

## Investigation of Single Nucleotide Polymorphisms in the Adipocyte Fatty-Acid Binding Protein (FABP4) Gene

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We found 8 single nucleotide polymorphisms (SNPs) in adipocyte fatty acid bonding protein (FABP4) gene as candidate gene of FAT1 locus on pig chromosome 4. With over 800 heads of major commercial pig breeds including Duroc, Landrace, Berkshire and Yorkshire, we analyzed SNPs of FABP4 gene to determine possible effects of FABP4 genotype to economically important traits. 400~800 bp amplicons in FABP4 gene were used PCR-RFLP for each SNPs and we found that the frequency of some SNPs of this gene was different among the breeds. According to the statistical analyses to determine possible associations of each genotype with economic traits, it was found that subgroup with different genotypes showed significant differences in daily gain, backfat thickness, lean percentage and feed conversion ratio ( $P < 0.05$ ). Thus, as a part of enhancing the selection competence related to swine growth rate and lean percentage, it is expected that FABP4 gene markers verified in this study will be useful to use for Korean commercial pig industry.

**Key words** : Economic traits, swine, SNP Detection, PCR-RFLP, polymorphism, FABP

### Introduction

FABP4 gene is located on pig chromosome 4 close to FAT1 loci which is considered one of the most important quantitative trait loci (QTL) and was reported as QTL from domestic animals for the first time. It has been reported that FABP4 has critical effects on obesity and growth [2]. Although many studies confirmed the FAT1 QTL on pig chromosome 4 from resource populations generated for QTL studies, causative gene (s) that might be responsible for the FAT1 QTL remains to be identified [3,7,9]. This situation contrasts well with other cloned loci like RN or IGF2. It is of interest why FAT1 has been not yet identified, but one of possible reasons is that more than 2 loci included in FAT1 make it more challenging to search for any causative mutation [10].

This hypothesis was supported with statistical QTL analysis including FABP4 gene in crossbred herds of

Landrace and Iberian (IBMAP).

Some studies reported that FABP4 is a major player for oxidation or esterification in plasma membrane to facilitate fatty acid to be conveyed.

According to conventional literatures, microsatellite analysis revealed that microsatellite within the first intron of FABP4 gene in Duroc is associated with fat content within muscle [4]. Another study reported that FABP4 is a candidate gene for lipid metabolism, so this study sought to analyze FABP4 polymorphisms in IBMAP crossing hybrids [8].

Genotypes of the most beneficial polymorphism (e.g. SNP in the 3rd intron, insertion/ deletion in the 1st intron, etc) were characterized in Iberian X Landrace cross hybrids. After analyzing QTL, single marker and haplotype, more than two quantitative trait genes were located in FAT1 region, and FABP4 polymorphisms were closely associated with obesity [1].

According to comparison of alleles from 4 breeds used herein, it was found that Del2634C polymorphisms occurred under indirect selection. In addition, it was also

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found that FABP4 is closely associated with obesity, not growth [6]. As indicated from previous studies [5], the FABP4 might be one of major genes in pigs controlling fat deposition. Here, we aimed to analyze the differences in the frequency of FABP4 mutations among commercial breeds, performed the association study to determine the possible effect of these SNPs of FABP4 gene on each trait. Thus, this study examined possible associations between economic traits of each swine breed (e.g. backfat thickness, daily gain, feed conversion ratio, and lean percentage) and thereby explored the feasibility of FABP4 gene in screening superior boars on the field of swine breeding industry.

## Materials and Methods

### Animal Material and Traits Analyzed

Experimental animals which consisted of Duroc, Landrace, Berkshire and Yorkshire were admitted and bred in the laboratory of Korea Swine Association (KSA) from January 2006 to January 2007. Phenotypic data of these animals which included daily gain, feed conversion ratio, backfat thickness and lean percentage, were measured and collected until body weight reached to 90kg from 30kg (Table Genomic DNAs were extracted from the bleeds of experimental animals using Genomic DNA Extraction Kit (Promega) and Toyobo MagExtractor Kit (Toyobo).

### Amplification and Sequencing of Swine FABP4 Gene

The sequence information of Landrace registered in National Center for Biotechnology Information (NCBI) was used (total 6300 bp) across 5'UTR to 3'UTR region, covering 5 exons and 5 introns (Fig 1). For PCR amplification, total 10 primers were designed using Oligo 6 software to generate 400~800 bp PCR products (Table 2).

Before PCR amplification, commercial breeds DNAs were amplified from the pooling DNA panel of 4 swine breeds (Duroc, Landrace, Yorkshire and Berkshire) by means of hot start PCR Taq polymerase. In terms of PCR products, each DNA panel's sequences could be obtained through ABI 3700 sequencer. Those sequences were analyzed using Sequencer 4.6 software to find out SNPs as index of differences in traits among breeds. To demonstrate restriction fragment length polymorphism (RFLP) of each SNPs, restriction enzymes were chosen using NEB cutter (<http://tools.neb.com/NEBcutter2/index.php>) (Table 3). PCR-RFLP test was conducted using DNA samples from total 800 swine bleeds (200 per breed) according to finding of total 8 single nucleotide mutations (Table 4).

### Statistical Analyses

In order to estimate the effects of FABP4 SNP genotypes on economic traits of commercial breeds studied, General

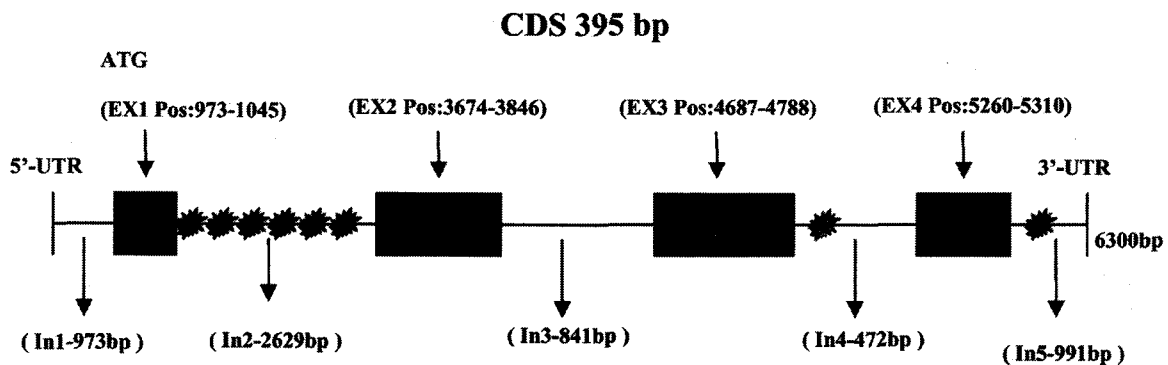


Fig. 1. Gene map and polymorphism in FABP4 on porcine chromosome 4. (Coding exon are marked by black block) \* - SNP SITE.

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

Breed	Daily Gain (g)	Feed Conversion (%)	Backfat (cm)	Meat Percentage (%)
DD(n=200)	1116.78±79.32	2.26±0.14	1.39±0.20	58.20±2.43
LL(n=200)	1020.10±92.89	2.34±0.14	1.40±0.26	55.53±2.99
YY(n=200)	1026.33±92.57	2.33±0.12	1.29±0.16	56.29±2.19
BB(n=200)	1044.91±96.90	2.29±0.14	1.37±0.21	57.25±2.89

DD: Duroc, LL: Landrace, YY: Yorkshire, BB: Berkshire

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

Amplicon	Primer		Annealing Temp.	product size
	Forward(5'→3')	Reverse(5'→3')		
<i>Fabp4-1</i>	TTA GAT TTT GAT GGC TTG TGA	CGG GTC ATT GGA CTT AGA G	56	644
<i>Fabp4-2</i>	GCC GAA CAC TAA ATG GTC AGA	GCA AAG AGC CAA GTA AGC CAA	60	700
<i>Fabp4-3</i>	TCT TAA AGG TGA ACT GAT ATG	CTT GGT CTC ATT CAT GC	58	726
<i>Fabp4-4</i>	TAT AGG CAA GAG GGT AAT CA	ATG GGA TGA ATA ATT TAC AGC	56	718
<i>Fabp4-5</i>	TGT GAT GGG CTG TAA AT	GCA GGT AAA TAA AGT AGG CTA	55	727
<i>Fabp4-6</i>	TGT TCC AGG AGA ATC TTT GT	CCT TAT TCC TCA CCT TGA CTT	60	697
<i>Fabp4-7</i>	CCT GGT TCC TAA AAG ACT GTT	TTT CTG TTT ATG GTG GTT GTC	63	885
<i>Fabp4-8</i>	GCA GAA GTG GGA TGG A	TAT TGA ACA AAA TCA GTC TGG	55	713
<i>Fabp4-9</i>	GAG AGC ATA AGC CAA ATT GTA	GCT GAG GCT GTG GTA TAG G	58	534
<i>Fabp4-10</i>	CAC AGC CTC AGC ACC TC	TTT GGA AAC CAC TAC AAG AAC	53	446

Table 3. The number of SNPs, polymorphic sequence and restriction enzyme for RFLP in pigs from Korea commercial line

Gene	No. of SNP	Polymorphic sequence	Different base in each breed	Restriction Enzyme for RFLP
FABP4	8	D/Y/B: TCA(A/G)TGA	D,Y,B: R/ L: A	<i>BapHI</i>
		D/L: TTT(T/G)AAC	D,L: K / Y: T / B: T	Not available
		D/Y: TTA(A/G)TAT	D/Y: R / L: A / B: A	Not available
		D/Y:GCA(A/G)CTT	D/Y: R / L: A / B: A	<i>Fnu4HI</i>
		D/L/Y: AGT(T/C)GAT	D/L/Y: Y / B: T	<i>Msl I</i>
		D/Y/B: AGT(T/C)GGC	D/Y/B: Y / L: T	<i>Fnu4HI</i>
		D/L: TGC(C/A)TAG	D/L: M / Y: C / B: C	<i>Bfa I</i>
		D/L: ACA(A/G)AAG	D/L: R / Y: A / B: A	Not available

DD: Duroc, LL: Landrace, YY: Yorkshire, BB: Berkshire

Table 4. FABP4 genotypes in four commercial pig breeds

Breed	A1761G (n=200)			A2521G (n=200)			T2713C (n=200)			T3182C (n=200)			C4858A (n=200)		
	AA	AG	GG	AA	AG	GG	TT	TC	CC	TT	TC	CC	CC	CA	AA
DD	5	152	43	25	85	90	11	169	20	6	111	73	2	160	38
LL	69	35	46	85	57	58	12	124	64	154	39	7	101	59	40
YY	90	102	3	20	50	130	32	96	72	9	161	30	70	53	70
BB	62	67	71	76	60	64	102	40	58	20	146	34	5	65	130

DD: Duroc, LL: Landrace, YY: Yorkshire, BB: Berkshire

Linear Model (GLM) analysis was performed using SAS 9.1 Package/PC with parameters of breeds and FABP4 genotypes. To determine possible effects of FABP4 genotypes on each trait, we conducted significance test between least square means (Table 5).

## Results and Discussion

### Analysis on the Frequency of FABP4 Genotypes in Each Breed

The frequency of FABP4 genotypes in major four commercial breeds admitted and bred in KSA test status is presented in Table 4. It was found that SNPs of each breed (Duroc, Landrace, Yorkshire and Berkshire) were highly

polymorphic in each trait. The effects of each SNP on traits of each swine breed were illustrated in Table 5. And FABP4 (A2521G) both Duroc and Yorkshire group, which showed higher growth rate than other 2 breeds, had higher frequency of FABP4 genotypes, and also showed higher significances in backfat thickness and lean percentage as 2 traits in potential association with growth rate than other 2 breeds.

### Analysis on Associations in Traits of FABP4 Genotypes

Table 5 shows possible effects of FABP4 genotypes on each trait. In Duroc group, it was found that there were significant differences in backfat thickness and lean percentage

Table 5. Least squares means and standard errors for the performance traits by FABP4 genotypes in four breeds combined data (n=800)

Region of SNP	Breed	FABP4 genotype	Daily Gain(g)	Feed Conversion(%)	Backfat(cm)	Meat Percentage(%)
A1761G	DD	AA	1192.333±39.289	2.161±0.062	<sup>a</sup> 1.330±0.058	<sup>a</sup> 58.500±1.085
		AG	1104.753±11.585	2.278±0.018	<sup>a</sup> 1.304±0.017	<sup>a</sup> 58.707±0.320
		GG	1104.625±17.012	2.298±0.027	<sup>b</sup> 1.381±0.025	<sup>b</sup> 57.321±0.470
	LL	AA	1013.125±16.643	2.367±0.023	1.466±0.056	56.656±0.697
		AG	989.115±18.463	2.392±0.026	1.589±0.062	55.319±0.774
		GG	960.461±26.111	2.382±0.037	1.541±0.088	56.138±1.094
	YY	AA	<sup>a</sup> 963.411±25.749	2.441±0.029	1.398±0.054	58.076±0.756
		AG	<sup>b</sup> 1040.204±16.005	2.368±0.018	1.384±0.033	57.279±0.470
		GG	<sup>ab</sup> 1035.000±22.634	2.369±0.025	1.320±0.054	57.677±0.664
	BB	AA	1003.523±58.123	2.289±0.109	1.301±0.056	56.545±0.982
		AG	1006.666±57.783	2.280±0.099	1.343±0.107	58.000±1.493
		GG	1009.941±24.274	2.295±0.041	1.247±0.045	57.417±0.627
A2521G	DD	AA	<sup>a</sup> 1066.166±14.131	<sup>a</sup> 2.348±0.022	1.344±0.022	58.109±1.421
		AG	<sup>a</sup> 1140.981±12.349	<sup>a</sup> 2.227±0.019	1.326±0.019	58.343±0.368
		GG	<sup>b</sup> 1119.700±28.961	<sup>b</sup> 2.262±0.045	1.278±0.046	58.660±0.863
	LL	AA	997.553±12.835	2.376±0.018	1.543±0.043	55.970±0.531
		AG	985.153±26.639	2.386±0.037	1.457±0.089	56.607±1.103
		GG	976.500±67.917	2.420±0.096	1.450±0.228	57.200±2.814
	YY	AA	<sup>a</sup> 1033.716±13.782	2.370±0.015	1.354±0.028	57.628±0.402
		AG	<sup>b</sup> 1011.950±23.872	2.407±0.027	1.397±0.050	57.555±0.697
		GG	<sup>a</sup> 885.000±61.637	2.493±0.069	1.503±0.129	55.900±1.800
	BB	AA	1009.312±25.022	2.301±0.042	1.284±0.045	57.256±0.635
		AG	1010.000±50.045	2.260±0.085	1.172±0.097	58.500±1.271
		GG	1008.941±30.474	2.200±0.065	1.257±0.033	56.917±0.891
T2713C	DD	TT	1080.111±22.971	2.325±0.036	1.305±0.034	58.072±0.643
		TC	1115.626±11.253	2.271±0.017	1.337±0.016	58.250±0.315
		CC	1115.111±26.047	2.251±0.041	1.311±0.039	58.072±0.729
	LL	TT	965.086±19.467	<sup>a</sup> 2.440±0.026	<sup>a</sup> 1.654±0.064	54.826±0.805
		TC	1013.476±14.406	<sup>b</sup> 2.345±0.019	<sup>b</sup> 1.485±0.047	56.442±0.596
		CC	976.666±38.114	<sup>b</sup> 2.380±0.052	<sup>ab</sup> 1.303±0.125	58.250±1.577
	YY	TT	1040.555±14.613	2.375±0.016	1.347±0.030	57.900±0.420
		TC	1000.272±22.895	2.393±0.026	1.432±0.047	56.677±0.658
		CC	960.142±40.589	2.415±0.046	1.348±0.084	57.571±1.167
	BB	TT	1009.426±21.102	2.307±0.072	1.382±0.068	56.216±0.835
		TC	954.500±23.148	2.430±0.117	1.460±0.234	55.800±1.786
		CC	1015.555±23.148	2.278±0.039	1.240±0.041	57.694±0.595
T3182C	DD	TT	1063.473±21.992	2.352±0.034	1.383±0.033	<sup>ab</sup> 56.968±0.610
		TC	1121.774±11.377	2.262±0.018	1.322±0.017	<sup>a</sup> 58.507±0.315
		CC	1110.470±23.250	2.261±0.036	1.291±0.034	<sup>b</sup> 58.805±0.645
	LL	TT	1003.000±12.903	2.366±0.018	1.519±0.044	56.409±0.531
		TC	981.500±33.525	2.436±0.047	1.578±0.114	53.812±1.381
		CC	956.555±31.608	2.405±0.044	1.578±0.114	56.055±1.302
	YY	TT	1024.804±17.266	2.390±0.019	1.349±0.035	57.800±0.482
		TC	1020.638±18.426	2.374±0.020	1.378±0.037	57.555±0.515
		CC	1026.166±45.135	2.395±0.050	1.463±0.091	55.783±1.262
	BB	TT	991.333±58.824	2.353±0.101	1.343±0.107	55.466±1.431
		TC	1009.500±25.471	2.280±0.043	1.258±0.046	57.956±0.619
		CC	1063.000±101.887	2.330±0.174	1.070±0.186	56.400±2.478
C4858A	DD	CC	1085.625±17.124	2.304±0.027	1.350±0.025	57.771±0.478
		CA	1121.150±11.377	2.265±0.018	1.321±0.017	58.460±0.316
		AA	1073.000±68.497	2.320±0.109	1.245±0.103	59.900±0.478
	LL	CC	956.555±31.608	2.405±0.044	1.508±0.108	56.055±1.302
		CA	981.500±33.525	2.436±0.047	1.578±0.114	53.812±1.381
		AA	1003.000±12.903	2.366±0.018	1.519±0.044	56.409±0.531
	YY	CC	<sup>a</sup> 967.500±37.304	2.445±0.042	1.301±0.079	58.100±1.103
		CA	<sup>ab</sup> 1041.396±13.293	2.367±0.015	1.384±0.028	57.390±0.393
		AA	<sup>b</sup> 964.083±30.459	2.429±0.034	1.341±0.097	58.008±1.103
	BB	CC	1009.250±48.472	2.347±0.085	1.275±0.083	<sup>a</sup> 56.657±0.840
		CA	1041.111±32.315	2.242±0.056	1.167±0.055	<sup>ab</sup> 58.966±0.741
		AA	968.857±36.642	2.328±0.064	1.375±0.063	<sup>b</sup> 56.657±1.112

<sup>a, b, c</sup> Values with different superscripts within column are significantly different, \*P<0.01 ; P<0.05

DD: Duroc, LL: Landrace, YY: Yorkshire, BB: Berkshire

between FABP4 (A1761G) GG subgroup and AG or AA subgroup ( $P < 0.05$ ). And in Duroc group, it was found that FABP4 (A2521G) AG subgroup showed significantly higher daily gain than AA or GG subgroup, while GG subgroup showed significantly higher feed conversion ratio than AA or AG subgroup. In addition, for FABP4 (T3182C) single nucleotide locus, CC subgroup showed more significant differences once again in lean percentage than TT or TC subgroup. On the other hand, in Landrace group, it was found that FABP4 (T2713C) TT subgroup showed significantly high involvement in feed conversion ratio and backfat thickness. And FABP4 TC or CC subgroup showed much less significances than TA subgroup ( $P < 0.02$ ). Also, Yorkshire group showed higher significances in daily gain en bloc than other breed groups. Here, FABP4 (A1761G) AG or GG subgroup showed more significant differences than AA subgroup. (A2521G) locus, (T2713C) locus and (C4858A) locus of Yorkshire group showed more significant differences in daily gain than those of other groups ( $P < 0.05$ ). In Berkshire group, it was found that FABP4 (C4858A) CA subgroup showed more significant differences in backfat thickness and lean percentage than CC or AA subgroup. As a result, it was found that there were more or less significant differences in daily gain, feed conversion ratio, backfat thickness and lean percentage among genotype subgroups in 4 breeds, which get involved in respective single nucleotides of FABP4. Therefore, FABP4 gene might be associated with increasing body fat which involves high level of daily gain, backfat thickness and lean percentage depending on genotypes. In addition, feed conversion ratio calculated now in KSA Laboratory is determined as total amount of feed taken by 2 head of black swine until they gain weight as heavy as 180 kg. That is why it fails to correctly reflect differences in feed conversion ratio depending on FABP4 genotype of each subgroup. According to the results of this study, it was found that different genotype subgroups showed excellent level of daily gain, backfat thickness and lean percentage depending on FABP4 SNP genotype and there are associations among high daily gain, high body fat and high lean percentage in subgroups of different FABP4 genotypes.

Hence, the results of this study demonstrated that FABP4 genotypes have significant effects on daily gain, backfat thickness, feed conversion ratio and lean percentage which are all measured in KSA Laboratory. So if they

are applied to screening index equation to measure the performances of black swine, FABP4 genotypes will be useful in accurate identification of superior swine. Moreover, there are significant differences in frequency of FABP4 genotype depending on swine breeds, so it will be helpful to use FABP4 genotypes for each breed in early screening superior swine.

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### 초록 : FABP4 유전자의 단일염기 다형성에 관한 연구

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본 연구는 돼지 4번 염색체에서 FAT1 좌위의 후보유전자인 Adipocyte Fatty-Acid 결합단백질 (FABP4) 유전자에서 8개의 Single Nucleotide Polymorphisms (SNPs) 를 발견하였다. Duroc, Landrace, Berkshire, Yorkshire를 기초 축으로 이용한 800두에 대해 FABP4 유전자의 단일염기 분석과 PCR-RFLP를 이용하여 그 다형성을 조사하고 돼지의 일당증체량, 등지방두께, 사료요구율, 정육율과 그 유전자형 간의 연관성을 규명하고자 실시하였다. FABP4 유전자에 대해 각 단일염기에 관한 PCR-RFLP를 이용하여 400~800 bp 산물을 증폭한후 각각의 제한효소로 사용하여, 얻어진 FABP4 유전자의 빈도는 품종별로 다르게 나타났다. 통계적 분석을 통하여 각 유전자형에 대한 경제형질과 연관성을 분석한 결과 일당증체량, 등지방두께, 정육율, 사료요구량은 다른 유전자형을 가진 개체들이 유의적으로 우수한 능력을 보였다 ( $P < 0.05$ ). FABP4 유전자는 일당증체량, 정육율, 등지방두께 에 높은 연관성이 있음을 관찰하였다. 따라서 돼지의 성장과 정육율에 관련된 선발력을 높이기 위해서 FABP4 유전자의 다형성 분석에서 검증된 PCR marker를 우량돼지육종 계획에 있어 분자생물학적 선발 marker로 사용할 수 있을 것으로 사료된다.