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Original Article

Association analyses of DNA polymorphisms in immune-related candidate genes GBP1, GBP2, CD163, and CD169 with porcine growth and meat quality traits

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Genetic polymorphisms within immunity-related candidate genes in pigs have been identified to control variations in immune functions and/or disease resistance. It has become necessary to evaluate the effects of other genetic markers of economically important traits prior to introducing them into marker-assisted selection programs. In this study, polymorphisms of porcine genes coding Interferon-induced Gunylate binding protein 1 (GBP1), GBP2, CD163, and CD169 were investigated for their association with growth and meat quality traits in a Korean native pig breed -Yorkshire inter-crossed F2 pig population (KY-F2). KY-F2 animals (n=346) have been successfully used for linkage mapping to identify quantitative loci that control meat quality, growth, and immunity traits. In our results, polymorphisms in genes GBP1 and GBP2 showed association with pig growth rate as well as meat quality traits such as crude fat, drip loss, and meat color (yellowness) in the KY-F2 population. The polymorphism in gene CD163 only showed association with crude fat, as a meat quality trait. CD169 gene was associated with pork tenderness. In conclusion, four immune-related genetic markers were validated for their association with growth and meat quality traits to gauge their potential use in a swine selection program. The results warrant further studies in other commercial pig populations.

Key words: pig, CD163, CD169, GBP1, GBP2

Introduction

In the swine industry, both production traits (such as meat quality, growth, carcass traits, and reproductive traits) and immune/health/disease resistance traits are of economic importance. Compared to production traits, animal health is an emerging area, with research aiming to establish a genetic basis for improvement of pig production. It has become necessary to identify genetic markers associated with animal health in order to improve disease prevention, as animals harboring variants with improved disease resistance could supply safe, healthy, and high quality meat to consumers, in addition to satisfying animal welfare concerns [1, 2].

Among swine diseases, porcine reproductive and respiratory syndrome (PRRS) is the most economically significant disease impacting commercial pig production worldwide [3, 4]. Its causative agent, known as PRRS virus (PRRSV) [5, 6], can affect all phases of production as well as maintain a relatively long-term subclinical infection [7-9]. In a previous nursery pig model and genome-wide association study (GWAS), a major quantitative trait locus (QTL) in SSC4 encoding GBPase sub-family was shown to be dramatically associated with host resistance to PRRSV infection. Furthermore, the most significant single nucleotide polymorphism (SNP), WUR10000125, is located in the 3'UTR of GBP1 gene [10].

Using a candidate gene approach, GBP1 and GBP2 genes were shown to be significantly up-regulated in PK15 cells after stimulation with poly (I:C) and associated with immune-related blood parameters (red blood cell count, hemoglobin concentration, and hematocrit) in a normal pig population [11]. Moreover, both porcine GBP1 and GBP2 genes have shown differential up-regulation after PRRSV infection in both cell and animal models via next generation sequencing (NGS) [12-14]. Porcine genes CD163 and CD169/ Sialoadhesin (SN)/ SIGLEC1 are two essential receptors for PRRSV invasion [15]. CD163 was shown to be involved in PRRSV entry, and CD169 has been confirmed as a PRRSV internalization receptor [16, 17]. Moreover, expression of porcine

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CD163 protein is correlated with replication of PRRSV in porcine alveolar macrophages (PAMs) [18]. CD163 is an important mediator of PRRSV infection in Marc-145 cells [19] and is associated with susceptibility to African swine fever infection [20]. In terms of genetics, porcine CD163 and CD169/SN are mapped to SSC5q21-q24 and SSC17q23 and expressed highly in lymph glands, spleen, and liver, which suggests their potential function in immunity. Moreover, a single nucleotide polymorphism (SNP), c.3534C>T in the 3'UTR of porcine CD163 gene, has shown association with immunoglobulin G content in a Chinese pig population comprised of both indigenous and crossed pig breeds [21]. The missense mutation c.878A>G (rs345830287) was identified in porcine CD169 exon4 as causing amino acid conversion of histidine (His) to arginine (Arg) and is associated with white blood cell counts [21]. More recently, new SNPs in CD163 and CD169 genes were identified in a commercial pig population, showing different allele frequencies between PRRSV-infected pigs and healthy pigs [22]. Both studies have proposed CD163 and CD169 genes as candidate genes for immune traits and disease resistance at the genetic level in pigs [21, 22].

The above studies suggest that porcine genes GBP1, GBP2, CD163, and CD169 are important candidate genes for pig resistance to PRRSV infection. Analysis of correlations between immune and production traits is a prerequisite before introducing immune traits into existing animal selection schemes [23]. Therefore, we investigated the association of polymorphisms [11, 21] in genes GBP1, GBP2, CD163, and CD169 with growth and meat quality traits in a Korean native pig breed-Yorkshire crossed F2 pig population (KY-F2) [24-26].

Materials and Methods

Samples and Traits

A total of 346 meat samples were collected from a crossed breed pig population (Korean native pig crossed with Yorkshire pig, F2 population (KY-F2) [24-26]. Genomic DNA was extracted from muscle tissue using a genomic DNA purification kit (Promega, UK), and DNA was diluted to 50 ng/μL after quality evaluation. One growth trait and 18 meat quality traits were evaluated, including average daily gain (ADG, kg/day), meat components (moisture%, crude protein%, crude fat%, crude ash%, cholesterol (mg/100 g)) indicating nutrient contents in pork, drip-loss% (fluid consisting of water and soluble proteins expelled from meat without any mechanical force other than gravity) causing weight loss during storage and retailing, cooking loss% (influences juiciness and appearance; high cooking loss results in lower meat quality), shear force (g) (gram force needed to shear a unit of muscle sample; represents tenderness), pH (24 hours post-mortem, normally ranges between 6.1 and 5.7; high or low pH influences meat color and firmness), marbling and texture affecting tenderness and juiciness of meat, meat color (redness, yellowness, and lightness), as well as meat taste or eating quality traits (total acceptance, tenderness, juiciness, and flavor). Detailed information on phenotypic measures has been described by Kim et al. [26].

Validation and genotyping of polymorphisms within candidate genes

Target SNPs within GBP1, GBP2, CD163, and CD169 genes (Table 1) were confirmed to be present in the KY-F2 population via PCR product sequencing. Information on primer pairs and restriction enzymes for each gene is listed in Table 2. PCR products from eight pigs were sequenced for each candidate gene. Briefly, each PCR reaction mixture (30 μL) contained 2 μL of DNA template (50 ng/μL), 15 μL of PCR premix (SolGent, South Korea), 0.5 μL of forward primer (10 pmoL/μL), 0.5 μL of reverse primer (10 pmoL/μL), and 12 μL of double distilled water. Thermal cycling conditions were an initial cycle of 10 min at 95ºC, followed by 28 cycles of 30 sec at 95ºC, 30 sec at TM temperature, and 40 sec at 72ºC. PCR product was determined by using 1.5 % agarose gel electrophoresis with DNA gel straining solution (Gen

Table 1. Polymorphism information in current study

Gene Symbol	Accession No.	Chromosome Location	Polymorphism	Gene location and Amino acid substitution
GBP1	NM_001128473	SSC4	c.[10A>G; 11A>G]	Exon2: p.Lys4Gly
GBP2	NM_001128474	SSC4	c.1382C>A	Exon9: p.Thr461Lys
CD163	DQ067278	SSC5	c.*146C>T	3'UTR
CD169	DQ176853	SSC17	c.878A>G	Exon4: p.His293Arg
WUR10000125	NM_001128473	SSC4	c.*580A>G (rs80800372)	3'UTR of GBP1

Note: Where c.=coding DNA reference sequence; p.=protein reference sequence; [] indicates an allele; * (asterisk)=translation termination (stop) codon. The above polymorphisms were named according to the nomenclature for the description of sequence polymorphisms (http://www.hgvs.org/mutnomen/).

DEPOT, South Korea), after which 20 μL of PCR product was sent for sequencing by a commercial service. Finally, sequences were aligned and compared using the Sequencher program (GENE Code Corporation, USA) to determine variants. Genotyping was conducted by the PCR-RFLP method as follows: 5 μL of PCR products were mixed with 1 μL of 10 × buffer, 1 U of restriction enzymes, and double-distilled water up to a volume of 10 μL and incubated at 37ºC overnight. Digested products were separated by performing 1.5% agarose gel electrophoresis with DNA gel staining solution (Gen DEPOT, South Korea) and were visualized under UV illumination.

Statistical analyses

Haploview (Version 4.2) [27] was employed to analyze the linkage disequilibrium (LD) between polymorphisms. Association test was conducted with the R package (http://www.r-project.org/) using a general linear model below:

$Y_{ijkl} = \mu + S_i + B_j + A_k + G_l + \varepsilon$

Where Y_{ijkl} is the response vector of phenotypes, μ is the population mean, S_i is the sex effect, B_j is the effect of slaughter batch, A_k is the age of animals when slaughtered, G_l is the genotype effect of each candidate gene, and ε is the random error. $P<0.05$ was considered statistically significant.

Results

Minor allele frequency and linkage disequilibrium (LD)

Target polymorphisms within candidate genes (Table 1) were confirmed in the KY-F2 population by PCR product sequencing using eight F1 animals. F2 pigs (n=346) of the KY-F2 population were genotyped by the PCR-RFLP method. Minor allele frequencies (MAF) were 27.31% for polymorphism GBP1:c.[10A>G; 11A>G] in exon2 of GBP1 gene and 26.45% for SNP GBP2:c.1382C>A in exon9 of GBP2 gene. These minor allele frequencies in the KY-F2 population are similar to those of Landrace and Duroc pigs [11]. Additionally, the LD was analyzed across three polymorphism pairs: First, polymorphisms GBP1:c.[10A>G; 11A>G] and GBP2:c.1382C>A were in high LD (r^2=0.87); Second, GBP1:c.[10A>G; 11A>G] and SNP WUR10000125 in GBP1, which were previously tested using a 60k SNP chip in the KY-F2 population, also showed high LD (r^2=0.94). Third, high LD was measured between GBP2:c.1382:C>A and SNP WUR10000125 (r^2=0.87). The MAF of CD163:c.*146C>T was 38% in the KY-F2 population, whereas the MAF of CD169 c.878A>G was 17%, which are similar and lower compared to a previous study, respectively [21].

Associations of polymorphisms in candidate genes with growth, meat quality, and immune traits

Associations were measured between polymorphisms in immune candidate genes (GBP1, GBP2, CD163, and CD169) versus 2 growth and 18 meat quality traits in KY-F2 animals. Polymorphisms in genes GBP1 and GBP2 were significantly associated with average daily gain (ADG) ($P<0.05$). Among the 18 meat quality traits, both polymorphisms were significantly associated with crude fat, drip loss, and yellow meat color ($P<0.05$). The homozygous genotype AA of GBP1:c.[10A>G; 11A>G] or genotype CC of GBP2:c.1382:C>A showed significantly lower crude fat, drip loss, and yellowness than genotypes AG and CA, respectively (Table 3). For genes CD163 and CD169, c.*146C>T within the 3'UTR of CD163 gene was significantly associated only with crude fat ($P<0.05$), and pigs with genotype CC showed a significantly higher crude fat content than TT pigs ($P<0.05$) (Table 4). The missense SNP c.878A>G within exon4 of gene CD169/SN was significantly associated only with tenderness, and pigs with genotype AA showed significantly higher tenderness than animals with genotype AG or GG ($P < 0.05$) (Table 4).

Table 2. Information on primers and restriction enzymes for PCR-RFLP

Primer Name	Sequence (5' to 3')	Tm (°C)	Product Size (bp)	Restriction Enzyme	Ref.
GBP1_F	GGATAACACTTCGGTAACTTGC	58	587	Bsu36I	[11]
GBP1_R	GAAGGGGAAACTGAGACACAAT				
GBP2_F	ACGGGAACTCCGAAGCAACT	60	325	Ssp I	[11]
GBP2_R	CAAGGGCTTTCCCACTGTCC				
CD163_F	GACCTGGACTATTGAATGGC	53	139	Fnu4h I	[21]
CD163_R	CATACAACTTTGAGTAGTCACTTC				
CD169_F	CGGCTGCTGTAGCTCTGATTG	61	530	Hinp1 I	[21]
CD169_R	GCCTTGGCCTGGTTTCCTTA				

Where, "F" and "R" in primer names represent "Forward" and "Reverse".

Discussion

In this study, we investigated associations between polymorphisms in immune candidate genes GBP1, GBP2, CD163, and CD169 (Table 1) and growth and meat quality traits in the KY-F2 pig population. Both GBP1 and GBP2 were associated with growth rate, ADG, and meat quality traits in the KY-F2 population (Table 3). According to our previous 60k SNP chip genotyping results, the SNP WUR10000125 and our GBP1 and GBP2 polymorphisms showed high LD associated with host resistance to PRRSV challenge [10]. In this case, several favorable genotypes (AG of GBP1:c.[10A>G; 11A>G], AB of SNP WUR10000125, and CA of GBP2:c.1382C>A) showed higher growth rates and lower viral loads post-PRRSV infection along with no negative impact on growth (ADG) or meat quality (crude fat and yellowness). In this study, among those traits significantly associated with GBP1 and GBP2, it appears that the minor allele G of GBP1 or A of GBP2 conferred an additive effect. Further, GG of GBP1 or AA of GBP2 was the best genotype based on both genotypes, showing the highest ADG, crude fat, and yellowness but reduced drip loss. Additionally, meat quality (drip loss)-associated QTLs were identified on SSC4 in the KY-F2 population in a previous study [26]. However, the polymorphisms in this study are not within those QTLs. The CD163 polymorphism was only associated with crude fat, whereas CD169/SN was significantly associated with tenderness in the KY-F2 population (Table 4).

Similarly, it has been reported that variants in porcine M307 of FUT1 gene, which are related to enterotoxigenic *Escherichia coli* F18 infection in pigs, are not only related to general disease resistance in piglets but also meat quality and growth [28]. Polymorphisms in signal transducer and activator of transcription 6 (STAT6) gene, which is tightly connected to IL-4 and IL-13 signaling, plays a key role in T(H)2 polarization of the immune system, and serves as a mediator of leptin signaling, have been associated with carcass traits and growth efficiency traits in cattle [29]. Improved growth and meat quality

Table 3. Associations of polymorphisms in GBP1 and GBP2 with growth and meat quality traits

Traits	GBP1			GBP2		
	AA (168)	AG (163)	GG (13)	AA (10)	CA (123)	CC (120)
ADG (kg/day)*	0.209 ± 0.081[a]	0.236 ± 0.094[b]	0.264 ± 0.049	0.264 ± 0.049	0.235 ± 0.095[a]	0.211 ± 0.081[b]
Moisture (%)	74.17 ± 1.52	73.78 ± 1.86	73.42 ± 2.01	73.43±2.19	74.14 ± 1.52	73.79 ± 1.87
Crude Protein (%)	22.16 ± 1.62	22.18 ± 1.63	22.48 ± 1.13	22.43±1.22	22.17 ± 1.60	22.18 ± 1.64
Crude Fat (%)	2.25 ± 1.23[a]	2.72 ± 1.63[b]	3.05 ± 1.54	3.11±1.64	2.71 ± 1.64[a]	2.27 ± 1.23[b]
Crude ash (%)	1.06 ± 0.14	1.06 ± 0.14	1.04 ± 0.14	1.04 ± 0.15	1.06 ± 0.14	1.05 ± 0.13
Cholesterol (mg/100g)	150.33 ± 86	138.59 ± 84.88	92.29 ± 40.07	93.22 ± 42.33	139.26 ± 85.19	148.84 ± 85.59
Drip loss (%)	4.80 ± 1.71[a]	5.42 ± 1.90[b]	5.44 ± 1.60	5.66 ± 1.64	5.44 ± 1.90[a]	4.78 ± 1.70[b]
Cook loss (%)	32.28 ± 3.49	32.13 ± 3.53	33.73 ± 4.09	33.66 ± 4.38	32.28 ± 3.49	32.16 ± 3.52
Sheer Force (g)	1736 ± 462	1732 ± 404	1739 ± 495	1750 ± 529	1732 ± 458	1736 ± 406
pH (24 hour)	5.67 ± 0.28	5.62 ± 0.24	5.55 ± 0.90	5.55 ± 0.10	5.67 ± 0.28	5.61 ± 0.23
Marbling	2.27 ± 0.95	2.51 ± 1.07	2.80 ± 1.13	2.97 ± 1.12	2.51 ± 1.07	2.27 ± 0.95
Texture	2.92 ± 0.42	2.82 ± 0.43	2.76 ± 0.30	2.70± 0.29	2.81 ± 0.43	2.92 ± 0.41
Redness	5.43± 2.04	5.99 ± 2.00	6.52± 2.04	6.79 ± 2.15	6.00 ± 2.01	5.43 ± 2.02
Yellowness	7.13 ± 1.84[a]	7.70 ± 1.78[b]	7.80 ± 1.54	7.99 ± 1.60	7.70 ± 1.78[a]	7.14 ± 1.83[b]
Lightness	52.13 ± 5.14	53.07 ± 5.91	52.99 ± 4.41	54.06 ± 5.65	53.07 ± 5.93	52.16 ± 5.11
Total Acceptance (%)	2.95 ± 0.30	2.87 ± .31	2.96 ± 0.14	2.94 ± 0.14	2.87 ± 0.31	2.95 ± 0.29
Tenderness	3.03 ± 0.74	3.11 ± 0.71	3.09 ± 0.78	3.15 ± 0.82	3.0 4 ± 0.74	3.10 ± 0.71
Juiciness	3.10 ± 0.39	3.13 ± 1.92	3.04 ± 0.31	3.05 ± 0.33	3.15 ± 1.87	2.98 ± 0.38
Flavor	2.95 ± 0.42	2.95 ± 0.35	2.92 ± 0.31	2.95 ± 0.27	2.94 ± 0.42	2.95 ± 0.35

Note: * ADG is average daily gain. For ADG only, numbers of animals are 92, 128, and 31 corresponding to genotypes AA, AG, and GG of GBP1 gene and 173, 79, and 1 corresponding to AA, CA, and CC of GBP2 gene, respectively. For meat quality traits, number of animals of each genotype for both genes is represented in brackets, and all traits are presented as mean ± standard deviation (S.D.). Within a single gene, columns with different superscript letters are significantly different at $P<0.05$.

are considered as a consequence of improved health conditions. Thus, it is possible that immune-related genes are associated with other important production traits such as meat quality and growth, although the biological mechanism is poorly understood. In this study, however, high linkage disequilibrium was detected in F2 animals, possibly creating a false association with growth and fat deposition QTLs, which were previously reported on SSC4 [10, 24, 26]. Therefore, it is necessary to investigate these associations in another commercial pig population.

In conclusion, polymorphisms in immune candidate genes might also be associated with growth and meat quality traits. Our results show that porcine genes GBP1 and GBP2 might be associated with growth, ADG, and meat quality traits such as crude fat, drip loss, and meat color (yellowness). The favorable genotype AG of GBP1 gene was shown to be consistent with pig resistance to PRRSV infection, as supported by other studies. CD163 was only associated with crude fat, and CD169/SN was significantly associated with only tenderness. These results might provide useful information for the development of a pig selection program.

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Table 4. Associations of polymorphisms in CD163 and CD169 with growth and meat quality traits

Traits	CD163			CD169		
	CC (121)	TC (182)	TT (40)	GG (231)	AG (110)	AA (2)
ADG (kg/day)*	0.228 ± 0.081	0.220 ± 0.097	0.231 ± 0.063	0.224 ± 0.094	0.227 ± 0.076	0.203 ± 0.00
Moisture (%)	73.69 ± 2.05	73.99 ± 1.57	74.55 ± 0.98	74.05 ± 1.64	73.75 ± 1.9	75.08 ± 0.22
Crude Protein (%)	22.03 ± 1.76	22.31 ± 1.54	22.11 ± 1.39	22.16 ± 1.54	22.28 ± 1.74	21.45 ± 0.01
Crude Fat (%)	2.84 ± 1.85[a]	2.41 ± 1.19[b]	1.92 ± 0.92	2.44 ± 1.35	2.65 ± 1.68	2.40 ± 0.46
Crude ash (%)	1.05 ± 0.14	1.07 ± 0.13	1.05 ± 0.14	1.06 ± 0.14	1.06 ± 0.14	1.07 ± 0.23
Cholesterol (mg/100 g)	131.84 ± 84.62	147.98 ± 86.96	152.65 ± 73.27	144.69 ± 85.18	139.39 ± 84.74	83.14 ± 78.31
Drip loss (%)	5.04 ± 1.65	5.27 ± 1.92	4.56 ± 1.7	5.23 ± 1.87	4.92 ± 1.71	4.44 ± 2.01
Cook loss (%)	32.57 ± 3.47	31.98 ± 3.53	32.51 ± 3.6	32.42 ± 3.40	31.94 ± 3.83	33.62 ± 2.79
Sheer Force (g)	1778 ± 410	1688 ± 445	1824 ± 457	1753 ± 425	1693 ± 452	2042 ± 862
pH (24 hour)	5.66 ± 0.25	5.63 ± 0.27	5.64 ± 0.19	5.63 ± 0.25	5.66 ± 0.28	5.63 ± 0.04
Marbling	2.59 ± 1.07	2.33 ± 1.01	2.20 ± 0.79	2.38 ± 0.99	2.43 ± 1.07	3.75 ± 1.77
Texture	2.89 ± 0.44	2.86 ± 0.39	2.83 ± 0.53	2.87 ± 0.42	2.86 ± 0.43	3.17 ± 0.24
Redness	5.66 ± 2.02	5.81 ± 2.11	5.62 ± 1.86	5.76 ± 2.04	5.68 ± 2.06	5.59 ± 2.79
Yellowness	7.31 ± 1.82	7.60 ± 1.86	6.94 ± 1.57	7.38 ± 1.79	7.52 ± 1.88	7.94 ± 3.19
Lightness	52.31 ± 5.60	53.12 ± 5.43	51.22 ± 5.09	52.48 ± 5.36	54.13 ± 7.82	53.01 ± 5.80
Total Acceptance	2.94 ± 0.29	2.90 ± 0.28	2.92 ± 0.38	2.91 ± 0.3	2.92 ± 0.3	3.00 ± 0.00
Tenderness	3.11 ± 0.74	3.07 ± 0.72	2.95 ± 0.68	3.06 ± 0.70[a]	3.07 ± 0.75[a]	4.42 ± 0.59[b]
Juiciness	3.01 ± 0.39	3.14 ± 1.82	2.95 ± 0.3	3.10 ± 1.63	2.99 ± 0.38	3.17 ± 0.47
Flavor	2.96 ± 0.39	2.96 ± 0.42	2.86 ± 0.27	2.93 ± 0.39	2.98 ± 0.39	2.83 ± 0.24

Note: * ADG is average daily gain. For ADG only, numbers of animals are 92, 128, and 31 corresponding to genotypes CC, CT, and TT of CD163 gene and 173, 79, and 1 corresponding to GG, AG, and AA of CD169 gene, respectively. For meat quality traits, number of animals of each genotype for both genes is represented in brackets, and all traits are presented as mean ± standard deviation (S.D.). Within a single gene, columns with different superscript letters are significantly different at $P<0.05$.

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