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Association of Microsatellite Marker in FABP4 Gene with Marbling Score and Live Weight in Hanwoo

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ABSTRACT

The bovine *fatty acid binding protein 4* (*FABP4*) plays an important role to uptake intracellular fatty acid. It has been previously reported as a positional candidate gene for marbling score in livestock. The re-sequencing of FABP4 gene detected a polymorphic AT repeated sequence in intron II of FABP4 gene. Allelic distribution for this microsatellite marker was examined in other cattle breeds. A total of 8 alleles were detected with diverse repeat units (14 to 21 AT repeat) in Hanwoo and 7 breeds. Of the 8 alleles, the predominant alleles were $[AT]_{16}$, $[AT]_{18}$ and $[AT]_{19}$ in the Hanwoo and 7 cattle breeds. The linear mixed model for genotypic effect (3237AT) on carcass traits showed a significant effect on marbling score (MAR P=0.025) and live weight (LWT; P=0.04) in the 583 Hanwoo cattle population. Live weight (LW) was highest in the homozygous $(AT)_{17}$ genotype (557.5 ± 6.94) and lowest in the heterozygous $(AT)_{16/17}$ genotype (521.7 ± 7.70). On the other hand, the homozygous $(AT)_{17}$ genotype (3.0 ± 0.15) has the highest effect on marbling score and the lowest effect was in homozygous $(AT)_{18}$ genotype (2.2 ± 0.15). The marbling score difference between both groups was 0.8 which is around two times higher than SNP genotype effect on marbling score in Limousin × Wagyu crosses.

(**Key words :** Fatty acid binding protein 4 (FABP4), Microsatellite marker, Marbling score (MAR))

INTRODUCTION

Marbling (intramuscular fat) is highly desired carcass trait for Korean consumer because it contributes the juiciness and tenderness of beef. For highly marbled meat production, grain based feeding strategies are performed in the Korean finishing farm sector. These feeding strategies produce a highly marbled meat but it lead to inefficiency, because steers produce an excess fat production. So far, genetic improvement program has been achieving a reliable meat production and, in particular, the identification of DNA marker might be used to marker assisted selection in the breeding program.

The *FABP4* gene plays an important role in lipid hydrolysis and intracellular fatty acid trafficking in different tissues (Damcott et al, 2004; Chmurzynska, 2006). The up take of an intracellular fatty acid plays an essential role as a signal molecule to trigger the process of preadipocyte differentiation and terminal differentiation-related gene expression (Amri et al, 1994; Duplus et al, 2000). So far, nine distinct members have been identified for the *FABP* gene family and three types of fatty acid *binding protein* (FABP) gene, *FABP3* (heart-type), *FABP4* (adipocyte-type) and *FABP5* (epidermal-type) gene have been reported to associate with fat traits in livestock (Gerbens et al, 2000; Calvo et al, 2004; Estelle et al, 2006). The *FABP4* gene was proposed as positional candidate gene for marbling score in Brahman × Hereford crosses (http://bovineqtl.tamu.edu/). In a previous study, we have reported 17 polymorphisms including 1 micro-satellite marker in the *fatty acid binding protein 4* gene. Of these polymorphisms, two SNPs of *FABP4* gene were significantly associated with marbling score and carcass weight in Hanwoo cattle (Park et al, 2006). Microsatellite marker polymorphism is more informative than bi-allelic individual SNP marker, because highly polymorphic microsatellite marker can explain a large portion of genetic variation for

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quantitative traits (Daw et al, 2005). Consequently, a highly polymorphic microsatellite marker could more explain genetic variation on quantitative traits in cattle.

In this study, the distribution of *FABP4* microsatellite (*3237AT*) genotype in Hanwoo and 7 cattle breeds was examined. We then estimated the *FABP4_μsat3237* marker effect on marbling score and live weight in Hanwoo steers.

MATERIALS AND METHODS

1. Animal and phenotype data

We obtained semen from 10-40 sires of 7 cattle breeds for comparison of allele frequencies. Phenotype data and blood samples for microsatellite marker genotyping were obtained from 583 Hanwoo cattle descending from 92 sires and unrelated dams (2-21 progeny number per sire) from two NIAS experimental stations, Dae-Kwan-Ryoung and Nam-Won. The Hanwoo received a total mixed diet of concentrate and rice straw for *ad libitum* intake with a ratio in total feed of about 1.5:1, 2:1 and 4.5:1 for growing period (4-12 months), finishing period I (13-18 months) and finishing period II (19-24 months), respectively. Crude protein (CP) and total digestible nutrients (TDN) of the concentrate were 14-16, 11-13 and 11% and 68-70%, 71-73% and 72-73% for growing period, finishing period I and finishing period II, respectively. Phenotypic data in this study included carcass weight (CWT), longissimus muscle area (LMA), back fat thickness (BF) and marbling score (MAR). Back fat thickness, eye muscle area and marbling score were measured at the 12^{th}-13^{th} rib junction after a 24 hour chill. The statistics for phenotypic data was summarized into Table 1. Marbling score was assessed on 1 to 7 scales, and the degree of marbling was evaluated based on the Korean Beef Marbling Standard (BMS) from Animal product Grading Service in Korea (APGS, 1995).

2. Genotyping

DNA samples were extracted from blood samples and semen. The DNA concentration was adjusted to 50 ng/μℓ. The primer for PCR was designed in Primer 3 (http://frodo.wi.mit.edu/) program using FABP4 gene sequence (TC 303523) and the primer sequence is *FABP4_μsat3237*-Forward primer: 5'-GGTGAATTTTCTCCCATTTA-3' and *FABP4_μsat3237*-Reverse primer: 5'-GAATAGCATCATAG-GGGTTTT-3'. PCR amplification for microsatellite marker in FABP4 gene was performed on 583 Hanwoo and foreign cattle breeds. The PCR amplification reaction was carried out in PCR solution including 1.5 mM $MgCl_2$, 2.0 mM dNTPs, 5 pM of each primer, 1 μℓ of the genomic DNA (50 ng) and 0.5 U Taq DNA polymerase (Promega, USA) in 20 μℓ volume. The PCR amplification reaction was carried out 94℃ for 5 min and 35 cycles of 94℃ for 1 min, 60℃ for 1 min and 72℃ for 1 min. After PCR amplification, the amplified DNA was diluted by 20 times and resolved by electrophoresis in capillary using an ABI 3730 XL sequencer (Applied Biosystems, Foster City, CA) and genotyped using Gene Mapper software (Applied Biosystems).

3. Statistical analysis

The statistical analysis for the *FABP4_μsat3237AT* was carried out using ASReml program (Gilmour et al, 2001). The following linear mixed model was used to estimate the *FABP4_μsat3237AT* effect:

$$Y_{ijk} = \mu + YS_i + L + (G)_k + bD_{ijk} + a_{ijk} + e_{ijk}$$

where Y_{ijk} is the observation for the trait, μ is overall mean, YS_i is the fixed effect of i^{th} year-season, $(L)_j$ is the fixed term of j^{th} location, $(G)_k$ is the fixed term of k^{th} genotypic effect of *FABP4_μsat3237AT*, D_{ijk} is the covariate term for

Table 1. The number of animals, phenotypic means and standard deviation (SD) for each trait

Traits	Mean	SD	Min	Max
Live weight (kg)	541.5	53.1	320	710
Carcass weight (kg)	313.5	34.1	174	423
Eye muscle area (cm^2)	57.9	2.07	52.6	79.8
Dressing percentage (%)	74.9	8.42	30	99
Marbling score (1-7)	7.45	3.03	2	21
Back fat thickness (mm)	2.05	1.27	1	7

the age of days at slaughter, $(a)_{ijk}$ was fitted as random effect and e_{ijk} is the random residual error. Genetic covariances among animals were fitted through a numerator relationship matrix (A): $Var\,(a) = A\sigma^2_e$.

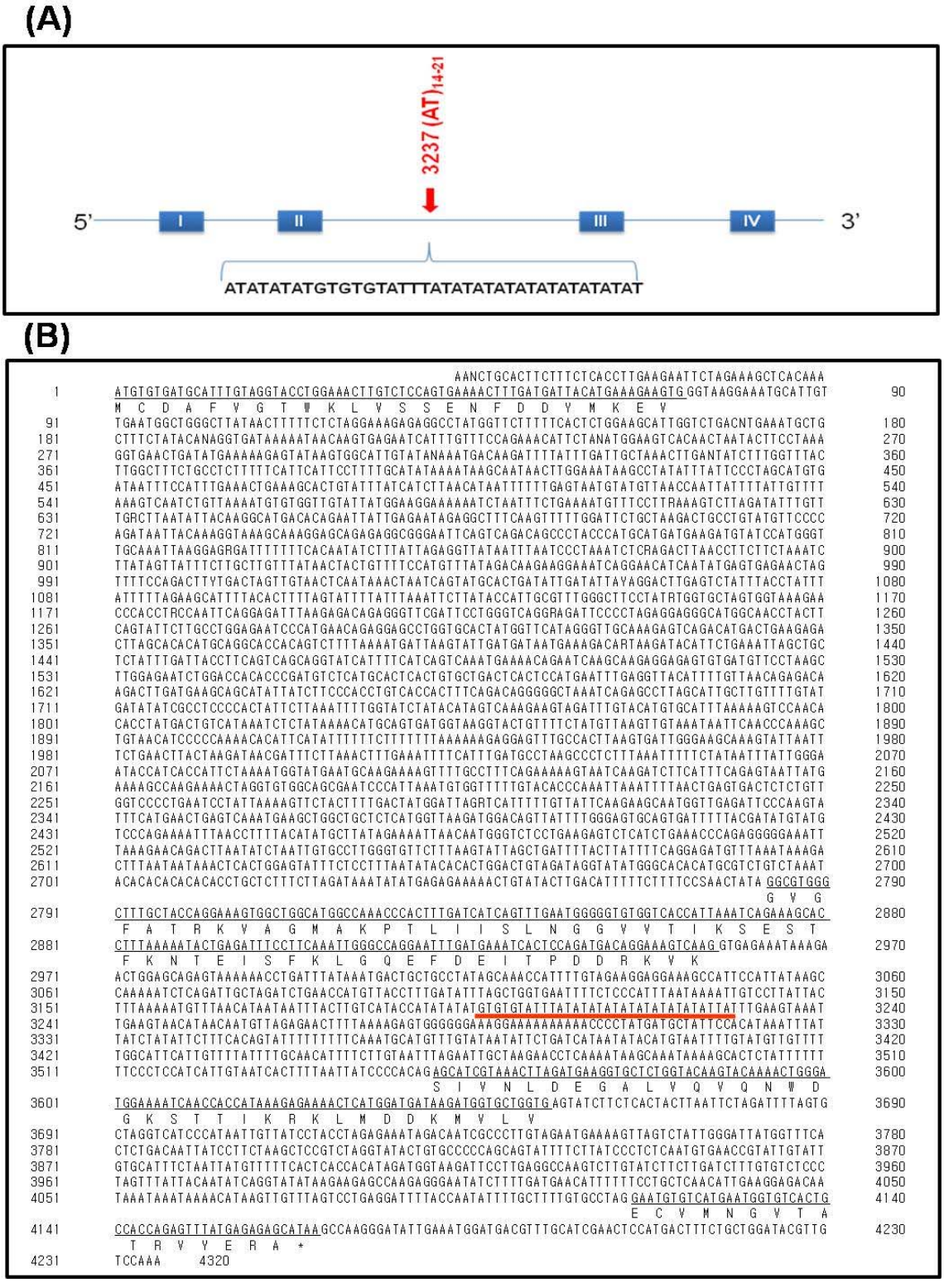

Fig. 1. A diagram of indicating the microsatellite marker of bovine FABP4 gene (GenBank acc.no NW_001493222.2) (A) and bovine fatty acid binding protein 4 gene sequence (B) with microsatellite marker underlining red line (*FABP4_μsat3237AT*).

RESULTS

1. Comparison of allele frequency among 7 cattle breeds

In previous study, we re-sequenced bovine FABP4 gene (4.2 kb) in 24 Hanwoo bulls to detect genetic polymorphism (Park et al. 2007). Within the intron II region of fatty acid binding protein 4 gene, a short interspersed repeat element (SINE), a $[AT]_{19}$ repeat was detected at 232 bp downstream of the exon II (Fig. 1). Using DNA panel of 24 Hanwoo bulls and 159 other cattle breeds, we detected 8 alleles with 14 to 21 AT repeat unit (Table 2). Of these 8 alleles, the predominant alleles were $[AT]_{16}$, $[AT]_{18}$ and $[AT]_{19}$ in the Hanwoo and other 7 cattle breeds. Particularly, in Simmental, the allele frequency of *FABP4_μsat3237* marker was highly diverse than in other cattle breeds (Table 2).

2. Estimation of *FABP4_μsat3237AT* marker effect on carcass traits

Based on the analysis of phenotypic data for carcass traits in 583 Hanwoo steers, Table 3 showed F-statistics and P-value for *FABP4_μsat3237AT* for live weight (LW), carcass weight (CWT), eye muscle area (EMA), dressing percentage (DP), marbling score (MAR) and back fat thickness (BF). As shown in Table 3, statistic analysis revealed the *FABP4_μsat3237AT* has a significant effect on marbling score (MAR P=0.025) and live weight (LW P=0.04) in the 583 Hanwoo, while the *FABP4_μsat3237AT* was not significant in other carcass traits such as carcass weight (*P* = 0.07), eye muscle area (P=0.28) and back fat thickness (*P* = 0.39). Live weight (LW) was highest in the homozygous $(AT)_{17}$ genotype (557.5 ± 6.94) and lowest in the heterozygous $(AT)_{16/17}$ genotype (521.7 ± 7.70) (Table 4). The difference in live weight between the highest and lowest genotype effect was 35.8 kg in Hanwoo steers. For marbling score, the homozygous $(AT)_{17}$ genotype has a highest effect (3.0 ± 0.15), and lowest effect was in homozygous $(AT)_{18}$ genotype (2.2 ± 0.15 Table 4). The difference in marbling score on these genotype effects was 0.8 which is two times higher than SNP genotype effect on marbling score (Park et al, 2006) in Hanwoo steers. These results indicated that microsatellite marker could be more informative in explaining variation for quantitative traits.

Table 3. *P-value* of all associations test of FABP4 microsatellite (*3237AT*) marker on carcass traits (n=543).

Traits	*F-statistics*	*P-value*
Live weight (kg)	1.98	0.04
Carcass weight (kg)	1.77	0.07
Eye muscle area (cm^2)	1.21	0.28
Dressing percentage (%)	0.18	0.99
Marbling score (1-7)	2.14	0.02
Back fat thickness (mm)	1.06	0.39

DISCUSSION

This study reveals a characterization of highly polymorphic microsatellite marker in FABP4 and its effect on carcass traits in Hanwoo cattle. FABP4 is known to play an essential role in the regulation of lipid homeostasis to interaction with peroxisome proliferators activated receptors (PPARs), which is a transcriptional factor to trigger differentiation at the early stage of preadipocyte cell (Damcott et al, 2004; Armi et al, 1994). Recently, Lee et al,

Table 2. The frequency of FABP4 microsatellite alleles in cattle breeds

Breeds	No	Number of AT repeat							
		14	15	16	17	18	19	20	21
Hanwoo	543	0.008	0.256	0.138	0.286	0.243	0.054	0.012	
Angus	13			0.35		0.46	0.1	0.07	
Charolais	12			0.5		0.46	0.04		
Simmental	28	0.04	0.02	0.36	0.02	0.28	0.26		0.01
Hereford	20			0.17		0.58	0.2	0.05	
Limousin	40			0.59		0.3	0.11		
Brahman	20			0.4		0.58	0.025		
Brown Swiss	25		0.02	0.92		0.02	0.04		

Table 4. Least squares means (LSM) and standard errors (SE) of FABP4 microsatellite (3237AT) genotype class on marbling score and live weight in Hanwoo cattle (n=543)

Genotype class	Animals	Marbling Score (1-7)	Live Weight (kg)
198/198	66	2.7 ± 0.14^{e}	542.9 ± 6.57^{c}
198/202	92	2.5 ± 0.12^{c}	536.9 ± 5.77^{b}
198/204	39	2.5 ± 0.18^{c}	548.3 ± 8.43^{d}
200/200	34	2.5 ± 0.19^{c}	552.1 ± 8.86^{e}
200/202	20	2.3 ± 0.25^{b}	548.6 ± 11.43^{d}
200/204	46	2.6 ± 0.17^{d}	521.7 ± 7.70^{a}
202/202	61	3.0 ± 0.15^{f}	557.5 ± 6.94^{f}
202/204	53	2.3 ± 0.16^{b}	544.1 ± 7.32^{c}
204/204	58	2.2 ± 0.15^{a}	538.5 ± 6.98^{b}
206/206	15	2.5 ± 0.29^{c}	542.9 ± 13.28^{c}
[1)]Others	59	2.4 ± 0.15^{b}	543.6 ± 7.04^{c}
Total	543		

[1)]Others indicated minor allele frequencies (*MAF < 0.001*).

(2008) reported that gene expression of FABP 4 was higher in highly marbled muscle than low marbled muscle of Hanwoo steers. The bovine FABP4 gene is assigned to BTA14 (Everts-van der wind et al, 2004), which is consistent with the localization of the human and porcine FABP4 gene on Chr8q21 (Prinsen et al, 1997) and Chr4 (Gerbens et al, 1998), respectively. The corresponding region was reported to have QTL effect for marbling score in a cross of *Bos indicus* × *Bos taurus* (Casas et al, 2003). These findings indicated that FABP4 gene is one of reliable candidate gene for marbling (intramuscular fat) in livestock.

Microsatellite marker is known to a neutral genetic polymorphism that does not significantly lead to gene expression change. However, if one microsatellite marker is in strong linkage disequilibrium (LD) with an invisible genetic polymorphism which cause either gene expression or phenotypic variation, it might be very useful indirect marker because of being able to explain a large genetic variation for quantitative traits from highly polymorphism (Daw et al, 2005). To determine FABP4 gene effect on marbling score in cattle, we examined microsatellite marker detected in intron II region of FABP4 gene. Statistical analysis revealed that the homozygous $[AT]_{17}$ genotype class of the FABP4 gene had approximately 0.8 unit higher marbling score than the homozygous $[AT]_{18}$ genotype class. Interestingly, the homozygous $[AT]_{17}$ genotype class had a considerably higher live weight (LWT) at 24 months of age than the homozygous $[AT]_{18}$ genotype class. Park et al. (2006) demonstrated two SNPs in FABP4 gene affecting marbling score and carcass weight in Hanwoo population. Michal et al. (2006) reported *7516G>C* of FABP4 also showed a significant effect on marbling score in Limousin × Waygu crosses. These results indicate that the FABP4 gene locus on BTA14 has a considerable effect on marbling score and body weight in cattle.

In conclusion, we have identified microsatellite marker $(AT)_n$ in FABP4 gene and revealed a significant effect on marbling score. Microsatellite marker $(AT)_{17}$ showed a significant association with marbling score (MAR $P = 0.024$) and live weight (LW $P = 0.04$). The phenotypic difference between highest and lowest genotypic effect was 0.8 and it is two times higher than SNP data in Hanwoo steers. This result shows that microsatellite marker might be able to explain a large genetic variation for quantitative traits and could be useful for applying to animal breeding program such as marker assisted selection (MAS).

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