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Discovery of genetic variants for fatty acid binding proteins of pig (Brief Report)

Entdeckung genetischer Varianten für Fettsäure bindenden Proteine vom Schwein (Brief Report)

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Background

Back fat thickness (BFT) and intramuscular fat (IMF) contents are known as major issues affecting meat performance. Several types of fatty acid-binding proteins (FABPs), which involve signal transduction pathways, are abundantly presented in tissues such as intestine, liver, kidney, mammary gland, heart, and red skeletal muscle (Nechtelberger *et al.* 2001). FABPs have been reported to be differentially expressed genes during porcine adipogenesis (Samulin *et al.* 2008) and related to fat deposition (Szczerbal *et al.* 2007). Accordingly FABPs may be candidate genes to explain variation of fat related traits in pigs. Therefore, it is an essential process to search genetic variants that may provide useful genetic information to study associations with quantitative loci (QTL).

Procedure

Amplification primers for FABP1, 2, 4, 5, and 7 genes were designed based on sequences from the GenBank (Table 1). PCR mixtures used were made up of 2 µl of 10 X reaction buffer (10 mM Tris, pH 8.3, 50 mM KCl, 0.1 % Triton X-100, and 1.5 mM $MgCl_2$), 25 mM of dNTP, 10 pmol of each primer, 50 ng of genomic DNA, and 0.2 units of Taq DNA polymerase (Gibco BRL, Grand Island, NY) in a final volume of 20 µl. After heating at 95 °C for 2 min, a total of 35 cycles were adapted for denaturation at 94 °C/1 min, annealing at 51 to 59 °C/1 min, and polymerization at 72 °C/1.5 min. After cleaning up PCR products using a PCR clean up kit (Nucleogen, Korea), the direct sequencing was performed with Big-dye terminator version 3.1 and an ABI3730XL Genetic Analyzer. Each experiment was duplicated for PCR amplification and sequencing reactions to minimize base calling errors. After identification of genetic variants, SNPs were detected for 355 Yorkshire pigs using the sequenom mass array system.

Results

This study aimed to search single nucleotide polymorphisms (SNPs) in FABPs as molecular markers accounting for variation of fat mechanisms of pigs. As shown in Table 2, a total of 26 SNPs were identified using 355 Yorkshire pigs, and the sequences with the newly identified SNPs were submitted to GenBank with accession numbers (FABP1, FABP2, FABP4, FABP5,

Table 1
Primer sequences, PCR conditions, and size of segments for the swine fatty acid binding proteins

Segment	Primer sequences[1]		Fragment	Annealing	Nucleotide position		GenBank
	Forward	Reverse	length (bp)	Tm (°C)	Forward	Reverse	Accession no.
FABP1-1	ACT TCT CCG GCA AAT ACC AA	CTC CCC TCC TCC AAC CAC AT	1 140	51	5-24	1 126-1 145	DQ182323
FABP1-2	GGA CTC CTG GCC AAA CAA CC	CCC AGC GCT AGG AGT CAC AA	1 194	54	855-874	2 030-2 049	"
FABP1-3	CAA AGG GGC AGT GAG ACA AG	CCC AAA GCT GCG AAG ACT A	1 303	57	1 166-1 185	2 451-2 469	"
FABP5-1	CTC GGC GTG TTG GGC TCA G	CTC TTT CAG GGG GCA CAG	1 470	57	680-698	2 133-2 150	NM_001039746
FABP5-2	TCC CTG GTG AAT CTT GAG TG	CCG TTT TGA TGG TGA GGT CT	1 233	57	1 959-1 978	3 173-3 192	"
FABP5-3	GGA ATG GCC CTA CGA AAA A	CCC CAC AGC CTC AGC AAC AC	1 501	57	3 108-3 126	4 590-4 609	"
FABP5-4	TGG AGG CAG AGA AGC AAG AT	TTA CTA ACC AAA GCG ATG AT	1 287	54	3 906-3 925	5 174-5 193	"
FABP4-1	GAG GGG GCT GAA ATA CAT AA	AAA GAA CAA GAA GCA AAG AG	1 339	54	22-41	1 342-1 361	EF061482
FABP4-2	TTT ATG GCT GTT TTT CAC TC	AAT TTA CAG CCC ATC ACA GA	1 520	54	1 102-1 121	2 603-2 622	"
FABP4-3	TGT TCC CAT TTT CAG AC	ATC ATA TCC CCA TTC ACA	1 366	51	2 381-2 397	3 730-3 747	"
FABP4-4	ATT TAC CTG CAG AAG ATG TT	GGA GGA ATA TTT AGA GGT CA	1 291	54	3 319-3 338	4 591-4 610	"
FABP4-5	ATT ATG TTA TGT TCT TTC AC	ATT CCG CAT TGC TAC TTT AC	1 912	51	4 178-4 197	6 071-6 090	"
FABP2-1	TGG TTT GGC ATT AGT GTA GT	ATA TTT TGA GGG TGG AGA AG	1 637	54	47-66	1 665-1 684	EU189034
FABP2-2	CCT GGG ATT TGT TTT ACT TA	ATG CTC ATC CCA CTC AAT AG	1 493	54	1 467-1 486	2 941-2 960	"
FABP2-3	TAA GTC AAT GGG AAT ACA CC	CTC CAA GAA CAA TGC TCA AT	1 359	57	2 389-2 408	3 729-3 748	"
FABP7-1	TTC TCG CCT CTG CCT CTT	TGT TCT TGA ATG TGC TTT GA	234	54	27-44	242-261	NM_001025229
FABP7-2	GGA AAT GTG ACT AAA CCA AC	GTC TCC ATC CAG GCT AAC A	170	57	180-199	332-350	"
FABP7-3	TTG ATG AAA CCA CAG CAG A	TTG AGT AAA CAG AAA GAT TG	240	54	292-310	513-532	"
FABP7-4	TCG CCA CTA TGA GAA GG	GAT TGC ACG TAG TAA CCA GT	194	57	458-474	633-652	"
FABP7-5	TGC TAC ACG GCT ATT AC	AGA TTT TTA TTC CAA CTT	247	54	540-556	770-787	"

[1]The primer selection based on GenBank accession numbers, and the newly identified sequences for FABP1, FABP2, FABP3, FABP4, FABP5, and FABP7 genes were submitted into GenBank with accession numbers (GU189560, GU189561, FJ755468, GU189562, GU189563, and GU189564), respectively.

Table 2
Identification of single nucleotide polymorphisms in the swine fatty acid binding proteins

Gene	SNP				Gene	SNP				Gene	SNP			
	Position[1]	NU[2]	AA[3]	Rate[4], %		Position	NU	AA	Rate, %		Position	NU	AA	Rate, %
FABP1	90	A/G	-	0.24	FABP1	903	A/G	-	0.13	FABP4	846	C/T	-	0.31
"	100	G/A	-	0.27	"	1 091	G/A	-	0.25	"	1 217	CA		(17-20)
"	182	T/C	-	0.14						"	1 328	A/G	R/H	0.12
"	221	A/G	-	0.16	FABP2	216	A/G	-	0.17	"	2 103	T/C	-	0.27
"	259	T/C	-	0.12	"	270	T/C	-	0.31	"	330	T/C	-	0.29
"	281	T/C	-	0.15	FABP5	272	G/A	-	0.33					
"	336	T/C	-	0.11	"	313	T/C	-	0.22	"	460	T/G	-	0.40
"	399	G/A	-	0.32						"	2 045	A/G	R/Q	0.11
"	510	C/T	-	0.27	FABP4	52	G/A	-	0.09					
"	588	C/T	-	0.12	"	168	T/C	-	0.28	FABP7	747	T/C	-	0.33

[1]The nucleotide positions based on the GenBank accession numbers that we have been submitted (FABP1, FABP2, FABP4, FABP5, and FABP7 for GU189560, GU189561, GU189562, GU189563, and GU189564, respectively), [2]Substitutions of nucleotides, [3]Substitutions of amino acids, [4]The rates of nucleotide substitution for 355 Yorkshire pigs

and FABP7 for GU189560, GU189561, GU189562, GU189563, and GU189564, respectively). Substitutions of amino acids detected with 2 SNPs at positions 1,328 (R/H) and 2,045 (R/Q) in FABP4 and FABP5 gene, respectively, and some of the amino acids revealed conservative patterns showing that the polymorphisms have not been observed in other species using the blast search. We also found di-repeat sequences, showing polymorphisms $(CA)_{17\text{-}20}$. The identified SNPs are the first report to help understanding of genetic structures for pig populations regarding fat related traits.

The existence of mutation sites in coding regions of FABP genes may give useful genetic information due to the relation to fat deposition (Szczerbal *et al.* 2007). In addition, as candidate genes associated with fatness (Estelle *et al.* 2009, Mercade *et al.* 2006), either genotypes or haplotypes of FABP genes in this analysis may help animal breeders when they select animals and change genetic structures in pig populations for commercial purposes.

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References

Nechtelberger D, Pires V, Söolknet J, Stur Brem G, Mueller M, Mueller S (2001) Intramuscular fat content and genetic variants at fatty acid-binding protein loci in Austrian pigs. J Anim Sci 79, 2798-2804

Samulin J, Berget I, Lien S, Sundvold H (2008) Differential gene expression of fatty acid binding proteins during porcine adipogenesis. Comp Biochem Physiol B Biochem Mol Biol 151, 147-152

Szczerbal I, Chmurzynska A, Switonski M (2007) Cytogenetic mapping of eight genes encoding fatty acid binding proteins (FABPs) in the pig genome. Cytogenet Genome Res 118, 63-66

Estelle J, Mercade A, Pérez-Enciso M, Pena RN, Silio L, Sanchez A, Folch JM (2009) Evaluation of FABP2 as candidate gene for a fatty acid composition QTL in porcine chromosome 8. J Anim Breed Genet 126, 52-58

Mercade A, Perez-Enciso M, Varona L, Alves E, Noguera JL, Sanchez A, Folch JM (2006) Adipocyte fatty-acid binding protein is closely associated to the porcine FAT1 locus on chromosome 4, J Anim Sci 84, 2907-2913

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