A genome-wide analysis of the ultimate pH in swine

H.Y. Chung*, K.T. Lee*, G.W. Jang, J.G. Choi, J.G. Hong and T.H. Kim

Animal Genomics and Bioinformatics Division, National Institute of Animal Science, Suwon, Korea

*These authors contributed equally to this study.
Corresponding authors: H.Y. Chung / T.H. Kim
E-mail: chung133@korea.kr / kth6160@korea.kr

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ABSTRACT. Meat pH is an important factor influencing meat quality traits in swine. This study evaluated a large number of genetic variants that covered all of the swine chromosomal regions. Approximately 68,000 single nucleotide polymorphisms (SNPs), found on Illumina Porcine SNP chips, were tested for associations with meat pH values. A genome-wide association study (GWAS) found that 19 SNPs on *Sus scrofa* chromosome 4 were significantly associated with pH. Two major candidate genomic regions were defined: a 1.08-Mb region (at nucleotide 30118313 to 31207050) contained 10 significant SNPs, based on an effect value of 5.0; and a 2.7-Mb genomic region (at nucleotide 73293076 to 76023681) contained 9 significant SNPs. Three putative genes - *PKHD1L1*, *VCPIP1*, and *LOC102166532* - were identified by GWAS near significant SNPs. These genes may account for variations in pH levels. Three pseudogenes and two non-coding RNAs were also detected by GWAS analysis. Estimations of expected and observed P values for pH revealed significant departures from the null hypothesis. A total of 9 haplotype blocks (HB) were constructed: HBs 1, 3, and 5 showed significant effects on pH_{24} and pH_{45}, whereas an association was not confirmed between pH_{24} and HBs 4, 6,

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and 8. Findings from this study indicate that the three genes identified may influence pH of pig meat.

Key words: Meat pH; Swine; Genome-wide association study; SNP

INTRODUCTION

In the pork industry, understanding meat product quality is complicated due to poorly defined standards of measurements and guidelines for genetic and environmental factors across international markets. Initial (pH_{45}) and ultimate (pH_{24}) pH values, which influence the extent of protein, meat color, and water-holding capacity (WHC), have significant impacts on purchasing decisions by consumers and processing yields of meat products (Offer and Knight, 1988). Several factors, including pH of muscle and WHC (Cannon et al., 1996), are believed to be major factors influencing sensory characteristics of meat products. At present, postmortem (PM) pH_{24} levels in pigs remain a major source of variation (Bidner et al., 2004) due to limited information about the relationships between *longissimus* muscle pH and meat palatability and processing characteristics. Therefore, monitoring of PM pH may be helpful in estimating meat quality traits of pigs.

The pH involves a hydrogen ion concentration of approximately 7.0 to 7.2 in live animals. Following death, pH declines due to the dissociation of lactic acids in muscle. The primary metabolic role of muscle glycogen, a complex carbohydrate, is the conversion of muscle proteins. When triggered by a lack of oxygen and nutrients, an enzymatic process (glycolysis) increases lactic acid in muscle and pH levels drop to around 5.5. Glycolysis is a very important factor in PM contribution to meat quality characteristics. Even though criteria for PM pH level are not clear, several studies have proposed that the threshold of pH_{24} to differentiate PSE (pale, soft, and exudative) meat from normal meat is less than 5.5 (Sellier and Monin, 1994; Forrest, 1998); 5.7 (van Laack et al., 2001); 5.69 (Kusec et al., 2005); and 5.8 (Sellier and Monin, 1994). However, studies have also reported that PM metabolism of glycogen can vary depending on environmental factors, such as elevated metabolism at slaughter (Rosenvold and Andersen, 2003), and PM meat chilling (Tomovic et al., 2008). In addition to environmental factors, genetic effects on pH should be carefully considered.

Particularly, the genetic mutation in the ryanodine receptor gene (*RN*) is a major example of a factor influencing pH, showing that negative alleles cause extremely low pH_{24} (Barbut et al., 2008).

Although studies have not characterized how many genes, or what kinds of genes may be responsible for changes in PM pH, utilization of genetic markers in pig populations are ideal tools for animal production systems, in terms of pH levels (Sellier and Monin, 1994).

It is essential to elucidate DNA variants that explain variation in meat quality traits and that can enhance traditional selection systems. However, a large number of genetic variants across the whole genome may be needed in selection programs due to the complicated mechanisms of genetic interactions. Thus, polygenic effects to explain genetic variation of carcass traits should use a large number of genes (Garnier et al., 2003). Genetic information at genome-wide levels should be verified for the determination of candidate genotypes. DNA chips containing numerous SNPs can be used to characterize genetic associations and pathways, and have been widely adapted in animal genomics. The aim of this study was to characterize individual variations of postmortem pH levels using associations at genome-wide levels.

MATERIAL AND METHODS

Animals

The study was approved by the ethics and welfare committee of the National Institute of Animal Science, Korea. To eliminate sex effects, 165 female Duroc pigs were randomly collected from NIAS. Animals were found to be free of significant inbreeding coefficients (less than 0.01) based on pedigree analyses. Pigs were provided standardized diets, based on reported requirements (NRC, 1998), and were tested for performance ability until 150 days of age. Once animals weighed approximately 100 kg (live weight), they were harvested at a packing facility in NIAS (National Institute of Animal Science), and muscle tissue samples (approximately 5 g) from the edge of the right shoulder were collected from each animal and stored at -70°C until further processing for genomic DNA extraction.

pH measurement

Two time points were used to measure meat pH: 45 min and 24 h PM, referred to as pH_{45} and pH_{24}, respectively. Forty-five minutes after slaughter, pH_{45} values were measured from the left *longissimus dorsi* muscle from primarily processed swine carcasses, between the fourth and fifth ribs. After 24 h of cooling, pH_{24} values were measured at these same anatomic points. To increase accuracy, and decrease measurement variation, two experienced operators measured pH values three times at 1 min intervals, using a pH meter (a portable needle-tipped combination electrode, Fisher AR50-155V NWK_binar pH-K21, Germany).

Genomic DNA preparation

Extractions were performed from approximately 1 g of each muscle sample. After chopping samples using blades, pieces were placed into a tube with extraction buffer, and genomic DNA was extracted using a kit and the manufacturer's guidelines (Wizard DNA extraction kit, Promega, USA). DNA quantity and purity (A_{260}/A_{280} ratio) were assessed for each sample using a NanoDrop 1000 spectrophotometer (Thermo Scientific, USA), and then samples were stored at -70°C until further processing for genotyping.

Genotyping

Genotyping was carried out using Illumina 60K Beadchip arrays from a genotyping service company (TNT Research Co.) in Korea. The analysis set the call rates as 0.95, determined using the Illumina Genome Studio software (Genotyping Module, version 1.0). For quality control, samples that diverged from the mean value by at least 3 standard deviations were excluded from analysis. For five individuals, no phenotype or genotype was detected, which was attributed to technical error; as a consequence, 160 female pigs were used for the genome-wide association study (GWAS). In addition, GWAS analysis excluded SNPs that deviated significantly from Hardy-

Weinberg equilibrium (HWE) and presented minor allele frequencies of less than 0.05. Overall, a total of 48,175 SNPs were finalized for GWAS analysis.

Statistical analysis

The statistical analysis was performed by comparing observed QTL (Quantitative Trait Loci) effects to the null distribution, obtained from permuting the phenotype labels related to genotyping labels 100 times. Genome wide association study analysis was performed on residuals, using a best linear unbiased prediction (BLUP) model, and SNPs (QTL effects > 5.0) at each locus were used for further association tests. Significance was estimated using the BLUP R package, to calculate the overall QTL effects, using the following model $Y = \mu + G + S + \beta A + e$, where Y is the vector of phenotypes; G is the fixed effect of genotypes; S is the random effect of sire; A is the effect of age as a covariate; and e represents random residual errors. Genomic regions containing genotypes of significant SNPs were used to construct haplotype blocks (HBs), using the HAPLOVIEW (V3.31) program with default parameters (http://www.broad.mit.edu/mpg/haploview/). Construction of HBs used two genomic regions that presented significant effects on pH, and contained 19 SNPs at 1.08 Mb (nucleotide positions 30118313 to 31207050) and 46 SNPs at 2.7 Mb (nucleotide positions 73293076 to 76023681) on *Sus scrofa* chromosome 4 (SC4). Statistical analysis used a general linear model (GLM) to confirm significant associations with haplotypes, and 9 HBs were tested for pH_{45} and pH_{24} levels using SAS Institute Inc. (2008) GLM procedures with a 0.05 significance level. Means of haplotypes in HBs were tested for differences using Duncan's multiple range procedure.

RESULTS

Genetic variation

The HWE analysis revealed that ASGA0019098 (P = 0.002) and DIAS0001443 (P = 0.042) markers in the 1.08 Mb genomic region (Table 1), and ASGA0020045 (P = 0.013) and ASGA0020070 markers (P = 0.004) in the 2.7 Mb genomic region (Table 2), departed from genetic equilibrium due to relatively low major allele frequency (MAF) levels, which ranged from 0.026 to 0.250. Findings corresponded to the highest and lowest MAF, which were 0.497 (ALGA0025622) and 0.026 (ASGA0020070), respectively (shown in Table 2). The expected heterozygosity estimates were highest (0.499) for ASGA0019108 and ASGA0019110, and lowest (0.05) for ASGA0020070. Tables 1 and 2 present estimates of genetic variability, including QTL effects on pH levels. According to the significant genetic effects on pH with a threshold level of 5.0 for QTL effects, 11 SNPs in the 1.08 Mb and 9 SNPs in the 2.7 Mb genomic regions were identified. The nearest genes around identified SNPs were *SYBU*, polycystic kidney and hepatic disease 1 (autosomal recessive)-like 1 (*PKHD1L1*), *TRHR*, *LOC100526166*, and *LOC102160264* in the 1.08 Mb region (Table 1); and *LOC102166532*, *LOC102161340*, *LOC102161575*, valosin containing protein (P97)/P47 complex interacting protein 1 (*VCPIP1*), *CYP7B1*, and *LOC102162782* in the 2.7 Mb region (Table 2). Nomenclature of the nearest gene (less than 1 Mb away from each of the identified SNPs) was in accordance with the Human Genome Organization (HUGO) Gene Nomenclature Committee (HGNC).

Table 1. Swine SNP probes in the 1.08-Mb genomic region (nucleotide positions 30118313 to 31207050) presenting signficant values in GWAS analysis.

ID	SNP	Variability			Nucleotide position		Allele		Nearest gene[3]	QTL effects	NCBI
Probe		Obs H	Exp H	HWE[1]	Porcine SNP60	Assembly[2]	MAF	Allele			
ALGA0024229	rs80995095	0.385	0.453	0.082	25840976	30118313	0.346	A:G	SYBU	9.989	NC_010446
ASGA0019098	rs80806218	0.179	0.251	0.002	25878165	30155502	0.147	C:A	SYBU	-	XM_001925055.2.
DIAS0001443	rs342656395	0.308	0.375	0.042	25963430	30240767	0.250	A:G	LOC100156275	2.013	XM_001925463.2.
DIAS0004696	rs341246446	0.417	0.458	0.315	25995426	30272905	0.356	A:G	PKHD1L1	10.048	XM_003125522.3.
ASGA0019102	rs81381564	0.423	0.479	0.182	26048203	30325682	0.397	A:G	PKHD1L1	9.053	XM_003125522.3.
INRA0013423	rs331833835	0.442	0.472	0.511	26086322	30363801	0.381	G:A	PKHD1L1	10.518	XM_003125522.3.
MARC0050770	rs80886930	0.436	0.470	0.431	26169228	30383118	0.378	C:A	PKHD1L1	10.588	XM_003125522.3.
MARC0039647	rs80838215	0.436	0.464	0.534	26350274	30597930	0.365	G:A	PKHD1L1	10.793	XM_003125522.3.
CASI0009594	rs319765754	0.372	0.355	0.760	26553765	30816873	0.231	G:A	TRHR	2.413	NM_001177488.1
ASGA0019105	rs81381581	0.468	0.495	0.567	26578999	30842196	0.452	G:A	TRHR	4.916	NM_001177488.1
ALGA0024245	rs81381587	0.474	0.496	0.671	26611326	30874523	0.455	A:G	TRHR	5.136	NM_001177488.1
ASGA0019108	rs81381592	0.462	0.499	0.409	26642261	30905458	0.481	A:G	LOC100526166	8.124	NC_010446
ASGA0019110	rs81381596	0.462	0.499	0.409	26663360	30926557	0.481	G:A	LOC100526166	8.124	NC_010446
ALGA0024252	rs81381599	0.16	0.179	0.349	26685942	30949139	0.099	A:G	LOC100526166	-	NC_010446
MARC0063249	rs81252242	0.468	0.495	0.567	26707437	30970634	0.452	G:A	LOC100526166	4.916	NC_010446
ALGA0024256	rs81381603	0.154	0.174	0.299	26727330	30990527	0.096	A:G	LOC102159736	-	XR_302857.1
ASGA0019112	rs81381605	0.321	0.381	0.072	26740454	31003651	0.256	A:G	LOC102159736	2.046	LOC102159736
ALGA0024267	rs81381627	0.410	0.460	0.221	26894620	31174732	0.359	G:A	LOC102160264	10.498	XR_302859
DRGA0004668	rs81302106	0.410	0.460	0.221	27019084	31207050	0.359	G:A	LOC102160264	10.498	XR_302859

[1]HWE: Hardy-Weinberg Equilibrium. [2]Assembly: the nucleotide positions were based on *Scrofa* version 10.2. [3]The nearest gene and gene symbol were based on HGNC (HUGO Gene Nomenclature Committee). MAF: major allele frequency.

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Table 2. Swine SNP probes in the 2.7 Mb genomic region (nucleotide positions 73293076 to 76023681) presenting signficant values in GWAS analysis.

ID	SNP	Variability			Nucleotide		Allele		Nearest gene[3]	QTL effect	NCBI
Probe		Obs H	Exp H	HWE[1]	Porcine SNP60	Assembly[2]	MAF	allele			
ASGA0020004	rs80949242	0.442	0.447	1	65491243	73293076	0.337	A:C	LOC102166532	9.685	CU151847.7
ALGA0025577	rs80991149	0.449	0.449	1	65524206	73326039	0.340	A:G	LOC102166532	9.671	CU151847.7
INRA0014602	rs339627487	0.449	0.449	1	65546885	73348718	0.340	A:G	LOC102166532	9.671	CU151847.7
ASGA0020008	rs80919420	0.449	0.449	1	65569851	73371684	0.34	G:A	LOC102166532	9.671	CU151847.7
ASGA0020014	rs80843298	0.494	0.491	1	65849712	73703545	0.433	G:A	CSPP1	1.379	XM_001926004.2.
ALGA0025591	rs80957272	0.494	0.491	1	65893922	73747755	0.433	A:G	CSPP1	1.379	XM_001926004.2.
H3GA0012873	rs80978339	0.494	0.491	1	65929955	73783788	0.433	G:A	CSPP1	1.379	XM_001926004.2.
DIAS0003279	rs80855249	0.494	0.491	1	65958782	73812615	0.433	G:A	COPS5	1.379	XR_302951.1
MARC0029339	rs80873235	0.532	0.497	0.488	65981576	73835408	0.458	A:G	LOC102166532	1.161	XM_001926086.1.
M1GA0005925	rs80812572	0.532	0.497	0.488	66019293	73873126	0.458	G:A	PPP1R42	1.161	XM_005663043.1
INRA0014618	rs322184622	0.532	0.497	0.488	66053176	73907009	0.458	A:G	LOC100524619	1.161	XM_003125607.2
DIAS0001140	rs345276418	0.532	0.497	0.488	66129891	74095578	0.458	A:G	LOC100524619	1.161	XM_005663058.1
MARC0017192	rs81284815	0.410	0.431	0.645	66259496	74150786	0.314	G:A	LOC102161340	10.562	XM_005663058.1
H3GA0012878	rs81382072	0.410	0.431	0.645	66342401	74177176	0.314	A:G	LOC102161575	10.562	XM_005663058.1
DIAS0001337	rs330787355	0.410	0.431	0.645	66408157	74259849	0.314	G:A	VCPIP1	10.562	XM_003355045.1
ASGA0020022	rs81382076	0.353	0.307	0.099	66428497	74280189	0.189	A:G	MYBL1	2.232	XM_003125609.4
MARC0005389	rs81224585	0.353	0.307	0.099	66462972	74329795	0.189	A:G	MYBL1	2.232	XM_003125609.4
ASGA0103404	rs81475447	0.104	0.099	1	66468936	63149423	0.052	G:A	PKIA	-	NM_214204.1
ALGA0025611	rs81382081	0.340	0.337	1	66490867	74357690	0.215	G:A	C4H8orf46	1.657	XM_003125610.2
ALGA0025613	rs81382085	0.340	0.337	1	66513055	74379878	0.215	C:A	C8orf46	1.657	XM_003125610.1
ALGA0025617	rs81382093	0.558	0.491	0.133	66544763	74411586	0.433	A:G	ADHFE1	0.561	XM_003125603.2
H3GA0012885	rs80854716	0.481	0.5	0.721	66688835	74555814	0.490	C:A	CRH	0.009	XM_005663048
ASGA0020028	rs80861851	0.462	0.47	0.914	66706247	74573226	0.378	G:A	CRH	1.504	XM_005663048
ALGA0025622	rs80950600	0.481	0.5	0.718	66718160	74585139	0.497	A:G	CRH	0.032	XM_005663048

Continued on next page

Table 2. Continued.

ID	SNP	Variability			Nucleotide		Allele		Nearest gene[3]	QTL effect	NCBI
Probe		Obs H	Exp H	HWE[1]	Porcine SNP60	Assembly[2]	MAF	allele			
ASGA0020031	rs80911632	0.462	0.453	0.982	66753298	74620277	0.346	G:A	CRH	0.771	XM_005663048
ALGA0025633	rs80926425	0.462	0.453	0.982	66774528	74641507	0.346	G:C	CRH	0.771	XM_005663048
SIRI0001105	rs344799531	0.462	0.453	0.982	66796676	74663655	0.346	G:A	TRIM55	0.771	NM_001244690.1
ALGA0025638	rs80790800	0.359	0.399	0.270	66827056	74694035	0.276	A:G	TRIM55	3.612	NM_001244690.1
ALGA0025649	rs80976751	0.391	0.345	0.169	67255621	75123448	0.222	A:G	MTFR1	0.411	XM_003125605.1
ASGA0020045	rs80921866	0.192	0.251	0.013	67384878	75252705	0.147	A:C	ARMC1	-	NM_001190196.1
ASGA0020046	rs80792551	0.481	0.485	1	67402831	75270658	0.413	G:A	ARMC1	0.641	NM_001190196.1
ALGA0025655	rs80849282	0.340	0.396	0.107	67433205	75301032	0.272	G:A	ARMC1	3.416	NM_001190196.1
ALGA0025656	rs80843219	0.346	0.405	0.099	67476646	75344473	0.282	G:A	LOC102161089	3.381	XR_302967.1
ALGA0025658	rs80878915	0.34	0.396	0.107	67496399	75387791	0.272	G:A	LOC102161089	3.416	XR_302967.1
H3GA0012892	rs80848027	0.34	0.396	0.107	67534024	75425416	0.272	G:A	LOC102161089	3.416	XR_302967.1
ALGA0025665	rs80973034	0.333	0.399	0.059	67564644	75451819	0.276	A:G	LOC102161089	3.998	XR_302967.1
M1GA0005931	rs80809599	0.555	0.497	0.216	67659453	75546628	0.465	A:G	LOC102161089	0.685	XR_302967.1
ASGA0020060	rs80839273	0.564	0.496	0.128	67676539	75563714	0.455	G:A	LOC102161089	0.679	XR_302967.1
ALGA0025668	rs80844879	0.551	0.495	0.219	67703686	75590861	0.449	C:A	LOC102161178	0.612	XR_302968.1
INRA0014665	rs326889316	0.25	0.273	0.400	67753401	75640576	0.163	A:C	LOC102161178	-	XR_302968.1
ALGA0025679	rs81382111	0.538	0.496	0.384	67904156	75934281	0.455	A:C	CYP7B1	5.294	XM_003481395.1
M1GA0005933	rs81382117	0.333	0.375	0.225	67961503	75991872	0.25	A:G	BHLHE22	3.929	XM_003481394.1
DRGA0004859	rs81302069	0.25	0.256	0.945	67974298	76004484	0.151	A:C	BHLHE22	-	XM_003481394.1
ALGA0025682	rs80961919	0.397	0.387	0.953	68026169	76150371	0.263	A:G	LOC102162782	5.037	XM_003481394.1
M1GA0005935	rs80893885	0.288	0.315	0.402	68067757	76138962	0.196	A:C	LOC102162782	1.881	XR_302971.1
ASGA0020070	rs80881055	0.026	0.05	0.004	68093767	76023681	0.026	A:G	LOC102162467	-	FP565677.1

[1]HWE: Hardy-Weinberg Equilibrium. [2]Assembly: The nucleotide positions were based on *Scrofa* version 10.2. [3]The nearest gene and gene symbol were based on HGNC (HUGO Gene Nomenclature Committee). MAF: major allele frequency.

pH

A quantile-quantile plot presenting pH_{24} levels for the observed versus expected P values is presented in Figure 1, confirming that some of measurements significantly deviated from the null hypothesis (y = x). The average pH_{45} and pH_{24} values were 5.74 and 5.78, respectively; these were not significantly different, but the average variance of pH_{24} (0.019) was slightly greater than that of pH_{45} (0.018). Results indicated that PM pH levels changed according to individual characteristics of muscle tissues. Tests for the normalization of pH measurements revealed that pH_{45} had a more normalized pattern compared to pH_{24}, but significant differences between pH_{24} and pH_{45} measurements were not observed. The pH_{45} and pH_{24} levels differed positively or negatively by 0.1 points in 44 and 29 animals, respectively, and increased or decreased by 0.3 points in 7 and 4 animals, respectively. A significant correlation (r = 0.406, P < 0.0001) between pH_{24} and pH_{45} was observed (Figure 2).

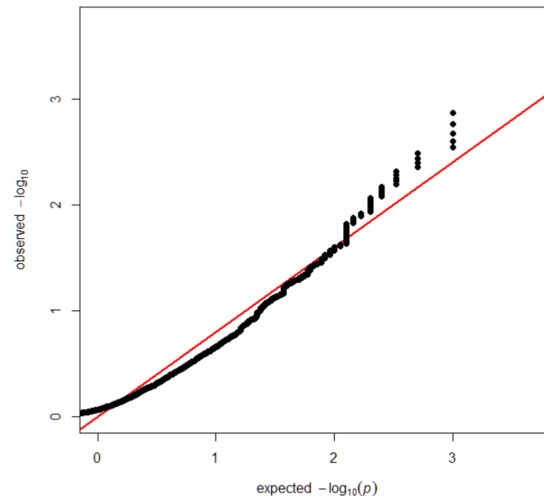

Figure 1. Quantile-quantile plot of observed versus expected P values for pH_{24}. The horizontal axis indicates the expected $-\log_{10}$ (P values) and the vertical axis indicates the observed $-\log_{10}$ (P values). The diagonal line represents y = x, which corresponds to the null hypothesis.

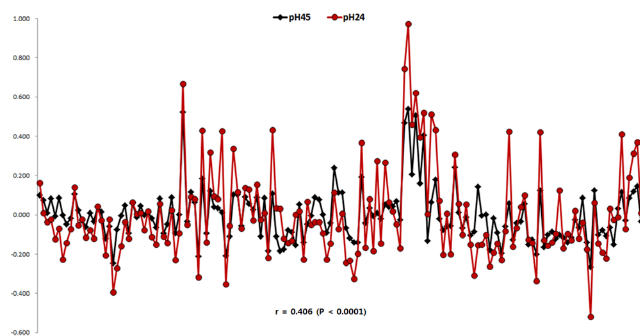

Figure 2. Distribution of variances between pH_{24} and pH_{45} presenting a signficant simple correlation.

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GWAS analysis

As shown in Figure 3, significant SNP effects on pH_{45} and pH_{24} were observed on SSC4 with an adaptation threshold of 8.0. Two major genomic regions, 1.08 Mb (nucleotide positions 30118313 to 31207050) and 2.7 Mb (nucleotide positions 73293076 to 76023681), were fully considered for candidate regions. In addition, chromosomes 1 and 14 also showed significant effects for pH_{45}, but significant marker effects for pH_{24} were not recognized on these chromosomes; for this reason, chromosomes 1 and 14 were not included for further GWAS analysis, and the analysis used a high threshold value (8.0) to minimize false-positive effects and maximize selection rates of the effective markers. The 10 SNP probes (ALGA0024229, DIAS0004696, ASGA0019102, INRA0013423, MARC0050770, MARC0039647, ASGA0019108, ASGA0019110, ALGA0024267, and DRGA0004668), which showed highly significant effects on pH levels, were densely located in an approximately 1.08 Mb region based on the genome assembly with a reference sequence GenBank accession number NC_010446.4 (UCSC**,** *Sscrofa* version 10.2). Candidate genes located around the significant SNPs showing QTL effects > 5.0 were identified as the *SBYU*, *TRHR*, *PKHD1L1*, *LOC100526166*, and *LOC102160264* genes. In addition, analysis of the2.7 Mb genomic region revealed significant effects on pH, presenting 9 SNP probes (bolded in Table 2 - ASGA0020004, ALGA0025577, INRA0014602, ASGA0020008, MARC0017192, H3GA0012878, DIAS0001337, ALGA0025679, and ALGA0025682). The nearby genes were *LOC102166532, LOC102161340, VCPIP1, CYP7B1*, and *LOC102162782*.

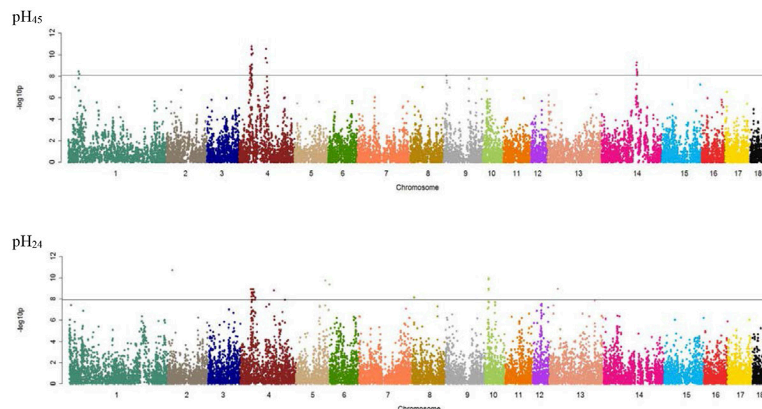

Figure 3. Distribution of significant association values of pH_{24} and pH_{45} in a GWAS analysis was placed on SSC4. Permutation tests defined a genome-wide significance level (8.0), and significant outcomes were observed in 1.08-Mb (nucleotide positions 30118313 to 31207050) and 2.7-Mb (nucleotide positions 73293076 to 76023681) genomic regions of chromosome 4.

Associations with haplotypes

Construction of haplotype blocks (HBs) presented 2 major blocks that were 354 and 13 kb in length in the 1.08 Mb genomic region based on genomic sequences in Figure 4, whereas the 2.7 Mb genomic region presented 7 major blocks (Figure 5) with lengths of 78, 280, 203, 107, 131, 17, and 12 kb.

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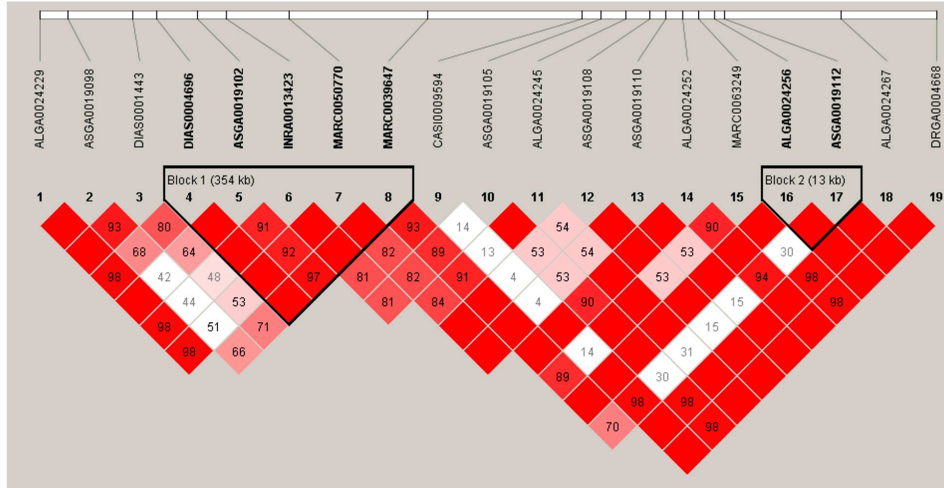

Figure 4. Construction of haplo-blocks 1 and 2 for the targeted genomic region from nucleotide positions 30118313 to 31207050 using HaploView version 4.1 (http://www.broad.mit.edu/mpg/haploview/). Positions were based on the UCSC Genome Browser on Pig Aug. 2011 (SGSC *S. scrofa*10.2/susScr3) Assembly. Linkage disequilibrium of the 10 significant SNPs in the 1.08-Mb genomic regions on SSC4 was determined for 160 Duroc female pigs.

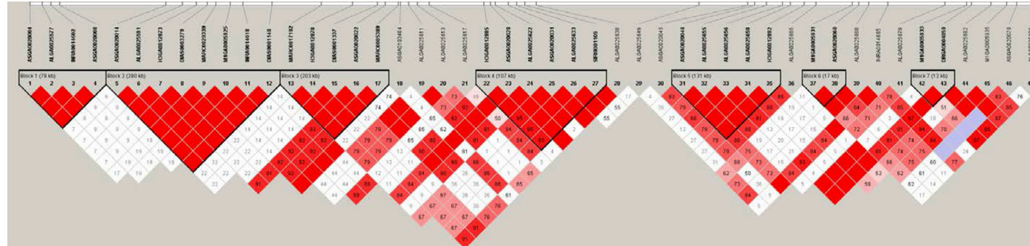

Figure 5. Construction of haplo-blocks 3 through 9 for the targeted genomic region from nucleotide positions 73293076 to 76023681 using HaploView version 4.1 (http://www.broad.mit.edu/mpg/haploview/). Positions were based on the UCSC Genome Browser on Pig Aug. 2011 (SGSC *S. scrofa*10.2/susScr3) Assembly. Linkage disequilibrium of the 9 significant SNPs in the 2.7-Mb genomic region on SSC4 was determined for 160 Duroc female pigs.

As shown in Table 3, HB 1 contained 5 haplotypes; the haplotype GGAAA (pH 5.923) had a significant effect on pH_{45} compared with other haplotypes such as AAAAG (pH 5.774), AAGCG (pH 5.766), AGAAG (pH 5.794), and AGGCG (pH 5.781). In addition, haplotype CGGA (pH 5.825) in HB 3 had a greater pH than haplotype AAAG (pH 5.737), and HB 5 showed statistically significant differences among haplotypes (AGAAA (pH 5.996) > AGAGG (pH 5.822) > GAGAA (pH 5.753)) for pH_{45}. All haplotype blocks had significant effects ($P < 0.05$) on pH_{24} except for HBs 4, 6, 7, and 8. Overall, HBs 1, 3, and 5 had significant genetic effects on both pH_{45} and pH_{24}. From results of GWAS and haplotype analyses, the present study confirmed significant haplotype block effects (1, 3, and 5), and, therefore, the candidate genes (*PKHD1L1*, *LOC102166532*, and *VCPIP1*) in this genomic region may be related to pH levels.

Table 3. Least square means and standard errors for pH_{45} and pH_{24} according to haplotype blocks (HB) on swine chromosome 4.

Block	Haplotype	N	pH_{45}				pH_{24}				Genomic region			No. of SNP[1]
			LSM		SE	P	LSM		SE	P	Range	Start	End	
HB1	AAAAG	3	5.774	±	0.11^{b}	0.0001	5.581	±	0.11^{b}	0.0001	325025	30272905	30597930	5
	AAGCG	123	5.766	±	0.04^{b}		5.697	±	0.04^{ab}					
	AGAAG	1	5.794	±	0.13^{b}		5.577	±	0.13^{b}					
	AGGCG	6	5.781	±	0.07^{b}		5.641	±	0.07^{b}					
	GGAAA	23	5.923	±	0.05^{a}		5.857	±	0.05^{a}					
HB2	AA	91	5.759	±	0.03	0.4790	5.779	±	0.03^{a}	0.0218	13124	30990527	31003651	2
	AG	40	5.742	±	0.03		5.741	±	0.03^{ab}					
	GG	25	5.721	±	0.03		5.692	±	0.03^{b}					
HB3	AAAG	138	5.737	±	0.04^{a}	0.0110	5.730	±	0.05^{a}	0.0071	78608	73293076	73371684	4
	CGGA	18	5.825	±	0.05^{b}		5.829	±	0.06^{b}					
HB4	AGAAGGAG	29	5.801	±	0.02	0.0930	5.826	±	0.02	0.5901	392033	73703545	74095578	8
	GAGGAAGA	126	5.757	±	0.01		5.776	±	0.01					
	GAGGGGAG	1	5.57	±	0.13		5.750	±	0.13					
HB5	AGAAA	6	5.996	±	0.05^{a}	0.0001	5.974	±	0.06^{a}	0.0057	179009	74150786	74329795	5
	AGAGG	15	5.822	±	0.03^{b}		5.770	±	0.04^{b}					
	GAGAA	135	5.753	±	0.01^{b}		5.786	±	0.01^{b}					
HB6	AGGACA	81	5.749	±	0.01	0.4950	5.762	±	0.02	0.0821	107841	74555814	74663655	6
	AGGGGG	17	5.786	±	0.03		5.810	±	0.03					
	CAAGGG	23	5.744	±	0.03		5.794	±	0.03					
	CGAGGG	34	5.797	±	0.02		5.824	±	0.02					
	CGGACA	1	5.88	±	0.13		5.680	±	0.14					
HB7	AGGGG	27	5.769	±	0.04	0.2990	5.753	±	0.04	0.0332	154758	75270658	75425416	5
	GAAAA	69	5.788	±	0.02		5.792	±	0.03					
	GGGGG	60	5.748	±	0.03		5.721	±	0.03					
HB8	AG	127	5.759	±	0.01	0.7550	5.783	±	0.01	0.9850	17086	75546628	75563714	2
	GA	27	5.779	±	0.03		5.786	±	0.03					
	GG	2	5.797	±	0.10		5.771	±	0.10					
HB9	AA	126	5.794	±	0.02	0.0550	5.785	±	0.02^{a}	0.0119	12612	75991872	76004484	2
	GA	26	5.833	±	0.03		5.876	±	0.03^{b}					
	GC	4	5.940	±	0.07		5.802	±	0.07^{ab}					

Different letters indicate significant differences between mean values of haplotypes. [1]No. of SNP: number of SNPs used to construct HB.

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DISCUSSION

pH

Changes in pH that occur after death may be difficult to estimate precisely, and prediction of PM pH levels may be made more difficult by such issues as low heritability, as well non-genetic variations such as feeding and management of animals). Once environmental variations are removed, it may be easy to define genetic differences regarding changes in meat pH levels after slaughter. However, physiological differences amongst individuals, based on genetic characteristics, are difficult to differentiate. For this reason, previous studies have attempted to understand physiological changes using pH values that can differentiate between normal and PSE meat. However, there are controversial issues to determine pH levels for PSE meat, and researchers have argued the pH_{45} value around 5.8 and the pH_{24} value around 5.5 to 5.7 (Forrest, 1998; van Laack et al., 2001). The pH is a general indicator of meat quality and can be used to monitor physiological parameters in both the anoxic and oxic phases. The pH level in the stomach is influenced by enzyme activities that are also dependent on the breakdown of all proteins. Generally, pH affects meat quality. For example, WHC and pale pork color are associated with low pH, whereas high pH levels cause a dark meat color. Pork with low pH is associated with a metallic and off-flavor taste, according to consumer preferences. On the other hand, dark, firm, and dry (DFD) pork with high pH levels (above 6.0 to 6.2) tends to have a reduced shelf life.

Changes in meat pH are related to the PM process of glycogen conversion into lactic acids. Variations in the extent of PM glycolysis are mainly responsible for the variation in WHC and color. In general, pork with normal color and WHC reaches a pH of 5.6 to 5.7 within 3 to 5 h of slaughter. The PSE pork shows rapidly decreasing pH_{24} even though muscle temperature is still high. Thus, the combined effects of low pH and high temperature reduce WHC and result in a pale meat color. In addition, low pH is caused by a combination of genetics, pre-slaughter stress, and PM handling processes (Forrest, 1998; van Laack et al., 2001). For example, a high carcass temperature results in a rapid decline in muscle pH_{45} during slaughter, and buildup of lactic acids in muscle due to excessive energy depletion (Ohene-Adjei et al., 2003).

Many studies have tried to understand mechanisms of PM pH and the association between PM meat pH and particular genes such as halothane (*HAL*) and rendement napole (*RN*), which are believed to be responsible for the extensive decline in muscle pH that results in acidic meat products (Ohene-Adjei et al., 2003; Estrade et al., 1993). Previous studies reported that RN genotypes are associated with meat yields (Lundstro et al., 1996), leaner carcasses (Milan et al., 2000; Ciobanu et al., 2001), high muscle glycogen stores, and an extended PM pH decline (Estrade et al., 1993). In the current study, we observed that pH levels differed between 45 min and 24 h post-slaughter. Le Roy et al. (2000) reported that the RN gene has no effect on pH_{45}, but the pH_{24} value is associated with WHC. Although studies have reported genetic effects on the decline in pH, such declines may be more fully explained by other genetic and environmental effects. Thus, the drop in pH may be more fully explained using genetic variants from the whole genome.

Polygenic effects

Several reports have indicated that heritabilities of most swine carcass traits are low (0.15-0.30) compared to heritability of intramuscular fat content (0.40 - 0.50; Sellier and Monin, 1994). Therefore, breeding schemes designed to alter muscle pH should be carefully designed to reduce

genetic defects such as PSE, which cause major economics losses for farmers. In terms of the known genes (*HAL* and *RN*) for PSE meat, it may be necessary to eliminate carries of alleles for these genes and to establish a new genetic pool, in order to solve the fundamental problem of producing PSE meat in the swine industry. To consider these genes, as well as others that may be significantly linked to production traits, genome wide studies should be included in the genetic analyses. However, up until now, the presence of both the *HAL* and *RN* gene is the only scientific way to determine pork quality regarding PSE meat.

Meat pH level at slaughter, which may be affected by environmental or genetic factors, is believed to be a major element determining meat quality. Previous studies have shown that pre-slaughter handling processes are a more significant factor affecting meat quality, compared with genetic effects (Kerth et al., 2001). It has also been previously shown that accelerated chilling affects animals carrying the undesirable *HAL* genotype (Kerth et al., 2001), while showing no effects on non-carriers of the gene (Bertram et al., 2000). One study suggested the use of gentle pre-slaughter handling to minimize environmental factors (Stoier et al., 2001). Hence, holistic studies with advanced molecular analysis using massive amounts of genetic information from the entire pig genome are required to verify effects of genes and gene variants on meat quality.

In consideration of the above points, we analyzed numerous SNPs from the whole genome in pigs, and found that the 14 most significant SNPs affecting pH levels of meat were harbored within *PKHD1L1*, *LOC102166532*, and *VCPIP1*. From these results, we assumed that genes such as *RN* and *HAL* would be shown in the GWAS analysis, a reasonable expectation considering the many reports that these genes are responsible for the production of PSE meat. However, underlying genetic determinants of PSE meat remain unknown at this point. It is still true that many genes in the genome may be correlated and interacted with other genes to determine phenotypic traits in pigs. Our analysis confirmed that several genes were related to pH_{24} even though meat pH was not an absolute determinant of PSE meat. The genes (*LOC102166532*, *PKHD1L1*, and *VCPIP1*) identified as being associated with meat pH in this study are responsible for pH_{24}, which is generally related to meat color and WHC. Although the GWAS analysis used with the current experimental design suggests a true effect, additional studies are required to define the precise mechanisms for the reported associations with identified genes on swine chromosome 4.

Gene functions

In general, SNP effects for a certain QTL are determined by the distribution of allele frequencies that have maximum probability to associate with the target traits. The 5 SNPs identified in this analysis that belong to *PKHD1L1* all had highly significant allele effects, and, therefore, this gene should be a candidate to explain phenotypic variations of pH_{24}. *PKHD1L1* (polycystic kidney and hepatic disease 1 (autosomal recessive)-like 1), which is a protein-coding gene, is known for associations with hepatitis. An approximately 16 kb large novel protein, the *PKHD1L1* transcript has a role in making fibrocystin, which spans the cell membrane of kidney cells. The protein may be involved in cell adhesion, repulsion, and proliferation (Ward et al., 2002). *PKHD1L1* is located on swine chromosome 4 (nucleotide positions 30266239 to 30648017) based on sequence data (NC_010446 chromosome 4 reference *S. scrofa* 10.2 Primary Assembly). This location contains neighboring genes such as *ENY2*, *NUDCD1*, *LOC100739513*, *LOC102160595*, *LOC102160679*, and *EBAG9*. *PKHD1L1* encodes a member of the polycystin protein family containing 11 transmembrane domains, and alternative splice variants have been described, although their biological nature has not been determined.

VCPIP1, a protein-coding gene, is associated with diseases such as pancreatic cancer and pancreatitis (Kuznetsova et al., 2007). Even though the function of this gene is not well defined in swine, it is known to act as a deubiquitinating enzyme, and is annotated as related to ubiquitin-specific protease activity in human (http://www.uniprot.org/uniprot/Q96JH7). The gene, which is 14,555 bp long and is located from 74,259,719 to 74,274,273 on SSC4, may have additional roles for processing peptides and proteins, but the nature of effects for these substrates regarding cleavage is not clear.

LOC102166532 (Carboxypeptidase A6, CPA6), which is a metallocarboxypeptidase enzyme, is expressed at high levels in the olfactory bulb of the brain, and in the embryonic brain, as well as in other tissues in the mouse (http://www.ncbi.nlm.nih.gov/gene?cmd=retrieveandlist_uids=57094). The protein belongs to a family of carboxypeptidases that catalyze the release of amino acids at C-terminals, having functions in biosynthesis of neuroendocrine peptides. Although the gene has not been reported in swine, a human study reported that polymorphic variants were associated with Duane retraction syndrome (Lyons et al., 2008).

It is well known that PM muscle pH influences freshness of pork, due to significant relationships between the rate of pH decline and meat quality. Factors that affect PM glycolysis are varied and include genetic determinants (Barbut et al., 2007), metabolism during slaughter (Rosenvold and Andersen, 2003), and PM meat chilling procedures (Tomovic et al., 2008). Eliminating all environmental effects that may influence changes in PM muscle pH would help to elucidate residual muscle pH variations that could be explained by genetic effects. The present analysis minimized environmental effects to account for variation in meat pH, and significant SNPs from the whole genomic were successfully determined based on pH_{24} and pH_{45} values. This study suggests that *LOC102166532*, *PKHD1L1*, and *VCPIP1* are candidate genes contributing to variation in pH of meat in swine. However, functions of these genes are not currently known, and, therefore, further experiments regarding these genes should be conducted. In addition, further functional studies using these genes to verify genetic effects on changes in muscle pH both *in vitro* and *in vivo* may be needed.

Conflicts of interest

The authors declare no conflict of interest.

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