 0.02**	**0.26 ± 0.02**	**0.0158**
Average daily gain on test (kg) (AGDTEST)	0.68 ± 0.01	0.68 ± 0.01	0.69 ± 0.01	0.4724
Birth weight (kg) (BIRTHWT)	1.52 ± 0.07	1.56 ± 0.07	1.58 ± 0.07	0.4585
16 day weight (kg) (SIXTHEWT)	**4.69 ± 0.29**	**5.03 ± 0.27**	**5.29 ± 0.28**	**0.0125**
Cholesterol (mg/100g) (CHOLES)	57.77 ± 1.57	57.83 ± 1.44	58.56 ± 1.47	0.7558
Marbling (MARB)	3.57 ± 0.15	3.68 ± 0.14	3.75 ± 0.15	0.2718
Total lipid (%) (TOTLIPPR)	3.16 ± 0.28	3.32 ± 0.26	3.32 ± 0.27	0.4748
Average drip loss (%) (AVDRIPPR)	6.10 ± 0.42	6.11 ± 0.38	5.75 ± 0.39	0.3919
Color (COLOR)	**3.42 ± 0.11**	**3.35 ± 0.10**	**3.24 ± 0.10**	**0.0889**
Firmness (FIRM)	3.46 ± 0.14	3.46 ± 0.12	3.39 ± 0.13	0.7048
Fiber type I (FTYPI)	0.10 ±0.03	0.10 ± 0.03	0.10 ± 0.03	0.9661
Fiber type II ratio (FTYPIIR)	**1.14 ± 0.18**	**1.06 ± 0.16**	**0.87 ± 0.17**	**0.1342**
Ham Hunter (HAMH)	40.40 ± 0.71	40.61 ± 0.65	40.84 ± 0.66	0.7400
Ham Minolta (HAMM)	16.44 ± 0.59	16.64 ± 0.54	16.75 ± 0.55	0.7705
Ham pH (HAMPH)	5.84 ± 0.04	5.83 ± 0.04	5.87 ± 0.04	0.3043
Hormel loin Hunter (HORMLH)	45.17 ± 1.14	45.39 ± 1.07	45.19 ± 1.09	0.8959
Hormel loin Minolta (HORMLM)	18.86 ± 0.98	19.05 ± 0.91	19.37 ± 0.93	0.7963
Hormel loin pH (HORMLPH)	5.78 ± 0.04	5.77 ± 0.03	5.78 ± 0.03	0.8265
Lab Loin Hunter (LABLH)	45.88 ± 0.75	46.23 ± 0.69	46.37 ± 0.71	0.6406
Lab loin Minolta (LABLM)	21.12 ± 0.72	21.45 ± 0.66	21.36 ± 0.68	0.6848
Lab loin pH (LABLPH)	5.79 ± 0.04	5.78 ± 0.03	5.78 ± 0.04	0.9963
Water holding capacity (g) (WHC)	0.18 ± 0.03	0.19 ± 0.02	0.18 ± 0.03	0.7250
Average Instron force (kg) (AVINSFOR)	4.75 ± 0.19	4.74 ± 0.17	4.73 ± 0.18	0.9833
Chew score (CHEWSCR)	**2.81 ± 0.21**	**2.64 ± 0.19**	**2.47 ± 0.20**	**0.1182**
Flavor score (FLAVSCR)	2.30 ± 0.35	2.29 ± 0.32	2.42 ± 0.32	0.8397
Juiciness score (JUICSCR)	6.02 ± 0.31	6.11 ± 0.28	6.27 ± 0.29	0.6036
Off Flavor score (OFFLAVSC)	2.19 ± 0.41	2.09 ± 0.37	1.70 ± 0.38	0.2714
Percent cooking loss (%) (PCCOKLOS)	18.91 ± 0.90	18.81 ± 0.81	18.39 ± 0.82	0.7485
Tenderness score (TENDSCR)	7.38 ± 0.27 a	7.57 ± 0.25	7.79 ± 0.25 b	0.1484
Average glycogen (µmol/g) (AVGGG)	**8.72 ± 0.74**	**9.21 ± 0.68**	**8.18 ± 0.68**	**0.0437**
Average glycolytic potential (µmol/g) (AVGP)	109.21 ± 3.90	108.41 ± 3.60	106.21 ± 3.65	0.5614
Average lactate (µmol/g) (AVLAC)	91.63 ± 3.05	89.94 ± 2.82	89.93 ± 2.85	0.5405

Table 10 - Association results of PLXNC1 gene with phenotypes in a Berkshire x Yorkshire pig including the three genes in the model.

	PLXNC1 genotype			
TRAIT	**11**	**12**	**22**	**Pr > F**
Average back fat (cm) (AVBFAT)	3.30 ± 0.09	3.25 ± 0.11	3.24 ± 0.20	0.7956
Carcass weight (kg) (CARCWT)	87.11 ± 0.37	87.41 ± 0.44	87.30 ± 0.88	0.6445
Last rib back fat (cm) (LASTRIB)	3.26 ± 0.09	3.19 ± 0.11	3.34 ± 0.21	0.5078
Loin eye area (cm^2) (LEA)	**35.19 ± 0.85**	**36.11 ± 0.98**	**38.72 ± 1.76**	**0.0639**
Carcass Length (cm) (LENGTH)	84.33 ± 0.32	84.14 ± 0.39	84.10 ± 0.78	0.7953
Lumbar back fat (cm) (LUMBAR)	3.57 ± 0.11	3.53 ± 0.13	3.48 ± 0.24	0.8729
Tenth rib back fat (cm) (TENTHRIB)	3.05 ± 0.11	3.01 ± 0.13	2.88 ± 0.24	0.7141
Average daily gain to weaning (kg) (ADGWT)	0.24 ± 0.01	0.24 ± 0.01	0.24 ± 0.03	0.9730
Average daily gain on test (kg) (AGDTEST)	**0.69 ± 0.01**	**0.67 ± 0.01**	**0.69 ± 0.02**	**0.0066**
Birth weight (kg) (BIRTHWT)	1.54 ± 0.05	1.53 ± 0.06	1.58 ± 0.11	0.8835
16 day weight (kg) (SIXTHEWT)	5.03 ± 0.23	5.05 ± 0.26	4.93 ± 0.47	0.9519
Cholesterol (mg/100g) (CHOLES)	58.29 ± 1.10	57.82 ± 1.34	58.05 ± 2.68	0.8898
Marbling (MARB)	3.73 ± 0.11	3.62 ± 0.13	3.65 ± 0.26	0.5344
Total lipid (%) (TOTLIPPR)	3.12 ± 0.22	3.32 ± 0.25	3.35 ± 0.44	0.4289
Average drip loss (%) (AVDRIPPR)	5.70 ± 0.30	5.81 ± 0.36	6.45 ± 0.71	0.5369
Color (COLOR)	3.30 ± 0.07	3.26 ± 0.09	3.45 ± 0.19	0.4955
Firmness (FIRM)	3.36 ± 0.10	3.37 ± 0.12	3.59 ± 0.23	0.5697
Fiber type I (FTYPI)	0.10 ± 0.02	0.09 ± 0.02	0.12 ± 0.05	0.8477
Fiber type II ratio (FTYPIIR)	1.02 ± 0.12	1.00 ± 0.15	1.05 ± 0.30	0.9764
Ham Hunter (HAMH)	41.35 ± 0.49	40.83 ± 0.60	39.67 ± 1.22	0.2512
Ham Minolta (HAMM)	17.19 ± 0.41	16.75 ± 0.50	15.89 ± 1.02	0.2865
Ham pH (HAMPH)	**5.90 ± 0.03**	**5.84 ± 0.04**	**5.81 ± 0.08**	**0.0692**
Hormel loin Hunter (HORMLH)	**44.27 ± 0.88**	**44.11 ± 1.00**	**47.37 ± 1.83**	**0.1333**
Hormel loin Minolta (HORMLM)	**21.04 ± 0.72**	**20.69 ± 0.85**	**15.56 ± 1.64**	**0.0018**
Hormel loin pH (HORMLPH)	5.77 ± 0.02	5.76 ± 0.03	5.80 ± 0.06	0.7308
Lab Loin Hunter (LABLH)	46.58 ± 0.54	46.63 ± 0.64	45.27 ± 1.27	0.5063
Lab loin Minolta (LABLM)	21.62 ± 0.52	21.74 ± 0.61	20.57 ± 1.21	0.5693
Lab loin pH (LABLPH)	5.80 ± 0.03	5.78 ± 0.03	5.78 ± 0.06	0.5443
Water holding capacity (g) (WHC)	0.18 ± 0.02	0.17 ± 0.02	0.20 ± 0.05	0.6381
Average Instron force (kg) (AVINSFOR)	4.72 ± 0.14	4.85 ± 0.17	4.66 ± 0.31	0.4265
Chew score (CHEWSCR)	**2.58 ± 0.15**	**2.84 ± 0.18**	**2.50 ± 0.36**	**0.1098**
Flavor score (FLAVSCR)	2.60 ± 0.24	2.40 ± 0.30	2.00 ± 0.60	0.4849
Juiciness score (JUICSCR)	6.04 ± 0.21	5.81 ± 0.26	6.55 ± 0.54	0.2057
Off Flavor score (OFFLAVSC)	1.66 ± 0.29	1.86 ± 0.35	2.48 ± 0.69	0.4139
Percent cooking loss (%) (PCCOKLOS)	18.83 ± 0.61	18.83 ± 0.76	18.45 ± 1.55	0.9662
Tenderness score (TENDSCR)	7.65 ± 0.19	7.37 ± 0.23	7.71 ± 0.46	0.2045
Average glycogen (μmol/g) (AVGGG)	8.25 ± 0.54	8.56 ± 0.64	9.29 ± 1.22	0.5814
Average glycolytic potential (μmol/g) (AVGP)	104.30 ± 2.90	106.46 ± 3.38	113.07 ± 6.34	0.2786
Average lactate (μmol/g) (AVLAC)	87.88 ± 2.26	89.32 ± 2.64	94.31 ± 4.96	0.3387

6. DISCUSSION

Linkage analysis was used to localize the PPARA, CRADD, SOC2 and PLXNC1 genes to SSC5 and PYY to SSC12. The mapping results of this study are in accordance with the results obtained by chromosomal painting between human and pig (Goureau et al., 1996).

Gene frequencies for the PPARA, CRADD and PLXNC1 were not very different between the Berkshire and Yorkshire grand parents and the QTL analysis could have limited power to detect QTL for meat quality and growth.

Malek et al. (2001 a,b) reported several QTL for body composition and meat quality in the distal arm of SSC5 in the Berkshire and Yorkshire population that was used in this study. The analysis of the Berkshire and Yorkshire F2 population showed evidence of associations of PPARA with meat quality traits (average drip loss, loin eye area, cholesterol, hormel loin Hunter and average lactate). Bossé et al. (2003) associated PPARA with reduced adiposity and oxidation of fatty acid that can relate with cholesterol index. Thus, PPARA is an interesting candidate gene for meat quality in swine (Table 3). But many significances can be decreased because a low frequency of the allele 11 (0.17) in the population of study. More studies need be made in others populations.

The PYY analysis in Berkshire and Yorkshire F2 population showed a very high association with marbling and also with other meat quality traits such as lumbar back fat, water holding capacity, average back fat, firmness, average glycolytic potential, total lipid, fiber type II, ham Hunter, flavor score and average glycogen.

Marbling scores correspond with intramuscular lipid content. Higher lipid content is generally considered more desirable because it adds to flavor and cooking properties and improves tenderness. Batterham et al. (2002) showed that PYY is involved in the control of digestive functions and in rats inhibits food intake and reduces weight gain. The PYY gene in this study appeared to be a good candidate gene in swine for meat quality traits mainly in the control of marbling in the swine meat.

The high growth genes in swine demonstrated a few associations with growth traits. Also, Malek et al. (2001 a, b) detected several QTL for meat quality traits (24-hr loin pH, 24-hr loin Minolta, 48-hr loin Minolta) and body composition traits (average backfat, lumbar, last rib backfat) on chromosome 5 in the pig. These results make this chromosome attractive for a positional candidate gene approach to eventually find the causative gene(s) associated with these QTL.

The CRADD analysis reveled associations with average daily gain on test and lumbar back fat, and also with meat quality traits (fiber type I, marbling and flavor score) with a high association with total lipid. This high association with total lipid continued in the analysis including all three genes as fixed effects in the model. Felmer et al. (2003) also related CRADD gene with adipocytes in mouse. This result suggests that CRADD gene is an interesting candidate gene in swine not only for growth traits but also for meat quality traits.

The SOCS2 results showed associations with weight on sixteen-day test, average daily gain to weaning, carcass weight and another also with meat quality (fiber type II and average glycogen). SOCS-2 is an essential down regulator of growth and its absence leads high growth phenotype in mouse (Metcalf et al., 2000).

SOCS-2 also was related with glycogen index. During the first 6 to 24 hours post-mortem, glycogen reserves in the muscle are reduced, lactic acid builds up, and muscle metabolism stops (Lundberg and Vogel, 1986). Greater amounts of glycogen in the tissue at harvest provide the potential for sustained glycolysis in the muscle after slaughter, which could result in lower ultimate pH. Firmness and fiber type could also be considered part of pre cooked tissue quality. This significant association was also found in the analysis including the three genes suggesting that the SOCS2 gene also is a good candidate for growth and meat quality traits in swine.

The PLXNC1 showed association with average daily gain on test and also with loin eye area, traits important for growth, and also with hormel loin Minolta, hormel loin Hunter traits related with color in the meat and ham pH. Also this significant association was found in the analysis including the three genes showing that this association is a good one to be used in the swine selection. It should be noted that no possible causative mutations were found and more research is needed to determine which gene is responsible for the trait differences.

7. CONCLUSIONS

The association results reported in this study yield important information that can be used to aid in directing future studies aimed at elucidating the underlying biological mechanisms behind the development of many quality and body composition traits.

The identified PPARA, PYY, CRADD, SOCS2 and PLXNC1 polymorphisms may be used as markers in order to track these QTL and to discover the differences in DNA sequences responsible for the phenotypic variation. But more studies need to be made to fully evaluate and validate these results to see if these associations still exist in another populations.

Therefore further study is needed to identify and investigate candidate genes for other regions of the swine genome for growth, body composition and meat quality.

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9. APPENDIX

Table 1 - Means and standard deviations for traits of interest measured on F_2 animals.

Traits analyzed for QTL mapping	Mean	Std Dev
Birth weight (kg)	1.55	0.325
16-Day weight (kg)	4.95	1.311
Average daily gain to weaning (kg/day)	0.24	0.074
Average daily gain on test (kg/day)	0.69	0.065
Carcass weight (kg)	87.08	5.733
Carcass length (cm)	84.16	2.454
Tenth rib back fat (cm)	3.19	0.779
Lumbar back fat (cm)	3.58	0.757
Last rib back fat (cm)	3.16	0.609
Average back fat (cm)	3.31	0.641
Loin eye area (cm^2)	35.59	5.684
Additional Traits:		
Live weight at slaughter (kg)	118.11	6.964
Dressing percent (%)	73.72	1.95

Table 2 - Means for traits of interest measured on F2 animals.

Trait (score range)	Score interpretation		Mean
	Low value	High value	
Subjective carcass evaluations			
Color score (1 – 5)	Pale	Dark	3.25
Marbling (1 – 5)	Low	High	3.80
Firmness (1 – 5)	Soft	Firm	3.42
Light Reflectance			
Hormel Ham Minolta (24-hour Semimembranosus Minolta L values)	Dark	Pale	17.47
Hormel Ham Hunter (24-hour Semimembranosus Hunter L values)	Dark	Pale	41.65
Hormel Loin Minolta (24 hour loin Minolta L values)	Dark	Pale	21.09
Hormel Loin Hunter (24 hour Hunter L values)	Dark	Pale	44.07
Lab Loin Minolta (48 hour loin Minolta L values)	Dark	Pale	22.07
Lab Loin Hunter (48 hour Hunter L values)	Dark	Pale	46.87
Muscle pH			
Hormel Ham pH (24 hour)	Pale	Dark	5.89
Hormel Loin pH (24 hour)	Pale	Dark	5.78
Lab Loin pH (48 hour)	Pale	Dark	5.83
Tissue Quality and Water Holding Capacity			
Drip Loss (%)	Low loss	High loss	5.84
Water Holding Capacity (g)	Low loss	High loss	0.21
Fiber Type I %			0.08
Fiber Type II Ratio			1.04
Glycogen Content of The Loin			
Average Glycogen (µmol/g)			8.68
Average Lactate (µmol/g)			86.67
Average Glycolytic Potential (µmol/g)			104.00

Table 2. (continued)

Trait (score range)	Score interpretation		Mean
	Low value	High value	
Fat Content			
Total Lipid (%)			3.23
Cholesterol (mg/100g)			57.72
Instrumental Tenderness			
Average Instron (Star Probe) Force (kg)	Tender	Tough	7.84
Cooking and Sensory Panel Evaluation			
Percent Cooking Loss (%)			18.23
Tenderness Score (1-10)	Tough	Tender	4.36
Juiciness Score (1-10)	Dry	Juicy	6.02
Chewiness Score (1-10)	Soft	Tough	2.42
Flavor score (1-10)	Little flavor	Intense flavor	2.85
Off Flavor Score (1-10)	No off flavor	High off flavor	1.59

Fonte: Malek et al. (2001a,b).