Journal of Life Science 2007 Vol. 17. No. 12. 1622~1626 ©JLS / ISSN 1225-9918

Investigation of Single Nucleotide Polymorphisms in Porcine Candidate Gene for Growth and Meat Quality Traits in the Berkshire Breed

Sang Wook Kim, Ji Hye Jung, Kyung Tag Do[1], Kwan Suk Kim[1], Chang Hee Do[2], Jun kyu Park[3], Young Kuk Joo[3], Tae Suk Kim[3], Bong Hwan Choi[4], Tae Hun Kim[5], Ki Duk Song[6] and Byung Wook Cho*

Department of Animal Science, Pusan National University, Kyung Nam, Miryang 627-706, Korea
[1]*Department of Animal Science, Chungbuk National University, Cheongju 361-763, Korea*
[2]*Department of Animal Science, Cungnam National University, Gyeongbuk 712-749, Korea*
[3]*Livestock Veterinary Research Institute, 15-1 Shinan, Sancheong, Gyeongsangnam-Do, 666-962, Korea*
[4]*Animal Genomics and Bioinformatics Division, National Livestock Research Institute, RDA, Suwon 441-707, Korea*
[5]*International Technical Cooperation Center, Rural Development Administration, RDA, Suwon 441-707, Korea*
[6]*NICHD/National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892, USA*

Received November 20, 2007 / Accepted December 18, 2007

This study was conducted to identify useful single nucleotide polymorphisms (SNPs) and determine their association with economically important traits in pig population. Four candidate gene analyses have identified important chromosomal regions and major genes associcated whit economic traits of the pig. For application of the chromosomal information to the pig industry using DNA technology, SNP markers were developed by comparative re-sequencing of polymerase chain reaction (PCR) products of 4 candidate genes (CSF2, IL4, MYOD, RIP140). PCR restriction fragment length polymorphism (PCR-RFLP) assays were developed for these 4 SNPs and used to genotype Berkshire pig populations in Korea.

Key words : Meat Quality, berkshire, PCR-RFLP, single nucleotide polymorphism, growth factor

Introduction

Several recent studies have shown that genetic markers are already being applied at a significant level in the pig industry today [11]. Animal breeders have started applying marker-assisted selection (MAS) using the genetic markers to improve the product quality and performance in their livestock. However, little number of markers associated with economically important traits in pig is available so far [6].

In order to find out these genetic markers, most of animal genotyping technology depends on the use of single nucleotide polymorphisms (SNPs). Several studies have reported quantitative trait loci (QTL) for meat quality on porcine chromosome (http://www.animalgenome.org/QTLdb/pig.html).

Early investigations to elucidate genetic variation of pork quality have discovered two major genes primarily involved in pale, soft, and exudative (PSE) meat condition (*HAL*) and cured-cooked ham yield (*RN*), respectively [3,7]. More recent developments of quantitative trait loci (QTL) studies have detected major chromosomal regions affecting various meat and eating quality traits in commercial pigs [4,5,8,12,13]. Many of the meat quality QTL were mapped to the intermediate region of SSC2 (*SW2445-S0565*). This identified chromosomal region spans about 60 cM, and contains syntheny groups homologous to four different human chromosomal fragments, HAS11, HSA19, HSA1, and HSA5 [9]. Porcine chromosome 13 is reported to contain quantitative trait loci (QTL) for growth rate, fat deposition, meat quality, and reproduction traits [1,10].

Therefore, the purpose of this study was to further characterize and implement the SNPs to improve the QTL map resolution and identify candidate genes to investigate meat quality QTL on SSC in Berkshire pig populations.

***Corresponding author**
Tel : +82-55-350-5515, Fax : +82-55-350-5519
E-mail : bwcho@pusan.ac.kr

Materials and Methods

Animal Material and Analyzed Traits

Experimental animals which consisted of Berkshire were admitted and bred in the laboratory of Livestock Veterinary Research Institute (LVRI) from January 2006 to January 2007. Phenotypic data of these animals such as reached 90 kg, daily gain, backfat thickness and weaning

weight, were measured and collected until body weight reached 90 kg (Table 1). Genomic DNAs were extracted from the bloods of experimental animals using Genomic DNA Extraction Kit (Promega) and Toyobo MagExtractor Kit (Toyobo). The sequencing DNA panel for SNP detection consisted of two individuals from each of Korean Berkshire breeds.

Primer Design and Polymerase Chain Reaction

A total of 4 primer pairs were tested for amplification and sequencing of 4 candidate genes (*CSF2, IL4, MYOD, RIP140).* These genes were selected for their known biological roles in skeletal muscle development, metabolism, and probable locations within the QTL region. Primer sequences of these candidate genes were obtained from Jungerius et al [3]. Polymerase chain reactions (PCR) were performed in 10 μl volumes contained 12 ng of genomic DNA, 10 pmol of each primer, 200 μM of each dNTP, 2.5 units of *Taq* DNA polymerase (Enzynomics™, Korea), and reaction buffer with 1.5mM $MgCl_2$. Thermocycling reaction was performed in a PTC-200 thermocycler (MJ Research, Watertown, MA, USA) with a 5 min initial denaturation at 94°C, 45 s at annealing temperature, 60 s at 72°C, and a final extension for 10 min at 72°C. The information for each primer sequence, annealing temperature, and fragment size are given in Table 2.

Sequencing, Polymorphism Identification, and Genotyping

A total of 4 PCR products were sequenced with both forward and reverse amplification primers at Genotech Co. (Daejeon, Korea). Sequencer software (Gene Codes, version 4.5, Ann Arbor, MI) was used to assemble the sequences and to identify polymorphism (Table 3). Polymorphic sites were analyzed for putative restriction fragment length polymorphism (RFLPs) using the NEBcutter program (http://tools.neb.com/NEBcutter2/index.php). All restriction enzymes were supplied by New England BioLabs (Ipswich, MA, USA) and restriction digestions were performed according to manufacturer's recommendations.

Table 1. Summary of overall means and standard deviations on performance traits

Gene	Breed	Days to 90 kg (day)	Daily Gain (g)	Backfat (cm)	Birth weight (kg)
CSF2	Berkshire (n=417)	159.736±17.397	569.970±61.504	18.019±2.770	1.337±0.250
IL4	Berkshire (n=400)	157.779±17.028	569.487±60.205	17.959±2.693	1.335±0.248
MYOD	Berkshire (n=597)	161.023±16.121	566.621±59.212	17.872±2.543	1.333±0.247
RIP140	Berkshire (n=476)	160.167±16.513	567.760±58.519	17.976±2.629	1.340±0.251

Table 2. PCR primers and conditions used for amplification and sequencing

Gene	STS name	Accesion no.	Primer		Annealing Temp.	product size
			Forward(5′→3′)	Reverse(5′→3′)		
CSF	CSF2sts1	BV079385	CAG CAT GTG GAT GCC ATC	GTA CAG CTT CAG GCG AGT CTG	56	973
MYOD1	MYOD1sts3	BV012581	GGT GAC TCA GAC GCA TCC A	ATA GGT GCC GTC GTA GCA GT	60	599
IL4	IL4sts1	BV079417	GAT CCC CAA CCC TGG TTC TGC T	GGC AGA AAG ACG TCG TCA C	56	433
RIP140	RIP140sts1	BV079372	TCCCTCCAAACGTCC	TCCCTCCAAACGTCC	56	286

* The STS name, accession .number and primer information used in the Table 2 is reported by Jungerius et al. (2002).

Table 3. Comparison of SNPs between two independent studies

Gene	STS name	Accession no.	product size (bp)	No. of SNPs in Jungerius et al (2003).	No. of SNPs in this study
CSF	CSF2sts1	BV079385	974	10	7 : 103(C/T), 104(A/G), 155(C/T), 192(A/G), 459(G/T)*, 650(C/G), 691(C/T)
MYOD1	MYOD1sts3	BV012581	599	2	2 : 343(A/C), 345(G/T)*
IL4	IL4sts1	BV079417	433	1	1: 321(C/T)
RIP140	RIP140sts1	BV079372	286	1	1 : 286(C/A)
total			2292 bp	14 SNP	11 SNP

* New SNPs.

Digested PCR products were analyzed on 2.5-4% agarose gels and each allele was scored manually. The restriction enzymes and polymorphic fragment sizes used for SNP genotyping were given in Table 4.

Statistical Analyses

In order to estimate the effects of SNP genotypes on economic traits of Korean Berkshire breeds studied, General Linear Model (GLM) analysis was performmed using SAS 9.1 Package/PC with parameters of breeds and SNP genotypes. To determine possible effects of SNP genotypes on each trait, we conducted significance test among least square means (Table 6).

Results and Discussion

Analysis on the Frequency of SNP Genotypes

The genotype (CSF2, IL4, MYOD and RIP140) frequency of Berkshire bred in Livestock Veterinary Research Institute (LVRI) is listed in Table 5. As the result of genotype frequency analysis, it was found that CSF2 DD, CSF2 DN and CSF2 NN frequency were 0.26, 0.25 and 0.49; IL4 DD, IL4 DN and IL4 NN frequency were 0.00, 0.01 and 0.99; MYOD DD, MYOD DN and MYOD NN frequency were 0.00, 0.22 and 0.78; RIP DD, RIP DN and RIP NN frequency were 0.01, 0.69 and 0.30. However it was found that IL4 genotype frequency did not show any polymorphism due to genotypic fixation of NN within Berkshire group. It is already known that the Berkshire is a breed characterized by thinner back fat and lower carcass percentage (%) than other pig breeds.

Analysis on Associations in Traits of Four SNP Genotypes

Table 6 shows possible genotypic effects of 4 candidate genes (CSF2, IL4, MYOD and RIP140) on performances examined in this study. Notably, in terms of possible effects of CSF2 genotype on characters, it was found that CSF2 CC group reached the weight of 90 kg in significantly earlier than TT group (163.036 days vs. 157.953 days). In addition, it was found that CSF2 CC genotype group weighted 100 g more in birth weight than CSF2 TT genotype group (1,364 kg vs. 1.285 kg). Therefore, it is estimated that CSF2 gene is possibly associated with TT group's growth rate, which is well demonstrate by heavier birth weight and earlier approach to 90 kg than other groups. However, it was found that Berkshire group's daily weight gain is not associated with its back fat thickness depending upon CSF2 genotype. For IL4 gene, it was impossible to deduce significance analysis, since IL4 genotype frequency in Berkshire breed group did not show any polymorphism due to fixed CC genotype. Indeed, CT genotype group is

Table 4. PCR primers and restriction enzymes used for SNP genotyping.

Gene	Primer sequences (5′→3′)	Fragment size (bp)	T_A (°C)	Restriction enzyme	Size (bp) of the allelic polymorphism
CSF2 (C691T)	GCTGTGATGGTGAGTGAGGA CCCTTGAATGCTAGGACTGC	362	56	*Mbo* II	362, 276
IL4 (C321T)	GATCCCCAACCCTGGTTCTGCT GGCAGAAAGACGTCGTCAC	434	56	*Alu* I	308, 194
MYOD1 (A343C)	GGTGACTCAGACGCATCCA ATAGGTGCCGTCGTAGCAGT	599	60	*Dde* I	599, 340
RIP140 (C286A)	TCGCTGACAGTAAAAAGAAA TCCCTCCAAACGTCC	286	56	*Dde* I	286, 159

* The four SNPs were genotyped in the Berkshire breed for linkage and association analyses.

Table 5. Four SNP genotypic and allelic frequencies in the Berkshire Pig Breeds

Gene	Breed	Number of pigs	Genotypic Frequency			Allelic Frequency	
			DD	DN	NN	1	2
CSF2	Berkshire	417	0.26	0.25	0.49	0.40	0.60
IL4	Berkshire	400	-	0.01	0.99	0.01	0.99
MYOD	Berkshire	597	-	0.22	0.78	0.11	0.89
RIP140	Berkshire	476	0.01	0.69	0.30	0.35	0.65

Table 6. Least squares means and standard errors for the performance traits by four SNP genotypes in Berkshire breeds combined data

SNP of Gene	Breed	Genotype	Days to 90 kg (day)	Daily Gain (g)	Backfat (cm)	Birth weight (kg)
CSF2(T/C)	Berkshire (n=417)	TT (DD)	[a]163.036±1.636	560.072±5.776	18.322±0.262	[c]1.285±0.023
		TC (DN)	[b]159.990±1.674	568.028±5.911	17.813±0.268	[b]1.335±0.024
		CC (NN)	[c]157.953±1.209	575.699±4.271	17.943±0.193	[a]1.364±0.017
IL4(T/C)	Berkshire (n=400)	TT (DD)	-	-	-	-
		TC (DN)	159.805±0.792	569.430±2.803	[b]18.038±0.120	1.336±0.010
		CC (NN)	161.200±1.621	561.400±26.944	[a]10.586±1.157	1.170±0.111
MYOD(C/A)	Berkshire (n=597)	CC (DD)	-	-	-	-
		CA (DN)	[a]152.125±0.685	563.540±2.912	17.983±0.222	1.362±0.121
		AA (NN)	[b]160.894±1.234	561.980±1.999	17.123±0.321	1.298±0.132
RIP140(C/A)	Berkshire (n=476)	CC (DD)	164.333±9.55	549.666±33.861	17.883±1.523	[a]1.063±0.144
		CA (DN)	160.435±1.022	567.041±3.623	18.005±0.163	[ab]1.361±0.015
		AA (NN)	159.533±1.615	569.523±5.723	17.911±0.257	[b]1.303±0.024

[a,b,c] Values with different superscripts within column are significantly different, P<0.05

relatively small in number, but it is expected that this group will have remarkably thicker back fat than CC genotype group in near future. Therefore, it will be necessary to conduct analytic experiments in comparison with other breed as a follow-up studies, so that they may come to more advanced findings. For MYOD gene, it was found that CA genotype group reached 90 kg in earlier days than AA genotype group (P<0.05). Particularly, it was found that RIP140 genotype had most significant effects on birth weight as one of characters (P<0.001). We was found that AA genotype group weighed 300 g more at birth than CC genotype group. Accordingly, the experiment results of this study confirmed that the genotype of these 4 candidate genes had more or less significant effects on days to 90 kg, daily weight gain, back fat thickness, and birth weight which are all tested in Livestock Veterinary Research Institute (LVRI). Although previous studies on Berkshire breed have limited findings due to difference in the number of Berkshire samples, this study is possibly the first report on such a lot of Berkshire breed samples around the nation. In future, it is expected that genotypic application of these 4 candidate genes (CSF2, IL4, MYOD and RIP140) to the selection index for evaluating the capacity of pig breed under test will be useful for correctly identifying excellent individuals. Moreover, it is found that Berkshire breed shows significant differences in frequency of CSF2 genotype, so the breed-specific adoption of CSF2 genotype will be helpful to screen excellent individuals in earlier days. Hence, as a part of improving selection index associated with pig growth rate and flesh, it is expected that PCR marker tested in polymorphism analysis on 4 candidate genes will be useful as a molecular selection marker for planning excellent pig breeding.

Acknowledgement

This work was supported by the "discovery of genes related to porcine meat quality" project in the National Institute of Animal Science and the "Development of DNA chip related to meat quality and its application technology for pig industry" of the Biogreen21 project (gn. 20050401-034-804-130-00-00).

References

1. Fahrenkrug, S. C., T. P. L. Smith, G. A. Rohrer and J. W. Keele. 2002. Single nucleotide polymorphism (SNP) discovery in porcine expressed genes. *Animal Genetics* **33,** 186-195.
2. Fujii, J., K. Otsu, F. Zorzato, L. S. de, V. K. Khanna, J. E. Weiler, P. J. O'Brien and D. H. MacLennan. 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science* **253,** 448-451.
3. Jungerius, B. J., A. P. Rattink, R. P. Crooijmans, J. J. Poel, B. A. Oost, M. F. Pas and M. A. Groenen. 2003. Development of a single nucleotide polymorphism map of porcine chromosome 2. *Anim. Genet.* **34,** 429-437.
4. Kim, J. J., M. F. Rothschild, J. Beever, S. Rodriguez-Zas and J. C. Dekkers. 2005. Joint analysis of two breed cross populations in pigs to improve detection and characterization of quantitative trait loci. *J. Anim. Sci.* **83,** 1229-1240.
5. Kim, T. H., K. S. Kim, B. H. Choi, D. H. Yoon, G. W. Jang, K. T. Lee, H. Y. Chung, H. Y. Lee, H. S. Park and J. W. Lee. 2005. Genetic structure of pig breeds from

Korea and China using microsatellite loci analysis. *J. Anim. Sci.* **83,** 2255-2263.
6. Kollers, S., K. Mégy and D. Rocha. 2005. Analysis of public single nucleotide polymorphisms in commercial pig populations. *Animal Genetics* **36,** 426-431.
7. Le, R. P., J. Naveau, J. M. Elsen and P. Sellier. 1990. Evidence for a new major gene influencing meat quality in pigs. *Genet. Res.* **55,** 33-40.
8. Malek, M., J. C. Dekkers, H. K. Lee, T. J. Baas, K. Prusa, E. Huff-Lonergan and M. F. Rothschild. 2001, A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. II. Meat and muscle composition. *Mamm. Genome* **12,** 637-645.
9. Meyers, S. N., M. B. Rogatcheva, D. M. Larkin, M. Yerle, D. Milan, R. J. Hawken, L. B. Schook and J. E. Beever. 2005. Piggy-BACing the human genome II. A high-resolution, physically anchored, comparative map of the porcine autosomes. *Genomics* **86,** 739-752.
10. Park, K. G., K. S. Park, M. J. Kim, H. S. Kim, Y. S. Suh, J. D. Ahn, K. K. Park, Y. C. Chang and I. K. Lee. 2004. Relationship between serum adiponectin and leptin concentrations and body fat distribution. *Diabetes Res. Clin. Pract.* **63,** 135-142.
11. Van, D. S. H., G. F. W. Prall and G. S. Plastow. 2005. Application of genomics in the pork industry. *Journal of Animal Science* **83,** E1-E8.
12. Van, W. H. J., B. Dibbits, E. E. Baron, A. D. Brings, B. Harlizius, M. A. Groenen, E. F. Knol and H. Bovenhuis. 2006. Identification of quantitative trait loci for carcass composition and pork quality traits in a commercial finishing cross. *J. Anim. Sci.* **84,** 789-799.
13. Wimmers, K., I. Fiedler, T. Hardge, E. Murani, K. Schellander and S. Ponsuksili. 2006. QTL for microstructural and biophysical muscle properties and body composition in pigs. *BMC Genet.* **9,** 7-15.

초록 : 버크셔 품종의 돼지 성장과 육질관련 후보유전자의 단일염기 다형성에 관한 연구

김상욱 · 정지혜 · 도경탁[1] · 김관석[1] · 도창희[2] · 박준규[3] · 주영국[3] · 김태숙[3] · 최봉환[4] · 김태헌[5] · 송기덕[6] · 조병욱*
(부산대학교 생명자원과학대학 생명자원과학부, [1]충북대학교 축산학과, [2]충남대학교 농업생명대학 동물자원학부, [3]경상남도 축산진흥연구소, [4]농촌진흥청 축산연구소, [5]농촌진흥청 국제기술협력과, [6]미국국립보건원)

4개의 후보 유전자를 분석해본 결과, 돼지의 주요 염색체 부위 및 유전자들이 주요 경제성 요인들과 관계가 있는 것으로 확인됐다. 양돈업계에서 DNA 기술을 이용한 염색체 정보를 활용하기 위해 본 연구에서는 4개의 후보 유전자에서 생성된 중합효소연쇄반응(PCR) 생성물을 비교 재 서열 함으로써 단일염기변이(SNP) 표지들을 개발했다. 또한 이들 4개의 SNP에 대해 PCR 제한효소 절편길이 다형 성(RFLP) 분석을 전개한 후, 이를 대한민국 내 버크셔 종 돼지 개체군의 유전자형을 분석하는데 활용했다. 본 연구는 유용한 단일염기변이를 식별하고 돼지 개체군 내 경제적으로 중요한 특성들과 SNP의 연관성을 확인하는 데 그 목적이 있다.