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Investigation of Single Nucleotide Polymorphisms in Porcine Candidate Genes for Economic Traits in the Commercial Pig Breed

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Several studies reported quantitative trait loci (QTL) for meat quality on porcine chromosome 2. For application of the chromosomal information to pig industry through using DNA technology, single nucleotide polymorphism (SNP) markers are developed by comparative re-sequencing of polymerase chain reaction (PCR) products of 13 candidate genes. A total of 34 SNPs were identified in 11 PCR products, an average of one SNP in every 296 bp.PCR restriction fragment length polymorphism (RFLP) assays were developed for 11 SNPs and used to genotype four commercial pig populations in Korea. The SNP markers were used to map candidate genes in QTL and to clarify the relevance of SNP and quantitative traits.

Key words : Economic traits, pig candidate gene, single nucleotide polymorphism, quantitative trait loci, commercial pig breed

Introduction

Past-day swine industry has improved production efficiency related traits, such as growth rate, reduced back fat thickness, and feed efficiency, through selective breeding of superior pigs based on available phenotypic information. Recently, consumer's demand for improved pork quality has increased, but the improvementof pork quality traits has been difficult with breeding schemes using phenotypic information because genetic improvement of meat quality traits requires extensive and expensive measurements of traits on slaughter relatives.

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 [2,7]. More recent development of quantitative trait loci (QTL) studies has detected major chromosomal regions affecting various meat and eating quality traits in commercial pigs [4,8,10,11,12]. According to these studies, pig chromosome 2 microsatellite markers are associated with several meat quality traits, such as marbling, lipid percent, drip loss, muscle pH, tenderness, meat color and muscle fiber diameter [5].

Many of the meat quality QTL were mapped to the intermediate region of SSC2 (*SW2445-S0565*). This identified chromosomal region spans about 60 cM, which contains four different human chromosomal fragments, HAS11, HSA19, HSA1 and HSA5 [9]. Previously, Jungerius [3] and coworkershave identified over 300 single nucleotide polymorphisms on SSC2 with a DNA panel of eight pigs which are one Meishan, one Pietrain, one Wild Boar and five Large Whites [1,6]. These SNPs might have a potential for the fine mapping of the observed QTL region on pig chromosome 2. Therefore, the purpose of this study was to further characterize and implement the SNP for improvement of map resolution and identification of candidate genes to investigate meat quality QTL on SSC2 in commercial pig populations.

Materials and Methods

Animal material

Experimental animals which consisted of Duroc, Landrace, Yorkshire and Berkshire were admitted and bred

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in laboratory of Korea Swine Association (KSA) from January 2006 to May 2007. Phenotypic data of these animals which included daily gain, feed conversion ratio, back fat thickness and lean percentage, were measured and collected until body weight reached to 90 kg from 30 kg. Sequencing DNA panel for SNP detection consisted of two individuals each from four commercial breeds in Korea, such as Berkshire, Duroc, Yorkshire and Landrace.

Primer design and polymerase chain reaction

A total of 15 primer pairs were tested for amplification and sequencing of 13 candidate genes (*PTH, CSF2, BDNF, LDHA, RPS13, ADM, WT1, FSHB, MYOD1, IL4, ADRB2* and *INSL3*). These genes were selected for their known biological roles in skeletal muscle development and metabolisms and probable locations within the QTL region. Primer sequences of these candidate genes were obtained from Jungerius [3] *et al.* Polymerase chain reactions 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, denaturation for 60 sec at 94°C, 45 sec at annealing temperature and 60 sec at 72°C for extension , with 35 cycle and a final extension for 10 min at 72°C. The each primer sequences, annealing temperatures and fragment sizes were given in Table 1.

Sequencing, polymorphism identification and genotyping

A total of 15 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. Polymorphic sites were analyzed for putative restriction fragment length polymorphism (RFLPs) using the NEBcutter program (http://tools.neb.com/NEBcutter2/index.php). Putative RFLPs were performed on individual DNA samples from four different pig breeds which include Duroc, Landrace, Yorkshire and Berkshire. All restriction enzymes were supplied by New England BioLabs (Ipswich, MA, USA) and restriction digestions were performed according to manufacturer's recommendations. 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 2.

Statistical analyses

In order to estimate the effects of SNP genotypes on economic traits of Korean commercial pig breeds, General Linear Model (GLM) analysis was performed using SAS 9.1 Package/PC with parameters of breeds and SNP genotypes. To determine possible effects of SNP genotypes

Table 1. 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′)		
PTH	PTHsts1	BV079397	ACC AGG AAG AGA TCT GTG AGT G	TGC CCT ATG CTG TCT AGA GC	56	311
CSF	CSF2sts1	BV079385	CAG CAT GTG GAT GCC ATC	GTA CAG CTT CAG GCG AGT CTG	56	973
	FOLR1sts1	BV012577	AGA CGG TCC TTC TGC CTG T	TTG AGG AGG AGC CTA TGG TTT	58	356
FOLR1	P006C12sts1	BV079398	TGA GTA CTC GTT ATG GAC GC	CTG TGC CTT TAG GAC TGA GG	56	506
	P006D12sts1	BV079401	CCA AGA TAC AGA AGT AGG AGC	TGC AGT CTT CTT GGT GCA GG	56	393
BDNF	P006A04sts1	BV079400	ATA TCA GGT GCT CAC AGT GC	GAC TTA ACT CTA GGA GTT CC	56	612
LDHA	LDHAsts2	BV012579	TTT CAC TGT CTA GGC TAC AAC AAG A	AGC TGG ATA GTT GGC TGC AT	56	517
RPS13	P005E11sts1	BV079399	CTT CCC CTA ATG TCA GTG	ATT AAG AGA CAG TAG AGT CC	45	799
ADM	ADMsts2	BV079387	ATT GAG AGA CCG AGA GTC CG	TTG CTA CTT CGC ATA TCA CCC	56	646
CAT	CATsts1	BV079378	TGC CTC TGA AAC AAA ACG TG	TTC AAA AGA CCC CAA AGC AT	58	458
WT1	WT1sts1	BV079371	TTA ACA TTC CTC CTG GCT CG	GCC TTG CCC TCT GAT TTA TTT	60	425
FSHB	FSHBsts2	BV079389	GCC AGC TTC AGG CTA ACA TT	GAC TTC ATC TTG GGG TGG AA	58	1101
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
ADRB2	ADRB2sts1	BV079372	CAA GTA CCA GAG CCT GCT GAC C	TAG AGA AGG GCA GCC AGC	62	455

* The STS name, accession number and primer information used in the Table 1 is reported by Jungerius et al. (2002).

Table 2. 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
CAT	TGCCTCTGAAACAAAACGTG TTCAAAAGACCCCAAAGCAT	458	56	*Msc* I	458, 353
PTH-1	ACCAGGAAGAGATCTGTGAGTG TGCCCTATGCTGTCTAGAGC	311	56	*APek* I	218, 136
PTH-2	ACCAGGAAGAGATCTGTGAGTG ATGGCTCTCAACCAGGACAT	201	56	*Tsp509* I	201, 108
WT1-a *WT1*-b	TTAACATTCCTCCTGGCTCG GCCTTGCCCTCTGATTTATTT	425	60	*Hpy188* I *Aci* I	137, 74 425, 251
**MYOD1*	GGTGACTCAGACGCATCCA ATAGGTGCCGTCGTAGCAGT	599	60	*Dde* I	599, 340
IL4	GATCCCCAACCCTGGTTCTGCT GGCAGAAAGACGTCGTCAC	434	56	*Alu* I	308, 194
FOLR1-1	CCAAGATACAGAAGTAGGAGC TGCAGTCTTCTTGGTGCAGG	393	58	*Dde* I	161, 126
FOLR1-2	TGAGTACTCGTTATGGACGC CTGTGCCTTTAGGACTGAGG	506	56	*Dde* I	233, 155
CSF2-1	CAGCATGTGGATGCCATC GTACAGCTTCAGGCGAGTCTG	974	56	*Hha* I	974, 692
**CSF2*-2	GCTGTGATGGTGAGTGAGGA CCCTTGAATGCTAGGACTGC	362	56	*Mbo* II	362, 276

* The two SNPs were genotyped in the four pig breeds for linkage and association analyses.

on each trait, we conducted significance test between least square means.

Results and Discussion

PCR amplification, sequencing and SNP detection

13 candidate genes were successfully amplified to a corresponding single band by using 15 primer sets, and they were used for subsequent sequencing of the amplicons. Ten animals from four different commercial pig breeds representing Duroc, Yorkshire, Landrace and Berkshire were sequenced for the 15 primer sets. A total of 29 SNPs were detected in the 11 primer sets (Table 3). A total of 11 SNPs were used to determine allelic frequencies of SNP among commercial pig populations using RFLP methods. Jungerius [3] *et al.* detected 62 SNPs from the 15 amplicons of eight animals made of one Meishan, one Pietrain, one Wild Boar and five Large White pigs, while we found 29 SNP from the 15 amplicons (Table 3).

Allelic variation of SNP in pigs.

In total, 15 primers pair amplicons were sequenced for SNP identification from Jungerius [3] *et al.* In the total contig length of 8,586 bp, 34 polymorphic positions were identified in our study, while Jungerius [3] *et al.* found 62 SNPs. The SNP density difference between the two data sets was largely due to three STSs (BV079398, BV079400 and BV079389). Jungerius [3] *et al.*, have reported BV079398 (*FOLR1*), BV079400 and (BDNF) STSs contained 18 and 5 SNPs respectively, but we found only one SNP in the BV079398 STS and no SNP in BV079400 STSs (Table 3). This result revealed that the number of SNP might be variable among the pig breeds in comparison, and the sources of sequence data used for SNP identification.

Allelic distributionof SNPs is important for commercial application of these polymorphisms. Although a small sample size was used in this study, it was possible to determine to some extent that the pattern of SNPs differed fromeach population. Of 11 SNPs tested for allelic distribution, 9 SNPs were fixed in at least one commercial population. Based on the information available in this study, it was suggested that Duroc and Berkshire pigs might be sharing a higher genomic similarity than Yorkshire or Landrace pigs on pig chromosome 2.

Table 3. PCR primers and conditions used for amplification and sequencing

Gene	STS name	Accesion no.	Product size	No. of SNPs in Jungerius et al. (2003)	No. of SNPs in this study
PTH	PTHsts1	BV079397	311	5	4: 111(C/T), 229(A/G), 246(A/G), 249(C/T)
CSF	CSF2sts1	BV079385	973	10	7: 103(C/T), 104(A/G), 155(C/T), 192(A/G), 459(G/T)*, 650(C/G), 691(C/T)
	FOLR1sts1	BV012577	356	2	2: 75(C/T)*, 256(A/G)
FOLR1	P006C12sts1	BV079398	506	18	1: 209-212(ATAC)**, 219(G/T)
	P006D12sts1	BV079401	393	8	5: 26(C/T)*, 31(A/G), 39(A/G)*, 69(A/C), 313(C/T)
BDNF	P006A04sts1	BV079400	612	5	0
LDHA	LDHAsts2	BV012579	517	1	1: 471(A/G)
RPS13	P005E11sts1	BV079399	799	1	1: 115(T)**, 487(A/C), 768(A)**
ADM	ADMsts2	BV079387	646	4	3: 157(A/G), 452(A/G) , 552~553(TG)**, 562(A/T)*
CAT	CATsts1	BV079378	458	1	2: 243(A/G)*, 356(A/G)
WT1	WT1sts1	BV079371	425	2	3: 64(A/G), 237(C/T), 252(C/T)*
FSHB	FSHBsts2	BV079398	1101	5	2: 335(A/G), 447(A/G), 518(G)**
MYOD1	MYOD1sts3	BV012581	599	2	2: 343(A/C), 345(G/T)*
IL4	IL4sts1	BV079417	433	1	1: 321(C/T)
ADRB2	ADRB2sts1	BV079372	455	0	0
Total			8584 bp	62 SNP	34 SNP

* New SNPs. ** Different nucleotide with public sequences.

A total of 11 SNPs were genotyped in four different commercial pig breeds and a summary of the frequencies is presented in Table 4. Two SNPs were polymorphic in all fourpig breeds. In Berkshire pigs, five SNPs out of 11 SNPs were fixed although the number of tested animals is relatively small.Six out of 11 SNPs were monomorphic in Duroc pigs, and two of the monomorphic SNPs were common ones between Berkshire and Duroc pigs. It is interesting to note that alternative alleles in PTH SNPs were also fixed between Berkshire and Duroc pigs. In Yorkshire pigs, three SNPs were fixed, but only one SNP was fixed in Landrace pigs. Berkshire were showed similar pattern of SNP genotypes, which four SNPs were monomorphic in the Berkshire breeds tested. SNP markers have high potential for detailed haplotype analysis and applications in association studies to identify the underlying genes responsible for the observed QTL effects. In this study, we report re-identification of SNPs on pig chromosome 2, SNP allelic frequencies between commercial breeds, and meat quality association of SNP alleles.

Table 4. Allele 1* frequencies of SNPs on pig chromosome2 in the four pig breeds

Gene	Berkshire (n=10)	Duroc (n=10)	Landrace (n=10)	Yorkshire (n=10)
CAT	0	0.25	0.16	0.25
PTH-1	1.00	0	0.20	0.13
PTH-2	0	1.00	0.80	0.87
WTI-a	0.33	0	0.60	0.13
WTI-b	0	0	0.17	0
MYOD1	0.49	0.50	0	0.57
IL4	0.20	0.16	0.30	0.40
FOLR1-1	0	0	0.16	0
FOLR1-2	0.42	1.00	0.95	1.00
CSF2-1	0.05	0.21	0.28	0.25
CSF2-2	0.25	0.25	0.63	0.65

*Uncut fragment

Analysis on associations in traits of two SNP genotypes

Table 5 shows possible genotypic effects of 2candidate genes (CSF2, MYOD) on characters. Notably, in terms of possible effects of CSF2 genotype on characters, it was found that CSF2 TT group back fat thickness was significantly higher than CC group (1.373 cm vs. 1.294 cm)

In addition, it was found that CSF2 TT genotype group weighed 10g more in daily gain than CSF2 CC genotype group (1030.755 g vs. 1026.084 g). For MYOD gene, it was found that CA genotype group weighed more daily gain than AA genotype group ($P<0.05$). Accordingly, the experimental results of this study confirmed that the genotype of these 2 candidate genes had more or less significant effects on daily weight gain, feed conversion ratio,

Table 5. Least squares means and standard errors for the performance traits by two SNP genotypes in commercial pig breeds combined data

SNP site & gene	Genotype	Daily Gain (g)	Feed conversion (%)	Back fat (cm)	Meat percentage (%)
CSF2	TT (DD)	a1030.755+5.698	2.098+1.629	a1,373+0.011	57.239+0.189
691(C/T)	TC (DN)	b1020.361+5.176	1.803+1.480	a1.346+0.010	57.617+0.170
n=1127	CC (NN)	ab1026.084+8.418	2.004+2.408	b1.294+0.016	57.759+0.278
MYOD	CC (DD)	a1029.807+6.875	2.106+1.987	1.352+0.014	57.371+0.229
343(A/C)	CA (DN)	a1020.903+4.666	3.845+1.348	1.341+0.009	57.490±0.155
n=1094	AA (NN)	b1040.588+6.975	2.729+2.016	1.352+0.014	57.755+0.231

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

back fat thickness and lean percentage which were all measured in KSA laboratory. So if they are applied to screening index equation to measure the performances of swine, two SNP genotypes will be useful in accurate identification of superior swine. In conclusion, comparative re-sequencing of 15 PCR products has identified 34 SNP markers which might be important for commercial pork production in Korea. More work is necessary with more genes and animals to utilize the DNA marker information on SSC2 for superior pork production, and the identified SNPs from this study would be a primary step for the identifying individual pigs with high pork quality as well as determining origin of the pig breed in commercial populations.

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초록 : 돼지 품종의 경제형질 관련 후보유전자의 단일염기 다형성에 관한 연구

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돼지 2번 염색체의 육질 관련 양적 경제형질에 관한 연구보고가 몇몇 이루어 지고 있다. 양돈업계에서 DNA 기술을 이용한 염색체 정보를 활용하기 위해 본 연구에서는 13개의 후보 유전자에서 생성된 중합효소연쇄반응(PCR) 생성물을 비교 재서열 함으로써 단일염기변이(SNP) 표지들을 개발했다. 11개의 중합효소연쇄반응 생성물에서 296 bp마다 에서 평균 하나의 SNP, 총 34개의 SNP를 발견하였다. 또한 11개의 SNP에 대해 PCR 제한효소 길이 절편길이 다형성(RFLP) 분석을 전개한 후, 이를 대한민국 상업돈 4품종 개체군의 유전자형을 분석하는데 활용하였다. 본 연구는 유용한 단일염기변이를 식별하고 돼지 개체군 내 경제적으로 중요한 특성들과 SNP의 연관성을 확인하는 데 그 목적이 있다.