ASSOCIATION ANALYSIS OF CANDIDATE GENES FOR FATTY ACID COMPOSITION IN PIG AND CATTLE

D. Maharani[1], S. L. Yu[1], K. S. Seo[2], S. H. Ryoo[3], Y. Jung[1], C. Jo[1], J. H. Lee[1],

[1]Department of Animal Science and Biotechnology, College of Agriculture and Life Sciences, Chungnam National University, Daejeon 305-764, Korea.
[2]Department of Animal Science and Technology, Suncheon National University, Suncheon 540-742, Korea.
[3]Government of Chungcheongnam-Do, Livestock Research Institute, Cheongyang 345-811, Korea.

Abstract. - **Fatty acid composition in meat is one of the health-related traits for human. In this study, three possible candidate genes for affecting fatty acid composition were analyzed. They are *Fatty acid synthase* (FASN), *fatty acid binding protein* 4 (FABP4) and *Liver X Receptor α* genes. The FASN genotypes were investigated in pig and FABP4 and LXR-alpha genes were investigated in Korean cattle (Hanwoo) and the association test has been being performed with fatty acid composition. The single nucleotide polymorphisms (SNP) c.265C>T in FASN, g.3693G>A in FABP4 and g.2376 G>A in LXR- *α* were genotyped using PCR-RFLP. The c.265C>T SNP in porcine FASN was significantly associated with C16:1 and C18:3 ($P<0.05$) and highly significant associated with C18:1, C18:2, monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) ($P<0.01$) However, the genotype of g.3693G>A SNP was highly significant with myristoleic acid (C14:1), palmitoleic acid (C16:1) and arachidonic acid (C20:4). In both of porcine FASN and bovine FABP4 have higher MUFA concentration and lower SFA. Our findings suggest that the c.265C>T SNP in FASN and g.3693G>A SNP in FABP4 genes can be useful markers for selecting animal having desirable fatty acid composition in pork and beef.**

Index Terms: **candidate gene, cattle, fatty acid composition, pig**

I. INTRODUCTION

The fatty acid composition of meat has an implication for human health. Saturated fatty acid (SFA) are considered to have the most harmful cardiovascular effect in human (Keys, Grande and Anderson, 1974). In contrast, the unsaturated fatty acid such as MUFA and PUFA is associated with reduced risk of cardiovascular disease (Lichtenstein, 2006). Moreover, MUFA & PUFA increase hepatic LDL (low density lipoprotein) receptor activity, thereby decreasing the circulating concentration of LDL cholesterol (Woollett, Spady and Dietchy, 1992).

Recently, several genes have been associated with quantitative trait loci for several traits in cattle. FASN gene has been reported has association with fatty acid in cattle (Abe et al., 2009, Morris et al., 2007, Ordovas et al., 2008, Zhang, Knight, Reecy and Beitz, 2008), chicken (Marrube et al., 2004) and pig (Munoz, Ovilo, Noguera, Sanchez, Rodriguez and Silio, 2003). The genotypes of FABP4 gene were also significantly associated with palmitoleic acids content in intramuscular fat in Japanese Black cattle (Hoashi et al., 2008). The genotypes of LXR alpha gene were significantly associated with linoleic acids content in intramuscular fat in Japanese Black cattle (Hoashi et al., 2008). However, these markers in the candidate genes can explain relatively small proportion of the genetic variation. Also, the candidate genes do not provide accurate and consistent evidence in each gene-trait association analysis in different population equally (Dekkers, 2004). Therefore, the objective of this study is to examine FASN effect in pig and FABP4, LXR-alpha effects in Korean cattle (Hanwoo) with fatty acid composition.

II. MATERIALS AND METHODS

A. Animals and Samples

Thirty eight Hanwoo were collected from shops around Daejeon in Korea with the help of regional Hanwoo brand, namely Tobawoo association. These cattle were reared under same feeding conditions and the fattening period was also controlled, and their slaughter ages were about 24 months. The *longissimus dorsi* muscle samples were collected for genomic DNA isolation and fatty acid composition analysis. Meanwhile, seventy one pigs were collected from slaughter house and the *longissimus thoracis at lumborum* muscle were also collected for genomic DNA isolation and fatty acid composition.

B. Fatty acid analysis

Total lipid in each sample was extracted by using chloroform-methanol (2:1, v/v) according to the procedure of Folch, Lees and Sloane Stanley (1957). The fatty acid methyl esters were prepared from the extracted lipids with BF_3-methanol (Sigma-Aldrich, USA). The fatty acid methyl esters were, then, separated on a gas chromatograph (HP-6890N, Palo Alto, USA). A split inlet (split ratio, 50:1) was used to inject samples into a capillary column (Omegawax 320, Supelco, Bellefonte, USA; 30 m x 0.25 mm x 0.25 μm), and ramped oven temperature was used (150°C for 3 min, increased to 180°C at 2.5°C /min and maintained for 5 min, then increased to 220°C at 2.5°C /min and maintained for 25 min). Inlet temperature was 210°C. Air was the carrier gas at constant flow of 0.7 mL/min.

C. DNA Extraction and genotyping

Genomic DNA samples were extracted from muscle sample and isolated by 20 mg/ml proteinase K digestion followed by phenol extraction. Primer sets and annealing temperature for PCR amplification and sequencing of FASN, FABP4 and LXR alpha genes were designed as shown in Table 1. The PCR mixture contained 50 ng genomic DNA, 10X Buffer mix and 10 mM dNTPs. Amplifications were performed under the following conditions: 10 min at 94ºC, 35 cycles of 30 s at 94ºC, 30 s at the annealing temperature, and 30 s at 72ºC and a final extension of 10 min at 72ºC.

The genotypes of polymorphism in FASN, FABP4 and LXR alpha genes were detected by PCR-RFLP using *Fnu*4HI, *Nla*III, *Hyp*CH4IV enzymes (New England Lab, USA) respectively. The digestions were performed in total 20 μl reaction volume mixtures with approximately 5 μg of PCR products and units of each restriction enzyme. The reaction was incubated at the proper time and temperature for each restriction enzyme. The 3% gel electrophoresis and sequencing were used to confirm the undigested products and sequenced homozygous and heterozygous.

Table 1. Primers for PCR amplification and restriction enzyme information for genotyping of FASN, FABP4 and LXR-alpha genes.

Gene	GenBank Access. No	Sequence (5' to 3')	Amplicon size (bp)	Annealing Temp (ºC)	Restriction enzyme
FASN	J02839	F: 5'-ATCAACCCTGCTTCCCTTCGTG-3' R: 5'-CGCGCTGGCAGCCTATCAT-3'	131 (exon 4)	58˚C	*Fnu*4HI
FABP4	NC_007312	F: 5'-ATTATCCCCACAGAGCATCG-3' R: 5'-ACAAGACTTGGCCTCAAGGA -3'	339 (exon 4)	58˚C	*Nla*III
LXR-alpha	NC_007313	F: 5'-CTAGGACAGCGTGGCGTATGAGAGC -3' R: 5'-ACAAGACTTGGCCTCAAGGA -3'	1716 (exon 4)	65˚C	*Hpy*CH4

D. Statistical Analysis

The effects of FASN, FABP4 and LXR-α genotypes on fatty acid composition traits were tested using ANOVA in SPSS program (ver. 15.0). In order to test the pair wise differences between the effects of genotype, Turkey's test was also performed.

III. RESULTS AND DISCUSSION

A. FASN genotyping and effect on porcine fatty acid composition

In current study, FASN gene was selected based on their biological functions for fatty acid composition in pig. The genotyping results using PCR-RFLP are shown in Fig. 1. The genotype frequencies were 0.94 (CC type) and 0.06 (CT type), and the estimation of allele frequencies were 0.97 and 0.03 for C and T allele, respectively. The c.265C>T SNP in FASN gene was highly significantly associated with C18:1, C18:2, MUFA and PUFA ($P<0.01$), and significantly associated with C16:1 and C18:3 ($P<0.05$) as shown in Table 2. Animals with genotype CC have lower MUFA than CT genotype, but higher for PUFA. This result can support the previous study that the FASN gene on SSC12 is a functional candidate gene underlying the QTL that affect fatty acid composition (Clop et al., 2003; Munoz et al., 2007).

B. FABP4 and LXR alpha genotyping and effects on fatty acid in Hanwoo cattle

Genotypes of FABP4 were determined by PCR-RFLP with the genotype frequencies 0.58, 0.37 and 0.05 for GG, GA and AA respectively (Fig. 1). The allele frequencies of G and A were 0.76 and 0.24, respectively. The FABP4 genetypes gave highly significant in myristoleic acid (C14:1), palmitoleic acid (C16:1) and arachidonic acid (C20:4) ($P<0.01$). The result of pair wise different mean showed that genotype AA is significantly different with genotype GG and GA in the proportion of C14:1, C16:1 and C20:4, respectively ($P<0.05$). No significant association was observed between the g.3693G>A SNP and SFA, MUFA and PUFA and similar study has been observed previously (Hoashi et al., 2008). However, the genotype GG had SFA higher than GA and AA genotypes. In contrast, MUFA and PUFA percentage in AA genotype was higher than in GG genotype. Our results were consistent with previous study described by Zembayashi et al. (1995) in term of higher MUFA and lower SFA in heifers. The genotyping of LXR alpha gene is currently under progress and the results were not included in this article.

Table 2. The effect of c.265C>T SNP in FASN gene in pig and the g.3693G>A SNP in FABP4 in Hanwoo beef for fatty acid composition.

Fatty acid composition	FASN		FABP4		
	c.265C>T		g.3693G>A		
	CC (n = 67)	CT (n = 4)	GG (n = 22)	GA (n = 14)	AA (n = 2)
Myristic acid (C14:0)	-	-	3.35 ± 0.13	2.92 ± 0.20	2.89 ± 0.62
Myristoleic acid (C14:1)	-	-	0.76 ± 0.06 [a]	0.62 ± 0.08 [a]	1.73 ± 1.08 [b]
Palmitic acid (C16:0)	25.75 ± 0.14	25.33 ± 0.43	26.41 ± 0.32	25.24 ± 0.90	23.67 ± 3.00
Palmitoleic acid (C16:1)	2.92 ± 0.05 [a]	3.49 ± 0.44 [b]	4.03 ± 0.15 [a]	3.58 ± 0.24 [a]	7.13 ± 3.61 [b]
Stearic acid (C18:0)	12.65 ± 0.14	11.63 ± 1.24	12.28 ± 0.35	12.32 ± 0.68	9.91 ± 4.90
Oleic acid (C18:1)	41.40 ± 0.54 [a]	48.21 ± 0.92 [b]	48.26 ± 0.63	47.75 ± 1.86	48.10 ± 2.08
Linoleic acid (C18:2)	13.54 ± 0.36 [a]	9.17 ± 0.39 [b]	4.05 ± 0.76	3.20 ± 0.22	4.78 ± 0.70
α Linoleic acid (C18:3)	0.46 ± 0.02 [a]	0.26 ± 0.04 [b]	0.17 ± 0.50	0.11 ± 0.01	0.11 ± 0.01
Arachidic acid (C20:0)	-	-	0.07 ± 0.004	0.09 ± 0.02	0.05 ± 0.03
Arachidonic acid (C20:4)	2.73 ± 0.16	1.76 ± 0.30	0.62 ± 0.06 [a]	0.60 ± 0.07 [a]	1.67 ± 1.08 [b]
Docosahexaenoic acid (C22:6)	0.56 ± 0.18	0.16 ± 0.07	-	-	-
SFA[1]	38.40 ± 0.23	36.95 ± 1.46	42.66 ± 0.53	41.08 ± 1.55	38.12 ± 7.44
MUFA[2]	44.31 ± 0.58 [a]	51.70 ± 1.19 [b]	53.05 ± 0.64	51.95 ± 2.02	56.96 ± 6.76
PUFA[3]	17.29 ± 0.53 [a]	11.35 ± 0.59 [b]	4.83 ± 0.84	3.90 ± 0.28	6.55 ± 1.79

Mean ± SE. are units of percentage fatty acid composition except for SFA, MUFA & PUFA.
The subcripts 1, 2, 3 were total of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA), respectively.
[a,b]Mean values with different superscript letters in the same row differ significantly at $P < 0.05$ (Turkeys' analysis).

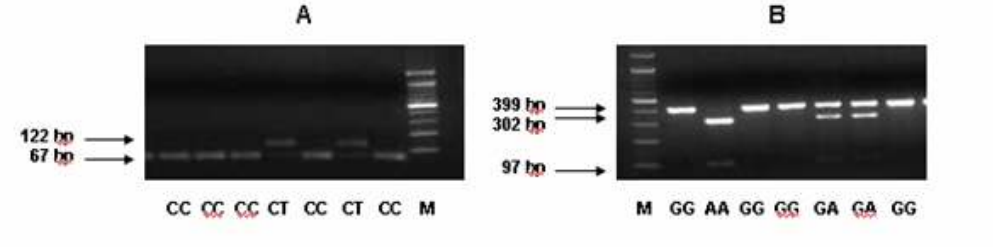

Figure 1. The Genotyping result of the c.265C>T SNP in FASN gene (A) and the g.3693G>A SNP of FABP4 gene (B). The arrowheads show the size of DNA fragment and M is the 100 bp size marker.

IV. CONCLUSION

The results of the current study indicated that genotyping of FASN, FABP4 and LXR-α gene might be good DNA markers for select pig and cattle having less SFA and more MUFA concentration. Moreover, by using these markers, enormous health related benefits will be achieved for the human health.

V. ACKNOWLEDGEMENT

This study was supported by Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

REFERENCES

Abe, T., Saburi J., Hasebe, H., Nagakawa, T., Misumi, S., Nade, T., Nakajima, H., Shoji, N., Kobayashi, M., & Kobayashi, E. (2009). Novel mutations of the FASN gene and their effect on fatty acid composition in Japanese Black beef. *Biochem Genet,* 47, 397-411.

Clop, A., Ovilo, C. M., Perez-Enciso, A., Cercos, A., Tomas, A., Fernandez, A. A., Coll, J. M., Folch, C. Barragan, I., Diaz, M., Oliver, A., Varona, L., Silio, L., Sanchez, A., & Noguera, J. L. (2003). Detection of QTL affecting fatty acid composition in the pig. *Mamm Genome,* 14, 650-656.

Dekkers, J. C. (2004). Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. *J Anim Sci,* 82 E-Suppl, E313-328.

Demeure, O., Duby, C., Desert, C., Assaf, S., Hazard, D., Guillou, H., & Lagarrigue, S. (2009). Liver X receptor alpha regulates fatty acid synthase expression in chicken. *Poultry Science,* 88, 2628-2635.

Folch, J., Lees, M., & Sloane Stanley, H.G. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226(1):497-509.

Hoashi, S., Hinenoya, T., Tanaka, A., Ohsaki, H., Sasazaki, S., Taniguchi, M., Oyama, K., Mukai, F., & Mannen, H. (2008). Association between fatty acid compositions and genotypes of FABP4 and LXR-alpha in Japanese black cattle. *BMC Genet,* 9, 84.

Keys, A., Grande, F., & Anderson, J. T. (1974). Bias and misrepresentation revisited: "perspective" on saturated fat. *Am J Clin Nutr,* 27, 188-212.

Lichtenstein, A. H. (2006). Thematic review series: patient-oriented research. Dietary fat, carbohydrate, and protein: effects on plasma lipoprotein patterns. *J Lipid Res,* 47, 1661-1667.

Marrube, G., Rozen, F., Pinto, G. B., Pacienza, N., Melo J. E., Huguet, M. J., Canet, Z., Zandomeni, R., & Miquel, M. C. (2004). New polymorphism of FASN gene in chicken. *J Appl Genet,* 45, 453-455.

Morris, C. A., Cullen, N. G., Glass, B. C., Hyndman, D. L., Manley, T. R., Hickey, S. M., McEwan, J. C., Pitchford, W. S., Bottema, C. D., & Lee, M. A. (2007). Fatty acid synthase effects on bovine adipose fat and milk fat. *Mamm Genome,* 18, 64-74.

Munoz, G., Ovilo, C., Noguera, J. L., Sanchez, A., Rodriguez, C., & Silio, L. (2003). Assignment of the fatty acid synthase (FASN) gene to pig chromosome 12 by physical and linkage mapping. *Anim Genet,* 34, 234-235.

Munoz, G., Alves, E., Fernandez, A., Ovilo, C., Barragan, C., Estelle ,J., Quintanilla, R., Folch, J. M., Silio, L., Rodriguez, M. C., and Fernandez, A. I. (2007). QTL detection on porcine chromosome 12 for fatty-acid composition and association analyses of the fatty acid synthase, gastric inhibitory polypeptide and acetyl-coenzyme A carboxylase alpha genes. *Anim Genet* 38(6):639-646.

Ordovas, L., Roy, R., Pampin, S., Zaragoza, P., Osta, R., Rodriguez-Rey, J. C., & Rodellar, C. (2008). The g.763G>C SNP of the bovine FASN gene affects its promoter activity via Sp-mediated regulation: implications for the bovine lactating mammary gland. *Physiol Genomics,* 34, 144-148.

Woollett, L. A., Spady, D. K., & Dietschy, J. M. (1992). Saturated and unsaturated fatty acids independently regulate low density lipoprotein receptor activity and production rate. *J Lipid Res,* 33, 77-88.

Zembayashi, M., Nishimura, K., Lunt, D. K., & Smith, S. B. (1995). Effect of breed type and sex on the fatty acid composition of subcutaneous and intramuscular lipids of finishing steers and heifers. *J Anim Sci,* 73, 3325-3332.

Zhang, S., Knight, T. J., Reecy, J. M., & Beitz, D. C. (2008). DNA polymorphisms in bovine fatty acid synthase are associated with beef fatty acid composition. *Anim Genet*, 39, 62-70.