



Effects of 7 SNPs on growth and meat quality traits in F₁ crossbred of Korean native pig × Duroc

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ABSTRACT

The study was designed to investigate genetic associations of single nucleotide polymorphism (7 SNPs located on 7 selected candidate genes) with growth and meat quality of F₁ Korean native pig × Duroc. Selected SNP sets (7) of 86 F₁ crossbred pig samples were genotyped by PCR-RFLP. The association analysis revealed that SNPs on *MC4R*, *PGK2* and *CTSD* genes were significantly associated with body weight. Average daily gain at 2 weeks (ADG2) showed significant associations with SNPs on *MC4R* and *PGK2*. On the other hand, ADG10 showed associations only with SNP on *MC4R*. In addition, backfat thickness and pH value also exhibited significant associations with the SNPs on *MC4R*, *TNNI1*, *CTSK*, *CTSZ*, and *PGK2* genes. Therefore, we concluded that a significant association exists between economic traits and SNPs located on 7 genes. However, further studies seem mandatory to establish the possibility that SNP markers tested here could be used as potential genetic markers for the crossbred Korean native pigs.

Key words: Crossbred, Korean native pig, PCR-RFLP, Pigs, SNP

Korean native pigs have shown lower growth rate and there are possibility that lower growth rate of Korean native pigs could be improved with effective genetic augmentation in the quantitative and qualitative traits through the use of marker-assisted selection (MAS). Yoo *et al.* (2012) notified several quantitative trait loci (QTL) linkage scans and phenotypically divergent breeds and candidate genes were also determined and analyzed for fatness and growth traits (Ai *et al.* 2012). Therefore, taking on the challenges from previous experimental data we designed the study to investigate association of major SNPs on 7 genes with economically important traits in crossbreds of Korean native pig.

MATERIALS AND METHODS

Crossbreds (86) of Korean native pig (♀) × Duroc (♂) populations were used. The economic traits, viz. birth weight, weaning weight, market weight, carcass weight, backfat thickness, average daily gain at 2, 4, 10, and 18

weeks and meat quality (water content, ash content, fat content, protein content, water holding content, pH value), were investigated. Genomic DNA was extracted from hairs of crossbred pigs using i-Genomic CTB DNA extraction mini kit. DNA concentration and purity (A260/A280 ratio) for each sample was assessed using spectrophotometer. The measured DNA samples were stored at –80°C until further analysis. Approximately 50 ng of genomic DNA was amplified in a final volume of 20 µl that contained 0.01 mM of each primer, 0.15 mM dNTPs, 10X PCR buffer 1.5 µl containing 15 mM MgCl₂/mL and 5U of Taq DNA polymerase. The PCR conditions were carried out as 95°C pre-denaturation for 5 min, 35 cycles of 95°C denaturation for 30 sec, 50–60°C annealing gel with 0.5 X TBE buffer to determine the size of DNA fragment. PCR products were genotyped by PCR-RFLP method. To genotype 7 SNPs, PCR products were digested directly with restriction enzymes (TaqI: 65°C, Mbo II, Xba I, Hin6 I, Hpy8 I, Msc I, FspB I, ScrF I: 37°C, Sma I: 25°C) in accordance with the manual, respectively and subjected to electrophoresis in 2% agarose gel stained with ethidium bromide. The allele frequency data for economic traits were analyzed by Cervus Ver. 2.0 program (Marshall *et al.* 1998). Statistical analysis of variance was accomplished by using the SAS Ver. 9.2 Package with PROC GLM procedures to evaluate effects of genotypes on economic and meat quality traits.

$$Y_{ij} = \mu + G_i + M_{ij} + e_{ij}$$

where Y_{ij} , observation for the economic traits; μ , whole average for each trait; G_i , effect of genotype; M_{ij} ,

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regression variable for measure age; and ϵ_{ij} , random error effect of each observation.

RESULTS AND DISCUSSION

We genotyped 7 selected SNPs in the crossbred pigs using the PCR-RFLP. All genotyped SNP's were analysed

for its association and relationship with economically important traits i.e. growth and meat quality. Tables 2 and 3 has illustration on genotype frequencies of SNP's on *MC4R*, *PGK2*, *TNNI1*, *TNNI2*, *CTSD*, *CTSK*, and *CTSZ* genes, respectively. Association study between the SNP genotypes on the 7 genes and economic traits were

Table 1. Primer sequence and PCR-RFLP information for 7 SNPs analysis

Gene(SNP)	Primer sequences (5'→3')	Product size (bp)	Annealing temp. (°C)	Enzyme	Reference
<i>MC4R</i> (c.1426A>G)	F: TACCCTGACCATCTTGATTG R: ATAGGAACAGATGATCTCTTT	226	64	<i>Taq</i> I	Kim <i>et al.</i> (2005)
<i>PGK2</i> (g.122T>G)	F: TTCCAATAACGATAAAT R: GATGTGGTTCCTTACTGTTCC	542	50	<i>Mbo</i> II	Jang <i>et al.</i> (2011)
<i>TNNI1</i> (g.5174T>C)	F: GAGTGGTAGGAGATTGACGGA R: GTAGCGAGCCTTCTCAGCC	190	60	<i>Xba</i> I	Xu <i>et al.</i> (2010)
<i>TNNI2</i> (g.1167C>T)	F: GGGTGGGACTTCGAAGGGA R: AGAGGAGACAGAGTCAGGGC	425	64	<i>Sma</i> I	Xu <i>et al.</i> (2010)
<i>CTSD</i> (g.70G>A)	F: GCTGTGCACCCTAGGAACC R: TCGTCAGGTCCAGGACAAAC	184	59	<i>Msc</i> I	Russo <i>et al.</i> (2008)
<i>CTSK</i> (g.15G>A)	F: CTCAAAGGCAGACAGCAATG R: TCATTTTGATCCCCAAGTCA	388	59	<i>Fsp</i> B I	Fontanesi <i>et al.</i> (2010a)
<i>CTSZ</i> (g.37A>G)	F: GGCCTCATGAGTACCTGTCC R: ATGTGCTGGTTCCTGGTGAC	100	59	<i>Scr</i> F I	Russo <i>et al.</i> (2008)

Table 2. Associations between 7 SNPs and economic traits in F₁ of Korean native pig × Duroc

Gene(SNP)	Genotype (n)	Traits*								
		BW	WW	MW	CW	BFT	ADG2	ADG4	ADG10	ADG18
<i>MC4R</i> (c.1426A>G)	AA(39)	1.26±0.05 ^b	6.24±0.22	98.35±1.53	76.91±1.36	24.56±0.73 ^b	0.45±0.02 ^A	0.24±0.01	0.59±0.01 ^a	0.68±0.01
	AG(33)	1.31±0.05 ^b	6.34±0.22	100.18±1.96	75.73±1.59	25.87±0.85 ^b	0.39±0.03 ^A	0.25±0.01	0.59±0.02 ^a	0.68±0.02
	GG(14)	1.48±0.07 ^a	5.88±0.31	101.58±1.90	76.34±1.36	28.73±0.83 ^a	0.26±0.04 ^B	0.21±0.02	0.51±0.03 ^b	0.658±0.01
	p-value	0.047	0.539	0.818	0.835	0.015	0.001	0.087	0.026	0.663
<i>PGK2</i> (g.122T>G)	GG(6)	1.60±0.06 ^A	7.02±0.90	106.38±4.43	81.34±3.966	27.82±2.58	0.54±0.08 ^a	0.29±0.03	0.66±0.04	0.72±0.03
	GT(53)	1.36±0.04 ^B	6.22±0.17	99.73±1.42	75.43±1.160	26.30±0.66	0.37±0.02 ^b	0.24±0.01	0.56±0.01	0.67±0.01
	TT(27)	1.14±0.05 ^C	6.02±0.29	97.43±1.97	74.38±1.776	24.05±0.93	0.40±0.02 ^b	0.23±0.01	0.57±0.02	0.67±0.01
	p-value	0.001	0.302	0.183	0.249	0.095	0.037	0.330	0.116	0.281
<i>TNNI1</i> (g.5174T>C)	CC(60)	1.34±0.04	6.30±0.16	99.24±1.30	74.80±1.09	25.20±0.63	0.39±0.02	0.24±0.01	0.57±0.01	0.67±0.01
	CT(26)	1.26±0.07	6.04±0.30	100.36±1.87	76.96±1.52	26.98±0.78	0.41±0.03	0.24±0.01	0.58±0.02	0.69±0.01
	p-value	0.273	0.402	0.635	0.268	0.107	0.609	0.998	0.576	0.258
<i>TNNI2</i> (g.1167C>T)	CC(18)	1.28±0.07	6.42±0.19	100.40±2.66	76.73±2.33	26.13±1.31	0.36±0.03	0.23±0.01	0.55±0.03	0.67±0.02
	CT(54)	1.32±0.04	6.17±0.20	98.96±1.31	75.04±1.12	25.52±0.61	0.40±0.02	0.25±0.01	0.58±0.01	0.67±0.01
	TT(8)	1.26±0.10	5.59±0.39	100.54±4.61	75.46±3.11	26.61±2.25	0.41±0.04	0.23±0.02	0.59±0.03	0.69±0.03
	p-value	0.808	0.358	0.837	0.774	0.795	0.661	0.410	0.605	0.805
<i>CTSD</i> (g.70G>A)	GG(2)	0.90±0.10 ^b	5.70±1.30	85.00±5.00	62.70±0.70	21.15±0.55	0.37±0.01	0.26±0.05	0.58±0.08	0.62±0.07
	AG(29)	1.23±0.06 ^{ab}	6.43±0.27	76.68±1.53	75.55±1.36	24.85±0.83	0.45±0.02	0.25±0.01	0.60±0.02	0.68±0.01
	AA(55)	1.37±0.04 ^a	0.14±0.17	100.53±1.41	75.65±1.17	26.26±0.65	0.37±0.02	0.24±0.01	0.57±0.01	0.68±0.01
	p-value	0.027	0.518	0.075	0.095	0.167	0.073	0.465	0.407	0.534
<i>CTSK</i> (g.15G>A)	AG(21)	1.30±0.05	6.36±0.23	99.17±2.23	75.68±1.79	24.75±0.92	0.36±0.03	0.24±0.01	0.56±0.03	0.67±0.02
	GG(65)	1.31±0.04	6.14±0.17	99.71±1.24	75.37±1.05	26.15±0.60	0.40±0.02	0.24±0.01	0.58±0.01	0.68±0.01
	p-value	0.797	0.495	0.829	0.884	0.237	0.274	0.735	0.703	0.537
<i>CTSZ</i> (g.37A>G)	AA(1)	1.60	8.10	104.30	75.20	20.60	0.52	0.33	0.69	0.69
	AG(4)	1.23±0.22	6.08±1.05	99.19±7.91	71.52±5.71	23.18±2.14	0.43±0.14	0.24±0.04	0.55±0.10	0.67±0.05
	GG(81)	1.32±0.03	6.20±0.14	99.54±1.08	75.65±0.91	25.93±0.52	0.39±0.02	0.24±0.01	0.57±0.01	0.67±0.01
	p-value	0.515	0.342	0.891	0.621	0.284	0.342	0.280	0.494	0.494

* Trait : BW, Birth weight; WW, weaning weight; MW, market weight; CW, carcass weight; BFT, back fat thickness; ADG 2, average daily gain 2 week; ADG 4, average daily gain 4 week; ADG 10, average daily gain 10 week; ADG 18, average daily gain 18 week

performed using the phenotype data on 86 F₁ Korean native pig × Duroc populations (Table 2). Primer sequence and PCR-RFLP information for 7 SNPs analysis are given in Table 1.

MC4R is a centrally expressed G-protein that mediated effect of leptin on food intake and energy homeostasis (Kim *et al.* 2005). Due to this fact, *MC4R* gene was considered to be an excellent candidate gene for fatness, feed intake or growth-related traits in animals (Kim *et al.* 2005). We observed significant differences on *MC4R* SNP's for body weight ($P<0.05$), back fat thickness ($P<0.05$), average daily gain of 2 ($P<0.001$), 10 ($P<0.05$) weeks and protein ($P<0.001$) on 86 F₁ crossbred pigs. Our observations were in absolute corroboration with Cho *et al.* (2007) who also demonstrated positive correlation of *MC4R* with economic traits.

In *PGK2* gene (g.122T>G), the observed frequencies of GG, GT and TT genotypes were 0.06, 0.63 and 0.31, respectively. Lately, phosphoglycerate kinase (PGK) was linked to have role in central metabolic pathway of glycolysis and increase angiogenesis (Wang *et al.* 2012). Due to its angiogenic process it helps in the building up of normal muscle mass formation (leading to significant ($P<0.05$) increase in the body weight and carcass weight). PGK is normally ubiquitously expressed as PGK1, PGK2 along with PGK1P1 and PGK1P2 that are considered as 2

nonfunctional pseudogenes (Ji *et al.* 2013). However, we observed SNP on *PGK2* gene that was interrelated with economically important traits including birth weight ($P<0.001$), average daily gain of 2 week ($P<0.05$), pH value ($P<0.05$) in F₁ population of Korean native pig × Duroc in this study.

The contractile protein troponin I (TnI), a constituent protein of the troponin complex located on the thin filaments of striated muscle brings about contraction by inhibiting calcium-induced myosin ATPase activity apparently improves the meat quality trait (Bottinelli and Reggiani 2000). In our study, we observed that genotypic frequencies of the *TNNI1* gene (g.5174T>C) were 0.70 for CC and 0.30 for CT, and *TNNI2* gene (g.1167C>T) were 0.23 for CC, 0.68 for CT and 0.09 for TT. Although, some studies have previously correlated the SNP's on *TNNI1* and 2 with fat content, backfat thickness in pigs (Munoz *et al.* 2013) but contrastingly we observed the SNP on *TNNI1* was interrelated with pH value ($P<0.05$) in the crossbred pigs.

In the present experiment we found *CTSD* (g.70G>A) and *CTSK* (g.37A>G) gene, and observed that frequencies of GG, GA genotypes in them were 0.01, 0.34 and 0.65, and 0.74 and 0.24, respectively, in the crossbred pigs. Role of cathepsin D was earlier emphasized in the pathogenesis of prion disease, alzheimers and in neuronal diseases (Zhou *et al.* 2012) by cleaving A β peptides and immunoreactive

Table 3. Associations between 7 SNPs and meat quality traits in F₁ of Korean native pig × Duroc

Gene	Genotype (n)	Traits*					
		Water (%)	Ash (%)	Fat (%)	Protein (%)	WHC (%)	pH
MC4R (c.1426A>G)	AA (6)	72.10±0.85	1.03±0.01	5.84±0.25	20.17±0.23 ^b	33.12±1.32	5.55±0.01
	AG (5)	72.67±0.56	1.07±0.01	6.47±0.03	20.60±0.12 ^{ab}	36.19±3.96	5.58±0.02
	GG (2)	72.16±0.91	1.06±0.02	5.94±0.24	22.45±0.72 ^a	31.54±1.59	5.54±0.01
	p-value	0.853	0.074	0.075	0.044	0.584	0.230
PGK2 (g.122T>G)	GG (2)	70.81±2.14	1.04±0.01	6.43±0.04	20.80±0.29	37.62±0.63	5.61±0.02 ^a
	GT (9)	71.95±0.71	1.05±0.01	6.19±0.17	20.44±0.19	33.10±1.29	5.56±0.01 ^{ab}
	TT (2)	71.11±2.45	1.04±0.01	5.84±0.55	20.60±0.49	35.73±2.52	5.54±0.02 ^b
	p-value	0.785	0.665	0.532	0.733	0.291	0.471
TNNI1 (g.5174T>C)	CC (10)	73.11±0.315 ^A	1.05±0.007	5.95±0.205	21.93±1.931	33.66±2.153	5.57±0.012
	CT (3)	70.34±0.916 ^B	1.03±0.032	6.40±0.034	20.79±0.176	34.71±1.766	5.55±0.018
	p-value	0.004	0.373	0.243	0.722	0.786	0.512
TNNI2 (g.1167C>T)	CC (4)	73.95±0.296	1.05±0.003	5.70±0.407	20.26±0.240	36.00±5.542	5.56±0.023
	CT (6)	72.41±0.428	1.06±0.014	6.25±0.235	20.17±0.232	34.13±0.986	5.57±0.017
	TT (3)	70.75±2.085	1.05±0.020	6.37±0.010	20.94±0.155	33.09±5.150	5.59±0.005
	p-value	0.093	0.927	0.347	0.197	0.876	0.808
CTSD (g.70G>A)	GA (7)	73.19±0.484	1.05±0.010	5.90±0.267	22.69±2.614	35.25±2.934	5.56±0.012
	AA (6)	71.65±0.713	1.04±0.015	6.23±0.183	23.23±2.501	33.17±1.615	5.57±0.015
	p-value	0.103	0.722	0.338	0.883	0.531	0.417
CTSK (g.15G>A)	AG (4)	71.06±1.058 ^b	1.04±0.021	6.15±0.288	20.42±0.249	33.04±2.930	5.55±0.015
	GG (9)	73.10±0.296 ^a	1.05±0.008	6.02±0.209	22.26±1.932	34.29±2.008	5.57±0.012
	p-value	0.034	0.334	0.736	0.528	0.747	0.293
CTSZ (g.37A>G)	AG (2)	71.82±1.255	1.01±0.030 ^b	5.84±0.520	28.11±7.625 ^a	31.36±1.415	5.54±0.015
	GG (11)	72.54±0.531	1.06±0.007 ^a	6.11±0.177	20.35±0.161 ^b	34.52±1.901	5.57±0.010
	p-value	0.592	0.048	0.568	0.016	0.474	0.175

lysosomes. However, several studies conducted earlier has related cathepsin D gene with fat deposition in pigs and have insisted on its usefulness in identifying DNA markers associated with fatness as well as other correlated traits (Fontanesi *et al.* 2012). In the *CTSD* (g.70G>A) and *CTSK* (g.37A>G) gene, the observed frequencies of GG, GA in the *CTSD* 2 gene, the g.70G>A SNP was associated with birth weight ($P<0.05$). The AA genotypes had higher birth weight (1.37 ± 0.04 kg) than those with the AG (1.23 ± 0.06 kg) and GG (0.09 ± 0.1 kg) genotype in the pigs. SNPs on *MC4R* (protein content), *PGK2* (pH), *TNNI* (water content), *CTSK* (water content), and *CTSZ* (ash and protein content) genes were significantly associated with meat quality traits in the crossbreds of Korean native pig $\times$ Duroc (Table 3).

Recently, *CTSK* was also found to affect the innate immune response to pathogens DNA by compromising TLR9 signaling. Additionally, another specific function of *CTSK* was also shown in adipocyte differentiation. Increased expression *CTSK* gene was earlier reported in adipose tissue of experimentally obese mice and in human with obese phenotype. SNPs on *CTSD*, *CTSK* and *CTSZ* gene were also reported earlier with a significant difference in backfat thickness, average daily gain in porcines (Fontanesi *et al.* 2012). However, our finding further confirmed that *CTSD*, *CTSK* and *CTSZ* gene affected birth weight ($P<0.05$), water content ($P<0.05$), ash content ($P<0.05$), and protein content ($P<0.05$) respectively in F_1 population of Korean native pig $\times$ Duroc.

Cathepsin B, *CTSF*, *CTSH*, *CTSL*, and *CTSZ* are cysteine lysosomal proteinases with endopeptidase, peptidyl dipeptidase, aminopeptidase, or carboxypeptidase activities and with expression in a wide range of tissues (*CTSB*, *CTSH*, *CTSL*, and *CTSZ*) and are mainly expressed in striated muscles (*CTSF*). Finally, *CTSZ* was previously linkage-mapped to SSC17 at about 110 cM and several QTL for growth, meat quality, and carcass traits were mapped on this chromosome (Piorkowska *et al.* 2012) and it was similarly traced in our population where we observed that *CTSZ* was associated with ADG, LC, HW and FGR. Several markers in cathepsin genes (*CTSD*, *CTSF*, *CTSH* and *CTSZ*) were found associated with growth, fat deposition, and production traits in the Italian Large White pig population (Piorkowska *et al.* 2012). We also received results for *CTSF* that revealed consistency with previously reported investigations in pigs. However, we are carrying out further investigations to verify the results obtained for *CTSD*, *CTSH*, and *CTSZ* and investigating whether the biochemical and physiological functions of the lysosomal proteinases, together with cathepsin genes affects economically important traits in pigs.

In conclusion, there was significant association with economic traits in F_1 Korean native pig $\times$ Duroc population. These results suggested that 7 SNP markers could be used as potential genetic markers for the crossbred breeding of

Korean native pigs. More Korean native pig $\times$ Duroc F_1 samples and expression analysis of these 7 genes in different pig samples might help to further solidify our results.

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