IDENTIFICATION AND EVALUATION OF QUANTITATIVE TRAIT LOCI INFLUENCING GROWTH, CARCASS COMPOSITION, AND MEAT QUALITY TRAITS IN PIGS

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Animal Science

2011

ABSTRACT

IDENTIFICATION AND EVALUATION OF QUANTITATIVE TRAIT LOCI INFLUENCING GROWTH, CARCASS COMPOSITION, AND MEAT QUALITY TRAITS IN PIGS

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A three-generation resource population for identifying quantitative trait loci (QTL) for growth, carcass and meat quality traits was previously constructed at Michigan State University by crossing pigs from the Duroc and Pietrain breeds. The initial genome scan of this population included 510 F_2 animals genotyped with 124 microsatellite markers and analyzed using a line-cross model. For the second scan, 20 additional markers on 9 chromosomes (SSC3 – 7, 12, 15, 16 and 18) were genotyped for 954 F_2 animals, and 20 markers used in the first scan were genotyped for 444 additional F_2 animals. Three least-squares Mendelian models for QTL analysis were fit to each trait: a line-cross (LC) model, a half-sib (HS) model, and a combined line-cross and half-sib (CB) model.

A total of 41 QTL for growth traits were identified on SSC4, 6 – 9, 11, 15, 16, and 18. The LC analysis revealed 26 QTL, including 7 new QTL (SSC7, 15 and 18) which were not detected in the first scan. The HS analysis revealed 12 QTL, and 3 additional QTL were detected using the CB model. A total of 91 QTL for carcass and meat quality traits were identified. The LC analysis revealed 50 QTL including 14 new QTL (SSC3, 6, 7, 12, 16, and 18). The HS analysis revealed 38 QTL including 13 QTL detected on SSC15. In addition, 3 QTL were detected using the CB model. Increasing the number of markers and animals facilitated detection of new QTL,

as well as confirmation of previously identified QTL. Also, three different least-squares models made it possible to detect new QTL segregating either between or within breeds.

Based on results of the initial genome scan, five putative QTL regions (SSC3, 6, 12, 15, and 18) for carcass and meat quality traits were selected for further evaluation in an US purebred Duroc population. A total of 81 gene-specific single-nucleotide polymorphisms (SNP) were genotyped of which 33 were segregating and were analyzed for associations with pH, color, marbling, days to 113 kg, backfat thickness, and LMA. The *MDH1* SNP on SSC3 was associated with pH traits. A combined genotype of *PRKAG3* T30N and I199V on SSC15 was associated with ultimate pH. The *HSPG2* SNP on SSC6 was associated with marbling score and days to 113 kg. Markers for *NUP88* and *FKBP10* on SSC12 were associated with 45-min pH and L*, respectively. Significant associations were observed for the SSC15 marker *SF3B1* with L* and LMA, and for the SSC18 marker *ARF5* with ultimate pH and color score. Thus, results observed for SNP markers in the US Duroc population were consistent with our previous genome scan.

Based on QTL detected on SSC18 and its biological function, corticotropin-releasing hormone receptor 2 (*CRHR2*) was evaluated as a potential candidate gene affecting stress response and influencing carcass and meat quality traits. Association analyses were performed for carcass and meat quality traits recorded for the MSU resource population and for a population exposed to a halothane challenge test, as well as for stress response phenotypes recorded for the halothane challenge population. *CRHR2* genotype was significantly associated with pH and temperature, drip loss, cook yield, moisture and protein percent, b*, ham weight, and dressing percent in the resource population, and *CRHR2* genotype exhibited a suggestive association with blotching, L* and color score in the halothane challenge population.

Identification and subsequent validation of QTL influencing growth, carcass and meat quality traits in resource and commercial populations will facilitate successful implementation of marker assisted selection programs in order to achieve genetic improvement.

This dissertation is dedicated to my father and mother.

Acknowledgments

A lot of support from many people made it possible for me to reach this moment. First of all, I would like to thank my major professor, Dr. Catherine Ernst, who gave me an opportunity to attend Michigan State University and has always encouraged and inspired me at every single step of the process during my whole Ph.D program. Without the tremendous efforts of Dr. Ernst, it is obviously impossible for me to finish my project. Dr. Ernst has provided me the opportunity with her efforts to perform outstanding research in the animal genetics area, present the outcomes at scientific meetings, and publish the results in peer-reviewed journals. Beyond the scientific aspects, Dr. Ernst was deeply involved in my life in many ways and her truly indescribable efforts are holding a special place in my heart and will stay forever.

I would also like to thank the members of my guidance committee for their valuable insight in my project and Ph.D program. Especially, Drs. Ronald Bates and Juan Pedro Steibel who gave me countless advice for every chapter presented in this dissertation. Dr. Bates and his family (Ms. Teresa Bates and Ms. A'Lana Bates) provided tremendous support to continue my program as well as Dr. Bates' scientific advice for my research project. Dr. Steibel helped with statistical analysis in detail, and his scientific view and rational thought deeply influenced me. Dr. Tempelman gave me a clear solution to construct the genetic map, even impressively asking other scientists to help me when faced with a difficult problem, as well as providing statistical ideas and help. Dr. Doumit provided me the opportunity to attend his classes as a student and teaching assistant, and his enthusiastic teaching inspired me to ruminate about research and teaching in academia. Dr. Gale Strasburg has always been available for listening and offering suggestions about my research and personal life.

I especially thank Ms. Nancy Raney for teaching and supporting me in everything starting from pipetting conducted in the laboratory. I thank Dr. David Edwards for providing answers to connect between previous and present research, and I also thank Ms. Emily Helman for working together. And I certainly appreciate Mr. Al Snedegar and Mr. Kevin Turner at the MSU Swine Teaching & Research Center as well as Routh Packing Company, National Swine Registry, Whiteshire Farm, Comparts Farm, and Waldo Farm who contributed to my research.

I would like to thank all faculty, staff and graduate students in the Department of Animal Science. Especially, Drs. Karen Plaut and Janice Swanson, former and present department chairpersons, and Dr. Janice Rumph who supported me to continue accomplishing my program. Also, Drs. Steve Bursian and Paul Coussens for their concern and encouragement during my program. I also would like to thank Mr. Thomas Forton and Dr. John Lee for concerns and prayers for me and my family.

The time shared with other students and researchers was invaluable. I would like to thank Bruna Sollero, Enrico D'Alessandro, Sombat Prasongsook, and Michelle Dawes, former students in our group, for working together. Especially, Enrico D'Alessandro and Sombat Prasongsook who helped to gas the pigs and collect data presented in this dissertation. Bruna Sollero helped me learn quantitative real-time PCR and Michelle Dawes worked together with me to extract DNA from muscle samples. Also, I thank Yvonne Badke for sharing the office and ideas.

Again, I deeply appreciate Drs. Ernst, Bates, and Steibel for their contributions to my research project and inspiring me to be a better scientist and man.

Finally, I would like to thank my children and my parents for all their support.

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LIST OF ABBREVIATIONS

BF10 = tenth rib backfat

BW = body weight

CB = combined line-cross and half-sib

CRHR2 = corticotropin-releasing hormone receptor 2

EBPRO = empty body protein

EBLIPID = empty body lipid

FFTOLN = fat-free total lean tissue

FDR = false discovery rate

GC = glucocorticoid

HCW = hot carcass weight

HS = half-sib

HPA = hypothalamic-pituitary-adrenal

HWE = Hardy-Weinberg equilibrium

LC = line-cross

LD = linkage disequilibrium

LMA = *longissimus* muscle area

LRF = last-rib backfat

NSR = National Swine Registry

pH_u = ultimate pH

PRKAG3 = protein kinase AMP-activated gamma 3

PSE = pale, soft, and exudative

PSS = porcine stress syndrome

QTL = quantitative trait loci

RFLP = restriction fragment length polymorphism

RYR1 = ryanodine receptor 1

SNP = single-nucleotide polymorphisms

SSC = *sus scrofa* chromosome

TOTFAT = total body fat tissue

INTRODUCTION

The advent of genomics has brought opportunities for quantitative trait loci (QTL) mapping and gene discovery. A comprehensive pig genome sequence, tens of thousands of molecular markers, and microarrays using several high-throughput technologies are being applied to understand the pig genome [1, 2]. As a result, QTL mapping in the pig has been remarkably successful and numerous publications have demonstrated the existence of QTL for major production traits at various positions in the pig genome [3]. The pork industry, however, has difficulties in applying QTL to commercial populations. The power of QTL mapping is reduced within commercial pig populations because only a limited proportion of parents will be heterozygous for any QTL [4]. Also, it is not practical to involve exotic breeds in commercial populations, although such breeds maximize the likelihood of detecting QTL due to large phenotypic variation within experimental resource populations. In addition, most economically important traits are multi-factorial polygenic traits influenced by interaction between environmental effects and many genes [5]. The confirmation and validation of QTL in commercial populations, and fine mapping and candidate gene analyses are promising strategies to overcome the limitations of QTL mapping [6].

1. Identification of quantitative trait loci for growth, carcass and meat quality traits

Genetic improvement of production efficiency such as growth traits has been shown to be remarkably successful by conventional breeding schemes. To obtain additional rapid and accurate genetic gain, marker-assisted selection (MAS) schemes are promising using QTL and candidate genes. Numerous studies have demonstrated the existence of QTL for growth traits at various positions in the pig genome. Putative QTL have been reported for birth weight on

chromosomes 1 [7-9], 3 [1, 5], 4 [10-13], 6 [14], 7 [10, 12, 15], 12 [9] and 13 [9, 16, 17]. QTL for backfat thickness at different developmental stages have been reported on chromosomes 1, 2 and 4 – 7 [10]. Also average daily gain has been documented on chromosomes 1 [5, 10, 12, 18], 2 [1, 19, 20], 3 [21, 22], 4 [1, 9, 12, 13, 23-25], 6 – 10 [5, 15, 26], 13 [9, 23], and X [27].

Genetic improvement of carcass composition and meat quality traits by conventional breeding methods is often challenging because these traits are difficult and expensive to measure, and they are expressed only late in life. Marker-assisted selection has been suggested as a strategy for genetic improvement of such recording intensive traits [28]. Swine industries are now paying more attention to meat quality and are including quality traits as an integral part of selection programs to make simultaneous improvements in both quality and production traits [29]. However, a limited number of studies have attempted to map QTL for meat quality traits in commercial populations [30], and the identification of the underlying genes causing the QTL effect still remains a challenging task. Putative QTL have been reported for backfat thickness on all chromosomes except 12, 16 and 17 [1, 14, 18, 20, 21, 31-36]. In addition, QTL for several meat quality traits have been reported for chromosomes 1 – 8, 12, 13, 15, 17 and X [32, 37-41].

A F_2 Duroc x Pietrain resource population has previously been developed at Michigan State University. An initial genome scan of this population using 124 microsatellite markers revealed many QTL regions for growth, carcass and meat quality traits [42, 43]. A total of 55 QTL for 22 of the 29 measured growth traits were found to be significant at the 5% chromosome-wise level. Of the 55 QTL, 16 were significant at the 1% chromosome-wise, 11 at the 5% genome-wise, and 10 at the 1% genome-wise significance thresholds. Putative QTL were discovered for 10th rib and last rib backfat on SSC6, body composition traits on SSC9, backfat and lipid composition traits on SSC11, and 10th rib and last rib backfat on SSC18. A total of 94 QTL for 35 of the 38

carcass and meat quality traits were found to be significant at the 5% chromosome-wise level. Of these, 43 were significant at the 1% chromosome-wise, 27 at the 5% genome-wise, and 14 at the 1% genome-wise thresholds. Putative QTL were discovered for 45 min pH and pH decline on SSC3, marbling score and carcass backfat on SSC6, carcass length and number of ribs on SSC7, marbling score on SSC12, and color measurements and tenderness score on SSC15.

2. Evaluation of SNPs located in putative QTL regions in commercial populations

Validation studies for identified QTL are necessary because QTL effects can be biased upward and the identification of the causative mutations underlying QTL by association analyses either in experimental or commercial populations is required to implement MAS. In pig breeding programs, commercially available SNPs are limited and include insulin-like growth factor 2 (*IGF2)* and melanocortin 4 receptor (*MC4R)* for growth traits [44, 45], and ryanodine receptor 1 (*RYR1)* and protein kinase adenosine monophosphate activated γ_3-subunit (*PRKAG3)* for carcass and meat quality traits [46, 47]. Numerous QTL have been identified over the past two decades, but there are several reasons for the limited number of QTL incorporated into MAS. The use of commercially relevant populations is important for transferring QTL results into breeding programs. Also, QTL identified in a specific population may not be detected across other populations because the segregation of a QTL gene or marker is not guaranteed, and the allele frequency at the causative locus can differ between populations [48]. In addition, low map resolution and small sample sizes have been recognized as limiting factors in QTL mapping. Evaluation of putative QTL regions in commercial populations using markers segregating in these populations is important for developing MAS programs for the industry.

3. Association of corticotropin-releasing hormone receptor 2 with carcass merit and meat quality traits

Pale, soft and exudative (PSE) pork was recognized and described a half century ago [49]. Numerous reports on the development of PSE pork have focused on major gene effects, including the halothane gene (*RYR1*) [46] and Napole gene (*PRKAG3*) [50, 51]. Concurrently, stress susceptibility of pigs was also described as a syndrome rather than a single disease entity and was identified as porcine stress syndrome (PSS) [52]. PSS is one of the problems to cause inferior meat quality. PSS has been directly associated with the HAL-1843 polymorphism caused by a substitution of T for C at nucleotide 1843 in the *RYR1* gene [46]. *RYR1* has been examined extensively and a genetic marker for HAL-1843 permits genetic testing, thus allowing producers to remove the allele deleterious to meat quality. Despite an attempt to eliminate HAL-1843 and considerable achievements in improving slaughter conditions, the frequency of PSE pork in the U.S. increased from 10.2% in 1996 to 15.5% in 2002 [53]. This implies that many genes contribute to pork quality. Corticotropin-releasing hormone receptor 2 (*CRHR2*) is a G-protein coupled receptor in the secretin family that functions in stress response and energy homeostasis. It is one of two receptors for CRH which plays a key role in mediating endocrine, autonomic, behavioral, and immune responses to stress trough the hypothalamus-pituitary-adrenal (HPA) axis [54]. *CRHR2* is a potential candidate gene affecting stress response and influencing carcass and meat quality traits in pigs.

4. Specific Aims

The research conducted for this dissertation project addresses the following specific aims:

1. Perform a second genome scan of the MSU Duroc x Pietrain resource population using additional microsatellite markers and F_2 animals, and applying different least-squares analysis models to identify quantitative trait loci (QTL) for growth, carcass merit and meat quality traits;
2. Conduct an evaluation study using gene-specific SNP markers located in five QTL regions identified from the genome scan of the resource population in an US purebred Duroc population; and,
3. Examine the association of corticotropin releasing hormone receptor 2 (*CRHR2*) with carcass merit and meat quality traits in the Duroc x Pietrain resource population and a population exposed to a halothane challenge test.

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Chapter I

Application of alternative models to identify QTL for growth traits in an F_2 Duroc x Pietrain pig resource population

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This chapter was published in the journal BMC Genetics in November, 2010.

Choi, I., Steibel, J. P., Bates, R. O., Raney, N. E., Rumph, J. M., & Ernst, C. W. (2010). Application of alternative models to identify QTL for growth traits in an F_2 Duroc x Pietrain pig resource population. *BMC Genetics, 11*(1), 97.

Abstract

Background

A variety of analysis approaches have been applied to detect quantitative trait loci (QTL) in experimental populations. The initial genome scan of our Duroc x Pietrain F_2 resource population included 510 F_2 animals genotyped with 124 microsatellite markers and analyzed using a line-cross model. For the second scan, 20 additional markers on 9 chromosomes were genotyped for 954 F_2 animals and 20 markers used in the first scan were genotyped for 444 additional F_2 animals. Three least-squares Mendelian models for QTL analysis were applied for the second scan: a line-cross model, a half-sib model, and a combined line-cross and half-sib model.

Results

In total, 26 QTL using the line-cross model, 12 QTL using the half-sib model and 3 additional QTL using the combined line-cross and half-sib model were detected for growth traits with a 5% false discovery rate (FDR) significance level. In the line-cross analysis, highly significant QTL for fat deposition at 10-, 13-, 16-, 19-, and 22-wk of age were detected on SSC6. In the half-sib analysis, a QTL for loin muscle area at 19-wk of age was detected on SSC7 and QTL for 10th-rib backfat at 19- and 22-wk of age were detected on SSC15.

Conclusions

Additional markers and animals contributed to reduce the confidence intervals and increase the test statistics for QTL detection. Different models allowed detection of new QTL which indicated differing frequencies for alternative alleles in parental breeds.

Background

A variety of analysis approaches have been applied to detect quantitative trait loci (QTL) in experimental populations. For an F_2 population design, a line-cross model is most commonly used to detect QTL segregating between divergent lines. This model assumes the founder lines are fixed for alternative QTL alleles [1] and under such assumption is the most powerful [2]. However, the QTL effects under the line-cross model can be biased downwards since not all QTL alleles are completely fixed, especially in domestic animals [3]. In addition, introgression of QTL detected using the line-cross model is difficult since genetic improvement in the pig breeding industry has been achieved largely by within breed selection [4]. To identify QTL segregating within parental breeds, a half-sib model that does not assume fixation of QTL alleles in the founder lines was introduced by Knott et al. [5]. A general model that accounts for between and within line segregation has been proposed [3], but it is computationally prohibitive to implement in many populations. Kim et al. [6] subsequently developed a combined model which accounts for both line effects and half-sib family effects.

Along with appropriate statistical methods for QTL mapping, marker density and sample size are also determining factors for estimating QTL locations and effects with accuracy and precision. Although increasing marker density is becoming routine for high resolution mapping [7], a two-step strategy of adding markers and animal genotypes into previously identified QTL regions is efficient and cost effective.

We have previously reported results for a whole genome scan of our Duroc x Pietrain F_2 population using a line-cross analysis [8, 9]. Both the Duroc and Pietrain breeds are used in commercial pig production and they exhibit variation in growth phenotypes [8]. The objective of

this study was to detect new QTL for growth traits using three different models, and to refine previously identified QTL regions with addition of new markers and additional F_2 animals.

Results

A linkage map was constructed with 136 microsatellite markers including 116 markers used in the first genome scan of the MSU Duroc x Pietrain population [8] distributed across the 18 autosomes and 20 additional markers located on 9 chromosomes (SSC3 – 7, 12, 15, 16 and 18; 1 to 4 markers per chromosome; Supplementary table I.1). All animals were genotyped for new markers, and 444 additional F_2 animals not included in the first scan were also genotyped for 20 of the markers used in the first scan located on the 9 targeted chromosomes. The total genome length excluding the sex chromosomes was 3,089.6 Haldane cM with an average marker interval of 19.5 cM for the 9 chromosomes having additional markers and 28.2 cM for other chromosomes. The information content was increased by adding markers and animals (Figure I.1).

Three least-squares Mendelian models for QTL analysis were fit to each trait for this study: a line-cross model, a half-sib model, and a combined line-cross and half-sib model, whereas only a line-cross model was applied to the first scan of this population which used 510 F_2 pigs [8, 9]. A total of 41 QTL were identified (Table I.1). The line-cross analysis revealed 26 QTL, including 7 new QTL not detected in the first scan. The half-sib analysis revealed 12 QTL, and three additional QTL were detected using the combined line-cross and half-sib model. A total of 23 QTL were identified with the combined model, but 20 of these were already detected with either the line-cross or half-sib model. Thus, the combined model used in tandem with the line-

cross and half-sib models facilitated identification of additional QTL not detected by either independent analysis. The significance threshold was determined by False Discovery Rate (FDR) and FDR was compared to conventional permutation tests for selected traits. A 5% FDR was more stringent than a 5% chromosome-wise level threshold and a 1% FDR was more stringent than a 5% genome-wise level threshold. For example, for 10^{th} rib backfat (BF10) at 19-wk of age the 5% FDR F-ratio of 6.79 was higher than the 5% chromosome-wise level threshold F-ratios of minimum 4.46 and maximum 5.69, and the 1% FDR F-ratio of 8.68 was higher than the 5% genome-wise level threshold F-ratio of 8.38.

Twelve highly significant QTL affecting fat deposition at different developmental stages were detected using the line-cross model on SSC6 between 164 and 174 cM (FDR ≤ 0.002), which is consistent with results of the first scan [8] (Table I.1). The estimates of the additive effects of these QTL indicated that the Duroc alleles contributed to higher fat deposition (Table I.1). For example, the QTL affecting BF10 at 22-wk of age had an estimated additive effect of 2.17 mm indicating that Duroc alleles contribute to larger measures of BF10. The addition of two markers into the *SW122 – SW18* interval on SSC6, as well as the addition of more F_2 pigs, narrowed the estimated QTL region and increased the statistical power. For last-rib backfat (LRF) at 19-wk of age, the 95% confidence interval decreased from 16 cM (160 cM – 176 cM) to 12 cM (163 cM – 175 cM) and the test statistic ($-\log_{10}P$) increased from 15.43 to 27.25 under the same model (Figure I.2). Similarly for BF10 at 19-wk, the 95% confidence interval narrowed from 11 cM (163.5 cM – 174.5 cM) to 9.5 cM (164.5 – 174 cM) and the test statistic ($-\log_{10}P$) increased from 14.89 to 25.29 under the same model (Figure I.2).

Results for SSC6 using the line-cross model also revealed significant QTL for 22-wk fat-free total lean tissue (FFTOLN) and 22-wk empty body protein (EBPRO) at 129 cM, consistent

with results of the first scan [8]. In addition, three new QTL were discovered under the half-sib model. QTL for 22-wk empty body lipid (EBLIPID) and 22-wk total body fat tissue (TOTFAT) were detected at 25 and 26 cM, respectively, and a QTL for FFTOLN was detected at 229 cM.

Additional markers and F_2 animals contributed to detection of new QTL using the line-cross model that were not detected in the first scan on SSC7, 15 and 18. In addition, QTL for fat traits detected in the first scan on SSC11 and SSC16 were confirmed in the second scan. For SSC7, QTL were detected for *longissimus* muscle area (LMA) at 19-wk of age (FDR < 0.002) and for LMA at 10- and 13-wk (FDR <0.04). Half-sib analysis of SSC7 revealed QTL for LMA at 10- and 19-wk of age in the *S0064 – SW1369* interval that differed in location by 125 cM and 90 cM, respectively (Table I.1). QTL for LMA at 19-wk were detected by both line-cross and half-sib analyses and were significant at the 1% FDR level, but their locations were in completely different positions (Figure I.3).

No QTL for growth traits were observed on SSC15 in the first QTL scan of this population [8]. However, the second scan of this chromosome using the line-cross model revealed QTL at 39 and 69 cM for LMA at 16- and 19-wk, respectively (FDR ≤ 0.04, Table I.1). Using the half-sib model for SSC15, significant QTL were detected for BF10 at 19- and 22-wk at 96 and 74 cM, respectively (FDR ≤ 0.005). However, the line-cross analysis did not detect significant BF10 QTL in this SSC15 region (Figure I.4).

A QTL for body weight at 22-wk was detected using the line-cross model in the *SW21 – SW983* marker interval at the proximal end of SSC9 (FDR ≤ 0.05). QTL for body weight at 19-wk and EBLIPID were also detected in this same chromosomal region using the combined model (FDR ≤ 0.05). A new QTL for BF10 at 16-wk was mapped by line-cross analysis at 4 cM between markers *SW2540* and *SW1023* on SSC18. The *SW1808* and *SW2540* markers were

added to SSC18 proximal to *SW1023*, thus extending the SSC18 map and facilitating further QTL detection on this chromosome.

Analysis of SSC4 using the line-cross model confirmed a QTL at 57 cM for 22-wk LMA that was observed in the first scan [8]. This QTL had an estimated additive effect of -1.1 mm^2 of LMA indicating that Pietrain alleles contribute to a larger LMA. Significant SSC4 QTL were identified using the half-sib model for BF10 at 22-wk and LRF at 16-wk in the *S0301 – SW871* region and also for TOTFAT at 25 cM.

New QTL were also detected using the half-sib model on SSC8 for EBLIPID at 137 cM (FDR $\leq$ 0.04) and on SSC16 for 19-wk BF10 at 93 cM (FDR $\leq$ 0.04). This position on SSC16 also included a QTL for TOTFAT detected using the line-cross analysis and a QTL for body weight at 22-wk detected using the combined analysis.

Discussion

Increasing the number of markers and animals for the genome scan of our Duroc x Pietrain resource population facilitated detection of new QTL (SSC7, 15 and 18), as well as confirmation of previously identified QTL affecting growth traits (SSC4, 6, and 16) using the line-cross analysis [8]. A QTL for LMA at 22-wk was confirmed on SSC4. On SSC6, 14 QTL affecting fat deposition traits were confirmed, although three QTL for LMA identified in the first scan were not significant in the second scan. On SSC7, three new QTL for LMA were identified, whereas a QTL for ADG located in the *S0115 – SWR773* interval in the first scan was not detected. Two new QTL for LMA were detected on SSC15. Six QTL on SSC16 had been detected in the first scan but only one QTL for TOTFAT was confirmed in the second scan. Many of these QTL which were significant at the 5% chromosome-wise level in the first scan were not detected in

the second scan, either because the 5% FDR threshold used for the second scan was slightly more stringent than the 5% chromosome-wise level threshold or because these were false positives in the first scan.

A QTL for LMA at 22-wk detected with the line-cross analysis, and QTL for LRF at 16-wk and BF10 at 22-wk detected with the half-sib analysis were localized in the *S0301 – SW871* interval on SSC4. A possible explanation for these results could be QTL alleles of Pietrain origin affecting LMA, and QTL alleles influencing backfat thickness segregating in each founder breed. A QTL affecting fatness in this interval has been confirmed by many previous studies [10-12], including a report by Andersson et al. [13] of the first pig QTL for growth and fatness on SSC4 in a Wild boar x Large White cross. Also, Cepica et al. [10] reported a QTL for LMA in this same region and demonstrated that Pietrain alleles were associated with increased meat and decreased fat content in a Wild boar x Pietrain cross.

Strong evidence for QTL affecting fatness was revealed at marker interval *SW1647 – SW1881* on SSC6. QTL for BF10 and LRF at different stages of growth (measured by ultrasound) were highly significant (FDR < 0.001). The estimates of the additive effects suggest that Duroc alleles contributed to larger measures of BF10 and LRF. However the estimates of the dominance effects for these QTL were negative. In the first scan, the predominant location for most of the BF10 and LRF QTL was estimated to be distal to marker *SW1881*. However, with the addition of marker *SW1647*, these QTL were determined to be located in the interval *SW1647 – SW1881* in the second scan. This region includes the *leptin receptor* (*LEPR*) gene located on SSC6q3.3-3.5 [14] and Óvilo et al. [15] reported a QTL for fatness in this same region. Muñoz et al. [16] reported the effect of QTL and *LEPR* alleles in this region to be significant for backfat thickness and also for body weight. Our results are in agreement with those of Muñoz et al. [16]

regarding backfat QTL. However, we did not find evidence for a QTL affecting body weight on SSC6, although body weight was positively correlated with BF10 and LRF in our population.

Three other regions on SSC6 included QTL for body composition traits determined by either the line-cross analysis or the half-sib analysis. In the line-cross analysis, QTL for FFTOLN and EBPRO at 22-wk mapped to 129 cM and this result is in agreement with a study reported by Mohrmann et al. [17]. Two additional QTL regions affecting EBLIPID and TOTFAT, and FFTOLN were detected by the half-sib analysis and were located at the proximal and distal ends of SSC6, respectively.

QTL for LMA at 10- and 19-wk of age were detected using both the line-cross and the half-sib models on different regions of SSC7. A newly detected QTL for LMA at 19-wk under the line-cross model was mapped to 138 cM with the contribution of the Pietrain allele increasing LMA, whereas the half-sib model revealed a QTL for LMA at 19-wk at 48 cM. Nagamine et al. [18] reported an LMA QTL in a Large White population that spanned most of SSC7 and Uemoto et al. [18] reported an LMA QTL segregating in a Duroc population with a relative peak location in between the QTL detected using the line-cross and half-sib models in the current study.

QTL for LMA located on SSC15 were also identified in the second scan. The QTL for LMA at 19-wk was detected in the *S0088 – SW1683* interval in a region that includes *MSTN* (*myostatin*) which is considered to be a candidate gene for muscle hypertrophy [19]. Stinckens et al. [20] reported a polymorphism in the porcine *MSTN* promoter region MEF3 binding site, which could potentially abolish enhancer activity, and that had a very high allele frequency in the Pietrain breed. Thus, its effect could be associated with the higher muscularity of the Pietrain breed. Two QTL influencing backfat thickness were identified on SSC15 using the half-sib analysis. A QTL for BF10 at 22-wk was located in the *SW1683 – SW906* interval and a QTL for BF10 at 19-wk

was located in the *SW1983 – SW1119* interval. This latter region is consistent with a QTL for 1st rib fatness detected in a four-way cross by Harmegnies et al. [21].

The marker *SW2517* located on SSC16q2.2 has been reported to be linked to a QTL affecting fatness at later stages of growth [22]. In the first scan, five QTL (body weight and BF10 at 19-wk, body weight and LRF at 22-wk, and TOTFAT) were detected near *SW2517.* A QTL for ADG was also identified on SSC16 distal to this region. In the second scan, QTL for TOTFAT detected with the line-cross analysis, BF10 at 19-wk detected with the half-sib analysis and body weight at 22-wk detected with the combined analysis were mapped to 93 cM at marker *SW2517*. In addition, suggestive QTL for EBLIPID (FDR < 0.06), TOTFAT (FDR < 0.066) and Age at 105 kg (FDR < 0.073) under the combined model were located at the same position. No additional *SW2517* animal genotypes were included in the second scan, although two new markers flanking *SW2517* were genotyped across the full population. For marker *SW2517,* three *SW2517* alleles were segregating and the number of phase known informative meioses was 385 of 510 animals. The allele associated with fatness and heavier body weight originated only from the Pietrain, whereas the other two alleles were segregating in both the Pietrain and Duroc founder breeds. Thus segregation patterns of alleles at this marker allowed detection of QTL by all three models. Liu et al. [23, 24] reported that a QTL influencing backfat thickness using both line-cross and combined analyses was located in the same SSC16 region for their Duroc x Pietrain population. The *prolactin receptor* (*PRLR*) gene located in this region [25] is well-known as a candidate gene affecting reproductive traits in pigs [26-29]. Prolactin also stimulates fat deposition and weight gain, and stimulates increases in white adipose tissue leptin mRNA and plasma leptin levels [30, 31]. Freemark et al. [32] provided evidence that the absence of *PRLR* in knockout mice was accompanied by reduced body weight gain after 16 weeks of age and

decreased abdominal fat mass. Recently, Lu et al. [33] demonstrated that polymorphisms in the *PRLR* gene were associated with growth traits in cattle. Based on results for other species and our localization of QTL on SSC16, *PRLR* may be a candidate gene for growth and fat deposition in pigs and further research is warranted.

A QTL for BF10 at 16-wk was identified at the proximal end of SSC18. Malek et al. [34] detected QTL for backfat thickness in the same SSC18 region in a Berkshire x Yorkshire population. A novel QTL for body weight at 22-wk was also identified on SSC9 in the second scan. This QTL had a dominance effect of 4.12 kg, which indicated that the heterozygous genotype contributed to heavier body weight at 22-wk. A QTL for body weight at 19-wk detected by the combined analysis was located in the same interval. A QTL for body weight at 3-wk was reported in the same SSC9 region [23], but no other body weight QTL have been reported in the *SW21 – SW983* interval.

Conclusions

Additional markers and animal genotypes contributed to refine QTL positions and increase the statistical power. The application of different QTL analysis models made it possible to detect new QTL segregating either between or within breeds. In total, 26 QTL with the line-cross model, 12 QTL with the half-sib model and 3 additional QTL with the combined line-cross and half-sib model were detected for pig growth traits. Analysis using the line-cross model was most powerful for detecting QTL, whereas the combined model which assumed QTL to be segregating at different allelic frequencies in the founder populations was less powerful than the line-cross or half-sib models. This result was not unexpected because the population was designed to exploit between breed differences and markers were selected in such a way that they were more

informative to declare breed of origin QTL than for detecting QTL using the half-sib analysis. However, our analysis shows that there is substantial segregation within breed that can be tracked (although to a lesser extent) by using the sire haplotype probabilities either alone (half-sib analysis) or jointly with the breed origin probabilities (combined analysis).

Methods

Animals and phenotypes

Animals from a three-generation Duroc x Pietrain resource population developed at Michigan State University and described by Edwards et al. [8] were used for this study. Animal protocols were approved by the Michigan State University All University Committee on Animal Use and Care (AUF# 09/03-114-00). The population was established from 4 F_0 Duroc sires and 15 F_0 Pietrain dams. The F_2 pigs were produced from 50 F_1 females and 6 F_1 males, and were born in 141 litters across 11 farrowing groups. The second genome scan for this study included the 510 F_2 animals used in the first genome scan along with an additional 444 F_2 animals. The 954 total pigs included all F_2 animals from this population for which complete growth phenotypes are available. Descriptive statistics for phenotypes used in this study are presented in Table I.2.

Markers and genotyping

Based on the first genome scan results using 124 markers [8, 9], 9 chromosomes (SSC3, 4, 5, 6, 7, 12, 15, 16 and 18) with significant QTL were selected for additional marker genotyping. Twenty new microsatellite markers were selected from the publicly available pig genome linkage

map [http://www.marc.usda.gov/genome/swine/swine.html] that map within the QTL regions on these chromosomes (Supplementary table I.1). New markers were confirmed to be informative in the MSU population by genotyping of F_0 pigs. All F_0, F_1, and the 954 F_2 pigs were genotyped for the 20 new markers, and the 444 additional F_2 pigs were also genotyped for 20 markers flanking the QTL regions on the 9 selected chromosomes. Genotyping was performed at a commercial laboratory (GeneSeek Inc., Lincoln, NE). Sex-averaged genetic linkage maps were constructed using CRI-MAP version 2.4 [35] and converted to the Haldane map function [36].

Statistical analysis

QTL mapping was performed using least-squares regression with line-cross, half-sib and combined line-cross and half-sib models. Genome-wise significance thresholds were determined by false discovery rate (FDR) [37]. QTL detected using the line-cross or half-sib model were declared using a FDR threshold of 5%, and then additional QTL detected with the combined line-cross and half-sib model were declared when such QTL had not previously been detected by either the line-cross or half-sib model.

Under the line-cross model it is assumed that the two founder lines are fixed for alternative alleles at the QTL affecting the traits of interest [1]. The QTL Express software [38] was used to estimate the probability of each F_2 individual being homozygous for two Duroc alleles (P_{11}), homozygous for two Pietrain alleles (P_{22}), or heterozygous (P_{12} or P_{21}) at fixed 1-cM intervals across the genome. By denoting the effect of P_{11} as positive additive (a), the effect of $P_{12} + P_{21}$

as dominance (d) and the effect of P_{22} as negative additive (-a), the following linear model was fitted at every cM across the genome.

$$y_j = X_j b + aPa_j + dPd_j + e_j$$

Where y_j is the phenotype of F_2 progeny j, X_j and b are the design matrix and solution vector for the fixed effects, respectively, a and d are the estimated additive and dominance effects of a putative QTL at the given location, respectively, Pa_j is the conditional probability of animal j to carry two Duroc alleles, Pd_j is the conditional probability of animal j to be heterozygous, and e_j is the residual error. The model for all traits included sex of animal and litter as fixed effects and the model for 10- to 22-wk ADG included 10-wk body weight as a covariate.

For the half-sib analysis, the F_2 individuals were treated as 6 paternal half-sib families which assumes no fixation of the QTL alleles in the founder lines. QTL Express [38] was used to calculate the probabilities of individuals of allele (P_1) or allele (P_2) from the common Duroc parent (P_{12} or P_{21}) [5]. In these analyses contrasts were made between the two haplotypes of every F_1 sire.

$$y_{ij} = X_{ij} b + s_i + \alpha \mathrm{HS}_i Ps_{ij} + e_{ij}$$

Where y_{ij} is the phenotype of F_2 progeny j of F_1 sire i, X_{ij} and b are the design matrix and the solution vector for fixed effects, respectively, s_i is the effect of the ith F_1 sire, $\alpha \mathrm{HS}_i$ is the substitution effect for the two putative QTL alleles (P_1 or P_2) carried by the ith F_1 sire, Ps_{ij} is

the probability that the F_2 individuals inherited the arbitrary allele (P_1) from F_1 sire i, and e_{ij} is the residual error.

The combined line-cross and half-sib model included,

$$y_{ij} = X_{ij}b + s_i + aPa_{ij} + dPd_{ij} + \alpha CB_i Ps_{ij} + e_{ij}$$

Where y_{ij} is the phenotype of F_2 progeny j of F_1 sire i, X_{ij} and b are the design matrix and the solution vector for fixed effects, respectively, s_i is the effect of the ith F_1 sire, a and d are the additive and dominance effects of breed-origin alleles, respectively, Pa_{ij} and Pd_{ij} are the corresponding breed-origin coefficients, αCB_i is the substitution effect for the two putative QTL alleles carried by the i th F_1 sire, Ps_{ij} is the probability that the F_2 individuals inherited the arbitrary allele (P_1) from F_1 sire i, and e_{ij} is the residual error. In this model, a and d account for the average effects of breed origin alleles through both the F_1 sire and the F_1 dam and αCB_i represents the difference between the two QTL alleles that a given F_1 sire received from the two parental breeds as a deviation from their average additive effect [6].

Authors' contributions

IC carried out the data analyses and drafted the manuscript. JPS participated in design of the study and supervised the data analyses. ROB coordinated development of the resource population and participated in design of the study. NER prepared the DNA samples and identified segregating markers. JMR contributed to design of the study. CWE coordinated the project, participated in design of the study and helped draft the manuscript. All authors read and approved the final manuscript.

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Acknowledgements

This project was supported by National Research Initiative Competitive Grant no. 2004-35604-14580 from the USDA National Institute of Food and Agriculture. We thank D. Edwards for technical contributions in development of the pig resource population.

Table I.1. Position and significance level of growth trait QTL.

Chr[1]	Position[2]	Trait	Type[3]	-log$_{10}P$[4]	FDR[5]	Flanking Markers	Additive[6]	Dominance[7]
4	25	22-wk total body fat tissue, kg	HS	3.17	0.0484	SW2509 – S0301		
	42	22-wk 10th-rib backfat, mm	HS	3.65	0.0209	S0301 – SW871		
	57	22-wk LM area, cm^2	LC	3.29	0.0351	S0301 – SW871	-1.10 (0.29)	-0.66 (0.51)
	65	16-wk last-rib backfat, mm	HS	3.97	0.0120	S0301 – SW871		
6	25	22-wk empty body lipid, kg	HS	3.76	0.0179	S0099 – SW2406		
	26	22-wk total body fat tissue, kg	HS	3.29	0.0398	S0099 – SW2406		
	129	22-wk fat-free total lean, kg	LC	5.30	0.0006	S0220 – SW122	-0.35 (0.09)	0.44 (0.14)
	129	22-wk empty body protein, kg	LC	4.85	0.0016	S0220 – SW122	-0.13 (0.03)	0.14 (0.05)
	164	13-wk 10th-rib backfat, mm	LC	21.81	0.0000	SW1647 – SW1881	0.98 (0.10)	-0.74 (0.15)
	164	22-wk 10th-rib backfat, mm	LC	18.49	0.0000	SW1647 – SW1881	2.17 (0.25)	-1.37 (0.36)
	164	22-wk last-rib backfat, mm	LC	22.25	0.0000	SW1647 – SW1881	1.55 (0.17)	-1.24 (0.24)
	165	10-wk 10th-rib backfat, mm	LC	22.90	0.0000	SW1647 – SW1881	0.70 (0.07)	-0.57 (0.11)
	165	13-wk last-rib backfat, mm	LC	21.85	0.0000	SW1647 – SW1881	0.58 (0.06)	-0.39 (0.09)
	165	16-wk last-rib backfat, mm	LC	22.26	0.0000	SW1647 – SW1881	0.86 (0.10)	-0.81 (0.14)
	166	10-wk last-rib backfat, mm	LC	20.30	0.0000	SW1647 – SW1881	0.43 (0.05)	-0.30 (0.07)
	166	19-wk last-rib backfat, mm	LC	27.25	0.0000	SW1647 – SW1881	1.32 (0.13)	-1.03 (0.18)
	168	22-wk total body fat tissue, kg	LC	4.69	0.0023	SW1881 – SW322	0.57 (0.13)	-0.38 (0.19)
	169	19-wk 10th-rib backfat, mm	LC	25.29	0.0000	SW1881 – SW322	1.78 (0.18)	-1.61 (0.27)
	172	16-wk 10th-rib backfat, mm	LC	23.45	0.0000	SW1881 – SW322	1.38 (0.14)	-0.97 (0.22)
	174	22-wk empty body lipid, kg	LC	8.61	0.0000	SW1881 – SW322	0.46 (0.09)	-0.50 (0.14)
	229	22-wk fat-free total lean, kg	HS	3.38	0.0343	SW607 – SW2419		
7	44	10-wk LM area, cm^2	HS	3.41	0.0325	S0064 – SW1369		
	48	19-wk LM area, cm^2	HS	4.78	0.0024	S0064 – SW1369		

Table I.1. (Cont'd).

Chr[1]	Position[2]	Trait	Type[3]	-log$_{10}P$[4]	FDR[5]	Flanking Markers	Additive[6]	Dominance[7]
7	138	19-wk LM area, cm^2†	LC	5.02	0.0011	SW859 – SW2040	-1.38 (0.29)	-0.55 (0.62)
	169	10-wk LM area, cm^2†	LC	3.51	0.0236	SW2040 – S0115	-0.43 (0.11)	0.05 (0.16)
	185	13-wk LM area, cm^2†	LC	3.22	0.0391	S0115 – SW632	-0.49 (0.13)	0.26 (0.20)
8	137	22-wk empty body lipid, kg	HS	3.30	0.0397	S0017 – SW2160		
9	7	22-wk body weight, kg†	LC	3.10	0.0485	SW21 – SW983	-0.52 (0.71)	4.12 (1.10)
	9	22-wk empty body lipid, kg	CB	3.55	0.0433	SW21 – SW983		
	12	19-wk body weight, kg	CB	3.78	0.0297	SW21 – SW983		
11	91	19-wk last-rib backfat, mm	LC	3.98	0.0093	S0230 – SW66	0.20 (0.19)	-1.11 (0.27)
	93	19-wk 10th-rib backfat, mm	LC	3.37	0.0307	S0230 – SW66	-0.63 (0.28)	-1.28 (0.41)
	106	22-wk empty body lipid, kg	LC	3.28	0.0356	S0230 – SW66	-0.22 (0.15)	-1.03 (0.29)
15	39	16-wk LM area, cm^2†	LC	3.19	0.0413	S0148 – SW1989	-0.75 (0.19)	0.05 (0.30)
	69	19-wk LM area, cm^2†	LC	3.28	0.0356	S0088 – SW1683	-0.86 (0.22)	-0.01 (0.35)
	74	22-wk 10th-rib backfat, mm	HS	4.43	0.0048	SW1683 – SW906		
	96	19-wk 10th-rib backfat, mm	HS	4.38	0.0053	SW1983 – SW1119		
16	93	22-wk total body fat tissue, kg	LC	3.64	0.0186	SW2517	-0.47 (0.18)	-0.89 (0.31)
	93	19-wk 10th-rib backfat, mm	HS	3.36	0.0355	SW2517		
	93	22-wk body weight, kg	CB	3.57	0.0424	SW2517		
18	4	16-wk 10th-rib backfat, mm†	LC	3.54	0.0222	SW2540 – SW1023	0.55 (0.14)	0.23 (0.20)

[1]Chr = chromosome

[2]Position in Haldane cM

[3]LC = QTL declared as line-cross type; HS = half-sib type; CB = combined type.

[4]Negative logarithm of the comparison-wise p value of the test statistic against the null hypothesis of no QTL at the most likely position for the inferred QTL model.

[5]FDR = false discovery rate

Table I.1. (Cont'd).

[6]Estimates of additive effects with standard errors for LC QTL. The effects are expressed as (DD-PP)/2, where D = Duroc allele and P = Pietrain allele.

[7]Estimates of dominance effects with standard errors for LC QTL. The effects are expressed as DP-PD, where D = Duroc allele and P = Pietrain allele.

[†]New QTL detected with the line-cross model in the second scan.

Table I.2. Number of records, means, and SD for growth traits measured.

Trait	N	Mean	SD
Birth weight (kg)	954	1.53	0.32
3-wk weight (kg)	954	5.69	1.48
6-wk weight (kg)	953	12.04	2.84
10-wk weight (kg)	954	26.43	4.84
10-wk 10th-rib backfat (mm)[1]	954	7.96	1.77
10-wk *longissimus* muscle area (cm^2)[1]	954	11.55	2.54
10-wk last-rib backfat (mm)[1]	954	6.11	1.06
13-wk weight (kg)	954	41.66	6.60
13-wk 10th-rib backfat (mm)[1]	954	9.74	2.68
13-wk *longissimus* muscle area (cm^2)[1]	954	16.98	3.35
13-wk last-rib backfat (mm)[1]	954	7.13	1.38
16-wk weight (kg)	954	62.28	8.27
16-wk 10th-rib backfat (mm)[1]	954	12.35	3.44
16-wk *longissimus* muscle area (cm^2)[1]	954	24.85	3.82
16-wk last-rib backfat (mm)[1]	954	9.57	2.28
19-wk weight (kg)	954	80.79	9.84
19-wk 10th-rib backfat (mm)[1]	954	15.90	5.02
19-wk *longissimus* muscle area (cm^2)[1]	954	31.39	4.19
19-wk last-rib backfat (mm)[1]	954	11.79	3.29
22-wk weight (kg)	954	100.05	10.87
22-wk 10th-rib backfat (mm)[1]	954	19.89	6.40
22-wk *longissimus* muscle area (cm^2)[1]	954	37.09	4.83
22-wk last-rib backfat (mm)[1]	954	14.35	4.16
10 - 22 wk ADG (g/d)	954	878.04	105.42
Age at 105 (kg)[2]	954	157.42	13.64
22-wk total body fat tissue (kg)[3]	954	24.94	6.96
22-wk fat-free total lean tissue (kg)[3]	954	38.35	4.45
22-wk empty body protein (kg)[3]	954	15.01	1.67
22-wk empty body lipid (kg)[3]	954	21.96	4.23

[1]10th rib backfat, last rib backfat, and *longissimus* muscle area at 10, 13, 16, 19, and 22 wk of age estimated using B-mode ultrasound (Pie Medical 200SLC, Classic Medical Supply Inc., Tequesta, FL).

[2]Age at 105 kg calculated following National Swine Improvement Federation guidelines [39].

Table I.2. (Cont'd).

[3]Total body fat tissue, fat-free total lean tissue, empty body protein, and empty body lipid at 22 wk of age calculated by using equations similar to those used by Wagner et al. [40].

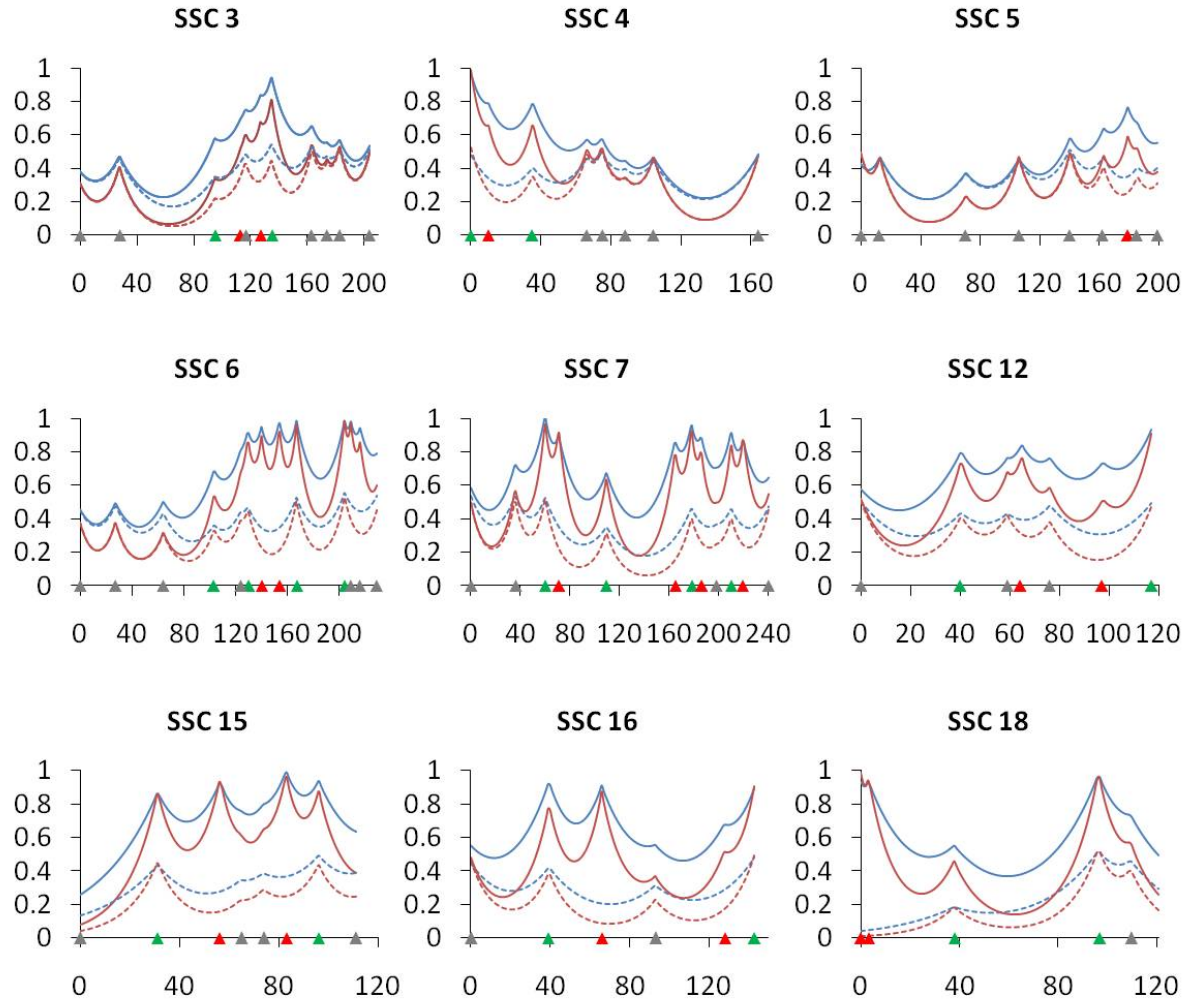

Figure I.1. Effect of additional markers and animals on information content.
Information content was determined for both the first QTL scan (dotted lines) and the second scan (solid lines) which include additional animals and markers. Blue lines indicate additive effects and red lines indicate dominance effects. Marker positions are shown as triangles on the X-axis (gray, markers used for both QTL scans and genotyped only in 510 animals; green, markers used for both QTL scans and genotyped in all animals; red, markers used for second scan only and genotyped in all animals).

For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

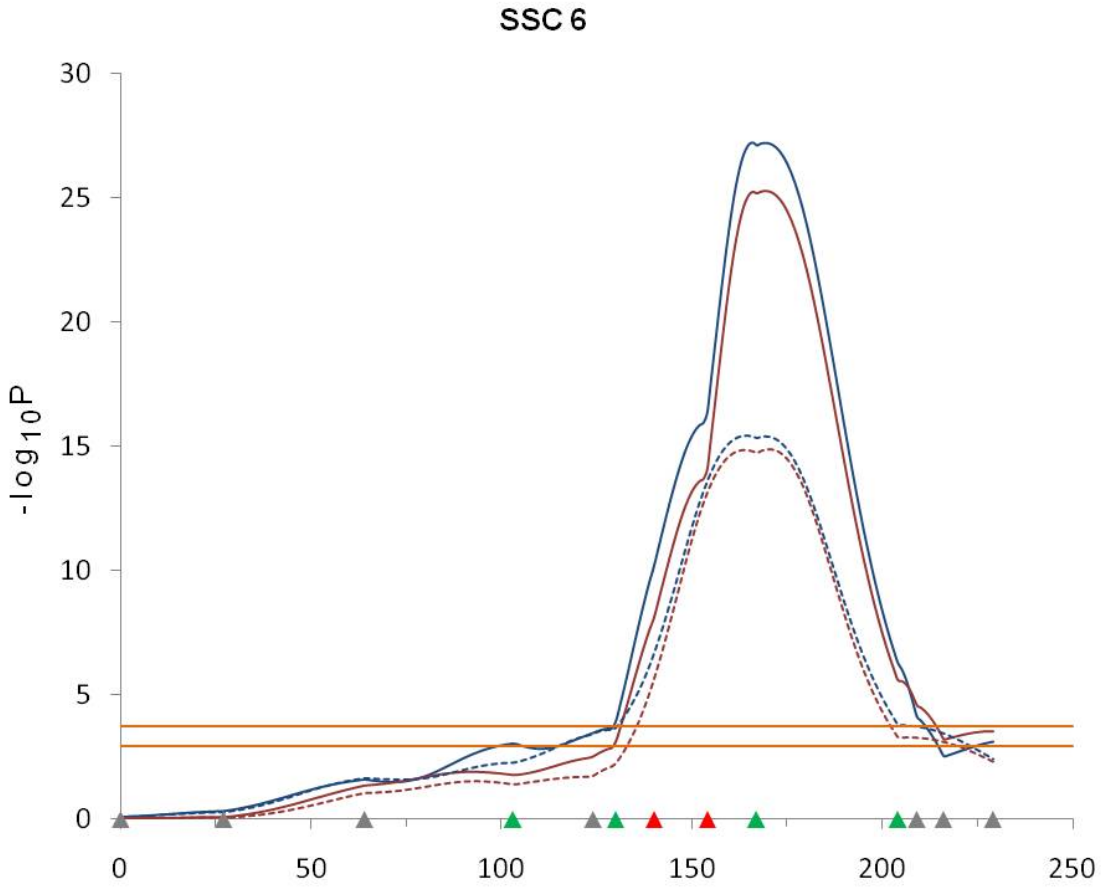

Figure I.2. Effect of additional markers and animals on statistical power for QTL detection. Blue lines indicate last rib fat (LRF) QTL at 19-wk and red lines indicate 10th rib backfat (BF10) QTL at 19-wk on SSC6. Solid lines are second scan results and dotted lines are first scan results. Marker positions are shown as triangles on the X-axis (gray, markers used for both QTL scans and genotyped only in 510 animals; green, markers used for both QTL scans and genotyped in all animals; red, markers used for second scan only and genotyped in all animals). Horizontal lines indicate significance thresholds (lower line, 5% FDR; upper line, 1% FDR).

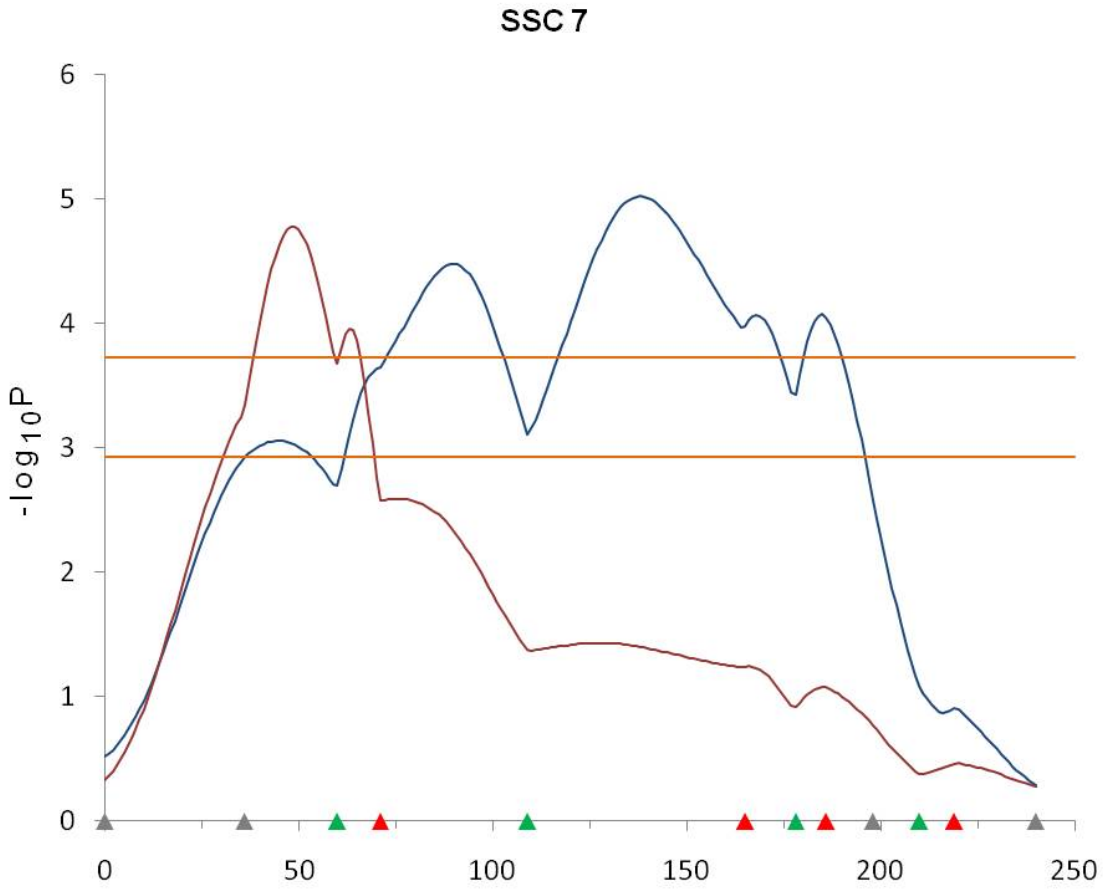

Figure I.3. QTL results determined by different models for *longissimus* muscle area (LMA) at 19 weeks of age on SSC7.
Blue and red lines indicate LMA QTL at 19-wk detected by line-cross and half-sib models, respectively (FDR ≤ 0.002). Marker positions are shown as triangles on the X-axis (gray, markers used for both QTL scans and genotyped only in 510 animals; green, markers used for both QTL scans and genotyped in all animals; red, markers used for second scan only and genotyped in all animals). Horizontal lines indicate significance thresholds (lower line, 5% FDR; upper line, 1% FDR).

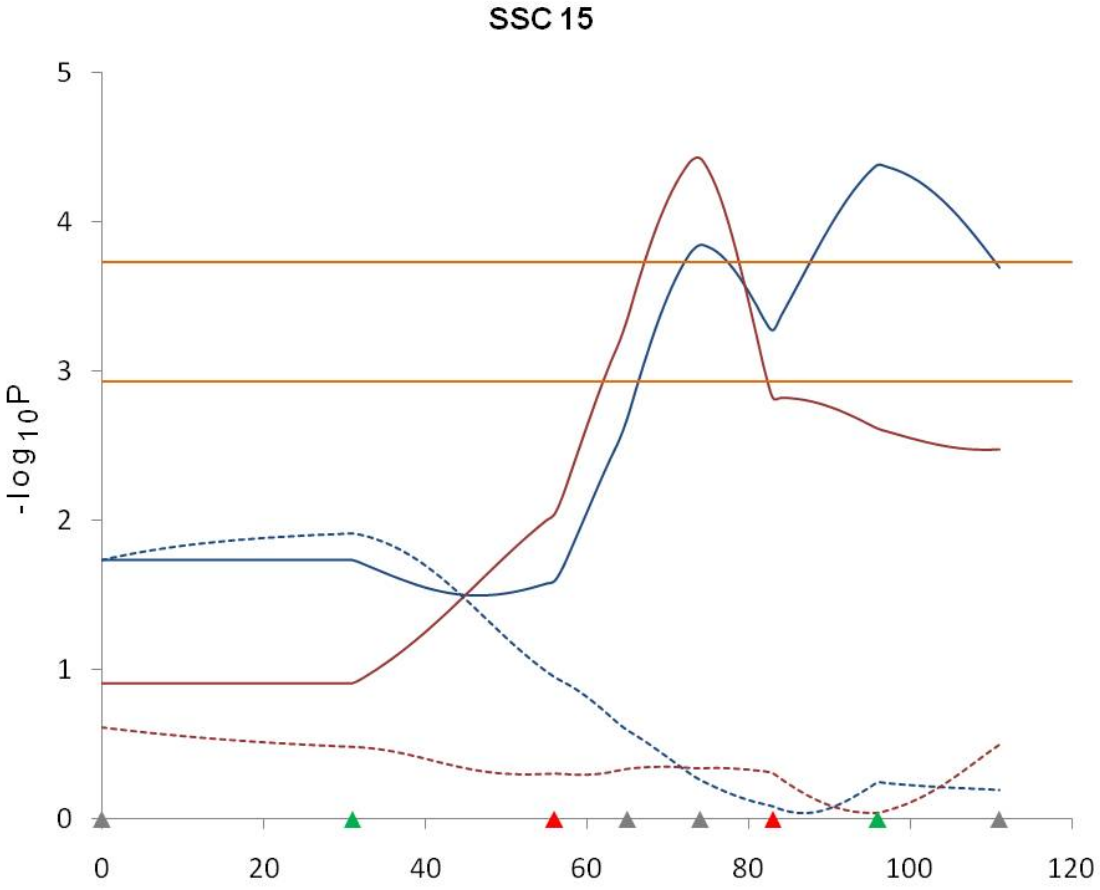

Figure I.4. QTL results determined by different models for 10th rib backfat (BF10) at 19 and 22 weeks of age on SSC15.
Blue and red solid lines indicate BF10 at 19- and 22-wk, respectively, detected with the half-sib model (FDR ≤ 0.005). Blue and red dotted lines indicate BF10 at 19- and 22-wk, respectively, detected with the line-cross model. Marker positions are shown as triangles on the X-axis (gray, markers used for both QTL scans and genotyped only in 510 animals; green, markers used for both QTL scans and genotyped in all animals; red, markers used for second scan only and genotyped in all animals). Horizontal lines indicate significance thresholds (lower line, 5% FDR; upper line, 1% FDR)

Supplementary Table I.1. Genetic maps constructed for the first and second genome scans of the Michigan State University Duroc x Pietrain resource population.

Chr[1]	Marker	1st scan[2] Kosambi cM	2nd scan Haldane cM
1	SW1514	0.0	0.0
	SW1515	21.1	25.4
	S0008	49.7	61.8
	S0331	77.5	97.0
	SW974	108.9	137.6
	S0056	179.7	247.4
	SW1301	235.1	328.7
2	SWR2516	0.0	0.0
	SW240	41.0	56.2
	S0170	53.7	70.5
	SW1026	64.8	82.8
	S0370	93.4	119.2
	SW1844	103.2	129.9
	S0378	110.1	137.3
	S0036	142.0	178.8
3	SW274	0.0	0.0
	SW2021	22.7	27.7
	S0206‡	68.7	94.8
	SWR978†		112.9
	ACTG2	86.4	116.5
	SW2141†		127.1
	SW2047‡	101.3	134.8
	SW2408	124.3	163.4
	S0002	132.4	174.1
	SW1327	141.2	182.8
	SW2532	159.6	204.5

Supplementary Table I.1. (Cont'd).

Chr[1]	Marker	1st scan[2] Kosambi cM	2nd scan Haldane cM
4	SW2404‡	0.0	0.0
	SW2509†		10.0
	S0301‡	29.2	35.3
	SW871	54.1	66.3
	SW2454	61.8	74.6
	S0107	73.8	88.2
	S0214	88.0	104.2
	S0097	131.3	164.5
5	SW413	0.0	0
	ACR	11.5	12.8
	SWR453	53.4	70.5
	SW2	81.5	106
	S0005	108.9	140.4
	S0018	127.4	162.8
	IGF1†		179.1
	SW995	147.6	185.5
	SW378	159.7	199.6
6	S0099	0.0	0.0
	SW2406	22.4	27.3
	SW2525	50.7	64.2
	S0087‡	81.4	103.4
	S0220	98.2	123.6
	SW122‡	103.9	129.5
	SW2173†		140.0
	SW1647†		153.7
	SW1881‡	135.8	167.0
	SW322‡	164.8	204.1
	SW1328†		208.9
	SW607†		216.1
	SW2419	181.1	229.6

Supplementary Table I.1. (Cont'd).

Chr[1]	Marker	1st scan[2] Kosambi cM	2nd scan Haldane cM
7	S0025	0.0	0.0
	S0064	28.1	35.6
	SW1369‡	48.0	59.8
	SW2019†		70.7
	SW859‡	92.5	109.2
	SW2040†		164.5
	S0115‡	135.8	177.7
	SW632†		185.5
	SWR773	151.6	197.7
	S0101‡	164.0	209.7
	S0212†		219.4
	SW764	186.7	240.7
8	SW2410	0.0	0.0
	SW905	22.9	28.0
	SWR1101	55.0	69.7
	S0017	95.1	124.4
	SW2160	110.7	142.4
	SW1085	124.4	158.0
	S0178	165.5	214.4
9	SW21	0.0	0.0
	SW983	13.2	14.9
	SW911	43.1	53.3
	SW2401	63.7	78.0
	SW539	72.4	87.5
	SW989	97.0	117.9
	SW2116	127.4	157.0

Supplementary Table I.1. (Cont'd).

Chr[1]	Marker	1st scan[2] Kosambi cM	2nd scan Haldane cM
10	SWR136	0.0	0.0
	SW249	20.2	24.2
	SWC19	44.3	53.9
	SW1041	56.8	67.9
	SW920	79.4	95.5
11	S0391	0.0	0.0
	S0071	53.0	77.0
	S0230	65.2	90.7
	SW66	119.0	169.1
12	SW2490	0.0	0
	SW957‡	31.2	40.4
	SW874	47.2	59
	SW37†		64.8
	S0090	61.0	76.2
	SWC23†		97.5
	SW2180‡	94.1	117.4
13	S0219	0.0	0.0
	SWR1941	13.7	15.6
	SW344	40.9	49.8
	SWR1008	54.2	64.9
	S0068	65.1	76.9
	SW398	85.8	101.8
	SW2440	103.2	122.2
	S0215	122.6	145.3
14	SW857	0.0	0.0
	SW510	26.6	33.4
	SW210	45.9	56.3
	SW886	64.7	78.6
	SW55	85.2	103.1
	SW1557	95.6	114.6
	SWC27	117.6	141.3

Supplementary Table I.1. (Cont'd).

Chr[1]	Marker	1st scan[2] Kosambi cM	2nd scan Haldane cM
15	SW1204	0.0	0.0
	S0148‡	24.7	31.2
	SW1989†		56.3
	S0088	54.8	64.5
	SW1683	64.5	73.6
	SW906†		82.8
	SW1983‡	82.2	95.9
	SW1119	96.0	111.4
16	S0111	0.0	0.0
	SW419‡	30.9	39.4
	SW1454†		66.0
	SW2517	64.8	93.0
	SW1897†		127.7
	S0061‡	99.5	143.0
17	SWR1004	0.0	0.0
	SW2441	22.7	27.7
	SW1031	42.9	51.9
	SW2427	94.9	127.1
18	SW1808†		0.0
	SW2540†		3.2
	SW1023‡	0.0	37.9
	SW1984‡	43.1	96.6
	S0062	54.9	109.6

[1]Chr = chromosome

[2]First scan reported in Edwards et al.

†Additional markers used for the second scan

‡Flanking markers used for the second scan

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Chapter II

Identification of carcass and meat quality QTL in an F_2 Duroc x Pietrain pig resource population using different least-squares analysis models

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This chapter was published in the journal Frontiers in Genetics (Livestock Genomics) in May, 2011.

Choi, I., Steibel, J. P., Bates, R. O., Raney, N. E., Rumph, J. M., & Ernst, C. W. (2011). Identification of carcass and meat quality QTL in an F_2 Duroc x Pietrain pig resource population using different least-squares analysis models. *Frontiers in Genetics, 2*, 18.

Abstract

A three-generation resource population was constructed by crossing pigs from the Duroc and Pietrain breeds. In this study, 954 F_2 animals were used to identify quantitative trait loci (QTL) affecting carcass and meat quality traits. Based on results of the first scan analyzed with a line-cross model using 124 microsatellite markers and 510 F_2 animals, 9 chromosomes were selected for genotyping of additional markers. Twenty additional markers were genotyped for 954 F_2 animals and 20 markers used in the first scan were genotyped for 444 additional F_2 animals. Three different Mendelian models using least-squares for QTL analysis were applied for the second scan: a line-cross model, a half-sib model, and a combined line-cross and half-sib model. Significance thresholds were determined by false discovery rate (FDR). In total, 50 QTL using the line-cross model, 38 QTL using the half-sib model and 3 additional QTL using the combined line-cross and half-sib model were identified ($q < 0.05$). The line-cross and half-sib models revealed strong evidence for QTL regions on SSC6 for carcass traits (e.g., 10^{th}-rib backfat; $q < 0.0001$) and on SSC15 for meat quality traits (e.g., tenderness, color, pH; $q < 0.01$), respectively. QTL for pH (SSC3), dressing percent (SSC7), marbling score and moisture percent (SSC12), CIE a* (SSC16) and carcass length and spareribs weight (SSC18) were also significant ($q < 0.01$). Additional marker and animal genotypes increased the statistical power for QTL detection, and applying different analysis models allowed confirmation of QTL and detection of new QTL.

Keywords: Pig, QTL, Carcass merit, Meat quality

1. Introduction

Quantitative trait loci (QTL) mapping has been conducted using numerous pig populations to identify genomic regions controlling phenotypic variation for hundreds of traits (http://www.animalgenome.org/cgi-bin/QTLdb/SS/index). Nevertheless, the implementation of QTL into breeding programs which is a major goal of QTL mapping has been limited not only due to insufficient numbers of identified causative mutations, but because of unknown linkage disequilibrium (LD) phase between markers and QTL resulting from cross breeding systems (Hayes et al. 2009; Spelman and Bovenhuis 1998). We have developed a F_2 Duroc x Pietrain resource population at Michigan State University (Edwards et al. 2008b) and reported QTL for carcass merit and meat quality traits (Edwards et al. 2008a). The Duroc and Pietrain breeds are used in breeding programs as sire breeds worldwide, and these breeds exhibit variation in carcass merit and meat quality phenotypes. Pietrain pigs have been shown to have less backfat (Affentranger et al. 1996; Edwards et al. 2003) and larger longissimus muscle area (LMA) (Edwards et al. 2003). Duroc and Duroc-sired pigs generally have more favorable meat quality (Affentranger et al. 1996; Edwards et al. 2003; Jeremiah et al. 1999; Langlois and Minvielle 1989), whereas Pietrain and Pietrain-sired pigs are leaner with average meat quality (Edwards et al. 2003).

A line-cross (LC) model, which assumes the founder lines to be fixed for alternative QTL alleles, has been most commonly used to identify QTL for F_2 population designs (Haley et al. 1994). The first genome scan for our Duroc x Pietrain population was performed using a LC analysis (Edwards et al. 2008a; Edwards et al. 2008b). However, for crosses between outbred lines such as domestic animals, not all QTL alleles are completely fixed so effects under the LC model can be biased downwards (Pérez-Enciso and Varona 2000). To identify QTL segregating

within parental breeds, a half-sib (HS) model that does not assume fixation of QTL alleles in the founder lines was introduced by Knott et al. (1996), and Kim et al. (2005) developed a combined line-cross and half-sib (CB) model that accounts for both line and half-sib effects. We have recently utilized LC, HS, and CB models to identify QTL for growth traits in our Duroc x Pietrain population (Choi et al. 2010). The objective of this study was to confirm previously identified carcass merit and meat quality QTL regions with addition of new marker genotypes and additional F_2 animals, and to detect new QTL for carcass merit and meat quality traits using three different least-squares models under different assumptions; 1) founders fixed for alternative QTL alleles (LC model), 2) segregation of QTL alleles at similar frequencies in founders (HS model), and 3) segregation of QTL alleles at different frequencies in founders (CB model).

2. Materials and methods

2.1 Animals and phenotypic data

A three-generation resource population developed at Michigan State University was used for this study. A detailed description of the animals and phenotypic data was previously reported (Edwards et al. 2008a; Edwards et al. 2008b). All grandparents were confirmed to be homozygous normal for the polymorphism at position 1843 in the *RYR1* gene (Edwards et al. 2008b). Animal protocols were approved by the Michigan State University All University Committee on Animal Use and Care (AUF# 09/03-114-00). A total of 954 F_2 pigs were used which included the 510 animals evaluated in the first genome scan. These pigs were produced from 6 F_1 boars and 50 F_1 sows which were retained from 4 F_0 Duroc sires and 15 F_0 Pietrain dams. The F_2 pigs were analyzed for 38 carcass and meat quality traits.

Details of carcass and meat quality phenotype collection were reported in Edwards et al.(2008a). Briefly, animals were slaughtered at the Michigan State University Meat Laboratory (East Lansing, MI) or a federally inspected commercial plant (DeVries Meats, Coopersville, MI). Slaughter age was 165.8 ± 9.2 days and the minimum off-farm body weight (BW) for slaughter was 82.54 kg. Hot carcass weight (HCW), and pH and temperature of the *longissimus* muscle (LM) at 45-min and 24-h postmortem were obtained. After overnight chilling, backfat thickness, number of ribs and carcass length were measured, and the weights of primal cuts were recorded. A single trained evaluator scored color, marbling and firmness using two 2.54-cm thick chops cut from the LM, and objective color scores of CIE L*, a*, and b* were obtained using a Minolta colorimeter. The remaining section of the LM was used to determine drip loss, cook yield, Warner-Bratzler shear force, proximate analysis measures, and sensory attributes. A trained sensory panel evaluated juiciness, tenderness, overall tenderness, connective tissue, and off-flavor using an 8-point hedonic scale. Descriptive statistics for phenotypes used in this study are presented in Table II.1.

2.2 Genotypic data

Nine chromosomes (SSC3 – 7, 12, 15, 16 and 18) were selected based on results of the first genome scan (Edwards et al. 2008a; Edwards et al. 2008b) which had been completed using 510 F_2 animals and 124 microsatellite markers. For the second scan 20 additional microsatellite markers were selected on these chromosomes (1 – 4 markers per chromosome; Choi et al. 2010) in order to increase the power of QTL detection and to narrow the QTL locations. All F_0, F_1, and the 954 F_2 pigs were genotyped for the 20 new markers, and the 444 additional F_2 pigs were also

genotyped for 20 markers flanking the QTL regions on the 9 selected chromosomes. Sex-averaged genetic linkage maps were estimated for all autosomes using CRI-MAP version 2.4 (Green et al. 1990) and converted to the Haldane map function (Choi et al. 2010).

2.3 Statistical analysis

Three different models using least-squares (LC, HS, and CB models) were adopted for QTL analysis (Kim et al. 2005) and analyses were performed using the methods described in Choi et al. (2010). Significance thresholds were determined by False Discovery Rate (FDR; Weller et al. 1998).

The LC analysis assumes the QTL to be fixed for alternative alleles in the founder lines. Probabilities of each F_2 individual being homozygous for two Duroc alleles (P_{11}), homozygous for two Pietrain alleles (P_{22}), or heterozygous (P_{12} or P_{21}) were estimated at fixed 1-cM intervals across the genome using the QTL Express software (Seaton et al. 2002). By denoting the mean of homozygous animals for the Duroc allele as positive additive (a), the mean of heterozygous animals as dominance (d) and the mean of homozygous animals for the Pietrain allele as negative additive (-a), the following linear model was fitted at every cM across the genome.

$$y_j = X_j b + aPa_j + dPd_j + e_j$$

Where y_j is the phenotype of F_2 progeny j, X_j and b are the design matrix and solution vector for the fixed effects, respectively, a and d are the estimated additive and dominance effects of a putative QTL at the given location, respectively, $Pa_j = P_{11} - P_{22}$ is the conditional

expectation of the number of Duroc alleles carried by animal j, $Pd_j = P_{12} + P_{21}$ is the conditional probability of animal j to be heterozygous, and e_j is the residual error.

The HS analysis assumes the QTL to be segregating in the parental breeds, and the 6 F_1 sires were regarded as common parents. QTL Express (Seaton et al. 2002) was used to calculate the probabilities of individuals inheriting allele (A_1) or allele (A_2) from the common F_1 sire (A_1 or A_2) at fixed 1-cM intervals (Knott et al. 1996). In these analyses contrasts were made between the two haplotypes of every F_1 sire.

$$y_{ij} = X_{ij}b + s_i + \alpha \mathrm{HS}_i Ps_{ij} + e_{ij}$$

Where y_{ij} is the phenotype of F_2 progeny j of F_1 sire i, X_{ij} and b are the design matrix and the solution vector for fixed effects, respectively, s_i is the effect of the i th F_1 sire, $\alpha \mathrm{HS}_i$ is the substitution effect for the two putative QTL alleles (A_1 or A_2) carried by the i th F_1 sire, Ps_{ij} is the probability that the F_2 individuals inherited the arbitrary allele (A_{i1}) from F_1 sire i, and e_{ij} is the residual error.

The CB model assumes the QTL to be segregating in the parental breeds.

$$y_{ij} = X_{ij}b + s_i + aPa_{ij} + dPd_{ij} + \alpha CB_i Ps_{ij} + e_{ij}$$

Where y_{ij} is the phenotype of F_2 progeny j of F_1 sire i, X_{ij} and b are the design matrix and the solution vector for fixed effects, respectively, s_i is the effect of the i th F_1 sire, a and d are the additive and dominance effects of breed-origin alleles, respectively, Pa_{ij} and Pd_{ij} are the corresponding breed-origin coefficients as described above, αCB_i is the substitution effect for

the two putative QTL alleles carried by the ith F_1 sire, Ps_{ij} is the probability that the F_2 individuals inherited the arbitrary allele (A_{i1}) from F_1 sire i, and e_{ij} is the residual error. In this model, a and d account for the average effects of breed origin alleles through both the F_1 sire and the F_1 dam and αCB_i represents the difference between the two QTL alleles that a given F_1 sire received from the two parental breeds as a deviation from their average additive effect (Kim et al. 2005). To avoid increasing Type I error rate due to multiple testing, a significance threshold of $q < 0.05$ was used, where q is the FDR corrected p-value. QTL detected using the LC, HS or CB models were declared using the following criteria:

1) LC QTL declared *if* $q_{LC} = min(q_{LC}, q_{HS}) < 0.05$

2) HS QTL declared *if* $q_{HS} = min(q_{LC}, q_{HS}) < 0.05$

3) CB QTL declared *if* $q_{CB} < 0.05$ *and* $q_{LC} > 0.05$ *and* $q_{HS} > 0.05$

A QTL was declared under the CB model only if it had not been previously detected using the LC or HS models.

3. Results

Three different models for QTL analysis revealed a total of 91 QTL for carcass and meat quality traits on all autosomes except SSC11 and 17. The LC analysis revealed 50 QTL (Table II.2) including 14 new QTL on 6 chromosomes (SSC3, 6, 7, 12, 16, and 18) which had not been identified in the first genome scan of this population (Edwards et al. 2008a). The HS analysis revealed 38 QTL, and 3 additional QTL were detected using the CB model (Table II.2). The thresholds used in this study were $-\log_{10}(P) = 3.78$ and $-\log_{10}(P) = 2.88$ at the 1% and 5% FDR

levels, respectively. As an example, the genome scan for ham weight is shown in Figure II.1. At the 1% FDR level, two QTL were identified using the LC model on SSC6 and 7, and one QTL was identified using the HS model on SSC7. At the 5% FDR level, additional QTL were revealed on SSC3 with the LC model and on SSC5, 8 and 9 with the HS model.

3.1 Line-cross analysis

A total of 50 significant QTL were identified on SSC1, 3-10, 12, 14, 16 and 18 using the LC model (Table II.2). Of these, 29 QTL were below the 1% FDR threshold on SSC1, 3, 6, 7, 12, 14 and 18. On SSC1, QTL affecting LMA and spareribs weight detected at 12 cM and 236 cM supported our previous results, but a QTL for dressing percent which was significant at the 1% chromosome-wise level in the first scan of this population (Edwards et al. 2008a) did not reach significance in the second scan. On SSC3, a QTL for 45-min pH was significant at the 1% FDR level, also confirming results from our first scan (Edwards et al. 2008a), and a QTL for ham weight was newly identified at the 5% FDR level.

On SSC6, QTL for moisture and firmness were located in the *S0087 – S0220* interval, QTL influencing meat quality traits were mapped to the *SW2173 – SW1647* interval, and QTL affecting fat deposition and carcass traits were identified in the *SW1647 – SW1881 – SW322* interval (Figure II.2). The QTL detected in these marker intervals showed additive pleiotropic effects indicating that the Duroc allele contributed to increased fat deposition and reduced muscularity. In contrast to SSC6, QTL affecting muscle mass located in the *SW2019 – SW859* interval on SSC7 showed negative additive effects, and the Pietrain allele was associated with higher muscularity. The incorporation of the new SSC7 marker *SW2019* in the *SW1369 – SW850* marker interval allowed refining the QTL position detected in the first scan, as well as increasing

the statistical power and narrowing the QTL interval. A QTL for LMA detected in the *SW859 – S0115* interval in the first scan was repositioned at 86 cM in the *SW2019 – SW859* interval in the second scan.

On SSC12, QTL for fat related traits including marbling score, belly weight and intramuscular fat percent detected in the *SW874 – S0090* interval in the first scan were identified in the second scan in the *SW957 – SW874* interval at the 1% FDR level. In addition, at the 5% FDR level, QTL for a* and b* not identified in the first scan were mapped to 93 cM and 110 cM of SSC12, respectively. A QTL for LMA mapped to 42 cM and QTL for intramuscular fat and moisture percent located at 143 cM were newly discovered on SSC16 in the second scan. In the *SW2540 – SW1023* interval of SSC18, not only was a QTL for spareribs weight confirmed from the first scan, but QTL for carcass length and last-lumber backfat were also newly identified in the second scan.

3.2 Half-sib analysis

HS analysis revealed a total of 38 QTL on SSC2, 3, 5, 7-10, 13-16 and 18 (Table II.2). Of these, 20 QTL indentified on SSC3, 5, 7, 8, 15, 16 and 18 were significant at the 1% FDR level including 13 QTL detected on SSC15.

A QTL affecting 45-min carcass temperature ($q < 0.01$) was detected at 47 cM on SSC3. On SSC5, a QTL for first-rib backfat was declared as a HS QTL ($q < 0.01$) in the second scan, whereas a first-rib backfat QTL had previously been identified in this location with the LC analysis in the first scan (Edwards et al. 2008a). On SSC8, a QTL affecting ham weight was identified at 39 cM, and QTL for off-flavor and cook yield were mapped to the distal region of SSC8 near *S0178*. In addition, a QTL for ham weight on SSC9 and a QTL for protein percent on

SSC10 were identified ($q < 0.01$). On SSC16, HS QTL were identified for L* ($q < 0.02$), a* ($q < 0.01$) and moisture percent ($q < 0.02$). A highly significant HS QTL influencing spareribs weight ($q < 0.0007$) was detected on SSC18 with an estimated location at 70 cM. The location of the LC QTL for spareribs weight on SSC18 was estimated at 24 cM so these QTL were considered to be unique QTL.

On SSC7, QTL affecting ham weight and number of ribs were identified in the *SW859 – SW2040 – S0115* interval ($q < 0.01$), and QTL for marbling score and loin weight significant at the 5% FDR level were located in the same interval. For ham weight, the QTL identified with the HS analysis was mapped to 139 cM ($q < 0.0002$), whereas the ham weight QTL revealed with the LC analysis was mapped to 104 cM ($q < 0.006$). Since these QTL detected by the different models mapped to distinct locations, they were considered to be separate unique QTL.

The HS analysis revealed evidence for QTL influencing meat quality traits in the *SW1683 – SW906 – SW1983* interval on SSC15 (Figure II.3). In the *SW1683 – SW906* interval, a QTL for protein percent had the highest test statistic ($-\log_{10}(P) = 27.55$; $q < 0.0001$) among the QTL detected on SSC15. In addition, a QTL for 24-h pH, a trait that is associated with many other meat quality traits, was highly significant ($q < 0.0001$; Figure II.3). The LC analysis also revealed significant QTL for these traits in the same interval, but the HS model showed much higher statistical evidence.

3.3 Combined analysis

In addition to QTL identified with the LC and HS analyses, three additional QTL exceeded the 5% FDR significance threshold using the CB analysis. A QTL for pH decline from 45-min to 24-h was mapped to 117 cM on SSC3, a QTL for spareribs weight was detected in the *SW2019 –*

SW859 interval on SSC7 and a QTL for 24-h carcass temperature was found in the *SW2540* – *SW1023* interval on SSC18. Although the statistical power was sufficient to detect QTL, the CB model revealed a small number of additional QTL because most QTL had been declared using either the LC or HS models due to higher test statistics with these analyses.

3.4 Effect of additional markers and animals on QTL detection

The QTL analyses under three different models revealed QTL for pH associated traits at different positions on SSC3 (Figure II.4). The first scan of this chromosome using the LC model had revealed QTL for 45-min pH and pH decline from 45-min to 24-h postmortem (Edwards et al. 2008a). The second scan included two additional markers and genotypes for additional F_2 pigs. A QTL for 45-min pH using the LC model ($q \leq 0.0076$) was mapped at 135 cM near marker *SW2047* (134.8 cM) and a QTL for pH decline from 45-min to 24-h was detected using the CB model ($q \leq 0.0469$) at 117 cM near marker *ACTG2* (116.5 cM) which did not reach the significance threshold in the LC analysis ($q \leq 0.065$). In addition, a QTL for 45-min carcass temperature was detected using the HS model ($q \leq 0.002$) located at 47 cM in the *SW2021* – *S0206* marker interval. These results confirm results of the first scan for 45-min pH and pH decline, and add new results for 45-min carcass temperature.

We have recently used LC, HS and CB models to identify QTL for growth traits in our Duroc x Pietrain resource population, and we reported that additional markers and animals contributed to reduce the confidence intervals and increase the test statistics for QTL detection (Choi et al. 2010). For the present study, QTL affecting the a* and b* objective color measures were newly detected on SSC12 ($q < 0.04$). In order to determine how the QTL peaks for these traits were changed, analyses were performed under 4 different scenarios; 5 and 7 markers with

510 and 948 animals (Figure II.5). The results indicated that increasing the number of animals or increasing the number of markers was effective in increasing the power to detect QTL on this chromosome, and that increasing the numbers of both animals and markers allowed detection of the a* and b* QTL.

4. Discussion

This study identified 91 QTL for pig carcass and meat quality traits located on all autosomes except SSC11 and 17 using three least-squares Mendelian analysis models. The LC analysis, which detected QTL segregating between breeds, revealed 50 QTL including 13 new QTL on 6 chromosomes (SSC3, 6, 7, 12, 16, and 18) that had not been identified in the first genome scan of this population (Edwards et al. 2008a). The HS analysis, which detected QTL segregating within breeds, revealed 38 QTL including 18 on SSC15. Three additional QTL were detected using the CB model (Kim et al. 2005).

Application of the three different models for SSC3 identified not only QTL influencing muscularity under the LC model, but also QTL affecting pH and carcass temperature using all three models. The LC QTL for 45-min pH detected at 135 cM near *SW2047* confirmed the 45-min pH QTL observed in the first scan (Edwards et al. 2008a). Beeckmann et al. (2003) reported a QTL for 45-min pH at the same interval in a Wild boar x Meishan F_2 population. Several studies (de Koning et al. 2003; Evans et al. 2003; Óvilo et al. 2002a; Wimmers et al. 2006) reported QTL affecting muscle pH in the *SW2021 – S0206* marker interval, a region where we identified a QTL for 45-min carcass temperature under the HS model. In addition, Duan et al. (2009) reported a QTL for pH decline from 45-min to 3-h in the *SW2021 – S0206* interval in a

White Duroc x Chinese Erhualian population. We also detected a QTL for pH decline from 45-min to 24-h under the CB model, however, our QTL was located at 117 cM near *ACTG2*.

Significant QTL affecting backfat thickness were located on SSC6 within the *SW1647 – SW1881 – SW322* marker interval at 160 – 174 cM. A 10,000 bootstrap permutation analysis showed the average QTL positions for each backfat trait to be located in the 160.12 – 167.96 cM region. The 95% confidence interval for 10th-rib backfat was estimated to be 159 – 165 cM (6 Haldane cM), which was considerably narrowed from the 38.5 Haldane cM interval observed for the first scan (Edwards et al. 2008a). Not only were QTL affecting fat deposition traits observed in this region, but QTL influencing muscularity were also identified at the same marker interval since Duroc alleles contributed to both fat accumulation and reduced muscle content.

Our results for backfat thickness traits were in agreement with other studies (Malek et al. 2001b; Óvilo et al. 2002b; Varona et al. 2002) that identified QTL for fatness traits in this region of SSC6. This region includes the leptin receptor (*LEPR*) gene which is considered as a potential candidate gene for fatness (Ernst et al. 1997; Mohrmann et al. 2006; Óvilo et al. 2005), and studies to identify a causal mutation in *LEPR* have been conducted (Mackowski et al. 2005; Muñoz et al. 2009). We also observed a QTL for intramuscular fat percent using the LC model in a position more proximal to this region of SSC6 at 141 cM, which coincided with a backfat thickness QTL detected with the HS model in a Duroc x Pietrain population by Liu et al. (2008). The SSC6 region affecting intramuscular fat percent also included QTL for marbling score and a*, which were all mapped to 141 – 152 cM in the *SW2173 – SW1647* interval. The confidence interval for these QTL did not overlap with the confidence interval for 10th-rib backfat. This result was consistent with previous studies (Óvilo et al. 2005; Szyda et al. 2003) which reported that QTL effects for backfat and intramuscular fat content resulted from different closely linked

loci on SSC6. QTL affecting intramuscular fat content have been reported (de Koning et al. 2000; Grindflek et al. 2001) in the same region where we detected a QTL for marbling score, although no other reports of subjective marbling score QTL in this SSC6 region have been reported. Also, Harmegines et al. (2006) identified QTL for a* as well as fat thickness in this same region.

The different models revealed distinct QTL regions on SSC7 with LC and HS QTL identified at 84 – 104 cM and at 130 – 178 cM, respectively. A highly significant QTL influencing muscle mass identified in the *SW2019 – SW859* interval had an additive effect for which Duroc alleles increased carcass length and decreased dressing percent, LMA and ham weight. In this region, Yue et al. (2003) found a 1% genome-wide level significant QTL influencing carcass composition traits such as carcass length in a Wild boar x Meishan population. Liu et al. (2008) reported QTL for carcass length and dressing percent with similar allelic substitution effect in their Duroc x Pietrain population as we observed in our study. However, Nezer et al. (2002) identified a QTL for carcass length at the more distal position from our QTL in a Pietrain x Large White population. In addition, Sato et al. (2003) detected a QTL for dressing percent in a Duroc x Meishan population in the same region as our study. A QTL for number of ribs was detected using the HS analysis. A QTL for number of ribs had been detected in this position at the 1% genome-wise significance level using the LC analysis in the first scan (Edwards et al. 2008a), however, evidence from the second scan suggests the HS model better describes the QTL allele frequency in the parental breeds. Also on SSC7, analyses using both the LC and HS models identified QTL for ham weight at different locations, which were in the *SW2019 – SW859* interval with the LC model and in the *SW859 – SW2040* interval with the HS model. Similarly, Milan et al (2002) also reported suggestive QTL for ham weight at different

positions using LC and HS models, and their LC QTL detected in the *SLA – S0102* marker interval was in a similar region to our LC QTL.

We have recently used LC, HS and CB models to identify QTL for growth traits in our Duroc x Pietrain resource population, and we reported that additional markers and animals contributed to reduce the confidence intervals and increase the test statistics for QTL detection (Choi et al. 2010). In the present study, genotyping of additional markers and animals increased the statistical power and facilitated discovery of new QTL which had not been observed in the first scan (Edwards et al. 2008a). For example, QTL for the objective color measures of a* and b* were identified on SSC12 with the addition of more F_2 pigs and more marker genotypes using the LC analysis. The LC analysis also identified QTL on SSC12 related to intramuscular fat percent and moisture at 47 – 50 cM and at 69 cM, respectively. A QTL for marbling was located in the *SW957 – SW874* marker interval, whereas the position of this QTL had been more distal for the first scan (Edwards et al. 2008a). The additive effects of these QTL indicated that Duroc alleles increased marbling and intramuscular fat percent, and decreased moisture percent. Harmegnies et al. (2006) also reported a QTL for a* although at a more distal position than our current result, and Malek et al. (2001a) detected a QTL for subjective color score in the same region as our result.

The half-sib analysis revealed strong evidence for QTL affecting meat quality traits on SSC15 at 74 – 90 cM in the *SW1683 – SW1983* marker interval where 13 and 2 QTL were significant at the 1% and 5% FDR levels, respectively, including a highly significant QTL for protein percent. Significant QTL had been identified in this region using the LC analysis in the first scan (Edwards et al. 2008a), and a negative additive effect had been seen for protein percent, color and tenderness traits suggesting contributions from segregation of Pietrain alleles. The

pleiotropic effects of Pietrain alleles contributing to leanness resulted in effects on other meat quality traits resulting in more muscularity, paler muscle color and less tenderness. QTL for 24-h pH, L* and tenderness significant at the 1% genome-wise level were identified in this region of SSC15 in a Berkshire x Yorkshire population (Kim et al. 2005; Malek et al. 2001a; Thomsen et al. 2004). Very few studies have measured the trait of protein percent and no QTL for protein percent have been reported on SSC15.

Several candidate genes such as myostatin (*MSTN*), Titin (*TTN*) and protein kinase AMP-activated gamma 3 (*PRKAG3*) are located in the *SW1683 – SW1983* interval (Davoli et al. 2003; Milan et al. 2000; Sonstegard et al. 1998). Stinckens et al. (2008) reported that pigs of the Pietrain breed had higher muscularity as a result of association between a polymorphism in the *MSTN* MEF3 binding site and muscle mass. Wimmers et al. (2007) reported that a polymorphism in *TTN* was associated with leanness in a Pietrain population. Also, Milan et al. (2000) mapped the *PRKAG3* gene in the *SW1683 – SW1983* marker interval and Ciobanu et al. (2001) identified three polymorphic sites in the *PRKAG3* gene that affect meat quality traits including 24-h pH.

5. Conclusion

This study used a Duroc x Pietrain F_2 resource population and identified a total of 91 QTL for carcass merit and meat quality phenotypes. Three different least-squares models were applied under different assumptions; 1) founders fixed for alternative QTL alleles (line-cross model), 2) segregation of QTL alleles at similar frequencies in founders (half-sib model), and 3) segregation of QTL alleles at different frequencies in founders (combined model). The addition of new marker and animal genotypes contributed to increasing the statistical power for QTL detection,

and the application of alternative models allowed confirmation of QTL and detection of new QTL segregating either between or within breeds.

Acknowledgements

This project was supported by National Research Initiative Competitive Grant no. 2004-35604-14580 from the USDA National Institute of Food and Agriculture. We thank D. Edwards for technical contributions in development of the pig resource population.

Table II.1. Number of records, means, and standard deviations for carcass and meat quality traits.

Traits	N	Mean	SD
Carcass measure			
Off-farm BW, kg	948	112.08	8.56
Hot carcass weight, kg	948	81.84	6.81
Dressing percent, %	948	73.01	2.11
45 min carcass temperature, °C	947	39.47	2.23
24 h carcass temperature, °C	945	2.91	1.19
45 min pH	934	6.37	0.22
24 h pH	927	5.51	0.14
45 min - 24h pH decline	914	0.86	0.22
carcass length, cm	947	78.72	2.51
Number of ribs	669	14.83	0.85
First rib backfat, mm	859	40.67	7.09
Last rib backfat, mm	947	28.69	6.38
Last lumbar vertebra backfat, mm	946	22.25	6.23
Tenth rib backfat, mm	941	24.16	7.35
Longissimus muscle area, cm^2	942	40.61	4.74
Primal cut weight			
Ham weight, kg	947	9.63	0.77
Loin weight, kg	947	8.28	0.83
Boston shoulder weight, kg	947	3.90	0.56
Picnic shoulder weight, kg	947	3.72	0.57
Belly weight, kg	947	5.03	0.67
Spareribs weight, kg	943	1.53	0.20
Meat quality evaluation			
Color, 1-6	945	3.16	0.82
Marbling, 1-10	946	2.82	0.84
Firmness, 1-5	932	2.86	0.79
L*	900	53.77	2.24
a*	900	17.25	1.83
b*	900	9.13	1.61
Proximate analysis			
Moisture, %	936	73.94	1.53
Fat, %	936	3.18	1.40
Protein, %	935	23.44	1.13
Laboratory analyses			
Drip loss, %	946	1.85	1.18
Cook yield, %	936	77.26	2.83
Warner-Bratzler shear force, kg	935	3.21	0.69
Sensory panel analyses			
Juiciness, 1-8	942	5.23	0.59
Tenderness, 1-8	942	5.55	0.62
Overall tenderness, 1-8	942	5.63	0.55
Connective tissue, 1-8	942	6.39	0.39
Off-flavor, 1-8	942	1.14	0.21

Table II.2. Position and significance level of carcass and meat quality trait QTL.

Chr[1]	Position[2]	Trait	Type[3]	-log$_{10}P$[4]	FDR[5]	Flanking Markers	Additive[6]	Dominance[7]
1	12	LM area, cm^2	LC	3.48	0.0177	SW1514 – SW1515	-1.27 (0.32)	0.45 (0.59)
	236	Spareribs wt, kg	LC	5.36	0.0005	SW974 – S0056	0.02 (0.02)	-0.12 (0.02)
2	81	Juiciness, 1 to 8	HS	2.98	0.0471	S0170 – SW1026		
	100	45-m carcass temperature, °C	HS	3.00	0.0460	SW1026 – S0370		
3	47	45-m carcass temperature, °C	HS	4.73	0.0020	SW2021 – S0206		
	97	Ham wt, kg	LC	3.68	0.0121	S0206 – SWR978	0.11 (0.03)	0.03 (0.05)
	117	45-min to 24-h pH decline	CB	3.21	0.0469	ACTG2 – SW2141		
	135	45-min pH	LC	3.92	0.0076	SW2047 – SW2408	-0.04 (0.01)	0.00 (0.02)
	151	Loin wt, kg	LC	3.36	0.0215	SW2047 – SW2408	0.02 (0.03)	-0.20 (0.05)
4	19	Off-farm BW, kg	LC	2.91	0.0473	SW2509 – S0301	-1.58 (0.45)	0.78 (0.77)
	21	HCW, kg	LC	3.11	0.0334	SW2509 – S0301	-1.30 (0.35)	0.56 (0.61)
5	94	First-rib backfat, mm	HS	3.95	0.0083	SWR453 – SW2		
	151	24-h carcass temperature, °C	LC	3.14	0.0317	S0005 – S0018	0.04 (0.03)	0.22 (0.06)
	173	Ham wt, kg	HS	3.13	0.0364	S0018 – IGF1		
6	103	Picnic shoulder wt, kg	LC	3.39	0.0204	SW2525 – S0087	-0.05 (0.02)	0.05 (0.02)
	114	Moisture, %	LC	5.12	0.0008	S0087 – S0220	-0.25 (0.08)	0.48 (0.13)
	124	Firmness, 1 to 5	LC	4.31	0.0037	S0220 – SW122	0.17 (0.04)	-0.03 (0.06)
	141	Fat, %	LC	19.03	0.0000	SW2173 – SW1647	0.56 (0.06)	-0.23 (0.09)
	146	Marbling, 1 to 10	LC	16.18	0.0000	SW2173 – SW1647	0.34 (0.04)	-0.14 (0.06)
	152	a*	LC	4.40	0.0031	SW2173 – SW1647	0.12 (0.05)	0.24 (0.07)
	160	First-rib backfat, mm	LC	6.71	0.0000	SW1647 – SW1881	1.54 (0.30)	-1.19 (0.47)
	162	Tenth-rib backfat, mm	LC	35.82	0.0000	SW1647 – SW1881	3.20 (0.25)	-1.65 (0.38)
	162	Carcass length, cm	LC	10.42	0.0000	SW1647 – SW1881	-0.46 (0.11)	0.55 (0.16)
	163	Loin wt, kg	LC	19.86	0.0000	SW1647 – SW1881	-0.21 (0.02)	0.18 (0.04)
	164	Last lumbar vertebra backfat, mm	LC	14.87	0.0000	SW1647 – SW1881	1.95 (0.25)	-1.51 (0.39)
	168	Belly wt, kg	LC	3.78	0.0101	SW1881 – SW322	0.06 (0.02)	-0.06 (0.02)
	174	Ham wt, kg	LC	10.38	0.0000	SW1881 – SW322	-0.16 (0.02)	0.08 (0.04)
	174	Last-rib backfat, mm	LC	7.47	0.0000	SW1881 – SW322	1.28 (0.27)	-1.62 (0.43)
	175	LM area, cm^2	LC	8.30	0.0000	SW1881 – SW322	-1.30 (0.21)	0.61 (0.35)
	179	Protein, %	LC	5.41	0.0005	SW1881 – SW322	-0.30 (0.06)	0.09 (0.10)
	182	HCW, kg	LC	3.10	0.0341	SW1881 – SW322	0.44 (0.35)	-2.23 (0.61)

Table II.2. (Cont'd).

Chr[1]	Position[2]	Trait	Type[3]	-log$_{10}P$[4]	FDR[5]	Flanking Markers	Additive[6]	Dominance[7]
6	183	24-h carcass temperature, °C	LC	4.98	0.0010	SW1881 – SW322	0.13 (0.03)	-0.11 (0.05)
7	15	Protein, %	LC	3.44	0.0186	S0025 – S0064	-0.27 (0.07)	-0.23 (0.15)
	75	Spareribs wt, kg	CB	3.40	0.0339	SW2019 – SW859		
	84	Dressing percent, %	LC	12.04	0.0000	SW2019 – SW859	-0.81 (0.11)	-0.05 (0.19)
	86	Carcass length, cm	LC	11.50	0.0000	SW2019 – SW859	0.88 (0.13)	0.29 (0.23)
	97	LM area, cm^2	LC	8.25	0.0000	SW2019 – SW859	-1.60 (0.26)	0.21 (0.46)
	104	Ham wt, kg	LC	4.05	0.0060	SW2019 – SW859	-0.12 (0.03)	-0.02 (0.04)
	130	Marbling, 1 to 10	HS	2.97	0.0480	SW859 – SW2040		
	139	Ham wt, kg	HS	6.13	0.0002	SW859 – SW2040		
	141	Loin wt, kg	HS	3.83	0.0102	SW859 – SW2040		
	178	Number of ribs	HS	9.23	0.0000	S0115 – SW632		
8	39	Ham wt, kg	HS	2.96	0.0490	SW905 – SWR1101		
	126	LM area, cm^2	LC	2.97	0.0429	S0017 – SW2160	-0.84 (0.24)	-0.40 (0.37)
	205	Off-flavor, 1 to 8	HS	4.48	0.0031	SW1085 – S0178		
	214	Cook yield, %	HS	3.73	0.0122	SW1085 – S0178		
9	0	Drip loss, %	LC	2.94	0.0449	SW21	-0.04 (0.07)	0.35 (0.10)
	25	Ham wt, kg	HS	3.11	0.0380	SW983 – SW911		
10	0	Overall tenderness, 1 to 8	LC	2.90	0.0484	SWR136	0.04 (0.04)	0.19 (0.05)
	21	Protein, %	HS	3.06	0.0414	SWR136 – SW249		
	72	Connective tissue, 1 to 8	LC	2.99	0.0410	SW1041 – SW920	0.08 (0.04)	0.17 (0.07)
12	47	Marbling, 1 to 10	LC	5.50	0.0004	SW957 – SW874	0.23 (0.04)	0.03 (0.07)
	50	Belly wt, kg	LC	4.14	0.0051	SW957 – SW874	0.09 (0.02)	-0.01 (0.03)
	50	Fat, %	LC	3.97	0.0070	SW957 – SW874	0.30 (0.07)	-0.11 (0.12)
	69	Moisture, %	LC	5.80	0.0002	SW37 – S0090	-0.36 (0.07)	0.08 (0.11)
	93	b*	LC	3.72	0.0112	S0090 – SWC23	0.17 (0.04)	0.08 (0.07)
	110	a*	LC	3.07	0.0360	SWC23 – SW2180	0.19 (0.05)	0.08 (0.08)
13	122	Last-rib backfat, mm	HS	2.96	0.0488	SW398 – SW2440		
14	62	a*	LC	3.30	0.0239	SW210 – SW886	-0.29 (0.07)	0.06 (0.13)
	73	Boston shoulder wt, kg	HS	3.18	0.0336	SW210 – SW886		
	136	Belly wt, kg	LC	4.26	0.0041	SW1557 – SWC27	0.06 (0.03)	-0.26 (0.06)
15	70	Loin wt, kg	HS	3.57	0.0160	S0088 – SW1683		

Table II.2. (Cont'd).

Chr[1]	Position[2]	Trait	Type[3]	-log$_{10}P$[4]	FDR[5]	Flanking Markers	Additive[6]	Dominance[7]
15	71	First-rib backfat, mm	HS	3.11	0.0383	S0088 – SW1683		
	72	Tenth-rib backfat, mm	HS	3.77	0.0115	S0088 – SW1683		
	74	Color, 1 to 6	HS	4.43	0.0033	SW1683 – SW906		
	76	L*	HS	4.74	0.0020	SW1683 – SW906		
	78	Juiciness, 1 to 8	HS	4.56	0.0027	SW1683 – SW906		
	78	Moisture, %	HS	5.59	0.0004	SW1683 – SW906		
	80	Warner-Bratzler shear force, kg	HS	5.51	0.0005	SW1683 – SW906		
	80	Overall tenderness, 1 to 8	HS	10.22	0.0000	SW1683 – SW906		
	80	Protein, %	HS	27.55	0.0000	SW1683 – SW906		
	80	Tenderness, 1 to 8	HS	9.72	0.0000	SW1683 – SW906		
	81	a*	HS	4.51	0.0029	SW1683 – SW906		
	81	24-h pH	HS	10.48	0.0000	SW1683 – SW906		
	82	Firmness, 1 to 5	HS	3.35	0.0240	SW1683 – SW906		
	83	Drip loss, %	HS	10.36	0.0000	SW906 – SW1983		
	85	Cook yield, %	HS	16.06	0.0000	SW906 – SW1983		
	87	Connective tissue, 1 to 8	HS	4.89	0.0015	SW906 – SW1983		
	90	Belly wt, kg	HS	3.38	0.0226	SW906 – SW1983		
16	42	LM area, cm^2	LC	3.58	0.0145	SW419 – SW1454	0.80 (0.20)	-0.23 (0.32)
	66	L*	HS	3.48	0.0191	SW1454		
	70	a*	HS	4.91	0.0015	SW1454 – SW2517		
	97	Moisture, %	HS	3.45	0.0199	SW2517 – SW1897		
	143	Fat, %	LC	3.24	0.0265	S0061	0.18 (0.06)	0.22 (0.09)
	143	Moisture, %	LC	3.35	0.0219	S0061	-0.14 (0.07)	-0.31 (0.09)
18	0	Tenth-rib backfat, mm	LC	3.00	0.0408	SW1808	0.96 (0.26)	0.04 (0.35)
	4	Carcass length, cm	LC	5.67	0.0003	SW2540 – SW1023	-0.49 (0.10)	-0.23 (0.14)
	24	Spareribs wt, kg	LC	5.13	0.0008	SW2540 – SW1023	-0.04 (0.01)	0.00 (0.02)
	30	Last lumbar vertebra backfat, mm	LC	4.40	0.0031	SW2540 – SW1023	1.60 (0.35)	0.01 (0.64)
	33	24-h carcass temperature, °C	CB	3.40	0.0339	SW2540 – SW1023		
	70	Spareribs wt, kg	HS	5.34	0.0007	SW1023 – SW1984		

[1]Chr = chromosome

Table II.2. (Cont'd).

[2]Position in Haldane cM

[3]LC = QTL declared as line-cross type; HS = half-sib type; CB = combined type.

[4]Negative logarithm of the comparison-wise p value of the test statistic against the null hypothesis of no QTL at the most likely position for the inferred QTL model.

[5]FDR=false discovery rate

[6]Estimates of additive effects with standard errors for LC QTL. The effects are expressed as (DD-PP)/2, where D = Duroc allele and P = Pietrain allele.

[7]Estimates of dominance effects with standard errors for LC QTL. The effects are expressed as DP-PD, where D = Duroc allele and P = Pietrain allele.

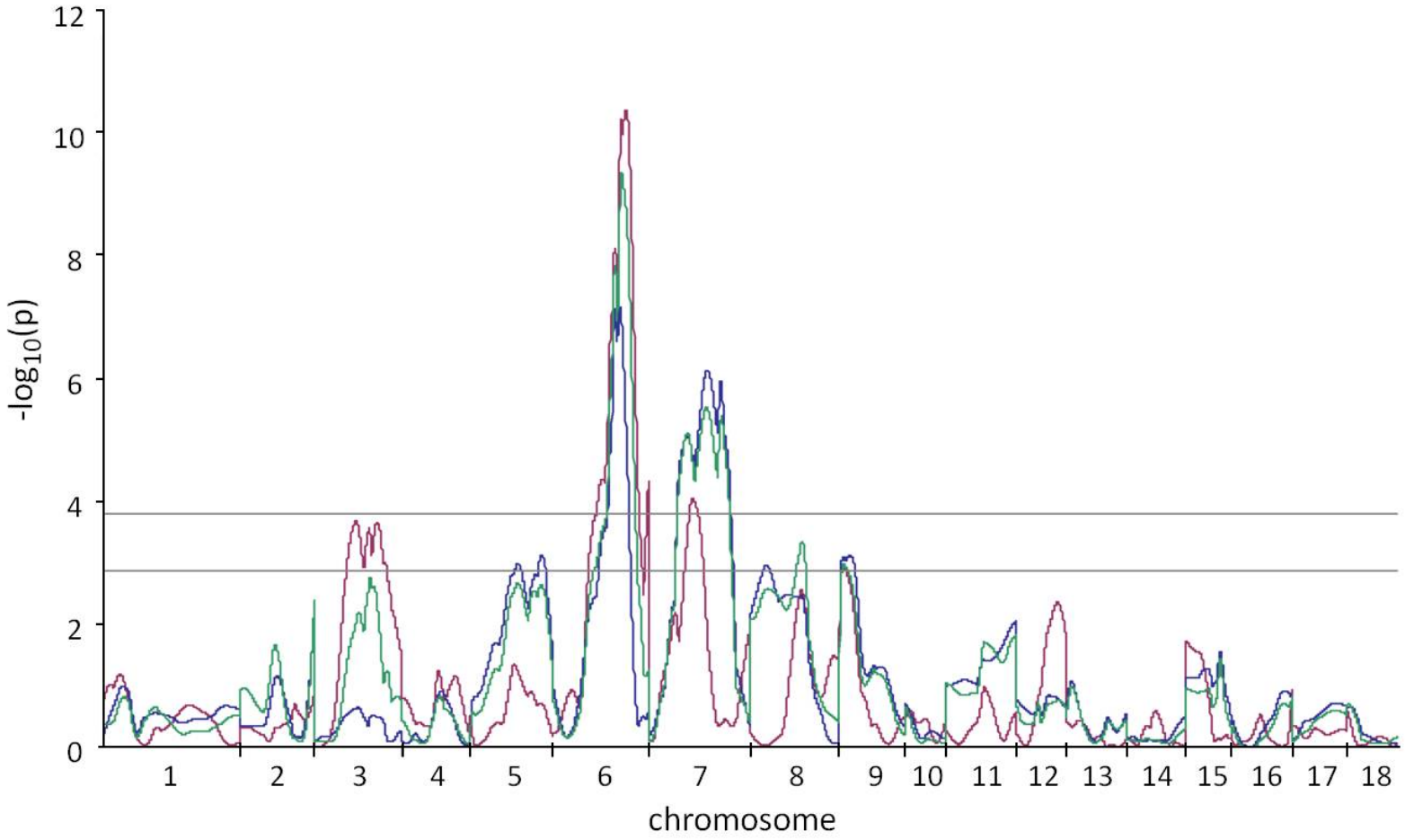

Figure II.1. Genome scan results for ham weight determined using different analysis models.
A whole genome scan to identify QTL for the trait ham weight was performed using three different analysis models (line-cross, red line; half sib, blue line; combined line-cross and half-sib, green line). The X-axis indicates positions of chromosomes 1 to 18. Horizontal lines indicate significance thresholds (lower line, 5% FDR; upper line, 1% FDR).

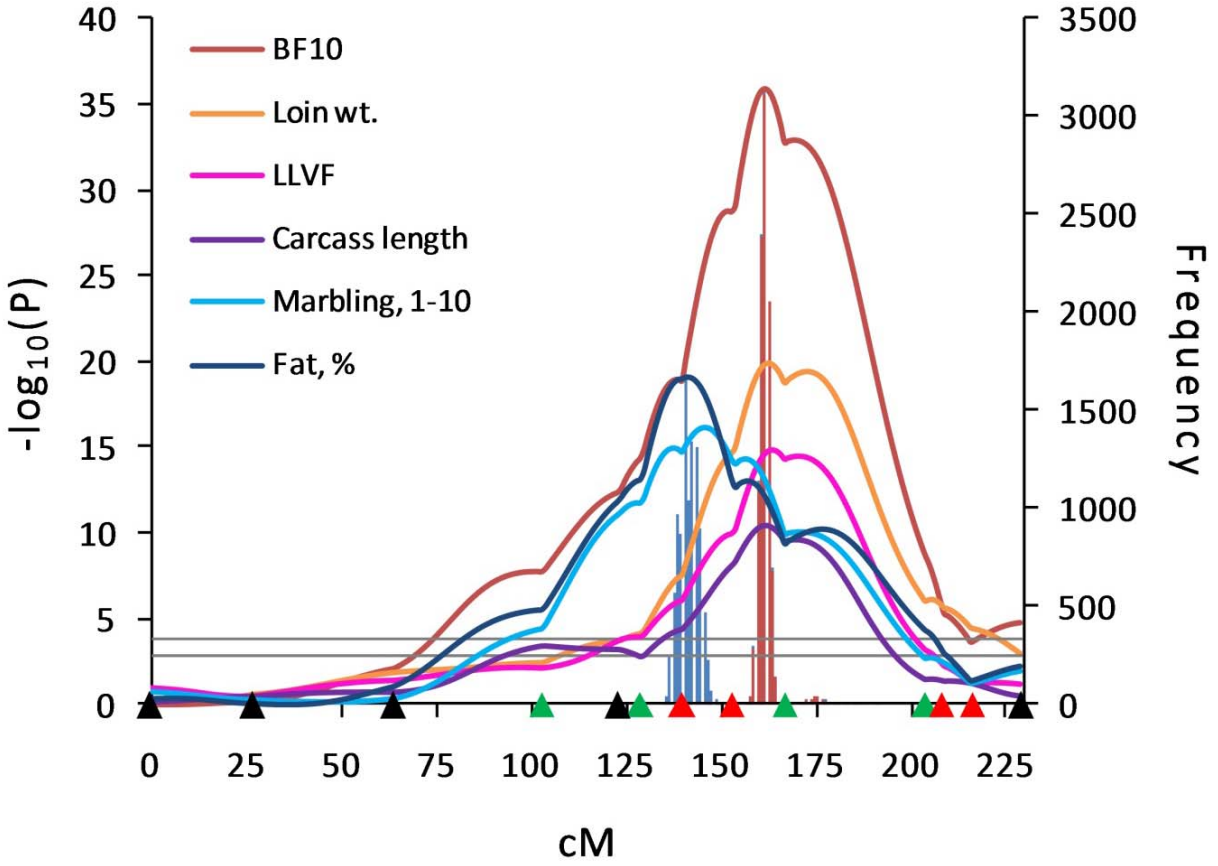

Figure II.2. Line-cross analysis indicated strong evidence for QTL influencing fat deposition traits on SSC6.
Highly significant QTL for traits related to fat deposition were identified on SSC6. Confidence intervals for fat percentage and 10th rib backfat (BF10) were estimated using 10,000 bootstrap permutations as 136 – 146 cM (blue bar) and 159 – 165 cM (red bar), respectively. Marker positions are shown as triangles on the X-axis (black, markers used for both QTL scans and genotyped only in 510 animals; green, markers used for both QTL scans and genotyped in all animals; red, markers used for second scan only and genotyped in all animals). Horizontal lines indicate significance thresholds (lower line, 5% FDR; upper line, 1% FDR).

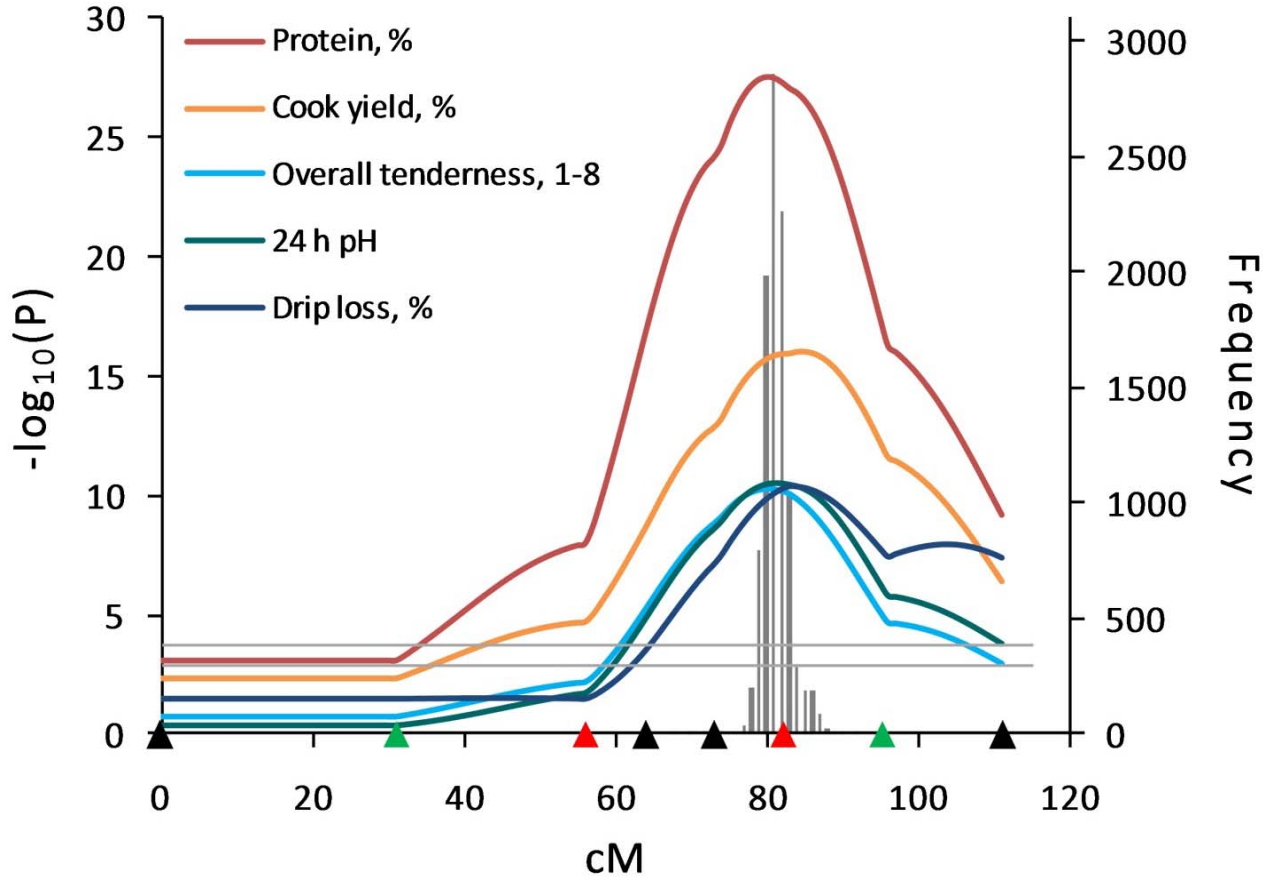

Figure II.3. Half-sib analysis indicated strong evidence for QTL influencing meat quality traits on SSC15.
Highly significant QTL for meat quality traits were identified on SSC15. Confidence interval for protein percentage was estimated by 10,000 bootstrap permutations as 77 – 85 cM (red bar). Marker positions are shown as triangles on the X-axis (black, markers used for both QTL scans and genotyped only in 510 animals; green, markers used for both QTL scans and genotyped in all animals; red, markers used for second scan only and genotyped in all animals). Horizontal lines indicate significance thresholds (lower line, 5% FDR; upper line, 1% FDR).

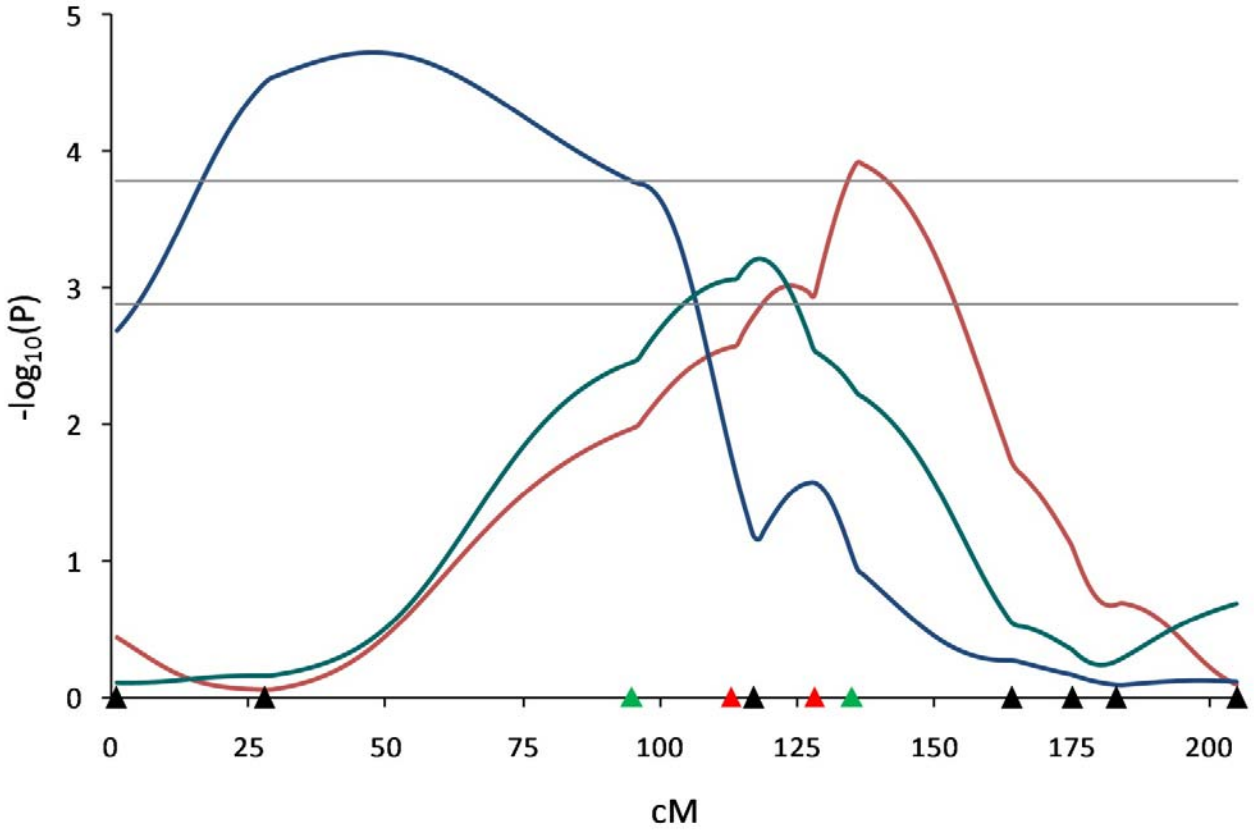

Figure II.4. QTL results determined by different models for pH related traits on SSC3.
Line-cross model detected a QTL ($q < 0.01$) for 45 min pH (red line) and half-sib model detected a QTL ($q < 0.01$) for 45 min carcass temperature (blue line). Combined model identified a QTL ($q < 0.01$) affecting pH decline from 45 min to 24 h (green line). Marker positions are shown as triangles on the X-axis (black, markers used for both QTL scans and genotyped only in 510 animals; green, markers used for both QTL scans and genotyped in all animals; red, markers used for second scan only and genotyped in all animals). Horizontal lines indicate significance thresholds (lower line, 5% FDR; upper line, 1% FDR).

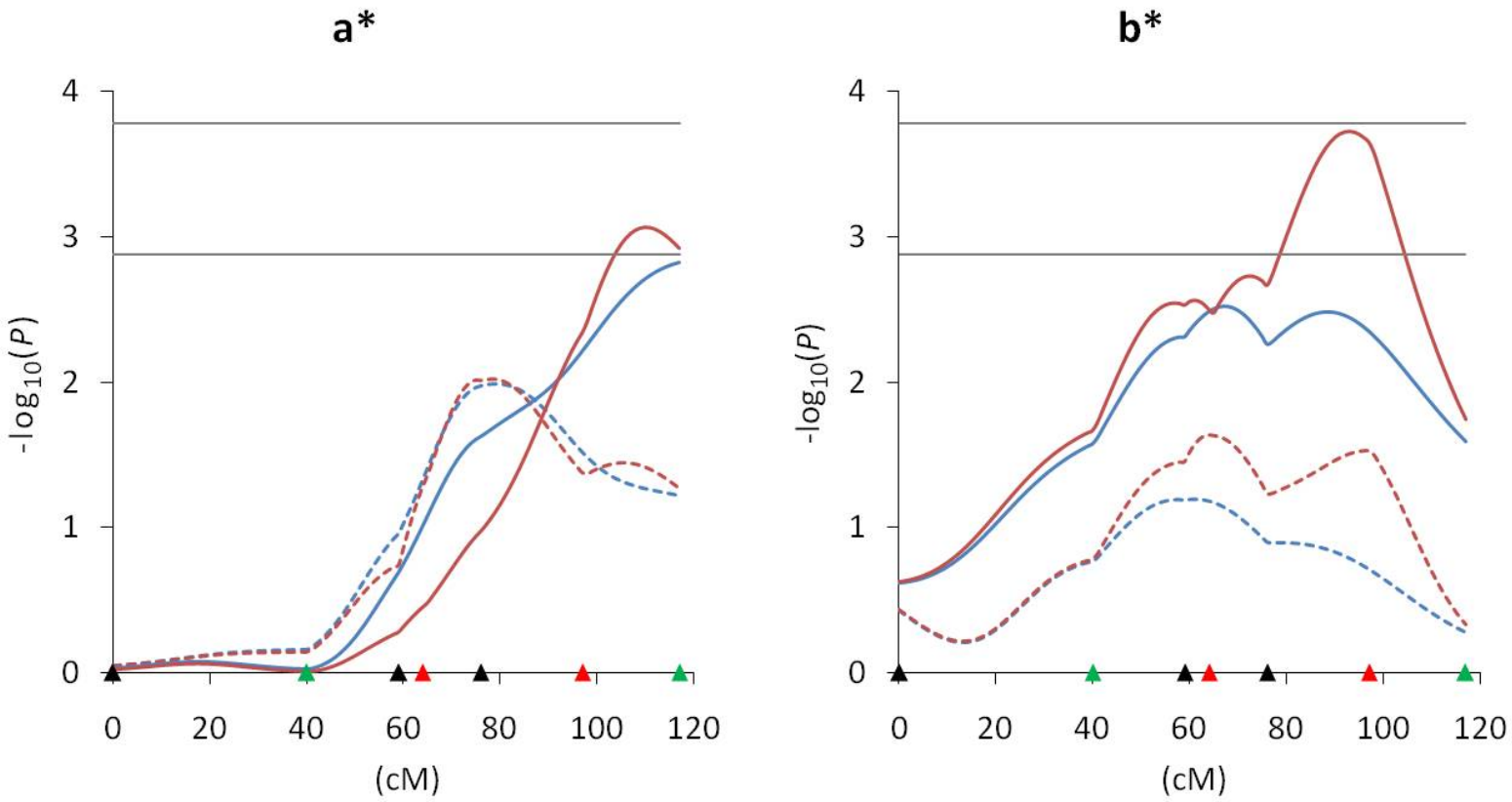

Figure II.5. Effect of additional markers and animals for detecting meat color QTL on SSC12.
Effects of additional marker genotypes and animals for detecting QTL for a* and b* objective meat color measures on SSC12 were compared under four different scenarios (5 and 7 markers with 510 F_2 animals, 5 and 7 markers with 948 F_2 animals). Blue lines indicate 5 markers (black and green triangles on the X-axis) and red lines indicate 7 markers (black, green and red triangles on the X-axis). Solid lines indicate 948 animals and dotted lines indicate 510 animals. Marker positions are shown as triangles on the X-axis (black, markers used for both QTL scans and genotyped only in 510 animals; green, markers used for both QTL scans and genotyped in all animals; red, markers used for second scan only and genotyped in all animals). Horizontal lines indicate significance thresholds (lower line, 5% FDR; upper line, 1% FDR).

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Chapter III

Evaluation of QTL for carcass merit and meat quality traits in an US commercial Duroc population

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This chapter formatted for submission to the journal Meat Science

Abstract

Efforts for deciphering the genetic basis of polygenic traits such as carcass merit and meat quality traits aim to identify genetic markers that can be used for marker assisted selection in the pig industry. Our previous genome scans revealed quantitative trait loci (QTL) influencing carcass merit and meat quality traits in a Duroc x Pietrain F_2 resource population. Putative QTL regions on 5 chromosomes (SSC3, 6, 12, 15, and 18) were selected for further evaluation in an US commercial Duroc population. A total of 81 gene-specific single nucleotide polymorphism (SNP) markers spanning these QTL regions were genotyped and 33 markers were segregating. These markers were evaluated for associations with pH, color, marbling, days to 113 kg, backfat thickness, and *longissimus* muscle area (LMA) in the Duroc population (n = 331). Association analyses were performed using a linear mixed model, and additive and dominance effects were estimated for each trait. The *MDH1* SNP on SSC3 was associated with 45-min and ultimate pH, and pH decline from 45-min to 24-h. A combined genotype of *PRKAG3* T30N and I199V on SSC15 was associated with ultimate pH. The *HSPG2* SNP on SSC6 was associated with marbling score and days to 113 kg. Markers for *NUP88* and *FKBP10* on SSC12 were associated with 45-min pH and L*, respectively. A significant association was observed for the SSC15 marker *SF3B1* with L* and LMA and for the SSC18 marker *ARF5* with ultimate pH and color score. The results of the present study in an US commercial Duroc population showed a general consistency with our previous genome scan.

Keywords: pig, carcass merit, meat quality, single nucleotide polymorphisms

1. Introduction

Meat quality has always been an important consideration for consumers along with cost, and the recent development of niche markets for pork represents consumers' various needs and concerns for meat quality (Brewer, Zhu, & McKeith, 2001; Honeyman, Pirog, Huber, Lammers, & Hermann, 2006). Conventional swine selection programs have been implemented mainly for production efficiency such as growth rate, feed conversion, and sow productivity since meat quality phenotypic data must usually be collected postmortem, and carcass value predictions varied with different carcass evaluation technology (Boland, et al., 1995). Selection for improvements in lean growth efficiency resulted in increased lean content and lean gain per day, but showed less backfat thickness, higher shear force and greater drip loss (Lonergan, Huff-Lonergan, Rowe, Kuhlers, & Jungst, 2001). Also, strong selection for leanness may give rise to the segregation of linked unfavorable alleles causing inferior pork quality. Suzuki et al. (2005) reported that selection for decreased backfat thickness had a negative influence on meat tenderness and color.

Rapid advancements in molecular genetics are contributing to localize quantitative trait loci (QTL) and to identify genes causing variation in economically important traits such as meat quality. Two well known achievements are the identification of substitutions within the ryanodine receptor 1 (*RYR1*) and protein kinase adenosine monophosphate activated γ_3-subunit (*PRKAG3*) genes. A recessive C1843T polymorphism (HAL-1843) in the *RYR1* gene was associated with the incidence of porcine stress syndrome (PSS) and an increase in the frequency of pale, soft, and exudative (PSE) pork (Apple, et al., 2005; Fujii, et al., 1991), and the identification of a dominant R200Q polymorphism in the *PRKAG3* gene was associated with reduced meat quality by high glycogen content in skeletal muscle (Milan, et al., 2000).

However, many genes are involved with additive effects even though assuming a single gene can have a larger effect on meat quality traits (Dekkers, 2004; van der Steen, Prall, & Plastow, 2005). In addition to *RYR1* and *PRKAG3*, Kim et al. (2000) reported a missense mutation D298N in the melanocortin-4 receptor gene (*MC4R*), a candidate gene for growth rate and fatness, and this gene was subsequently associated with darker meat and less drip loss (Otto, et al., 2007). In addition, Ciobanu et al. (2004) reported a haplotype of calpastatin (*CAST*; R249K and S638R) to be associated with decreased shear force and increased juiciness.

In order to identify a causative mutation, the positional candidate gene approach using QTL mapping has been conducted by many research groups and results have been confirmed in various populations (de Vries, Sosnicki, Garnier, & Plastow, 1998). After completion of the pig genome sequencing, it will be more feasible to identify genes underlying complex traits such as meat quality (Archibald, et al., 2010; Gao, Zhang, Hu, & Li, 2007; Mullen, Stapleton, Corcoran, Hamill, & White, 2006).

In our previous studies, putative QTL regions for meat quality traits were identified through two genome scans of our Duroc x Pietrain F_2 resource population (Choi, et al., 2011; Edwards, Ernst, Raney, et al., 2008). The objective of this study was to determine if five QTL regions identified from our previous studies were segregating in an US commercial purebred Duroc population. For this study 81 gene-specific single-nucleotide polymorphisms (SNP) on 5 chromosomes (SSC3, 6, 12, 15, and 18) were selected that map to QTL regions identified from our first genome scan result (Edwards, Ernst, Raney, et al., 2008) and association analyses were performed to evaluate the genetic relationship with meat quality traits in an US purebred Duroc population.

2. Materials and methods

2.1. Animals

A commercial purebred Duroc population was used. Pigs were from three herds as suggested by the National Swine Registry (NSR; http://www.nationalswine.com) in the United States. Animal protocols were approved by the Michigan State University All University Committee on Animal Use and Care (AUF#04/06-048-00). A total of 331 pigs were produced from three sire families per herd which were chosen to represent a cross-section of the sire families within the US Duroc breed. Pigs across 103 litters were born from December 2006 to July 2008 and harvested from June 2007 to December 2008. The NSR provided muscle tissue samples taken at harvest for DNA extraction as well as records of live animal traits. The B-mode ultrasound estimates of 10th rib backfat thickness and loin muscle area (LMA) on test day were determined. The number of days to 113 kg was calculated, and backfat thickness and LMA were also adjusted to a common weight of 113 kg based on National Swine Improvement Federation guidelines (2006). After harvesting, 45-min pH and 24-h pH (Ultimate pH; pH_u) were measured on *longissimus* muscle adjacent to the 10th rib, and pH decline from 45-min to 24-h was calculated. The fresh loin was cut at the 10th rib and allowed to bloom for a minimum of 10 min and evaluated for subjective color and marbling scores (NPPC, 2000). The color score scale ranged from 1 (pale pinkish gray) to 6 (dark purplish red) and the marbling score scale was 1 (rarely marbled) to 10 (best marbled) corresponding to approximately 1 – 10% intramuscular lipid (NPPC, 2000). Objective color score of CIE L* was also measured using a Minolta colorimeter. Descriptive statistics for phenotypes used in this study are presented in Table III.1.

2.2. Genotyping

Genomic DNA was extracted from muscle tissue using a PureLink Genomic DNA kit (Invitrogen, Carlsbad, CA, USA) and quality of DNA was verified using a Quant-iT^{TM} Assay Kit (Invitrogen, Carlsbad, CA, USA) with an Invitrogen Qubit fluorometer. Gene-specific SNP markers were chosen from our previous work or from publicly available sources. A total of 81 SNPs from 80 candidate genes on 5 chromosomes (SSC3, 6, 12, 15 and 18) were selected that mapped to putative QTL regions identified in our first genome scan using 510 F_2 animals (Edwards, Ernst, Raney, et al., 2008; Edwards, Ernst, Tempelman, et al., 2008). Markers were included in an Illumina Goldengate 96 plex SNP multiplex custom assay panel (Illumina, Inc. San Diego, CA), which was read using the Illumina BeadXpress Reader. The Illumina Goldengate assay was performed at the Michigan State University Research Technology Support Facility. Genotype segregation was determined using the Illumina Genome Studio software. A total of 6 SSC3 (out of 18), 5 SSC6 (out of 19), 10 SSC12 (out of 18), 8 SSC15 (out of 20) and 4 SSC18 (out of 6) markers were found to be segregating in the commercial Duroc population (minor allele frequency > 0.05). Information about these 33 SNPs is presented in Table III.2 and further details regarding the SNP assay probes are presented in Supplementary Table III.1.

2.3. Statistical analyses

Association analyses between SNP genotypes and meat quality traits were performed using linear mixed models. The model specifications are as follows;

1) For pH traits, L*, color and marbling:

$$Y_{ijklmno} = \mu + S_j + \sum_k G_{kl} + H_m + D_{mn} + L_o + e_{ijklmnop}$$

where $Y_{ijklmno}$ is the trait measured on animal *i*, μ is the overall mean for the trait, S_j is the sex (*j* = 1 or 2), $\sum_k G_{kl}$ is the *l*th genotypic effects of all *k*th SNPs (*k* = 1 – 33, *l* = 1 – 3), H_m is herd (*m* = 1 – 3), D_{mn} is harvest date within herd (*n* = 1 – 14), L_o is the random effect of litter (*o* = 1 – 103), and $e_{ijklmnop}$ is the residual term,

2) For days to 113 kg, backfat thickness and LMA:

$$Y_{ijklmo} = \mu + S_j + \sum_k G_{kl} + H_m + L_o + e_{ijklmop}.$$

For all analyses, only trait-gene associations for which a SNP was significant in the model were considered further for comparison among genotype classifications. Additive genetic effects were estimated as half of the difference between homozygous genotypes and dominance genetic effects were estimated as the deviation of the heterozygous genotype from the mean of the homozygous genotypes. For *PRKAG3*, a combined genotype was considered in the analysis because the T30N and I199V markers were in high linkage disequilibrium, and these loci are physically separated by only 98 kb. All statistical analyses were completed using the SAS software (SAS Inst., Inc., Cary, NC).

3. Results

3.1. Allele and genotype frequencies

The SNP allele and genotype frequencies are shown in Table III.3. Eighteen of 33 SNPs in this analysis were in Hardy-Weinberg equilibrium and overall allele frequencies showed a range of segregation patterns. Five markers, *RNF40*, *PCGF1*, *CEBPZ*, *COL16A1*, and *SF3B1*

exhibited only two of three possible genotypes. As these SNPs were not prescreened in the Duroc population and this population is under selection, it was not unexpected for some markers to exhibit unequal genotype frequencies.

3.2. Association analyses

The malate dehydrogenase 1 (*MDH1)* marker located on SSC3 was significantly associated with 45-min pH and pH_u, and pH decline from 45-min to 24-h ($P < 0.01$; Table III.4). Pigs with the GG genotype exhibited a 45-min pH of 6.87 compared with 6.46 and 6.50 for pigs with the AA and AG genotypes, respectively. For pH_u, pigs with the AG genotype were significantly higher than pigs with the AA genotype ($P < 0.05$) (Table III.4). Consequently, GG genotype pigs also had a significantly greater pH decline from 45-min to 24-h than AA and GG genotype pigs. Pigs with the GG genotype exhibited higher 45-min pH and greater pH decline from 45-min to 24-h compared to pigs with the AA genotype, and pigs with the AG genotype exhibited lower 45-min pH and smaller pH decline compared to the average value of pigs with the AA and GG genotypes. The polycomb group ring finger 1 (*PCGF1*) marker also located on SSC3 showed a significant association with 45-min pH ($P < 0.01$; Table III.4) with pigs of the AG genotype exhibiting higher pH than pigs of the GG genotype. The nucleoporin 88 kDa (*NUP88*) marker on SSC12 was also associated with 45-min pH ($P < 0.05$; Table III.4) with AC heterozygotes exhibiting higher 45-min pH than AA homozygotes, and the dominance effect at this locus was significant ($P < 0.05$; Table III.4). Similarly pigs with the heterozygous AG genotype for the ADP-ribosylation factor 5 (*ARF5*) marker on SSC18 showed higher pH_u values than pigs with the AA genotype ($P < 0.05$) and

again the dominance effect at this locus was significant ($P < 0.05$; Table III.4). For *PRKAG3* the markers for T30N and I199V were combined (6 genotypes observed; CC/AA, CC/AG, CC/GG, AC/AG, AC/GG, AA/GG) and evaluated. The combined *PRKAG3* genotypes were associated with pH_u ($P < 0.05$; Table III.5). Pigs with the CC/AG genotype had significantly higher pH_u (5.95) than pigs with the AC/AG (5.84), AC/GG (5.84) or CC/GG (5.81) genotypes ($P < 0.05$).

The FK506 binding protein 10, 65 kDa (*FKBP10*) marker on SSC12 and the splicing factor 3b, subunit 1 (*SF3B1*) marker on SSC15 were significantly associated with L* ($P < 0.01$). Pigs with the heterozygous AG genotype at the *FKBP10* locus were lighter in color ($P < 0.05$; Table III.4) than pigs with the AA genotype, and the observed dominance effect of 1.34 was significant ($P < 0.01$). Pigs with the TT genotype at *SF3B1* on SSC15 had higher L* values than pigs with the AT genotype ($P < 0.01$). The *PCGF1* SNP on SSC3, which was associated with 45-min pH, also exhibited a significant effect on L* ($P < 0.05$) and on color score ($P < 0.01$). Pigs with the *PCGF1* GG genotype exhibited lighter colored meat than pigs with the AG genotype as measured by both L* and subjective color score (Table III.4). The *ARF5* marker on SSC18, which was associated with pH_u, was also associated with color score ($P < 0.05$; Table III.4). For this marker a significant dominance effect ($P < 0.01$) was observed and pigs with the heterozygous genotype exhibited significantly higher color scores than pigs with either homozygous genotype ($P < 0.05$).

For heparan sulfate proteoglycan 2 (*HSPG2*) on SSC6, pigs with the GG genotype exhibited higher marbling scores and took more days to reach 113 kg ($P < 0.05$) than pigs of the CC genotype (Table III.4). Pigs with the AG genotype of the 95 kDa retinoblastoma

protein binding protein (*RNF40*) SNP on SSC3 exhibited more backfat thickness than pigs with the GG genotype ($P < 0.05$; Table III.4). Two SSC15 and one SSC12 markers were significantly associated with LMA (Table III.4). Pigs with the GG genotype for the mitogen-activated protein kinase kinase kinase 2 (*MAP3K2*) marker had smaller LMA ($P < 0.01$) than pigs of the AG genotype. For the *SF3B* SNP, pigs of the TT genotype had larger LMA than pigs of the AT genotype ($P < 0.05$), and for the phospholipase D2 (*PLD2*) marker pigs of the AA genotype had less LMA than pigs of either the AG or GG genotypes ($P < 0.05$).

4. Discussion

We have previously reported results for whole genome scans of our Duroc x Pietrain F_2 population (Choi, et al., 2010; Choi, et al., 2011; Edwards, Ernst, Raney, et al., 2008; Edwards, Ernst, Tempelman, et al., 2008). For the present study, five QTL regions were selected for further evaluation in an unrelated US Duroc population through analysis of gene-specific SNP markers that are located within the QTL regions. Significant QTL for 45-min pH and pH decline from 45-min to 24-h were identified in the middle of SSC3 in the marker interval *S0206* to *SW2408* (Edwards, Ernst, Raney, et al., 2008) and this result was confirmed in a subsequent QTL analysis (Choi et al., 2011). Eighteen SNP markers spanning this region of SSC3 were selected for evaluation, and 6 of the 18 markers were found to be segregating in the Duroc population. Our QTL analyses revealed numerous QTL on SSC6 influencing carcass length, LMA, backfat thickness, marbling score, fat and protein percent, and ham and loin weight (Choi, et al., 2010; Choi, et al., 2011; Edwards, Ernst, Raney, et al., 2008; Edwards, Ernst, Tempelman, et al., 2008). Nineteen SNPs were selected in the SSC6 *S0087 – SW322* marker interval although only 5 SNPs were used in the analysis because 13

SNPs were not segregating and 1 SNP was excluded because it was in complete LD with a neighboring SNP marker and thus would not have contributed any additional information. QTL on SSC12 were identified for pH_u, fat and moisture percent, marbling score and belly weight in the *SW957 – SW2180* marker interval (Edwards, Ernst, Raney, et al., 2008). Ten of the 19 selected SSC12 SNP markers were segregating in the Duroc population and were used in the analysis. A region on SSC15 in the marker interval *S0148 – SW1983* was considered as a strong QTL region affecting many meat quality traits (i.e., protein percent, meat color, sensory panel tenderness, shear force, and cook yield; Edwards, Ernst, Raney, et al., 2008). Twenty SNPs were selected in this region and 8 SNPs segregating in the Duroc population were analyzed. On SSC18, we detected QTL for backfat thickness and pH_u. Six SNP markers were selected spanning a large portion of SSC18, and 4 of these markers segregating in the Duroc population were used in the analysis.

Of the 33 segregating SNPs in the five putative QTL regions, 11 SNPs exhibited significant associations with one or more carcass merit or meat quality traits measured in the purebred Duroc population. The association of *MDH1* genotypes with 45-min pH and pH decline from 45-min to 24-h was in agreement with our previous results (Choi, et al., 2011; Edwards, Ernst, Raney, et al., 2008). The *MDH1* gene encodes a 334 amino acid cytoplasmic malate dehydrogenase which catalyzes the oxidation of malate to oxaloacetate utilizing the NAD/NADH cofactor system in the citric acid cycle and catalyzes the oxidation of NADH, H^+ into NAD^+ in glycolysis. Campbell et al. (2001) found the *MDH1* gene in mice to be expressed 2.2 fold higher in red skeletal muscle than in white skeletal muscle consistent with its role in glycolysis. In Duroc sires, the expression of *MDH1* protein in *longissimus*

lumborum muscle was highly correlated with pH_u (r = 0.629) which resulted from increased glycolysis by *MDH1* protein (Kwasiborski, et al., 2008). Our observation of *MDH1* genotype associations with 45-min pH, pH_u and pH decline from 45-min to 24-h in Duroc pigs continue to support the potential of *MDH1* as a candidate gene for *longissimus* muscle pH traits.

The *PRKAG3* gene, which encodes a subunit of AMP-activated protein kinase (AMPK), is well known as a candidate gene affecting meat quality caused from increased glycogen level in skeletal muscle. Milan et al. (2000) identified 7 SNPs (at amino acid positions 30, 53, 193, 194, 199, 200, and 372) in the *PRKAG3* gene and found the R200Q substitution to be associated with a higher glycogen content and a consequent lower pH_u which corresponded to the dominant RN^- allele of the Napole gene (Fernandez, Lafaucheur, Gueblez, & Monin, 1991). An additional missense mutation (G52S) in *PRKAG3* was revealed from putative QTL regions affecting muscle glycogen content and pH_u in a Berkshire x Yorkshire F_2 population in which the R200Q substitution was not segregating, and the effects of G52S along with T30N and I199V were investigated in commercial populations (Ciobanu, et al., 2001). The large effects of the I199V substitution showed a significant association with pH and meat color across populations (Landrace, Large White, Berkshire, Duroc, and Duroc Synthetic breeds), and the T30N substitution showed a significant association with pH_u exclusively in a Duroc population where the genotype frequencies of TT, TN, and NN were 0.34, 0.46, and 0.2, respectively (Ciobanu, et al., 2001). Huang et al. (2004) reported that the frequency of favorable alleles 30T and 199I were 0.35 and 0.24 respectively in Duroc, and the 200Q allele was found to segregate only in Hampshire. In the present study, the allele

frequencies of 30T (C allele at this locus) and 199I (A allele at this locus) were 0.66 and 0.37, respectively. Evaluation of combined genotypes for the T30N and I199V markers revealed an association with pH_u. Animals with the CC genotype at T30N in combination with at least one A allele at the I199V locus (AA or AG) tended to have higher pH_u compared to other allelic combinations. This provides evidence of the favorable role of these two *PRKAG3* SNPs on pH_u in the US Duroc breed.

The *PCGF1* gene consists of 9 exons and encodes a 259 amino acid polypeptide that is a component of the polycomb group multiprotein complex (Nunes, et al., 2001). The *PCGF1* mRNA which was ubiquitous in adult tissue and abundant in nervous tissue, heart and testis in mouse was highly expressed in the developing nervous system and acts as a transcriptional repressor via protein kinase C phosphorylation (Gong, et al., 2005; Nunes, et al., 2001). Genotypes of the *PCGF1* SNP marker located on SSC3 were significantly associated with 45-min pH, L*, and color score. Pigs with the AG genotype exhibited a higher 45-min pH and consequently darker meat (i.e., lower L* and higher subjective color score) than pigs with the GG genotype. The associations of *MDH1* and *PCGF1* with pH on SSC3 were consistent with our previous studies which identified QTL for 45-min pH and pH decline from 45-min to 24-h (Choi, et al., 2011; Edwards, Ernst, Raney, et al., 2008). Also, Beeckmann et al. (2003) reported a QTL for 45-min pH at the same interval in a Wild boar x Meishan F_2 population. In addition, Harmegnies et al. (2006) and Ma et al. (2009) reported QTL affecting L* in a similar region of SSC3. Other color traits QTL have also been identified on SSC3. Harmegnies et al. (2006) reported a QTL for a* in the same region in a commercial four-way cross population and Soma et al. (2011) reported QTL for L* and b* at

the proximal end of SSC3 as well as a QTL for color score located in a position proximal to that of *PCGF1*.

FKBP10 encodes a 65kD rough endoplasmic reticulum FK506 binding protein that is part of a large family of FK binding proteins which possesses both peptidyl-prolyl cis-trans isomerization and chaperone functions (Davis, Broekelmann, Ozawa, & Mecham, 1998). The *FKBP10* SNP on SSC12 was associated with L* in this study. Li et al. (2010) evaluated an *FKBP10* SNP in a Korean Native Pig x Landrace F_2 population and found associations with growth traits at 21 days, although they did not identify an association with meat color or other meat quality traits. We had identified QTL influencing the objective color parameters of a* and b* at a more distal position on SSC12 in our second genome scan (Choi, et al., 2011), but significant QTL influencing meat color were not observed in our first genome scan in this region (Edwards, Ernst, Raney, et al., 2008). Ma et al. (2009) reported a QTL for color score in this region of SSC12 in a Duroc x Erhualian population, and Harmegnies et al. (2006) reported a QTL for a* at a position distal to *FKBP10* in a commercial four-way cross population.

The *HSPG2* gene encodes a large proteoglycan that is a component of the extracellular matrix. It is located on SSC6 (Aldenhoven, Spötter, & Distl, 2005) and was associated with marbling score and days to 113 kg. Our results indicated that animals with the GG genotype grew more slowly and had higher marbling scores. Schwab et al. (2010) reported a positive moderate genetic correlation between intramuscular fat and growth rate in a Duroc population, which agreed with the genetic control of this gene for these two traits. The location of the *HSPG2* gene is within the confidence interval of a highly significant QTL region for fat percent and marbling score that was identified by both our first and second

genome scans (Choi, et al., 2011; Edwards, Ernst, Raney, et al., 2008). Also, detection of significant QTL for fat-free total lean and empty body protein at 22 wk of age was reported in this same region in our previous genome scans (Choi, et al., 2010; Edwards, Ernst, Tempelman, et al., 2008) and supports *HSPG2* as a possible candidate gene for fat deposition traits.

On SSC15, the *MAP3K2* and *SF3B1* markers were associated with LMA and the *SF3B1* marker was also associated with L*. The *MAP3K2* gene maps to human chromosome 2 and encodes a 619 amino acid protein that activates MAP2K which regulate MAPK in a protein kinase signal transduction cascade (Blank, Gerwins, Elliott, Sather, & Johnson, 1996; W. Chen, White, & Cobb, 2002). The MAPK signaling cascade in the c-Jun N-terminal kinase (JNK) and extracellular signal regulated kinase (ERK) pathways regulates cell differentiation, proliferation and apoptosis, and is involved in cardiac muscle defects during development (Regan, et al., 2002; Widmann, Gerwins, Johnson, Jarpe, & Johnson, 1998). The *SF3B1* gene encodes subunit 1 of the splicing factor 3B protein which plays an essential role for pre-mRNA processing (Golas, Sander, Will, Luhrmann, & Stark, 2003). An association of *SF3B1* with L* in the present study was consistent with our genome scan results identifying a QTL for L* on SSC15, and also associations of *MAP3K2* and *SF3B1* with LMA were consistent with our previous studies identifying QTL affecting LMA on SSC15 (Choi, et al., 2011; Edwards, Ernst, Raney, et al., 2008).

Based on genome scans using our Duroc x Pietrain F_2 resource population, gene-specific SNP markers located in the regions of five putative QTL (SSC3, 6, 12, 15, and 18) were selected for evaluation in an US purebred Duroc population. Of the 81 gene-specific SNPs genotyped, 33 were found to be segregating and were analyzed for associations with carcass

merit and meat quality traits in the Duroc population. While the Duroc population had fewer phenotypic traits recorded than had been measured for the resource population, 11 of the SNP markers showed a significant association with at least one trait. Significant associations were observed for the SSC3 marker *MDH1* with all pH traits and for the SSC3 marker *PCGF1* with 45-min pH, L* and color score. The SSC6 marker *HSPG2* was associated with marbling score and days to 113 kg. On SSC12, significant associations were found for *NUP88* with 45-min pH, and *FKBP10* with L*. The SSC15 marker *SF3B1* was associated with L* and LMA, and markers for the SSC15 gene *PRKAG3* showed a significant association with pH_u. On SSC18, *ARF5* was associated with pH_u and color score. The results obtained in the present study were consistent with our previous genome scans for pH on SSC3, marbling score on SSC6, pH on SSC12, L* on SSC15 and pH on SSC18. Further study is warranted to confirm segregation of QTL for these traits using larger commercial Duroc populations with these SNPs as well as additional SNPs in order to facilitate incorporation into pig breeding programs.

Acknowledgments

This project was supported in part by National Research Initiative Competitive Grant no. 2004-35604-14580 from the USDA National Institute of Food and Agriculture. We thank the National Swine Registry for phenotypic data and samples.

Table III.1. Number of records, means, and standard deviation for 9 traits evaluated in this study.

Trait	N	Mean	SD
45 min pH	245	6.33	0.43
24 h pH (Ultimate pH; pH_u)	326	5.86	0.23
45 min - 24 h pH decline	224	0.58	0.36
L*	313	51.19	2.69
Color score, 1-6	326	2.99	0.63
Marbling score, 1-10	327	2.37	1.16
Backfat thickness, mm	277	13.12	2.74
Longissimus muscle area, cm^2	277	47.15	4.76
Days to 113 kg	277	166.56	15.27

Table III.2. Information for SNP markers evaluated in this study.

Chr[1]	Gene symbol	Full name	Position[2]	SNP type	SNP source[3]
3	*RNF40*	95 kDa retinoblastoma protein binding protein	16,106,853	A/G	ss23130356
	IFT172	Intraflagellar transport 172 homolog	43,823,993	T/C	ss23131537
	PCGF1	Polycomb group ring finger 1	63,084,541	T/C	ss23133110
	TIA1	TIA1 cytotoxic granule-associated RNA binding protein	66,216,145	A/G	ss16337136
	MDH1	Malate dehydrogenase 1, NAD	72,272,863	A/G	ss23132846
	CEBPZ	CCAAT/enhancer binding protein (C/EBP), zeta	96,014,633	T/C	ss23129373
6	*MLL4*	Myeloid/lymphoid or mixed-lineage leukemia protein 4	30,161,015	A/C	ss16337525
	HSPG2	Heparan sulfate proteoglycan 2	54,946,906	C/G	ss23130687
	COL16A1	Collagen, type XVI, alpha 1	60,507,310	A/G	ss23129728
	LEPR	Leptin receptor	103,462,536	T/C	Chen et al. 2004
	STMN1	Stathmin 1	–	A/G	ss99306908
12	*FKBP10*	FK506 binding protein 10, 65 kDa	18,725,878	T/C	ss23129646
	CACNB1	Calcium channel, voltage-dependent, beta 1 subunit	21,096,363	A/G	ss86353491
	CACNA1G	Calcium channel, voltage-dependent, T type, alpha 1G subunit	24,128,357	T/G	ss86353487
	DLX4	Distal-less homeobox 4	24,905,314	T/C	ss23129839
	PPM1E	Protein phosphatase, Mg^{2+}/Mn^{2+} dependent, 1E	33,289,660	T/C	ss23130731
	SLC6A4	Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	44,338,635	T/G	ss99306907
	SGSM2	Small G protein signaling modulator 2	45,906,070	T/G	ss23130795
	PLD2	Phospholipase D2	49,274,975	T/C	ss23131423
	NLE1	Notchless homolog 1 (Drosophila)	–	A/G	ss23132413
	NUP88	Nucleoporin 88kDa	–	T/G	ss16336982
15	*PPAPDC1B*	Phosphatidic acid phosphatase type 2 domain containing 1B	45,833,191	A/G	ss23131638
	OCA2	Oculocutaneous albinism II	53,553,470	A/G	Fernandez et al. 2006
	ABCB11	ATP-binding cassette, sub-family B (MDR/TAP), member 11	70,887,762	G/C	ss23131708

Table III.2. (Cont'd).

Chr[1]	Gene symbol	Full name	Position[2]	SNP type	SNP source[3]
15	*PRKAG3* T30N	Protein kinase, AMP-activated, gamma 3 non-catalytic subunit	113,850,723	A/C	Milan et al. 2000
	PRKAG3 I199V	Protein kinase, AMP-activated, gamma 3 non-catalytic subunit	113,949,491	T/C	Milan et al. 2000
	MAP3K2	Mitogen-activated protein kinase kinase kinase 2	–	A/G	ss23130119
	SF3B1	Splicing factor 3b, subunit 1	–	A/T	MSU
	TTN	Titin	–	T/G	ss16337371
18	*ARF5*	ADP-ribosylation factor 5	18,821,285	T/C	MSU
	ING3	Inhibitor of growth family, member 3	24,115,025	T/C	MSU
	CRHR2	Releasing hormone receptor 2	40,666,448	A/G	ss86353512
	H2AFV	H2A histone family, member V	49,063,850	A/G	ss23132945

[1]Chr = chromosome

[2]Position is estimated based on pig genome sequence (Build 9; http://www.ensemble.org) and – indicates unidentified position in the current pig genome sequence.

[3]SNP were obtained from public database sources or publications, or were developed in our laboratory, and are identified by dbSNP accession number, reference citation or as MSU for new SNP

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Table III.3. Genotypic and allelic frequencies for SNP markers.

Chr[1]	Gene symbol	Genotype frequency			Allele frequency		P value (χ^2 test)
3	*RNF40*	0.85(GG)	0.15(AG)	–	0.93 (G)	0.07 (A)	HWE
	PCGF1	0.32(GG)	0.68(AG)	–	0.66 (G)	0.34 (A)	<0.05
	TIA1	0.05(AA)	0.35(AG)	0.6(GG)	0.77 (G)	0.23 (A)	HWE
	MDH1	0.85(AA)	0.13(AG)	0.02(GG)	0.92 (A)	0.08 (G)	<0.05
	CEBPZ	0.79(AA)	0.21(AG)	–	0.9 (A)	0.1 (G)	<0.05
	IFT172	0.32(AA)	0.52(AG)	0.16(GG)	0.58 (A)	0.42 (G)	HWE
6	*MLL4*	0.22(AA)	0.47(AC)	0.32(CC)	0.55 (C)	0.45 (A)	HWE
	HSPG2	0.28(CC)	0.44(CG)	0.28(GG)	0.5 (G)	0.5 (C)	<0.05
	COL16A1	0.88(AA)	0.12(AG)	–	0.94 (A)	0.06 (G)	HWE
	LEPR	0.52(AA)	0.37(AG)	0.11(GG)	0.7 (A)	0.3 (G)	<0.05
	STMN1	0.61(AA)	0.38(AG)	0.02(GG)	0.8 (A)	0.2 (G)	<0.05
12	*FKBP10*	0.08(AA)	0.34(AG)	0.58(GG)	0.75 (G)	0.25 (A)	HWE
	CACNB1	0.13(AA)	0.31(AG)	0.56(GG)	0.71 (G)	0.29 (A)	<0.05
	CACNA1G	0.24(AA)	0.38(AC)	0.38(CC)	0.57 (C)	0.43 (A)	<0.05
	DLX4	0.08(AA)	0.31(AG)	0.61(GG)	0.76 (G)	0.24 (A)	<0.05
	PPM1E	0.77(AA)	0.21(AG)	0.02(GG)	0.88 (A)	0.12 (G)	HWE
	SLC6A4	0.05(AA)	0.48(AC)	0.47(CC)	0.71 (C)	0.29 (A)	<0.05
	SGSM2	0.02(AA)	0.34(AC)	0.64(CC)	0.81 (C)	0.19 (A)	<0.05
	PLD2	0.33(AA)	0.53(AG)	0.14(GG)	0.59 (A)	0.41 (G)	HWE
	NLE1	0.51(AA)	0.26(AG)	0.22(GG)	0.64 (A)	0.36 (G)	<0.05
	NUP88	0.18(AA)	0.49(AC)	0.34(CC)	0.58 (C)	0.42 (A)	HWE
15	*PPAPDC1B*	0.4(AA)	0.48(AG)	0.12(GG)	0.64 (A)	0.36 (G)	HWE
	OCA2	0.57(AA)	0.35(AG)	0.08(GG)	0.74 (A)	0.26 (G)	HWE
	ABCB11	0.01(CC)	0.1(CG)	0.9(GG)	0.94 (G)	0.06 (C)	HWE
	PRKAG3 T30N	0.09(AA)	0.5(AC)	0.41(CC)	0.66 (C)	0.34 (A)	<0.05
	PRKAG3 I199V	0.11(AA)	0.52(AG)	0.37(GG)	0.63 (G)	0.37 (A)	HWE
	MAP3K2	0.15(AA)	0.42(AG)	0.43(GG)	0.64 (G)	0.36 (A)	HWE
	SF3B1	0.23(TT)	0.77(AT)	–	0.62 (T)	0.38 (A)	<0.05
	TTN	0.36(AA)	0.5(AC)	0.14(CC)	0.61 (A)	0.39 (C)	HWE
18	*ARF5*	0.2(AA)	0.47(AG)	0.33(GG)	0.56 (G)	0.44 (A)	HWE
	ING3	0.2(AA)	0.48(AG)	0.32(GG)	0.56 (G)	0.44 (A)	HWE
	CRHR2	0.26(AA)	0.51(AG)	0.24(GG)	0.51 (A)	0.49 (G)	HWE
	H2AFV	0.26(AA)	0.44(AG)	0.29(GG)	0.51 (G)	0.49 (A)	<0.05

[1]Chr = chromosome

Table III.4. LS means of significant SNP markers for carcass and meat quality traits.

Trait	Gene	Chr[1]	Homozygote	Heterozygote	Homozygote	Additive	Dominance
pH45	*MDH1*	3	6.46± 0.12^{a} (AA)	6.50± 0.13^{a} (AG)	6.87± 0.17^{b} (GG)	-0.21±0.06**	-0.17±0.07**
	PCGF1	3	6.42± 0.13^{a} (GG)	6.53± 0.13^{b} (AG)			
	NUP88	12	6.42± 0.13^{d} (AA)	6.53± 0.13^{e} (AC)	6.49± 0.13^{de} (CC)	-0.03±0.03	0.08±0.03*
pH_u	*ARF5*	18	5.83± 0.10^{d} (AA)	5.91± 0.1^{e} (AG)	5.89± 0.1^{de} (GG)	-0.03±0.02	0.05±0.02*
	MDH1	3	5.86± 0.09^{d} (AA)	5.95± 0.1^{e} (AG)	5.81± 0.13^{de} (GG)	0.02±0.05	0.12±0.06*
pH decline	*MDH1*	3	0.61± 0.18^{a} (AA)	0.55± 0.18^{a} (AG)	1.08± 0.23^{b} (GG)	-0.24±0.08**	-0.30±0.09**
CIE L*	*FKBP10*	12	53.17±1.52^{d} (AA)	54.88±1.43^{e} (AG)	53.90±1.39^{de} (GG)	-0.38±0.42	1.34±0.47**
	SF3B1	15	54.67±1.46^{a} (TT)	53.30±1.36^{b} (AT)			
	PCGF1	3	54.44±1.44^{d} (GG)	53.53±1.37^{e} (AG)			
Color	*PCGF1*	3	2.15± 0.37^{a} (GG)	2.45± 0.35^{b} (AG)			
	ARF5	18	2.13± 0.36^{d} (AA)	2.46± 0.35^{e} (AG)	2.31± 0.37^{d} (GG)	-0.09±0.07	0.24±0.09**
Marbling	*HSPG2*	6	1.59± 0.57^{d} (CC)	1.87± 0.55^{de} (CG)	2.07± 0.56^{e} (GG)	-0.23±0.10*	0.04±0.13

Table III.4. (Cont'd).

Trait	Gene	Chr[1]	Homozygote	Heterozygote	Homozygote	Additive	Dominance
Days	*HSPG2*	6	156.71± 8.58[d] (CC)	158.15± 8.22[d] (CG)	162.5± 8.26[e] (GG)	-2.77±1.34*	-1.46±1.70
Backfat	*RNF40*	3	13.36± 1.74[d] (GG)	15.01± 1.84[e] (AG)			
LMA	*MAP3K2*	15	51.40± 2.78[ab] (AA)	51.71± 2.66[a] (AG)	49.57± 2.72[b] (GG)	0.79±0.53	1.29±0.61*
	SF3B1	15	51.95± 2.76[d] (TT)	49.84± 2.65[e] (AT)			
	PLD2	12	49.67± 2.67[d] (AA)	51.1± 2.66[e] (AG)	51.3± 0.82[e] (GG)	-1.16±0.50*	0.20±0.62

[1]Chr = chromosome

[a,b]LS mean $P < 0.01$

[d,e]LS mean $P < 0.05$

*$P < 0.05$ and ** $P < 0.01$

Table III.5. LS means of combined *PRKAG3* SNP genotypes for ultimate pH.

	CC/AA	CC/AG	CC/GG	AC/AG	AC/GG	AA/GG
pH_u	5.93± 0.11^{ab}	5.95± 0.10^{a}	5.81± 0.11^{c}	5.84± 0.10^{c}	5.84± 0.10^{bc}	5.88± 0.09^{abc}
No. of animals	37	73	21	95	68	31

^{a,b,c}LS mean $P < 0.05$

Supplementary Table III.1. Probe sequences spanning SNP positions used for Illumina Goldengate assays.

chr[1]	SNP	Probe sequence
3	*RNF40*	CCCCCTTCTG GTCCCCTTGC CTCTGAATGT TGGAATTCTC CAAACTTCTT [A/G] TAGTAGTACA CAGCCTGCAT TTGCTCAGTG GTTCCTCGGT CAGCCGTGTG
	PCGF1	GAGAAACGGA TCCGAGAATT CTACCAGTCC CGAGACTTGG ACAGGATCAC [T/C] CAGCCCAGTG GGGAAGAGCC AGCCCTGAGC AACCTCGGCC TCCCCTTCAG
	TIA1	CCTTCGATCG TAGTACCATT CACTGAGACA ATGGCATGGG CTGCACTTTC [A/G] TGGGTGGAAA ATCTAAGAGA AAGAGAATGA GGTTTTTTTG TTTTTGATTT
	MDH1	TTAAAGCCTC TATTGGGCCA TAGCTCCTCT GTTTATAAGA TGAGGGTTAT [A/G] TTTCTCTGGC CTGCTTCACA CATAGATCAG AAGAGTAGAA TGAGATAATA
	CEBPZ	TTGATGATAA ATACGGGAGG AAGAAAGGAA AGCAACAAAG GGGGTTTGCA [T/C] AGTGGATGGC AGATGACCTT AGGCTGTGGC AGACCAGTTC CTGTTACTTA
	IFT172	GGTCACGGGT GTCTCTGAAA AACGTCCACA CCTACACACG AAATTTCACA [T/C] ATGATTTCAT CATCTTATTA CCATCTTTCC TGCTTTTTAG GGCCGAACCA
6	*MLL4*	GGCACCACAC CCCAGACCTC TCACCGGAGA TAGACCTCAG CGCGGGCTGC [A/C] CCATGGGGAT TCAGGGGTGG TTCCTCTTGG CCCTCTCCCT GCTGGTGGTA
	HSPG2	TCTGTGTGAC TGGAAGCAAG TCCCTGGATC TCTCAGGTTC CTCTGGGACA [C/G] CCTAGACAGG AAGGAAGTTC CCCATCTGCT CAAGTTCCAG GAGGGGGTGG
	COL6A1	GAGGCGCCTA GCTCTTCTCT GAGGCAGGGA GGAGAGCTGG GAGTGTCAGG [A/G] GCCGATTTTG ATATCCCAGG CTGGGGTGGG GACAGTGGAG AGACAGAGCT
	LEPR	CTCAAAGAAC ACTTCAACTT TGAATGGACA TGATGAGGCA GTTGTTGAAA [T/C] GGAACTTAAT TCAAGTGGTA CCTACTTATC AAACTTATCT TCTAAAACAA
	STMN1	GAAGGTTGGT GTCTCTTCGT GGCTTTGAGG GGGTTAAGCG CTTGGAGTTC [A/G] ATGCACCGCT GAAGTGGCTT GAACTCTTGA CCTTGATCAC ACGCTTGGTG
12	*FKBP10*	GGTGGCTCCC GGGAAAAACT CAGCTTAGGT CTCGCAAGGG AAGAACCAAC [T/C] GGGGCTGCTG ATGAGCAGGA AAGCGCTCTG GAAACCAGGG TTGGCGCCTT
	CACNB1	AGACCCGGGG GAAGGGAAGG GGGGACGTTT GGGTTCTCCT GGTTTCCTCT [A/G] GCCTGCATGA GAGAGTGACC AGGAACACCT GGCCCGGGTC CTTAGGTGCA
	CACNA1G	AGCCGGGACC CCTCAGGGAG CGCTGGGCAC CCTACAGGGC GTCCACCTCT [T/G] GGGGTGGGTC TCCTCTTGGT TTTACAGGTG AGAACACTGA GGCTCAGAGA

Supplementary Table III.1. (Cont'd).

chr[1]	SNP	Probe sequence
12	*DLX4*	GAGCCACGCC CGTCCACACC TACACTCCTC CTGACCTTTC TTTCAATCCT [T/C] CATTTTTCCT CCTTTCCCCC CATAGGTAAA GATCTGGTTT CAGAACAAAC
	PPM1E	AGCCACACAA ACCTGACAGA GAGGTAAGTA TGGTCTGTTC TGTTAGGACA [T/C] ATGCCACAAA GGCAGTTTTC TAATTATTAA TTTTCATTTA TCTCAGCAAC
	SLC6A4	TCGGGTGGAT GGAGGGGTCT TCCAAGAAAG TGGTGATTTC CATCAGTGTT [T/G] CGGGGAGCTA GGGACCTCCT GGAATCAGTC AGGCCTTAGG TATCCCCAGG
	SGSM2	AGCCTGTTCC TTTGGGGCAG GGAGATGGGG AGCCAGGCCA CAGGAACCCA [T/G] GGAGGGGCCC TTGGAAACCT CAGTCCCTTC CAGCTAGAAA CAGGGCAGAG
	PLD2	GACTTCAGCC CGCTGCCCCA GCCCCCTCTC CCTGACCCGA GAGCCCCCC [T/C] GCAGAGCCCT CCTCTCCCGG GGTCACCCAC CACGTCGTAT TCCCCGTCCC
	NLE1	CAAGTAAGGG ACTTCCCCAG TGGAGTTTGG TCCCCAAAGG CCAAGGATAC [A/G] TGCCCTGGAA GAGCCCTTCC TGGACTTGAC CTTGGGGTTG GGGTTCCTAG
	NUP88	TTCTTATGTT GAGATAGCTT AGAAGTGCTC TGCTAAAATG ACTGGCTTAA [T/G] GTTTCCGACG GGTAGGGACG TAATTCACGG AAGGCCAGCA GCAGCAAGTA
15	*PPAPDC1B*	TCCACTCTGG CTCAAGATAC GGGTTCTGAG GACGCTTTAG GGCGCCCGG [A/G] GTAACTGGTC ATTTAGTATA CGGGTGATGG GTGCTCCTGG TGGTACTGTG
	OCA2	GCTTGGAAGT TTCTCCCAAG AGGAAGGGTC ACGGTGCATA CCTGTTTACC [A/G] TCCAGAGTTC ATCACTGCTG ATGAACCTTG GGAGAACAGC TCAGCTGAGT
	ABCB11	GTCTGCAGAG AAAAGATGGA AAGGTTGAGG CAAT [C/G] ACGCATGCTT GCTCCCTGGC CCACCATGGT GAGAGTTCTG CCTCATACC
	PRKAG3 T30N	CATCCCGAGC TGTAACCACC AGCTCAGAAA GAAGCCATGG GGACCAGGGG A [A/C] CAAGGCCTC TAGATGGACA AGGCAGGAGG ATGTAGAGGA AGGGGGGCCT CC
	PRKAG3 I199V	ACACCATGCT GGAGATCAAG AAGGCCTTCT TTGCCCTGGT GGCCAACGGC [A/G] TCCGAGCGGC ACCTTTGTGG GACAGCAAGA AGCAGAGCTT CGTGGGGATG CTGAC
	MAP3K2	TTGAGAGAAC AAATACCGCC CACAAACCCA TCCCCTCTCC CACAGTCTGC [T/C] GTCTCCCAGC ACGGGAGTGC CACCAACCCA AGGCTTAGCC AGCATTGTGG
	SF3B1-1	CTAATGGTTT GGTGTTTTTA ACATAAAAGG AATTTGTGGA CTTTTTTTTT [A/T] AACCAGCTTA TTAGATGTAT TTATGAAAAT TTAAAACTCT AAAACAATAC

Supplementary Table III.1. (Cont'd).

chr[1]	SNP	Probe sequence
15	*TTN*	TCGTAAGTAC ACCTTTACCA TTTTCATTAC ATGCTTAAGG CCTCTATGTG [T/G] TTTTTATTTT TGGCTTTTGT CTTCTGAGCA CGGCAAATCC TCAGAAACCT
18	*ARF5*	AGCTGGGAAA AGGGAGCCTG GGGGCCCCCA GAGACCCCCC TCAGATGCCA [T/C] CGCCTCTGCC TTCTCTCAGT TGCAGGAAGA CGACGTGCGC GATGCGGTGC
	ING3-1	CACGACTGAT TGAGAAACAA ATATCCCTCC CCTTTTATTT TGTGAGATAT [A/G] TTTTTTCATG CAATTACTGG TGGATTTATC TTCTTCATTT GGGAA
	CRHR2	CTCCACCTGC GGCTGGCACA CTCAAAGCCG TGCA [T/C] GCCTTGAACCC GGGCCCCAGC CCCGTCACGC CTACCGCCC
	H2AFV	TGAAAATTGA TTCCTGTATA TAACTTAGTG AATTTTTACT TCTGCATACT [T/C] TGGCCTCACA TTTAAAGACT TCTTCGAAGT TGAACGTTAA ACTGTCATGG

[1]Chr = chromosome

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Chapter IV

Association of a corticotropin-releasing hormone receptor 2 (*CRHR2*) polymorphism with carcass merit, meat quality and stress response traits in pigs

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This chapter formatted for submission to the journal Molecular Biology Reports

Abstract

Corticotropin-releasing hormone receptor 2 (*CRHR2*) was evaluated as a potential candidate gene affecting stress response, carcass merit and meat quality traits in pigs. *CRHR2* is one of two receptors for corticotropin-releasing hormone and plays a key role in mediating stress response through the hypothalamic-pituitary-adrenal (HPA) axis. A single nucleotide polymorphism (SNP) was identified within the pig *CRHR2* gene and its position was localized to pig chromosome 18. The *CRHR2* SNP was used to screen a Duroc x Pietrain F_2 resource population and a Landrace-Yorkshire population that was exposed to a halothane challenge test. Association analyses were performed for carcass merit and meat quality traits recorded for each population, and for stress response phenotypes recorded for the halothane challenge population. For the resource population, *CRHR2* genotype was significantly associated with 45-min pH, ultimate pH (pH_u), 45-min temperature, drip loss, cook yield, moisture percent, protein percent, CIE b*, ham weight, and dressing percent. Pigs with the GG genotype had a lower 45-min carcass temperature which led to maintenance of a higher 45-min pH and pH_u. Consequently, early postmortem temperature and pH may have influenced cook yield and protein percent. For the halothane challenge population, *CRHR2* genotype exhibited a suggestive association with CIE L* and color score, as well as with blotching although no other stress response phenotypes showed an association with *CRHR2* genotype. This study supports an association of *CRHR2* genotype with meat quality and suggests additional research with additional pig populations may be warranted.

Keywords: Corticotropin-releasing hormone receptor 2, stress response, carcass merit, meat quality

Introduction

Tremendous efforts to obviate inferior meat quality resulting from animal stress have been continuing in the pig breeding industry since pale, soft, exudative (PSE) pork was recognized in the 1950s. Thereafter the prevalence of the porcine stress syndrome (PSS) condition and its effect on the occurrence of PSE pork became a concern, which the National Pork Producers Council affirmed as the most important quality problem in the 1990s [1-4]. PSE pork induced from the rate and extent of muscle pH decline postmortem and high muscle temperature [5] is influenced by genetic, biochemical and environmental factors. PSS is a non-pathological disorder affected by diverse stressors such as transportation, temperature, restraint, weaning and handling [6], and its symptoms are characterized by rapid respiration, muscle tremors and rigidity, reddish blotches on the skin, increases in body temperature, and even death. In order to identify stress susceptible pigs, a halothane challenge test has been used [7]. This test was unable to distinguish heterozygous (N/n) stress carriers from homozygous normal (N/N) individuals. Identification of a polymorphism at position 1843 in the skeletal muscle ryanodine receptor 1 (*RYR1*) gene that was highly associated with PSS facilitated development of a DNA-based assay for detecting the C1843T mutation which allowed identification of heterozygous carriers (N/n) [8,9]. However, despite efforts to reduce the incidence of PSE pork, PSE pork was 15.5 percent of US slaughter pigs in 2002 which corresponds to $90 million per year in lost revenue [1].

Another potential candidate gene for animal stress may be corticotropin-releasing hormone receptor 2 (*CRHR2*). Corticotropin-releasing hormone (*CRH*) plays a key role in mediating the endocrine, autonomic, behavioral, and immune responses to stress through the hypothalamic-pituitary-adrenal (HPA) axis stimulating cortisol release [10,11]. *CRHR1* and *CRHR2*, two receptors of *CRH*, are expressed in distinct locations implying different physiological functions

[12]. *CRHR2* is found in skeletal, smooth, and cardiac muscle as well as brain, whereas *CRHR1* is found in ovary, testis, skin, and adrenal gland. At the DNA level, a C/T polymorphism at position 233 within the *CRH* gene was associated with meat color in Pietrain pigs [13]. In addition, mRNA abundance of *CRH* in brain of stressed pigs was higher than in unstressed pigs [14]. A relationship of *CRHR2* and meat quality is plausible, but no studies have been done to consider potential associations between *CRHR2* and meat quality.

In the present study, *CRHR2* was evaluated as a potential candidate gene affecting stress response and influencing polygenic carcass and meat quality traits. The effect of *CRHR2* genotype was investigated using an experimental F_2 resource population and a commercial pig population that was exposed to a halothane challenge test.

Materials and methods

Animals and traits

Two independent pig populations, a Duroc x Pietrain F_2 resource population and a halothane challenge population, were used for this study. Animal protocols were approved by the Michigan State University (MSU) Institutional Animal Care and Use Committee (AUF# 09/03-114-00 and AUF# 02/07-011-00, respectively). The MSU resource population was derived from 4 F_0 Duroc sires and 15 F_0 Pietrain dams, and 438 F_2 pigs were genotyped for this study. All grandparents were confirmed to be homozygous normal for the polymorphism at position 1843 in the *RYR1* gene [14]. A detailed description of the animals and phenotypic data was previously reported [15,16]. Briefly, animals were slaughtered at the Michigan State University Meat Laboratory (East Lansing, MI) or a federally inspected commercial plant (DeVries Meats, Coopersville, MI).

Slaughter age was 165.8 ± 9.2 days and the average off-farm body weight (BW) at slaughter was 111.15 ± 8.98 kg. Hot carcass weight (HCW), and pH and temperature of the *longissimus* muscle (LM) at 45-min and 24-h (pH_u) postmortem were obtained. After overnight chilling, backfat thickness, number of ribs and carcass length were measured, and the weights of primal cuts were recorded. A single trained evaluator scored color, marbling and firmness using two 2.54-cm thick chops cut from the LM, and objective color scores of CIE L*, a*, and b* were obtained using a Minolta colorimeter. The remaining section of the LM was used to determine drip loss, cook yield, Warner-Bratzler shear force, proximate analysis measures, and sensory attributes. A trained sensory panel evaluated juiciness, tenderness, overall tenderness, connective tissue, and off-flavor using an 8-point hedonic scale. Descriptive statistics for phenotypes used in this study are presented in Table IV.1.

The second population was comprised of 363 pigs produced in four farrowing groups from Yorkshire-Landrace F_1 sows bred to Landrace boars at the MSU Swine Teaching and Research Center. Pigs were subjected to a halothane gas challenge test at 56 ± 3 d of age with the test repeated 2 d after the first test. Pigs were chosen at random for halothane gas challenge which consisted of pig exposure to 5% halothane gas in a closed system at a rate of 2 L/min for three minutes per pig for the first farrowing group and two minutes per pig for subsequent farrowing groups. After 1 min inhalation of halothane, limb rigidity, blotching of the skin, and muscle tremors were recorded on a binary scale (0 = no response; 1 = response) and body temperature was measured. Animals were slaughtered at a federally inspected commercial facility (J.H. Routh Packing Co, Sandusky, OH). The pH of the LM at 45-min and 18-h (pH_u) postmortem were measured and pH decline from 45-min to 18-h was calculated. After overnight chilling,

subjective color and marbling scores were evaluated, objective color scores of CIE L*, a*, and b* were measured using a Hunter colorimeter, and drip loss was estimated from LM using filter paper [17]. Descriptive statistics for phenotypes used in this study are presented in Table IV.2.

CRHR2 genotyping

DNA was isolated from white blood cells for pigs from the resource population using standard procedures as previously reported [14]. Primers for use in the polymerase chain reaction (PCR) were designed from heterologous *CRHR2* sequences using the OLIGO® 5.0 Primer Analysis Software (NBI, Plymouth, MN) to amplify a portion of the pig *CRHR2* gene (forward primer 5′-GCA TCA CCT ACA TGC TCT TCT T-3′; reverse primer 5′-CTC TCC ATT GAA GAA GCA GTA GA-3′), and identity of the pig *CRHR2* sequence-tagged site (STS) was confirmed by sequencing. A single nucleotide polymorphism (SNP) in *CRHR2* was identified by sequencing [18] and information on the SNP and the pig STS has been deposited into appropriate databases (dbSNP ID: ss86353512, GenBank accession no. BV727800). A PCR restriction fragment length polymorphism (RFLP) approach was used to genotype *CRHR2* in the MSU resource population. Approximately 25 ng of genomic DNA was amplified in a final volume of 10 µl containing 0.5 µM each primer, 200 µM dNTPs, 1.5 mM $MgCl_2$, 1x PCR buffer, and 0.05 units Taq DNA polymerase. Amplifications were performed in a MJ Research thermal cycler with a profile that included an initial denaturation at 94 °C for 10 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 58 °C for 1 min, and extension at 72 °C for 1 min followed by a final 10 min extension at 72 °C. Subsequently, the 422 bp amplicons were digested with the endonuclease *NlaIII* resulting in two alleles (cut fragments 258 bp and 164 bp designated allele A; uncut fragment 422 bp designated allele G).

For the halothane challenge population, DNA was purified from white blood cells using an Invitrogen PureLink Genomic DNA minikit (Invitrogen Corp., Carlsbad, CA) following the manufacturer's instructions. Quantity and quality of purified DNA was measured on a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA). An Invitrogen Quant-iT dsDNA assay was also used to quantify extracted DNA, according the manufacturer's instructions, and samples were read using an Invitrogen Qubit fluorometer. In order to more rapidly genotype this population, the *CRHR2* SNP was included in an Illumina Goldengate 96 plex SNP multiplex custom assay panel (Illumina, Inc. San Diego, CA). Pigs were genotyped with the custom assay, and the assay was read using an Illumina BeadXpress Reader at the MSU Research Technology Support Facility. Genotype segregation was then determined using the Illumina Genome Studio software.

Statistical analysis

Allele and genotype frequencies for the *CRHR2* polymorphism were estimated by the ALLELE procedure of SAS (version 9.1.3). Hardy-Weinberg equilibrium (HWE) was tested by using 10,000 permutations to approximate p-values for HWE, and 1,000 bootstrap samples were used to calculate 95% confidence intervals for allele frequency and Hardy-Weinberg disequilibrium coefficients. The association analyses between *CRHR2* genotypes and traits were performed using the MIXED or GLIMMIX procedure software of SAS (version 9.1.3), depending on the response variable distribution. The phenotypic traits for which *CRHR2* genotype was significant in the model were considered further for comparison of least-squares means. Additive effect of *CRHR2* was estimated as half of the difference between two homozygous genotypes ($a = ½$ (AA – GG)) and dominance effect was estimated as the

difference between the heterozygous genotype and the average of the two homozygous genotypes (d = AG – ½(AA + GG)).

For the resource population, the statistical model was as follows;

$$Y_{ijklmn} = \mu + Sex_j + CRHR2_k + Farrowing_l + Harvest_m + Litter_n + e_{ijklmno}$$

where Y_{ijklmn} is the trait measured on animal i, μ is the overall mean for the trait, Sex_j is the fixed effect of the animal's gender (j = barrow or gilt) and $CRHR2_k$ is the fixed effect of *CRHR2* genotype (k = AA, AG or GG). $Farrowing_l$, $Harvest_m$, and $Litter_n$ are random effects of farrowing group (l = 11 levels), harvest date (m = 33 levels) and litter (n = 55 levels), respectively, and $e_{ijklmno}$ is the residual term.

For the halothane challenge population, a generalized linear mixed model with a logit link function was used to analyze the binomially distributed response traits (blotching, rigidity and tremor) and then estimates were transformed back to the original scale via the inverse link function in order to compare least-squares means. The statistical model for halothane response traits was as follows [1];

$$Y_{ijklm} \sim Bernoulli(P_{ijklm})$$

$$logit(P_{ijklm}) = \mu + Sex_j + CRHR2_k + Farrowing_l + Litter_m + e_{ijklmn} \quad [1]$$

where P_{ijklm} is the probability of the i th animal exhibiting a positive response for the measured trait, μ is the overall mean for the trait, Sex_j and $CRHR2_k$ are the fixed effects. $Farrowing_l$, and $litter_m$, are random effects of farrowing group (l = 4 levels) and litter (m = 38 levels), respectively. Body temperature was analyzed with the same fixed and random effects as [1] using a linear mixed model.

For carcass and meat quality traits in the halothane challenge population, the summation of binary recorded blotching, rigidity and tremor per animal was treated as an overall halothane effect (denoted as Gas in the model) and used as a fixed effect along with sex and *CRHR2* genotype. The statistical model used was as follows [2];

$$Y_{ijklmno} = \mu + Sex_j + CRHR2_k + Gas_l + Farrowing_m + Harvest_n + Litter_o + e_{ijklmnop} \quad [2]$$

where $Y_{ijklmno}$ is the trait measured on animal i, μ is the overall mean for the trait, Sex_j, $CRHR2_k$ and Gas (l = 0 – 3) are the fixed effects. $Farrowing_m$, $Harvest_n$ and $litter_o$, are the random effects of farrowing group (m = 4 levels), harvest date (n = 8 levels) and litter (o = 38 levels), respectively.

For growth traits in the halothane challenge population, the model was as follows [3];

$$Y_{ijklmn} = \mu + Sex_j + CRHR2_k + Gas_l + Farrowing_m + Litter_n + e_{ijklmno} \quad [3]$$

where Y_{ijklmn} is the trait measured on animal i, μ is the overall mean for the trait, Sex_j, $CRHR2_k$ and Gas_l are the fixed effects. $Farrowing_m$, and $litter_n$, are the random effects of farrowing group (m = 4 levels) and litter (n = 38 levels), respectively. For average daily gain from weaning to off-farm date, body weight of age at weaning was included as a covariate.

Results

Allele and genotype frequencies

Allele and genotype frequencies for *CRHR2* in both the resource population and the halothane challenge population are shown in Table IV.3. The frequency of the *CRHR2* allele A was 0.38 and that of allele G was 0.62 in the resource population, whereas the frequency of allele

A was 0.60 and that of allele G was 0.40 in the halothane challenge population. Frequencies in both populations were in agreement with Hardy-Weinberg equilibrium.

Resource population

Based on the location of *CRHR2* on human chromosome 7 (GenBank accession no. NM_001883), pig *CRHR2* was expected to be located on SSC18. This was previously confirmed using the *CRHR2* STS by both linkage mapping with the PigMap reference families and radiation hybrid (RH) mapping using the INRA University of Minnesota porcine RH panel [18]. The position of *CRHR2* was determined to be near marker *S0062* (lod score = 16.83) on SSC18 by RH mapping and *CRHR2* also showed evidence of linkage with marker *S0062* (lod score = 4.86) in the PigMap families. In our Duroc x Pietrain resource population, *CRHR2* was localized to 95.4 Kosambi cM which was at the distal end of SSC18 on the map for this population (*SW1808 – SW2540 – SW1023 – SW1984 – S0062 – CRHR2*), and was consistent with the results obtained by RH mapping and linkage mapping in the PigMap population. Localization of the pig *CRHR2* STS to the pig genome sequence (Bulid 9; http://www.ensemble.org) indicates a start position for the STS of 40,666,139 bp on SSC18.

Results of the association analysis of *CRHR2* genotypes with carcass merit and meat quality traits for the resource population are presented in Table IV.4. Pigs exhibiting the GG genotype had significantly higher LM 45-min pH and pH_u values than pigs with the AA genotype ($P < 0.05$), and consequently GG pigs also had a lower moisture percent and a greater cook yield ($P < 0.05$). The GG pigs also exhibited a higher LM protein percent than the AA and AG pigs ($P < 0.05$) consistent with the decreased moisture percent. A significant additive effect was observed for these traits such that the addition of a G allele increased pH values, cook yield and protein

percent, and decreased moisture percent. Pigs with the GG genotype also exhibited a lower LM drip loss than pigs with the AG genotype ($P < 0.05$), although pigs with the AA genotype were intermediate and not significantly different from either AG or GG pigs. In addition pigs with the GG genotype exhibited a lower 45-min carcass temperature than pigs with the AG genotype ($P < 0.05$), with no significant difference from AA pigs observed.

Higher values for CIE b* indicating more yellowness for LM color were observed for pigs with the GG and AG genotypes compared to pigs with the AA genotype ($P < 0.05$), and this trait exhibited a significant additive effect. Pigs with the GG genotype also exhibited a lower ham weight compared to pigs with the AA or AG genotypes ($P < 0.05$). Interestingly, a significant dominance effect was observed for dressing percent ($P < 0.05$) such that pigs with the AG genotype exhibited a higher dressing percent than pigs with either homozygous genotype.

Halothane challenge population

Results of the association analysis of *CRHR2* genotypes with stress response, carcass merit and meat quality traits for the halothane challenge population are presented in Table IV.5. Association analyses were performed to investigate the relationship between *CRHR2* genotypes and four halothane challenge response traits: blotching, rigidity, tremor and body temperature. Pigs with the GG genotype exhibited less blotching ($P = 0.07$) than pigs with the AG genotype. For carcass and meat quality traits, meat from pigs with the GG genotype exhibited a lighter muscle color as measured by CIE L* compared to AG genotype pigs ($P = 0.07$). Subjective color score also tended toward significance for this population such that AG genotype pigs had higher scores (darker color) than AA pigs ($P = 0.08$). A significant dominance effect ($P < 0.05$) was

observed for both CIE L* and color score indicating that heterozygous animals exhibited darker meat than homozygous animals.

Discussion

CRHR2 is one of two receptors for *CRH* ,and it is a G-protein-coupled receptor that functions in stress response and energy homeostasis [12]. The major ligands for *CRHR2* are *urocortin II* and *urocortin III* which are two *CRH*-related neuropeptides and selective agonists [19,20]. *CRHR2* contributes to an animal's ability to cope with stressful situations such as feeding suppression, hypotension, and anxiolysis, and it is also involved in weight regulation. The *CRHR2* gene contains 17 exons, and three expression variants have been reported in human and rodents: $CRHR2_{\alpha}$, $CRHR2_{\beta}$ and $CRHR2_{\gamma}$ [21-23] with $CRHR2_{\beta}$ being the major *CRHR2* isoform expressed in skeletal muscle tissue in rodents [24].

Glucocorticoid (*GC*) that is synthesized and secreted from the adrenal cortex as a negative feedback response to stress has been shown to induce skeletal muscle proteolysis [25], and to differentially regulate the expression of the *CRHR*s [26]. Elevated *GC* under stress response stimulates muscle proteolysis and affects meat quality [27-29]. Shaw et al. [25] reported that elevated cortisol levels in skeletal muscle were highly correlated with cortisol levels in plasma, and were also associated with meat quality traits exhibiting a positive correlation with ultimate pH and color score and a negative correlation with drip loss [27]. A positive relationship has also been shown between plasma cortisol level and muscle rigidity in humans [30].

For the present study *CRHR2* was evaluated as a candidate gene for potential effects on carcass merit and meat quality traits, and also effects on response to stress induced by a halothane gas challenge test. In the F_2 resource population, *CRHR2* genotypes were significantly

associated with 45-min pH, pH_u, 45-min carcass temperature, drip loss, cook yield, moisture percent, protein percent, CIE b*, ham weight, and dressing percent. In the halothane challenge population, *CRHR2* genotype influenced blotching, CIE L* and color at a suggestive significance level.

The *CRHR2* gene was highly associated with pH_u in the resource population ($P < 0.002$). A previous genome scan of this population had revealed a QTL for pH_u on SSC18, although the position of this QTL did not overlap the map position of *CRHR2* [16]. Other QTL studies have identified QTL for pH_u in the region where *CRHR2* is located [31,32]. A significant effect of *CRHR2* genotype on carcass temperature and pH supports a potential role for this gene in meat quality since early postmortem temperature and pH influence variation in expression of other meat quality traits [33]. For pigs with the GG genotype, a lower 45-min carcass temperature led to maintaining a higher 45-min pH and pH_u, thus resulting in higher cook yield. An interesting allelic interaction known as overdominance was observed for dressing percent which surpasses both homozygous genotypes. No QTL have been reported for dressing percent in this region of SSC18.

In the halothane challenge population, *CRHR2* genotype exhibited only a suggestive association with L* and color score, and with blotching. Muráni et al. [34] also evaluated *CRHR2* as a potential candidate gene for stress response phenotypes. In contrast to the present study that used halothane gas as a measure of stress susceptibility, Muráni et al. [34] induced stress by mixing of individuals with different aggressive temperaments. They measured plasma concentrations of cortisol, creatine kinase, glucose and lactose, adrenal weight, and also lesion

scores as an assessment of aggressive behavior. No significant associations were identified between *CRHR2* genotypes and stress response traits [34].

The present study used two populations to evaluate *CRHR2* as a potential candidate gene affecting carcass merit, meat quality and stress response phenotypes. Results for the Duroc x Pietrain F_2 resource population support an association of *CRHR2* with meat quality, and indicate the potential for animals with the GG genotype to have more favorable meat quality. The halothane challenge population had fewer carcass and meat quality trait phenotypes recorded than were available for the resource population. However, there was little evidence for association of *CRHR2* genotypes with meat quality in this population, with only L* and color score exhibiting a suggestive association. Differences in response between the two populations could be due to breed (genetic background) and other differences between the populations. In addition, stress response phenotypes measured in the halothane challenge population did not exhibit significant associations with *CRHR2* genotype with only blotching showing a suggestive relationship. This result taken together with a previous study of *CHRH2* associations with stress response [32] suggest that *CRHR2* may not be a strong candidate for stress response traits. Further research considering the relationship of *CRHR2* with pig carcass merit and meat quality phenotypes in additional populations is warranted.

Acknowledgements

This project was supported in part by MSU AgBioResearch, and the halothane challenge population was developed through a MSU Animal Industry Initiative Coalition Grant. The authors thank M.E. Doumit, E.E. Helman, and D.B. Edwards for assistance with data collection for the pig populations, and J.P. Steibel for advice on statistical analysis.

Table IV.1. Number of records, means, and SD for MSU Duroc x Pietrain F_2 resource population.

Trait	N	Mean	SD
Off-farm BW, kg	438	111.15	8.98
Hot carcass weight, kg	438	80.91	7.18
Dressing percent, %	438	72.77	2.01
45-min carcass temperature, °C	437	39.15	2.36
24-h carcass temperature, °C	436	2.74	1.18
45-min pH	431	6.37	0.23
24-h pH	430	5.53	0.15
45-min to 24-h pH decline	424	0.84	0.23
Carcass length, cm	437	78.82	2.57
Number of ribs	294	14.83	0.89
First-rib backfat, mm	373	40.49	7.21
Last-rib backfat, mm	437	28.47	6.27
Last-lumbar vertebra backfat, mm	436	22.15	6.67
Tenth-rib backfat, mm	433	23.66	7.10
LM area, cm^2	434	40.98	4.66
Ham weight, kg	437	9.52	0.81
loin weight, kg	437	8.18	0.87
Boston shoulder weight, kg	437	3.72	0.63
Picnic shoulder weight, kg	437	3.84	0.60
Belly weight, kg	437	4.92	0.70
Spareribs weight, kg	436	1.48	0.21
Color, 1 to 6	436	3.26	0.80
Marbling, 1 to 10	437	2.88	0.80
Firmness, 1 to 5	423	2.86	0.80
CIE L*	421	53.60	2.17
CIE a*	421	17.42	1.96
CIE b*	421	8.98	1.64
Moisture, %	429	73.94	1.51
Fat, %	429	3.27	1.32
Protein, %	428	23.33	1.11
Drip loss, %	436	1.77	1.19
Cook yield, %	434	77.40	2.91
Warner-Bratzler shear force, kg	434	3.18	0.70
Juiciness, 1 to 8	435	5.28	0.59
Tenderness, 1 to 8	435	5.58	0.61
Overall tenderness, 1 to 8	435	5.65	0.56
Connective tissue, 1 to 8	435	6.39	0.39
Off-flavor, 1 to 8	435	1.14	0.21

Table IV.2. Number of records, means, and SD for halothane challenge population.

Trait	N	Mean	SD
Weight at birth, kg	363	1.49	0.33
Weight at weaning, kg	363	6.48	1.43
Weight at slaughter, kg	348	116.30	13.13
45-min pH	323	5.93	0.24
18-h pH	323	5.49	0.17
45-min to 18-h pH decline	326	0.43	0.24
CIE L*	327	55.15	4.15
CIE a*	327	7.74	1.36
CIE b*	327	15.39	1.01
Subjective color score, 1-6	327	2.12	0.88
Subjective marbling score, 1-10	325	1.95	0.71
Drip loss, %	324	5.08	1.87
ADG, g/day	348	692.45	99.44
Body temperature on day 1 of halothane challenge, °C	363	39.65	0.37
Limb rigidity score on day 1 of halothane challenge, 0 or 1	363	0.74	0.44
Front leg tremors on day 1 of halothane challenge, 0 or 1	363	0.17	0.37
Blotch score on day 1 of halothane challenge, 0 or 1	363	0.21	0.41

Table IV.3. Allele and genotype frequencies for *CRHR2* in two pig populations.

Population	Allele frequency		P-value	Genotype frequency		
	A	G	(χ^2 test)	AA	AG	GG
Resource population	0.38	0.62	0.64	0.14	0.48	0.38
Halothane challenge	0.60	0.40	0.62	0.37	0.47	0.17

Table IV.4. Least squares means by *CRHR2* genotype for carcass and meat quality traits significant in resource population.

Trait	obs	AA (n=61)	AG (n=211)	GG (n=171)	Additive[1]	Dominance[1]
24-h pH (ultimate pH)	430	5.50 ± 0.02 [a]	5.51 ± 0.02 [a]	5.57 ± 0.02 [b]	-0.03 ± 0.01 **	-0.03 ± 0.02
45-min pH	431	6.30 ± 0.04 [a]	6.37 ± 0.03 [b]	6.42 ± 0.03 [b]	-0.06 ± 0.02 **	0.01 ± 0.03
45-min carcass temp	437	39.28 ± 0.43 [ab]	39.36 ± 0.37 [a]	38.92 ± 0.38 [b]	0.18 ± 0.16	0.26 ± 0.18
CIE b*	421	8.90 ± 0.38 [a]	9.23 ± 0.37	9.23 ± 0.37 [b]	-0.16 ± 0.07 *	0.16 ± 0.09
Cook yield, %	434	76.67 ± 0.49 [a]	77.29 ± 0.31 [a]	78.01 ± 0.34 [b]	-0.67 ± 0.28 *	-0.05 ± 0.34
Dressing percent	438	72.56 ± 0.35 [ab]	73.03 ± 0.26 [a]	72.51 ± 0.27 [b]	0.02 ± 0.17	0.49 ± 0.20 *
Drip loss, %	436	1.79 ± 0.20 [ab]	1.90 ± 0.14 [a]	1.53 ± 0.15 [b]	0.13 ± 0.10	0.24 ± 0.13
Ham weight, kg	437	9.62 ± 0.08 [a]	9.56 ± 0.05	9.44 ± 0.06 [b]	0.09 ± 0.04 *	0.03 ± 0.05
Moisture percent	429	74.26 ± 0.28 [a]	73.98 ± 0.22 [a]	73.58 ± 0.23 [b]	0.34 ± 0.13 **	0.06 ± 0.16
Protein percent	428	23.15 ± 0.18 [a]	23.32 ± 0.12 [a]	23.55 ± 0.13 [b]	-0.20 ± 0.10 *	-0.03 ± 0.13

[1] Significant additive and dominance effect at the * $P < 0.05$ and ** $P < 0.01$

[a,b] LS mean different at $P < 0.05$

Table IV.5. Least squares means by *CRHR2* genotype for traits significant in halothane challenge population.

Trait	obs	AA (n=129)	AG (n=171)	GG (n=60)	Additive[1]	Dominance[1]
Blotch	345	0.07 ± 0.08^{ab}	0.08 ± 0.09^{a}	0.03 ± 0.04^{b}	0.36 ± 0.26	0.47 ± 0.34
Color score	324	2.01 ± 0.16^{a}	2.20 ± 0.15^{b}	2.00 ± 0.19^{ab}	0.01 ± 0.08	0.19 ± 0.10*
CIE L*	324	55.55 ± 1.13^{ab}	55.01 ± 1.10^{a}	56.11 ± 1.20^{b}	-0.28 ± 0.33	-0.82 ± 0.42*

[1] Significant additive and dominance effect at the * $P < 0.05$

[a,b] LS mean different at $P < 0.10$

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Discussion

Discussion

Quantitative trait loci (QTL) are genomic regions that affect phenotypic variation and that are generally influenced by several polymorphic genes and environmental factors. QTL mapping performed with phenotypic and genotypic data has often been underestimated for its important role in contributing to the understanding of the genetic basis of phenotypic variation due to an emphasis on the limitations of QTL mapping. This is because not only have a limited number of causative genes been identified as a result of coarse map resolution and different gene segregation patterns across or within breeds, but also in pigs more than 6,000 QTL have been reported for growth, carcass, meat quality, reproduction and animal health traits (http://www.animalgenome.org/cgi-bin/QTLdb/SS/index). However, according to the Pig QTL database, a limited number of pig resource populations have been constructed for QTL mapping in the United States [1-8]. Furthermore, few studies have used the Duroc breed in their resource population, and Duroc is one of the most popular sire breeds in the United States. In addition, the size of many Duroc-containing populations was relatively small with relatively few phenotypic traits measured. Among US populations that include Duroc parentage and have data reported, our Duroc x Pietrain resource population developed at MSU is larger and more comprehensive [6-9].

A Duroc x Pietrain F_2 resource population was previously constructed at MSU to discover quantitative trait loci (QTL) influencing growth, carcass merit and meat quality traits. The Duroc and Pietrain breeds were selected because they are utilized in commercial pig breeding worldwide and they exhibit widely different phenotypic variation [10, 11]. In our previous study,

Edwards et al. [7, 9] revealed 55 QTL for growth traits and 94 QTL for carcass and meat quality traits through a genome scan which used a line-cross analysis model with 124 microsatellite markers in 510 F_2 animals.

In order to identify additional QTL, three least-squares Mendelian models for QTL analysis were applied for a second genome scan (SSC1 – 18). This scan included all of the marker data from the first scan along with 20 new markers on 9 selected chromosomes (SSC3 – 7, 12, 15, 16 and 18) and 20 markers from the first scan for 444 additional F_2 animals. Increasing the number of markers and animals facilitated detection of new QTL (SSC7, 15 and 18), as well as confirmation of previously identified QTL affecting growth traits (SSC4, 6, and 16) using the line-cross analysis [7]. Also, the second scan narrowed the confidence intervals of QTL and increased the statistical power compared to results of the first scan. The half-sib analysis for which F_2 individuals were treated as 6 paternal half-sib families revealed 12 QTL for growth traits on SSC4 – 8, 15 and 18. For carcass and meat quality traits, the line-cross analysis revealed 50 QTL including 14 new QTL on 6 chromosomes (SSC3, 6, 7, 12, 16, and 18) which had not been identified in the first genome scan [9]. The half-sib analysis revealed 38 QTL, and 3 additional QTL were detected using the combined line-cross and half-sib model.

In order for QTL results to lead to applications for the industry, putative QTL identified in resource populations require validating the segregation of QTL in commercial populations. Based on the first genome scan, QTL regions on 5 chromosomes (SSC3, 6, 12, 15, and 18) were selected for further evaluation. Gene-specific SNP markers located in the QTL regions were genotyped in an U.S. commercial purebred Duroc population for a SNP association study. Thirty-three of 81 gene-specific SNPs were segregating and were evaluated for associations with

pH, color, marbling, days to 113 kg, backfat thickness, and LMA. Eleven SNPs exhibited significant effects on one or more carcass merit or meat quality traits measured in this population. The SNP evaluation study showed a consistency with our previous genome scans for pH on SSC3, marbling score on SSC6, pH on SSC12, L* on SSC15 and pH on SSC18. As expected, the effect of the SNP markers for the analyzed traits was not as significant as the genome scan. There are several reasons to explain the reduced statistical power. First, a limited number of SNP markers were genotyped across the putative QTL regions. For example, the confidence interval (CI) for the 10^{th}-rib backfat QTL on SSC6 which was highly significant in the genome scan was estimated to span a 30 cM interval (118 – 148 cM) [9], but only 5 SNP markers were segregating and used to analyze traits in the Duroc population. Considering a genetic distance of 1 cM roughly corresponds to about 1 million base pairs (bp) or 10 genes in mammals [12], the use of 5 SNP markers was not enough to expect high linkage disequilibrium with the QTL. Second, there were genetic differences between the MSU resource population and the purebred Duroc population. Finally, the size of the Duroc population was relatively small and many more animals would be needed to do a thorough QTL validation study.

Based on QTL detected on SSC18 and the biological function of corticotropin-releasing hormone receptor 2 (*CRHR2*), *CRHR2* was evaluated as a potential candidate gene affecting stress response and influencing polygenic carcass and meat quality traits in the MSU resource population and also in a halothane challenge population. To prevent inferior meat quality caused by animal stressors, mutations within the *RYR1* and *PRKAG3* genes have been used for selection in commercial breeding programs [13, 14]. However, these two mutations cannot explain all of the phenotypic variation in pork quality because pale, soft, exudative (PSE) pork resulting from animal stress is still a major concern in the pig industry. *CRHR2* contributes to an animal's

ability to cope with stressful situations such as feeding suppression, hypotension, and anxiolysis, and it is also involved in weight regulation. *CRHR2* genotype was associated with carcass and meat quality in the MSU resource population supporting it as a potential candidate gene for the relationship between stress and meat quality traits. However, *CRHR2* genotype showed only a suggestive association with blotching, L* and color score in the halothane challenge population. Reasons for this difference include breed differences between the two populations as well as the relatively small size of the halothane challenge population.

Genome scans in the Duroc x Pietrain F_2 population revealed many QTL influencing growth, carcass merit and meat quality traits and also, evaluation of identified QTL using SNP markers and candidate gene analysis were performed in commercial populations. With the pig genome sequence, the availability of high-throughput SNP genotyping will help to overcome the limitations of traditional QTL mapping. Putative QTL regions identified in the genome scan can be narrowed with the high density SNP chip. Also, confirmation of QTL effects using SNP genotyping in large commercial populations will be promising. In addition, comparison of QTL results with the results of expression QTL analyses in the resource population and conducting genome-wide association studies will facilitate understanding of the genetic basis of complex traits and incorporation into pig breeding programs.

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