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Kwon, Kang
Department of Animal Science and Biotechnology, College of Agriculture and Life Sciences, Chungnam National University

Cahyadi, Muhammad
Department of Animal Science and Biotechnology, College of Agriculture and Life Sciences, Chungnam National University | Department of Animal Science, Faculty of Agriculture, Sebelas Maret University

Park, Hee-Bok
Department of Animal Science and Biotechnology, College of Agriculture and Life Sciences, Chungnam National University

Seo, Dong Won
Department of Animal Science and Biotechnology, College of Agriculture and Life Sciences, Chungnam National University

他

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# Association of Variation in the *MC4R* Gene with Meat Quality Traits in a Commercial Pig Population

**Kang KWON[1], Muhammad CAHYADI[1,2], Hee–Bok PARK[1], Dong Won SEO[1], Shil JIN[1], Sang–Wook KIM[3], Yang–Il CHOI[3], Kwan Suk KIM[3], Takafumi GOTOH[4] and Jun Heon LEE[1]***

Kuju Agricultural Research Center, Kyushu University,
Kuju 4045–4, Taketa City, 878–020, Oita, Japan
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The *melanocortin–4 receptor* (*MC4R*) gene is known to encode a membrane–bound receptor protein and is a member of the melanocortin receptor family of genes. In mammals, these genes are involved in energy homeostasis and in regulating feeding behavior and body weight. The objective of the present study was to examine if there were any associations between variations in the *MC4R* gene with meat quality traits in a commercial pig population in Korea. Among the total of 593 commercial pigs, sire information was retrieved from 232 pigs. These animals were successfully genotyped for the c.892A>G (p.Asp298Asn) single nucleotide polymorphism (SNP) by using *Taq*I PCR–RFLP methods. Association analyses between this SNP and meat quality traits were performed using a general linear model (GLM) including sire effect. This SNP was significantly associated with backfat thickness ($P<0.05$), marbling ($P<0.01$). Interestingly, this SNP marker was also associated with volatile basic nitrogen after 14 days of storage ($P<0.05$). To our knowledge, it is the first results observed for the *MC4R* genotypes with volatile basic nitrogen after 14 days of storage in the commercial pig population. Therefore, these results suggest that the *MC4R* gene can be targeted in marker–assisted breeding for selecting pigs with good meat quality.

**Key words**: commercial pig, *MC4R*, meat quality, sire effect, single nucleotide polymorphism

## INTRODUCTION

Two major aspects of meat quality are nutritional quality and sensory quality. The former is usually objective because most nutritional traits are measurable quantities. On the other hand, sensory quality, including flavor, juiciness, tenderness, and color, is mostly qualitative, which is highly subjective; it is dependent on consumer preferences (Bender, 1992). These aspects are important because meat should be nutritious and healthy, satisfy consumer preferences, and be economically valuable. Over the last few decades, the improvement of live body weight while maintaining meat quality traits has become a major focus of the swine industry (Cisneros *et al*., 1996). Meat quality traits such as marbling, backfat thickness, juiciness, tenderness, color, pH, fatty acid composition, and water holding capacity are important traits in pork because they are key factors that determine the compatibility of pork for storage, marketing display, packaging, and further manufacturing processes (Rosenvold and Andersen, 2003).

Thousands of quantitative trait loci (QTL) pertaining to economically important traits in swine have been released by the National Animal Genome Research Program (NAGRP) (http://cn.animalgenome.org/cgi–bin/QTLdb/index). Of these, 5406 QTLs are related to meat and carcass qualities. The existence of these data allows researchers to investigate and compare QTLs in diverse breeds and different populations. Additionally, this has made it possible to trace mutations in functional candidate genes for traits of interest. Utilization of whole–genome molecular data in swine breeding schemes has been conducted to ascertain the identities of loci that are responsible for phenotypic traits associated with meat quality and to develop marker assisted selection (MAS) techniques in the livestock industry (Dekkers, 2004). The use of MAS in swine breeding programs has been proven to be useful in the improvement of disease resistance, pork quality, carcass composition, reproductive traits, and growth related–traits (Ernst and Steibel 2013).

The *melanocortin–4 receptor* (*MC4R*) gene encodes a G–protein–coupled receptor expressed in the appetite–regulating areas of the brain that interact with adrenocorticotropic hormone and melanocyte–stimulating hormone (MSH) and is a member of the melanocortin receptor family. This gene family is involved in feed intake regulation and energy balance (Bruun *et al*., 2006; Fan *et al*., 1997). The *MC4R* gene is also expressed in placental and intestinal tissues. Mutations in the *MC4R* gene have been reported to be associated with obesity in mice and humans (Huszar *et al*., 1997; Vaisse *et al*., 1998). In pigs, the *MC4R* gene is one of the important candidate genes exhibiting positive correlations with meat quality traits. This gene has been mapped on Sus scrofa chromosome 1 (SSC1) by Kim *et al*. (2000a) located at 82.5 cM (Rohrer *et al*., 2012), where a QTL region for meat qual-

[1] Department of Animal Science and Biotechnology, College of Agriculture and Life Sciences, Chungnam National University, Daejeon 305–764, Korea
[2] Department of Animal Science, Faculty of Agriculture, Sebelas Maret University, Surakarta 57126, Indonesia
[3] Department of Animal Science, Chungbuk National University, Cheongju 361–763, Korea
[4] Kuju Agricultural Research Center, Kyushu University, Kuju 4045–4, Taketa City, 878–020, Oita, Japan
* Corresponding author: (E–mail: junheon@cnu.ac.kr)

**Table 1.** Descriptive statistics for meat quality traits observed in this study

| Trait | Mean | SD | Min. | Max. |
|---|---|---|---|---|
| Carcass weight (kg) | 86.95 | 5.19 | 74.00 | 102.00 |
| Backfat thickness (mm) | 23.03 | 5.11 | 13.00 | 39.00 |
| Moisture (%) | 74.30 | 0.82 | 71.87 | 76.59 |
| Crude fat (%) | 2.18 | 0.73 | 0.74 | 5.11 |
| Crude ash (%) | 1.18 | 0.16 | 0.76 | 1.66 |
| Water holding capacity (%) | 57.07 | 4.85 | 41.60 | 72.90 |
| Loin pH | 5.57 | 0.12 | 5.37 | 6.16 |
| Drip loss (%) | 4.93 | 1.45 | 2.15 | 10.27 |
| Cooking loss (%) | 31.31 | 2.96 | 22.20 | 40.73 |
| Shear force | 1192.53 | 319.51 | 513.33 | 2393.33 |
| Lightness (L) | 60.53 | 4.22 | 49.60 | 76.61 |
| Redness (a) | 6.80 | 1.86 | 1.96 | 13.39 |
| Yellowness (b) | 9.39 | 1.10 | 6.30 | 13.41 |
| Marbling score | 2.60 | 0.68 | 1.17 | 4.67 |
| Color score | 3.00 | 0.40 | 1.83 | 4.17 |
| Tenderness score | 2.87 | 0.31 | 1.83 | 3.67 |
| Volatile basic nitrogen (VBN) | 12.12 | 2.60 | 7.14 | 21.00 |
| VBN7 | 15.70 | 3.22 | 10.71 | 25.39 |
| VBN14 | 18.70 | 3.09 | 12.63 | 28.41 |
| Thiobarbituric acid (TBA) | 0.20 | 0.10 | 0.05 | 0.53 |
| TBA7 | 0.25 | 0.18 | 0.04 | 0.96 |
| TBA14 | 0.34 | 0.15 | 0.05 | 0.64 |

Abbreviation: VBN7 and VBN14 are volatile basic nitrogen at 7 and 14 days of storage, respectively. TBA7 and TBA14 are thiobarbituric acid at 7 and 14 days of storage, respectively.

ity traits such as color, pH, intramuscular fat traits, and marbling, was detected (Malek *et al*., 2001; Rohrer *et al*., 2012). Polymorphisms within the *MC4R* gene have been reported and are known to be associated with significant variation in pork quality. For example, a missense mutation that replaces aspartic acid (Asp) with asparagine (Asn) at position 298 of the protein sequence of the *MC4R* gene product (p.Asp298Asn) was associated with lean meat and backfat thickness in a Belgian commercial pig population, and a swine breed composite ($DIV_2$) population in China (Chao *et al*., 2012; Van den Maagdenberg *et al*., 2007). However, the effects of this mutation were not observed empirically and could not be validated in all investigated populations (Muñoz *et al*., 2012; Park *et al*., 2002; Stachowiak *et al*., 2006; Stinckens *et al*., 2009). Therefore, the objective of this study was to determine if the *MC4R* gene was associated with meat quality traits in a commercial pig population in Korea. To this end we genotyped the c.892A>G (p.Asp298Asn) single nucleotide polymorphism (SNP) by using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis.

## MATERIALS AND METHODS

### Animals and phenotypes

Among the total of 593 pigs sampled from a commercial population (generated by the three–way cross breeding system using Landrace, Yorkshire, and Duroc, from seven different farms), sire information was retrieved from 232 pigs. These animals were used in this association study. The pig sub–population investigated was obtained from six different farms, which used 10 sires to generate a total of 232 piglets (121 castrated males and 111 females). Sire, sex, and carcass weight were included as covariates in the linear model to analyze carcass and meat quality traits (Table 2). They were reared and managed in controlled conditions in each farm by intensively concerning animal rights. Carcass traits were collected immediately after slaughtering.

Backfat thickness data were collected at the $10^{th}$ and $11^{th}$ of ribs at *Longissimus dorsi* (LD) area. Furthermore, meat quality traits including pH, marbling, tenderness, water holding capacity (WHC), cooking loss, drip loss, shear force, and proximate composition analysis were measured at 24 hours after the slaughter, while volatile basic nitrogen (VBN) and thiobarbituric acid (TBA) were calculated at 1, 7 and 14 days after slaughtering. Loin steak was used and weighed before and after cooking at 70˚C in 40 minutes for both cooking loss and shear force measurement using Warner–Bratzler shear force meter. In addition, meat colors, lightness (L), redness (a), yellowness (b), were also observed following Commission International de Leclairage (CIE) protocol.

**DNA extraction and genotypes**

Muscle tissue was used to extract and isolate genomic DNA using 20 mg/mL proteinase K digestion followed by phenol extraction. A primer pair previously described by Meidtner *et al*. (2006) and Fan *et al*. (2009), 5'–TCGATTGCAGTGGACAGGTA–3' as a forward primer and 5'–GAAAATGCTGTTGTTGAAGCA–3' as a reverse primer, were used to isolate 663 bp of exon one of the *MC4R* gene. The PCR was carried out to isolate the *MC4R* gene in 25 $\mu$L volume containing 25 ng per $\mu$L DNA genome, 0.01 $\mu$M primers, 5 mM of dNTP, 2.5 $\mu$L of 10X PCR reaction buffer, 0.625 units of *h–Taq* DNA polymerase (Solgent, Korea), and water. The PCR reaction was performed using PTC–200 thermocycler machine (MJ Research, USA). Reaction condition was pre–denaturation at 95˚C for 15 minutes, and followed by 40 cycles of denaturation at 95˚C for 20 seconds, 20 seconds at 62˚C of annealing temperature, extension at 72˚C for 30 seconds, final extension was carried out at 72˚C for 5 minutes. Then, the PCR products were visualized using 2% agarose gel electrophoresis. To verify the SNPs, previously PCR products were purified using Geneclean turbo kit (MP Biomedicals, USA), and then they were sequenced using Applied Biosystems 3730 DNA sequencer (PE Applied Biosystems, USA). Furthermore, sequence data were anlyzed to investigate point of mutation using Sequencher ver 4.7 (Gene codes, USA).

Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) was performed to genotype a dbSNP (dbSNP Acc. No. 107793985) which is also known as c.892A>G SNP (Asp298Asn). This marker was recognized by *Taq*I restriction enzymes. The PCR products were digested using 2 units of each restriction enzymes for more than 6 hours under optimal reaction conditions (Biolabs® Inc., USA). Then, digested PCR products were separated and visualized by 2.5% of MetaPhor® agarose gel (LONZA, USA) electrophoresis for 40 minutes.

**Statistical Analysis**

Association between the SNP marker of *MC4R* and meat quality traits was evaluated using a general linear model (GLM) implemented in MINITAB version 14.0 (Minitab Inc., USA). The mathematical model used in this study was as follows:

$$Y_{ijklm} = \mu + Sex_i + Genotype_j + Farm_k + Sire_{l(k)} + b_1 CW_{ijklm} + \varepsilon_{ijklm}$$

Where, $Y_{ijklm}$ is the phenotype of the m$^{th}$ animal, $\mu$ is the overall mean, $Sex_i$ is the effect of sex, $Genotype_j$ is the effect of the genotype, $Farm_k$ is the effect of the farm, $Sire_{l(k)}$ is the effect of the l$^{th}$ *Sire* nested within the k$^{th}$ *Farm*, $CW_{ijklm}$ is the effect of carcass weight as a covari-

**Table 2.** Significance effect of sire, sex and carcass weight (CW) on the meat quality phenotypes

| Trait | Sex | | Farm | | Sire | | CW | |
|---|---|---|---|---|---|---|---|---|
| | Adj MS§ | *F*–value | Adj MS | *F*–value | Adj MS | *F*–value | Adj MS | *F*–value |
| BF thickness | 506.49 | 35.39*** | 124.57 | 8.70*** | 235.92 | 16.48*** | 182.86 | 12.78*** |
| Moisture | 0.2326 | 0.42 | 2.8232 | 5.10*** | 4.7889 | 8.65*** | 0.6186 | 1.12 |
| Crude fat | 2.9301 | 5.98* | 1.2430 | 2.54* | 1.2023 | 2.45* | 0.8786 | 1.79 |
| Crude ash | 4.289 | 0.96 | 1.492 | 0.34 | 4.840 | 1.09 | 1.364 | 0.31 |
| WHC | 59.42 | 3.49 | 267.16 | 15.69*** | 67.47 | 3.96** | 14.32 | 0.84 |
| Loin pH | 0.0305 | 2.47 | 0.0303 | 2.45* | 0.0353 | 2.86* | 0.0088 | 0.71 |
| Drip loss | 0.025 | 0.01 | 13.910 | 7.54*** | 3.922 | 2.13 | 0.407 | 0.22 |
| Cooking loss | 4.923 | 0.90 | 100.368 | 18.36*** | 70.771 | 121.95*** | 0.209 | 0.04 |
| Shear force | 11768 | 0.15 | 540261 | 7.11*** | 1213139 | 15.97*** | 791 | 0.01 |
| L | 37.23 | 2.22 | 46.16 | 2.76* | 43.33 | 2.59* | 38.60 | 2.30 |
| a | 0.679 | 0.23 | 15.799 | 5.37*** | 3.759 | 1.27 | 4.698 | 1.60 |
| b | 7.830 | 6.94*** | 3.370 | 2.99* | 1.107 | 0.98 | 0.069 | 0.06 |
| Marbling score | 0.5411 | 1.26 | 0.8666 | 2.01 | 1.5232 | 3.54** | 0.0936 | 0.22 |
| Color score | 0.3917 | 2.58 | 0.2168 | 1.43 | 0.1795 | 1.18 | 0.1971 | 1.30 |
| Tenderness score | 0.0281 | 0.31 | 0.2945 | 3.30** | 0.0763 | 0.85 | 0.1559 | 1.75 |
| VBN | 1.321 | 0.89 | 85.818 | 57.77*** | 305.187 | 205.43*** | 3.119 | 2.10 |
| VBN7 | 1.84 | 1.05 | 174.87 | 99.68*** | 368.92 | 210.29*** | 2.98 | 1.70 |
| VBN14 | 6.47 | 2.86 | 239.64 | 106.02*** | 107.55 | 47.58*** | 1.29 | 0.57 |
| TBA | 0.0107 | 2.31 | 0.07695 | 16.62*** | 0.19478 | 42.07*** | 0.01306 | 2.82 |
| TBA7 | 0.00053 | 0.05 | 0.75087 | 71.23*** | 0.25360 | 24.06*** | 0.01109 | 1.05 |
| TBA14 | 0.02604 | 4.39* | 0.59972 | 101.20*** | 0.09520 | 16.07*** | 0.00059 | 0.10 |

§Adjusted mean squares; *$P$<0.05; **P$P$<0.01; ***$P$<0.001

ate, and $\varepsilon_{ijklm}$ is random error. The Tukey's test was also performed to differentiate between genotypes. The threshold for the significance was set at $P<0.05$.

## RESULTS

The c.892A>G SNP of the *MC4R* gene has been successfully genotyped by PCR–RFLP method. All three known genotypes of this SNP, AA (71), AG (111), and GG (50), were detected, and they obeyed Hardy–Weinberg equilibrium ($\chi^2$= 0.7339, d.f. = 1, P>0.10). Therefore, this SNP marker was used for subsequent association study.

Descriptive statistics, including mean, standard deviation (SD), and minimum (Min.) and maximum (Max.) values, of 22 meat quality and carcass traits are summarized in Table 1. Furthermore, this study revealed that sire and farm were robustly affecting pork quality traits and sex was affecting backfat thickness, yellowness, and TBA14 (Table 2).

Association analysis between the *MC4R* gene polymorphism and carcass and pork quality traits was performed using all 232 animals (Table 3). This SNP marker was associated with marbling score ($P<0.01$), significantly affected backfat thickness at the 10th and 11th ribs ($P<0.05$), and was significantly associated with volatile basic nitrogen (VBN) at 14 days of storage ($P<0.05$). Individuals carrying an A allele of the c.892A>G SNP presented higher marbling scores than those carrying the G allele (Table 3). The mean of marbling score of homozygous AA individuals was 2.8, as opposed to 2.4 in homozygous GG individuals. These scores suggest that this population has abundant marbling in intramuscular fat. At the same time, pigs with the AA genotype presented higher backfat thickness than homozygous GG individuals. In addition, heterozygous AG pigs exhibited lower VBN in pork after 14 days of storage compared to other genotypes (Table 3).

## DISCUSSION

A number of confirmation studies have been reported regarding polymorphisms which are spread on the promoter and exon regions of the *MC4R* gene. A total of 41 variants have been compiled at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/snp). Some of these porcine *MC4R* gene variants have been discussed in the literature, and their

**Table 3.** The effects of MC4R c.892A>G SNP on meat quality traits in the commercial pig population

| Trait | Genotype | | | *P*–value |
|---|---|---|---|---|
| | AA† | AG† | GG† | |
| Backfat thickness (mm) | 23.20±0.48[a] | 22.76±0.38a | 21.29±0.55b | 0.024* |
| Moisture (%) | 74.20±0.095 | 74.26±0.075 | 74.40±0.110 | 0.363 |
| Crude fat (%) | 2.2543±0.090 | 2.166±0.075 | 2.098±0.085 | 0.243 |
| Crude ash (%) | 1.203±0.014 | 1.205±0.011 | 1.202±0.017 | 0.987 |
| WHC (%) | 57.28±0.530 | 57.64±0.414 | 57.43±0.604 | 0.849 |
| Loin pH | 5.579±0.014 | 5.554±0.011 | 5.536±0.016 | 0.119 |
| Drip loss (%) | 4.839±0.173 | 4.954±0.137 | 4.830±0.199 | 0.805 |
| Cooking loss (%) | 31.61±0.298 | 31.68±0.236 | 31.79±0.343 | 0.920 |
| Shear force | 1210±35.11 | 1199±27.74 | 1211±40.35 | 0.954 |
| L (CIE–L) | 60.96±0.521 | 60.30±0.413 | 60.80±0.560 | 0.547 |
| a (CIE–a) | 6.753±0.218 | 6.616±0.173 | 6.809±0.251 | 0.771 |
| b (CIE–b) | 9.366±0.137 | 9.521±0.107 | 9.398±0.156 | 0.603 |
| Marbling score[1] | 2.790±0.084[a] | 2.630±0.066[ab] | 2.397±0.096[b] | 0.008** |
| Color score[2] | 2.974±0.050 | 2.997±0.039 | 3.003±0.057 | 0.906 |
| Tenderness score[3] | 2.917±0.038 | 2.872±0.030 | 2.838±0.044 | 0.370 |
| VBN (mg%) | 12.32±0.148 | 12.14±0.117 | 12.10±0.170 | 0.522 |
| VBN7 | 15.60±0.161 | 15.48±0.127 | 15.56±0.185 | 0.835 |
| VBN14 | 18.97±0.183a | 18.38±±0.144[b] | 18.76±0.211[ab] | 0.026* |
| TBA (mgMA/kg) | 0.179±0.009 | 0.183±0.007 | 0.173±0.010 | 0.728 |
| TBA7 | 0.222±0.013 | 0.226±0.010 | 0.213±0.015 | 0.770 |
| TBA14 | 0.326±0.010 | 0.322±0.008 | 0.318±0.0113 | 0.844 |

[1]Marbling, 1 indicates extremely low in intramuscular fat and 5 indicates very abundant in intramuscular fat
[2]Meat color, 1 indicates very pale and 5 indicates very dark
[3]Tenderness, 1 indicates very tough, very dry, and very mild. 5 indicates very tender, very juicy, and very intense.
†Represents Least Square Means (LSM) ± Standard Error (SE)
*Represents that the genotypes affect the traits significantly (*$P<0.05$) and highly significant (**$P<0.01$)

effects to the pork quality have shown different results (Bruun *et al*., 2006; Fan *et al*., 2009; Kim *et al*., 2000b; Meidtner *et al*., 2006; Muñoz *et al*., 2012; Ovilo *et al*., 2006; Park *et al*., 2002; Stachowiak *et al*., 2006; Stinckens *et al*., 2009). In this study, c.892A>G SNP was mapped in order to investigate its effects on economically important quantitative traits. The minor allele frequency of c.892A>G SNP was almost equal to that of the major allele frequency, and was also in Hardy–Weinberg equilibrium, therefore, it was used for subsequent association study.

Association analysis between the *MC4R* gene polymorphism and carcass and pork quality traits was performed using all 232 animals (Table 3). The c.892A>G SNP marker was tightly associated with marbling score ($P<0.01$) and significantly affected backfat thickness at the 10th and 11th ribs ($P<0.05$). Individuals carrying an A allele of the c.892A>G SNP presented higher marbling scores than those carrying the G allele (Table 3). The mean of marbling score of homozygous AA individuals was 2.8, as opposed to 2.4 in homozygous GG individuals. These scores suggest that this population has abundant marbling in intramuscular fat. At the same time, pigs carrying AA genotype presented higher backfat thickness than homozygous GG individuals. Van den Maagdenberg *et al*. (2012) reported that the c.892A>G SNP of the *MC4R* gene was associated with fat thickness, with pigs carrying the AA genotype presenting higher fat thickness. The same effect was also discovered in the commercial pig population assessed in this study. Moreover, the significance association of c.892A>G SNP to the marbling score implied that it may play an important role in swine fat deposition, particularly in the muscle (Davoli *et al*., 2012). In addition, the c.892A>G SNP was also significantly associated to the volatile basic nitrogen (VBN) at 14 days of storage ($P<0.05$) which is heterozygous AG pigs contained lower VBN in pork after 14 days of storage compared to other genotypes (Table 3). It may be the first report of an association between variants of the *MC4R* gene with VBN content. Huang *et al*. (2014) explained that VBN is one of the most important reference indexes to evaluate pork freshness. A lower VBN content indicates fresher pork. Therefore, this novel finding may be useful as a marker for selecting for pork freshness.

In summary, our findings confirmed that the c.892A>G SNP is a promising molecular marker for pork fatness, especially high marbling score and backfat thickness. With respect to VBN content, further studies are necessary to confirm and validate the results of our study in other swine populations.

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