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Association of single nucleotide polymorphism (SNP) markers in candidate genes and QTL regions with pork quality traits in commercial pigs☆,☆☆

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ABSTRACT

Numerous reports have described genetic markers or genomic regions (QTL) associated with pork quality and/or palatability but few validation studies have been reported. Therefore, 156 SNP markers from 45 candidate genes and eight QTL regions were analyzed for association with pork quality and palatability traits from 888 pork loins. Loins were collected at three slaughter facilities and selected to represent a wide range of pork color, pH and marbling. Phenotypic data recorded included objective and subjective measures of color and marbling, purge loss, shear force, and cooking loss. Data were analyzed with SAS PROC MIXED where loin was fit as a random effect. Results indicated some of the markers tested should be useful in industry, while others are not segregating in all populations or linkage disequilibrium between markers and causative genetic variation fluctuates among populations limiting their universal utility. Genes with the largest effects on pork quality were MC4R, IGF2, CAST and PRKAG3.

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1. Introduction

Numerous reports of genetic markers for pork quality have been published. These studies include both genome scans and candidate gene approaches implemented in populations ranging from experimental F2 populations using exotic breeds to standard commercial populations. Unfortunately, few associations have been validated in additional populations of commercial pigs. To date, the markers most consistently associated with pork quality include ryanodine receptor 1 (RYR1; Fujii et al., 1991; Leach, Ellis, Sutton, McKeith, & Wilson, 1996), protein kinase adenosine monophosphate-activated γ_3 subunit (PRKAG3; Milan et al., 2000; Ciobanu et al., 2001), MC4R (Kim, Larsen, Short, Plastow, & Rothschild, 2000a) and recently calpastatin (CAST; Ciobanu et al., 2004; Lindholm-Perry et al., 2009; Nonneman et al., 2011).

Genetic markers that are predictive of pork quality could be used for genetic selection programs or enable processors to determine the best market for specific pork products. Most genetic selection in commercial swine is conducted within specific commercial proprietary lines where markers specific to each line may be the most economical application of the technology. However, independent swine producers or pork processors do not have access to this information, and genetic markers that are the causative genetic variant (quantitative trait nucleotide, QTN) or in strong linkage disequilibrium with the QTN are needed. To determine a marker's utility in multiple commercial populations requires a broad sampling of market animals with relevant phenotypic data.

The objective of this study was to test markers in candidate genes and within reported QTL regions for associations with measures of pork quality in a group of pork samples collected at three different abattoirs harvesting commercial market hogs.

2. Material and methods

2.1. Samples

Loin selection and processing is discussed in greater detail by Moeller et al. (2010). Briefly, loins were sampled from three different commercial abattoirs during the fall and spring with a total of 20 different sampling dates. To ensure a broad sampling of commercial germplasm and farms, each day the loins selected were harvested over an eight hour timeframe. Tissue samples were available for DNA extraction from fresh boneless loins sampled from two facilities (222 and 219 loins each) while a third facility provided fresh loins (n = 223) as well as loins enhanced by injection of a solution intended to improve tenderness and juiciness (n = 224). In an attempt to uniformly represent the range of pork quality observed commercially, an initial classification of high, medium or low for muscle pH, Minolta

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Table 1
Descriptive statistics of traits measured before enhancement.

Trait	Number of records	Mean	Standard deviation	Minimum	Maximum
Minolta a*	906	17.26	1.40	11.70	21.02
Minolta b*	906	4.94	1.33	1.90	10.60
Minolta L*	906	52.90	4.34	40.91	67.50
Loin pH 24 h post-mortem	906	5.77	0.24	5.34	6.65
Color score	906	3.15	1.02	1.00	6.00
Marbling score	906	2.56	1.27	1.00	6.00
Intramuscular fat content	904	3.09	1.38	0.22	6.93

L* and marbling was determined. Loins were selected to uniformly fill all cells of a $3\times3\times3$ design; however, due to a strong negative correlation between Minolta L* and pH, cells containing loins with high pH and Minolta L* values as well as cells containing loins with low pH and Minolta L* values were not equally represented. No information was available on pre-harvest management of these pigs and sex determination was not attempted.

2.2. Phenotypic data

Description of procedures used to collect pork quality measurements were presented in Moeller et al. (2010). Briefly, whole boneless loins were collected approximately 24 h post-mortem, cut near the seventh rib and allowed to bloom for 10 min. Then loin pH and L*, a* and b* color measurements using a Minolta colorimeter were recorded. Subjective visual color and marbling scores (1 to 6 scale) were collected as outlined by the National Pork Producer Council (NPPC, 2000). A loin sample was obtained to measure intramuscular fat content (IMF) by an ether extract method (AOAC, 2007).

Enhanced loins were injected with solution prior to aging. Loins were then weighed, vacuum packaged and aged at 2 °C for 7 to 10 days. Loins were removed from packaging, weighed to determine loin purge, and sliced into 2.54 cm thick chops and frozen at −28.8 °C for storage.

Four frozen chops from each loin were used for Warner–Bratzler shear force determination. Each chop was weighed frozen and thawed to determine thaw purge and then one chop from each loin was selected to be cooked to 145, 155, 165 or 175° F (62.8, 68.3, 73.9 or 79.4 °C, respectively) internal temperature. Cooking time and final temperature was recorded along with cooked weight to determine cooking loss. Chops were cooled to 22 °C for 4 h. Six 1.27 cm diameter cores were removed from each chop parallel to the longitudinal orientation of the muscle fibers and sheared with a Warner–Bratzler shearing device. The average of all six cores was analyzed. Mean, range and standard deviation are presented for each phenotype in Tables 1 and 2.

2.3. Genotypic data

Candidate gene SNPs were selected from literature reporting polymorphisms within or near genes expected to affect pork quality and/or composition. Genes affecting composition were included as adiposity is highly correlated with intramuscular fat content. Genes were included even if no association had previously been shown to affect the targeted traits. A complete list of all candidate genes genotyped is presented in Table 3.

Eight QTL regions were targeted. A primary factor for inclusion was genomic regions with convincing evidence of QTL from a Landrace × Duroc F2 population reported by Rohrer, Thallman, Shackelford, Wheeler, and Koohmaraie (2005) and corroborated in other studies. Chromosome 6 was also studied due to numerous associations reported for pork quality despite not being identified by Rohrer et al. (2005). For each selected QTL region, at least six SNP markers were selected, where two SNPs spanned the region 5–10 cM prior to the QTL peak, two SNPs were located over the QTL peak and two SNPs spanned the region 5–10 cM after the peak. If sufficient SNPs were available for a region, markers were selected based on anticipated information content within commercial pig populations. However, for some regions all SNPs within the range were tested. The QTL regions studied are presented in Table 4.

Sequence information available in GenBank on pork quality candidate gene SNP markers were compiled in a file, along with SNP markers flanking QTL regions based on the current USMARC porcine linkage map and processed through MassARRAY Assay Designer 3.1.2.2 (Sequenom Inc., San Diego, CA) to group SNPs into assay groups of approximately 30 SNPs. Oligonucleotides used for each SNP assay are presented in Supplemental Table 1. Assays were run according to manufacturer's protocols, analytes detected with mass spectrometry and genotypes called using MassARRAY TYPER 3.4 software (Sequenom Inc., San Diego, CA). Manual evaluation of all scored genotypes was performed. Assays that failed to provide a sufficient number of genotypes or where their genotypic distributions were clearly inconsistent with the Hardy–Weinberg equilibrium ($X^2_{2df}>20.0$; $p<4.5\times10^{-5}$) were eliminated from the study. Assays with low minor allele frequency (MAF<0.05) or that mildly deviated from the Hardy–Weinberg equilibrium ($6.0<X^2_{2df}<20.0$; $0.05>p>0.000045$) were left in the study, but their results should be considered with caution.

2.4. Statistical analyses

Data were analyzed using SAS version 9.2 (Cary, NC). The PROC MIXED procedure was used for all measurements. For traits recorded prior to enhancement (a*, b*, L*, pH, IMF, color and marbling scores) the model included fixed effects for PLANT, DATE and GENOTYPE and LOIN as a random effect. The analyses of purge loss included a fixed effect for ENHANCEMENT as well as the previous effects. Each SNP marker was analyzed independent of all other markers. Haplotype analyses were not attempted as genomic regions tested were too broad (20 or more cM).

There were four measurements for each loin for traits measured after freezing. The statistical model for thaw purge loss included the fixed effects of PLANT, DATE and ENHANCEMENT, LOIN was considered

Table 2
Descriptive statistics of traits measured after enhancement.

Trait	Natural loins					Enhanced loins				
	Number of records	Mean	Standard deviation	Minimum	Maximum	Number of records	Standard mean	Deviation	Minimum	Maximum
Purge loss-aging, %	674	1.96	1.91	0	10.62	227	3.84	1.78	1.08	19.96
Purge loss-Thawing, %	676	2.83	1.32	0	7.86	227	0.78	0.48	0	3.63
Cooking loss-62.8 °C	675	9.65	0.41	0.97	3.41	227	5.56	1.57	2.46	12.41
Cooking loss-68.3 °C	676	10.59	3.17	0.02	21.6	227	6.07	1.75	3.14	15.83
Cooking loss-73.9 °C	676	12.76	3.41	0	23.61	227	7.02	2.06	2.73	15.92
Cooking loss-79.4 °C	674	15.08	4.27	0	36.57	225	8.64	3.19	0	19.4
Shear force-62.8 °C	678	2.51	0.6	1.26	4.97	227	1.67	0.41	0.97	3.41
Shear force-68.3 °C	672	2.64	0.76	1.23	6.84	227	1.65	0.43	1	3.45
Shear force-73.9 °C	676	2.75	0.78	1.24	7.02	227	1.62	0.37	0.88	3.31
Shear force-79.4 °C	676	2.88	0.85	1.46	6.43	225	1.72	0.42	1.04	3.55