

UPDATE ON GENETIC MARKERS FOR PORK QUALITY

GA Rohrer, J Holl, AK Lindholm-Perry, DJ Nonneman,
SD Shackelford, T Wheeler and M Koohmaraie

USDA-ARS US Meat Animal Research Center
Clay Center, NE 68933

Introduction

Nearly 20 years ago the animal genetics field was rapidly trying to develop genetic markers that would be useful for marker assisted selection (MAS) programs. At that time, meat quality and reproduction were at the top of the list of traits that would be greatly improved by this new technology. Large research programs were developed and many promises were made. Yet today the swine industry would like to see more information on the application of genetic markers for economically important traits.

Cattle genomics research initially began research on meat quality, as there was a clear message from the industry that tenderness was a serious problem with some of the cattle slaughtered. However, research in pigs focused more on reproductive performance rather than on meat quality. This was fostered by the recent importation of Chinese germplasm into the US at the time as well as a belief among researchers that pork quality was not a problem. Pork quality prior to the 1990's was usually considered acceptable by consumers, but the dramatic move to leaner pigs was beginning to show its negative effects on pork quality.

Initial genome scans on carcass traits in pigs focused on carcass composition and utilized exotic (Meishan or Wild Boar) germplasm (for example Andersson et al., 1994; Rohrer and Keele, 1998). There were some striking similarities among these studies for backfat depth with QTL residing on SSC 1, 2, 4, 7 and X. Interesting features were that the QTL on SSC 2 was imprinted (paternal expression) and the QTL on SSC 7 was transgressive (Meishan contributing the leaner allele). Later genome scans aimed at pork quality traits used commercially relevant germplasm. Most studies used either Duroc or Berkshire crossed animals (Malek et al., 2001; Stearns et al., 2005; Rohrer et al., 2006). Similar QTL results across studies have been found for Hunter L* (SSC 1, 2 and 14) as well as measures of tenderness (SSC 2 and 15).

Causative genetic variation underlying marker associations have been identified for at least three different genes. First was the identification of a mutation in RYR1 that in the homozygous form resulted in porcine stress syndrome (PSS) or malignant hyperthermia (Fujii et al., 1991). While the mutation caused PSS and sudden death, the heterozygotes and homozygous mutant animals also had significantly lower quality pork resulting in pale, soft and exudative meat due to low ultimate pH (Boles et al., 1991). A gene which caused reduced pork quality in processed products, known as Rendement Napole (RN), was determined to be PRKAG3 (Milan et al., 2000). After a mutation that caused the classical RN phenotype was discovered, additional variation in the gene associated with pork quality was reported (Ciobanu et al., 2001). Finally, subsequent research on a SSC 2 QTL (primarily affecting growth and fat deposition) resulted in

the identification of a likely causative variant located near the IGF2 gene (Van Laere et al., 2003).

Candidate gene analyses have also been reported for pork quality traits. Some studies were an analysis of a single, non-coding polymorphism near a gene of interest tested in a group of phenotyped pigs, while others evaluated a large portion of a gene and tested the polymorphisms in a large group or groups of pigs. Genes with the most convincing evidence of significant associations include AFABP (Gerbens et al., 1998), HFABP (Gerbens et al., 1999), CAST (Ciobanu et al., 2004), ASIP (Kim et al., 2004), DLK1 (Kim et al., 2004) and GYS1 (te Pas et al., 2003).

Objectives

The objective of this study was to test the robustness of reported marker associations with pork quality traits. To accomplish this objective, genotyping assays were designed for as many polymorphisms from the literature as possible and typed across pig populations with pork quality data available. Genetic marker-phenotype associations were determined using three different statistical models typically used in marker association studies. Results were compared across populations as well as statistical models.

Methodology

Animal Populations

Four different populations were used. One population was comprised of a Duroc-Landrace F2 population that was described by Rohrer et al. (2006) and used for a genome scan of QTL (DLF2). These two breeds were selected to maximize genetic variation for tenderness and marbling among the four most commonly used breeds in the US pig industry. The F2 population contained 370 phenotyped pigs with barrows and gilts represented. These pigs were produced by a local cooperator and slaughtered at a commercial abattoir.

The other three populations were developed at USMARC from the commercial pig research population. The commercial research population was developed by mating Yorkshire-Landrace composite females to either Duroc or Landrace boars selected from the industry. Twelve boars of each breed were selected and approximately 10 barrows per boar were slaughtered at the USMARC abattoir and phenotyped during this first cross generation (TC1; n=228). The Landrace-sired pigs were crossed to Duroc-sired pigs to develop a Landrace-Duroc-Yorkshire composite population (LDY). Gilts in the F4 generation of LDY composite population were slaughtered at the USMARC abattoir and tenderness data collected (n=530). The final population was developed by mating LDY females to a commercial terminal sire selected for either extreme pork quality or leanness (TC2). Barrows and gilts were slaughtered at the USMARC abattoir and tenderness data collected (n=163).

Phenotypic measurements recorded were intramuscular fat content (%), Hunter L*, a*, b*, purge loss, cooking loss, slice shear force (SSF) after aging (7 or 14 days) and pH at 24 or 48 hour post-mortem. Intramuscular fat content, Hunter L*, a* and b* were not measured in the LDY population.

Genotypic Data

Twelve assays were successfully designed for the Sequenom MassArray system to genotype single nucleotide polymorphisms (SNP) for seven genes reported in the literature. One SNP for ASIP, DLK1, GYS1, MC4R and RYR1 were genotyped along with three SNP for CAST and 4 SNP for PRKAG3. The SNP marker assays typed in this study are listed in Table 1.

Statistical Analysis

Each marker system was analyzed separately. Three different models were fitted. Model G included the fixed effects of slaughter group, sex (when appropriate) and genotype. Model GS was similar to model G but also included sire as a fixed effect. Model GA fitted an animal model using MTDFREML software programs and included fixed effects of genotyped, slaughter group and sex (when appropriate). Each model was used to analyze each population separately as well as a final analysis that combined all data sets together. Contrasts were developed to estimate the additive and dominance effects for each assay. Nominal significance values were computed, not taking multiple tests into account.

Results

The RN causative mutation identified by Milan et al. (2000) was not segregating in our populations. This result was expected as the causative mutation was reported to only be found in Hampshire pigs and there were no animals with Hampshire breeding tested. Similarly, the stress-causing allele of RYR1 was only found at an extremely low frequency of one population resulting in too few heterozygotes to detect a significant association with pork quality traits.

The ASIP assay was only successfully genotyped in the DLF2 population. The only significant association found with model GA was with b* reflectance ($p < .04$).

The remaining nine assays were genotyped across all of the animals. Analyses of the combined dataset for the eight traits found 26, 17 and 14 significant ($p < .05$) associations using model G, model GS and model GA, respectively (Table 2). Ten of the 14 significant associations from model GA were significant in all three models. Typically, the significant associations in the GA model appeared quite definitive, as they were highly significant and results similar to those of G and GS models. For some associations, accounting for more genetic variation improved the significance as it appeared to remove noise in the analysis. However, many associations significant in model G did not hold up once more genetic variation was parameterized. Frequently the results of the GS model were intermediate to those of G and GA.

Model G results within each population appeared quite sporadic. There were 16 significant ($p < .05$) in the DLF2 population, 11 in the TC1 population, 11 in TC2 population and 18 in LDY population (only 5 phenotypes). In the DLF2 population, 11 of the associations were significant in the combined data set under model G and 6 were significant with model GA. In the TC1 population, 5 associations were significant in the combined data under Model G and only 1 with model GA. In the TC2 population, 7 significant associations were significant in the combined data set under model G and 3 under model GA. Finally, 12 of the significant associations in the

LDY population were significant in the combined data set under model G and 9 under model GA.

Discussion

The combined data set should reveal the markers that would be the most useful for the general swine industry, as it is based on the most observations and broadest sampling of germplasm. In addition, the results of the GA model appear to be the most robust. Therefore, the associations that would appear to be useful for industry applications are the CAST assays (23795.1 and Ser66Asn) for pork tenderness, GYS1 for pH, MC4R for purge loss and PRKAG3 (PRK-T30N and PRK-I199V) for pH and water holding capacity. The associations detected for CAST, GYS1 and PRKAG3 are similar to those reported. While MC4R was originally reported for effects on growth rate and fat deposition, these traits were not analyzed in this study.

The CAST assays were consistently associated with tenderness in all four populations and the alternate homozygotes differed by approximately 10% of the mean. The additive effects for 23795.1 ranged from .27 to .82 kg shear force (overall estimate .57 kg) among the four tested populations while Ser66Asn estimates ranged from .49 to 2.40 kg shear force (overall estimate .71 kg). The next most consistent associations were seen for PRKAG3-T30N where additive effects on pH ranged from .004 to .047 (overall estimate .031). Both GYS1 and MC4R were only tested in three populations and both had one estimate of virtually zero and two other estimates that showed a consistent effect.

Associations identified within populations using model G did not appear to be very robust across other statistical models nor across populations. Many of these associations were not significant when sire was fitted, indicating that the association could be due to unique data structures. A possible scenario is when a marker has a very low minor allele frequency, most heterozygous animals may be related via a common sire or grandsire. Associations that were not consistent across populations could also indicate different linkage disequilibrium between the assayed polymorphism and the causative genetic variation. Therefore these markers could be useful in some industry populations but, not in all populations.

As more studies are conducted and higher marker densities applied, useable markers for industry should be developed at a much faster pace in the near future. However, results from association analyses fitting animal models to account for other genetic variation appeared to be more robust and should be preferred analyses for reporting results.

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Table 1. SNP markers genotyped for pork quality.

Marker name	Gene (Symbol)	Reference
ASIP	Agouti signaling protein (ASIP)	Kim et al. (2004b)
23795.1	Calpastatin (CAST)	Nonneman et al., unpublished
Ser66Asn	Calpastatin (CAST)	Ciobanu et al. (2004)
Ser638Arg	Calpastatin (CAST)	Ciobanu et al. (2004)
DLK1	Delta-like 1 (DLK1)	Kim et al. (2004a)
GYS1	Muscle glycogen synthase (GYS1)	te Pas et al. (2003)
MC4R	Melanocortin receptor 4 (MC4R)	Kim et al. (2000)
PRK-RN	Protein kinase, AMP-activated,g3 (PRKAG3)	Milan et al. (2000)
PRK-T30N	Protein kinase, AMP-activated,g3 (PRKAG3)	Ciobanu et al. (2001)
PRK-G52S	Protein kinase, AMP-activated,g3 (PRKAG3)	Ciobanu et al. (2001)
PRK-I199V	Protein kinase, AMP-activated,g3 (PRKAG3)	Ciobanu et al. (2001)
RYR1	Ryanodine receptor 1 (RYR1)	Fujii et al. (1991)

Table 2. Significance of genotypic effects and genetic contrasts of marker associations with pork quality in the combined data set.

SNP	Trait ¹	Model GA	Model GA	Model GS	Model GS	Model G	Model G
		Genotypic p-value	Significant Contrast ²	Genotypic p-value	Significant Contrast ²	Genotypic p-value	Significant Contrast ²
23795.1	imf	0.5965		0.2679		0.0053	d
23795.1	L*	0.8320		0.8319		0.9844	
23795.1	a*	0.0844		0.1005		0.0716	d
23795.1	b*	0.2508		0.2036		0.2419	
23795.1	purge	0.6526		0.3872		0.4177	
23795.1	ckloss	0.2184		0.3109		0.0080	a
23795.1	SSF	0.0017	a	0.0081	a	<.0001	a
23795.1	pH	0.9315		0.9919		0.6767	
Ser66Asn	imf	0.5208		0.2653		0.5918	
Ser66Asn	L*	0.5313		0.2616		0.3950	
Ser66Asn	a*	0.6559		0.4778		0.4942	
Ser66Asn	b*	0.9782		0.8054		0.9594	
Ser66Asn	purge	0.8711		0.8843		0.3600	
Ser66Asn	ckloss	0.1775		0.1871		0.1646	
Ser66Asn	SSF	0.0002	a	<.0001	a	0.0005	a
Ser66Asn	pH	0.9067		0.9965		0.8855	
Ser638Arg	imf	0.1213		0.0327		0.0291	a
Ser638Arg	L*	0.0992		0.0238	d	0.1959	
Ser638Arg	a*	0.2641		0.2197		0.3615	
Ser638Arg	b*	0.1576		0.0798	a,d	0.4190	
Ser638Arg	purge	0.7657		0.4012		0.7312	
Ser638Arg	ckloss	0.4151		0.3252		0.1642	
Ser638Arg	SSF	0.6874		0.4474		0.6570	
Ser638Arg	pH	0.9305		0.8647		0.7298	
DLK1	imf	0.1681		0.0022		0.0001	a
DLK1	L*	0.2073		0.7094		0.3131	
DLK1	a*	0.0934		0.1341		0.0153	a
DLK1	b*	0.3388		0.3800		0.3806	
DLK1	purge	0.6122		0.4231		0.2026	

DLK1	ckloss	0.0995		0.2343		0.4205	
DLK1	SSF	0.9541		0.9343		0.5748	
DLK1	pH	0.5446		0.4746		0.2278	
GYS1	imf	0.0153	d	0.0597	d	0.0995	d
GYS1	L*	0.0869		0.1266		0.0511	
GYS1	a*	0.0254	a	0.0337		0.0142	a
GYS1	b*	0.0003	d	0.0007	d	<.0001	a
GYS1	purge	0.0004	a	0.0002	a	<.0001	a
GYS1	ckloss	0.3041		0.0943	a	0.0049	a
GYS1	SSF	0.7970		0.7035		0.5765	
GYS1	pH	0.0153	a	0.0029	a	0.0002	a
MC4R	imf	0.5024		0.6194		0.0258	
MC4R	L*	0.9012		0.9738		0.6237	
MC4R	a*	0.4330		0.2739		0.4215	
MC4R	b*	0.3666		0.4759		0.0527	d
MC4R	purge	0.0003	a	<.0001	a	0.0007	a
MC4R	ckloss	0.9695		0.9882		0.7571	
MC4R	SSF	0.1725		0.1630		0.0152	a,d
MC4R	pH	0.1981		0.2912		0.3291	
PRK-T30N	imf	0.1367	a	0.0724	a	0.1301	
PRK-T30N	L*	0.1886		0.3261		0.1898	
PRK-T30N	a*	0.2475		0.0940	d	0.0641	
PRK-T30N	b*	0.3039		0.1656		0.0808	a
PRK-T30N	purge	0.0016	a	0.0024	a	0.0001	a
PRK-T30N	ckloss	0.0645	a	0.0488	a	0.1751	
PRK-T30N	SSF	0.0816		0.0194	a	0.0023	a
PRK-T30N	pH	0.0000	a	<.0001	a	<.0001	a
PRK-G52S	imf	0.1208		0.2878		0.4440	
PRK-G52S	L*	0.8412		0.8258		0.4093	
PRK-G52S	a*	0.5529		0.6208		0.6651	
PRK-G52S	b*	0.8221		0.9343		0.6638	
PRK-G52S	purge	0.0289	a	0.0748	a	0.0093	a
PRK-G52S	ckloss	0.0676		0.2558		0.0206	a
PRK-G52S	SSF	0.6493		0.6367		0.1754	
PRK-G52S	pH	0.0513		0.0572		0.0140	
PRK-I199V	imf	0.7292		0.6066		0.4797	
PRK-I199V	L*	0.2057		0.2289		0.1581	

PRK-I199V	a*	0.0357		0.1889		0.0477	a
PRK-I199V	b*	0.0217		0.2076		0.0381	
PRK-I199V	purge	0.1096		0.1238		0.0279	
PRK-I199V	ckloss	0.0165	a	0.0074	a	0.0616	
PRK-I199V	SSF	0.2001		0.0658	a	0.0166	a,d
PRK-I199V	pH	0.0001	a	0.0001	a	<.0001	a

¹Traits are: imf-intramuscular in the longissimus; L*-Minolta L* of longissimus; a*-Minolta a* of longissimus; b*-Minolta b* of longissimus; purge-purge loss of longissimus; ckloss-cooking loss of longissimus chop cooked for slice shear force analysis; SSF-slice shear force; pH-longissimus pH at 24 or 48 hours post-mortem.

²Significance reported was $p < .05$. Genetic contrasts were: a-additive; d-dominance.