VALIDATION OF MOLECULAR DIAGNOSTIC MARKERS FOR CARCASS TRAITS IN COMMERCIAL HANWOO STEERS

S. C. Shin, J. P. Heo, K. Y. Chung, and E. R. Chung*

Division of Animal Science and Resources, Sangji University, 660 Usandong, Wonju, Gangwondo, 220-702, South Korea

*Corresponding author (phone: +82-33-730-0541; fax: +82-33-730-0541; e-mail: erchung@sangji.ac.kr)

***Abstract*— Associations between 8 available molecular diagnostic markers for carcass traits (*TG g.371T>C, APM1 g.1454G>A, CPE g.601T>C, FABP4 g.2834C>G, FABP4 g.3533T>A, FABP4 g.3691G>A, SCD g.10153A>G, SCD g.10329T>C*) and carcass traits (meat quality and quantity trait) were validated by the large-scale farm field evaluations in commercial Hanwoo steers (n=586). In this validation study, Genotypes of eight SNP markers were analyzed using PCR-RFLP method, respectively. At the *AMP1 g.1454G>A, FABP g.3691G>A, SCD g.10153A>G, CPE g.601T>C* SNP markers, there were a significant effect of the on carcass traits (MC, FC, BF, MI, QG, MT and CW, respectively), but no significant associations were observed between effect of the *TG g.371T>C, FABP4 g.2834C>G, FABP4 g. 3533T>A SNP and carcass trait in Hanwoo steers.* We reconstructed haplotypes across three SNP loci (*APM1 g.1454G>A, SCD g.10153A>G and CPE g.601T>C*) using the PHASE program for the multiple DNA marker composition, analyzed association between haplotypes and carcass traits. The haplotypes were significantly associated with BF (*P* = 0.13), MI (P = 0.035) and QG (P=0.030). Animals with the GGC haplotype had higher MI, QG score than those with the GGT, GAT and AGT haplotypes. The multiple DNA marker by haplotype of available molecular diagnostic markers for carcass traits may be useful as a genetic marker for Hanwoo breeding using marker assisted selection.**

***Index Terms*—carcass traits, Hanwoo (Korean cattle), multiple DNA marker, SNP, validation**

I. INTRODUCTION

Meat quality and carcass composition are the most economically important traits in beef cattle production. Carcass and meat quality traits, which are under the control of multiple genes, are economically important traits in beef cattle. Gene mapping and discovery programs have resulted in the detection of a plethora of QTL for various beef cattle production traits. Because of the cost of collecting phenotypes and genotypes from large animals, discovery populations often involve a relatively small sample size. Before moving genetic markers from discovery populations to commercialization, it is important to validate their purported effects on the trait of interest in different breeds and environments, and assess them for correlated responses in the associated traits (Barendse, 2005). One of the biggest challenges in achieving this objective is the paucity of cattle populations with sufficient phenotypic data to assess the association between various traits and newly discovered genetic markers, and this makes it difficult and expensive to do large-scale field evaluations(Eenennaam et al., 2007). Therefore, the objective of this study was to validated 8 available molecular diagnostic markers for carcass traits (*TG g.371T>C, APM1 g.1454G>A, CPE g.601T>C, FABP4 g.2834C>G, FABP4 g.3533T>A, FABP4 g.3691G>A, SCD g.10153A>G, SCD g.10329T>C*) by large-scale farm field evaluations in commercial Hanwoo steers. And we studied for development of new diagnostic multiple DNA marker for carcass trais using these markers.

II. MATERIALS AND METHODS

A. Animals and carcass data

586 commercial Hanwoo steers (*Hoengseong Hanwoo* brand) with pedigree information and carcass data were used in this study. The carcass data included were marbling score (MS), meat color (MC), fat color (FC), meat texture (MT), maturity score (MA), grade of meat quality (MG), backfat thickness (BF), M. *Longissimus dori* area (EMA), carcass weight (CW), meat quantity index (MI) and grade of meat quantity (QG).

B. SNP marker genotyping using PCR-RFLP analysis

For each animal (n=586), genomic DNA was extracted from hair root using E-prep kit (Prepgene, Korea). SNP marker genotyping of molecular diganostic markers for carcass traits were carried out using PCR-restriction fragment length polymorphism (RFLP) method. The primer sequences, fragment size and restriction enzyme and GenBank accession number are shown in Table 1.

C. Statistical Analysis

Allele and genotype frequencies were calculated by simple allele counting method (Falconer and Mackay, 1996). Hardy-Weinberg equilibrium in examined population was tested by comparing expected and observed genotype frequencies using a chi-square test. The PROC GLM procedure of SAS (SAS, Inst. Inc., Cary NC) was used to test the association between SNP marker genotypes of the candidate genes and carcass and meat quality traits. The linear model used was as follows: $Y_{ijklm} = \mu + S_i + YS_j + SP_K + A_l + G_m + e_{ijklm}$

Where Y_{ijklm} is the observation of the carcass traits, μ is the overall mean for each trait, S_i is the effect of sire (n=19), YS_i is the effect of i_{th} year and season of calving, SP_k is the effect of slaughter place, A_l is the effect of age at slaughter(covariate), G_m is the fixed effect of SNP genotype and e_{ijklm} is the random residual effect.

We reconstructed haplotypes across three SNP loci (*APM1 g.1454G>A, SCD g.10153A>G and CPE g.601T>C*) using the PHASE program (Stephens et al. 2001) for the multiple DNA marker composition, analyzed association between haplotypes and carcass traits.

Table 1. Primer sequences, fragment size, restriction enzyme and GenBank accession number of SNP markers

SNP marker	Primer sequences (5' - 3')	Fragment size (bp)	Restriction enzyme	GenBank accession no.
TG g.371T>C	F-GGGGATGACTACGAGTATGACTG R-GTGAAAATCTTGTGGAGGCTGTA	545	*Mbo* Ⅰ (↓GATC)	AY615525
APM1 g.1454G>A	F-CGCTGTTGTAAGAGGCAAAGAT R-TTGAATCAGTCGTCCTTACCCT	323	*Pas* Ⅰ (CC↓CWGGG)	DQ156119
FABP4 g.2834C>G	F-GCTGCTCTCATGGTTAAGATGG R-CCTTGACTTTCCTGTCATCTGG	591	*Hpy188* Ⅰ (TCN↓GA)	NC_007312
FABP4 g.3533T>A	F-ACTGCTGCCTATAGCAAACCAT R-TACGATGCTCTGTGGGGATAAT	555	*Csp6* Ⅰ (G↓TAC)	
FABP4 g.3691G>A	F-ACCCCTATGATGCTATTCCACA R-ATACGGTTCACATTGAGAGGGA	565	*Nla*Ⅲ (CATG↓)	
SCD g.10153A>G	F-GATGAAACATTCCAGTCCTTGC R-GGAGAGGGGTCATAAAACAGGT	600	*Nco* Ⅰ (C↓CATGG)	AY241932
SCD g.10329T>C	F-TTATGACAAGACCATCAACCCC R-AGCAAGACTACCACCCAGATCA	363	*Aci* Ⅰ (C↓CGC)	
CPE g.601T>C	F-CCTTACTGTCTTCCCAAGTCCA R-GTCGTTCCTTCTACAAAGCTGC	450	*BspH* Ⅰ (T↓CATGA)	AY970663

III. RESULTS AND DISCUSSION

A. SNP marker genotyping

As shown figure 2, Genotypes of eight SNP markers were analyzed using PCR-RFLP method, respectively. We were detected three genotypes in all SNP markers (*SCD g.10329T>C SNP* excepted, because it was detected only two genotypes).

B. Associations test

Results of the SNP markers association analysis are presented Table 2. At the *AMP1 g.1454G>A* SNP, there was a significant effect of the on MC, FC, BF, MI and QG. At the *FABP g.3691G>A* SNP, there was a significant effect of the on MS, MG, MI and QG. At the *SCD g.10153A>G* SNP, there was a significant effect of the on MS, MT and MG. At the *CPE g.601T>C* SNP, there was a significant effect of the on CW and MI. But no significant associations were

observed between effect of the *TG g.371T>C, FABP4 g.2834C>G, FABP4 g. 3533T>A* SNPs and carcass trait in Hanwoo steers. Of seven reconstructed haplotypes, four haplotypes (GGT, GGC, GAT and AGT) were predominant (total frequency 97.1%) in the 586 Hanwoo steers(Table 3). As shown table 4, the haplotypes were significantly associated with BF (P = 0.13), MI (P = 0.035) and QG (P=0.030). Animals with the GGC haplotype had higher MI, QG score than those with the GGT,GAT and AGT haplotypes. On the other hand, Animals with the GGC haplotype had lower BF score than those with the GGT,GAT and AGT haplotypes.

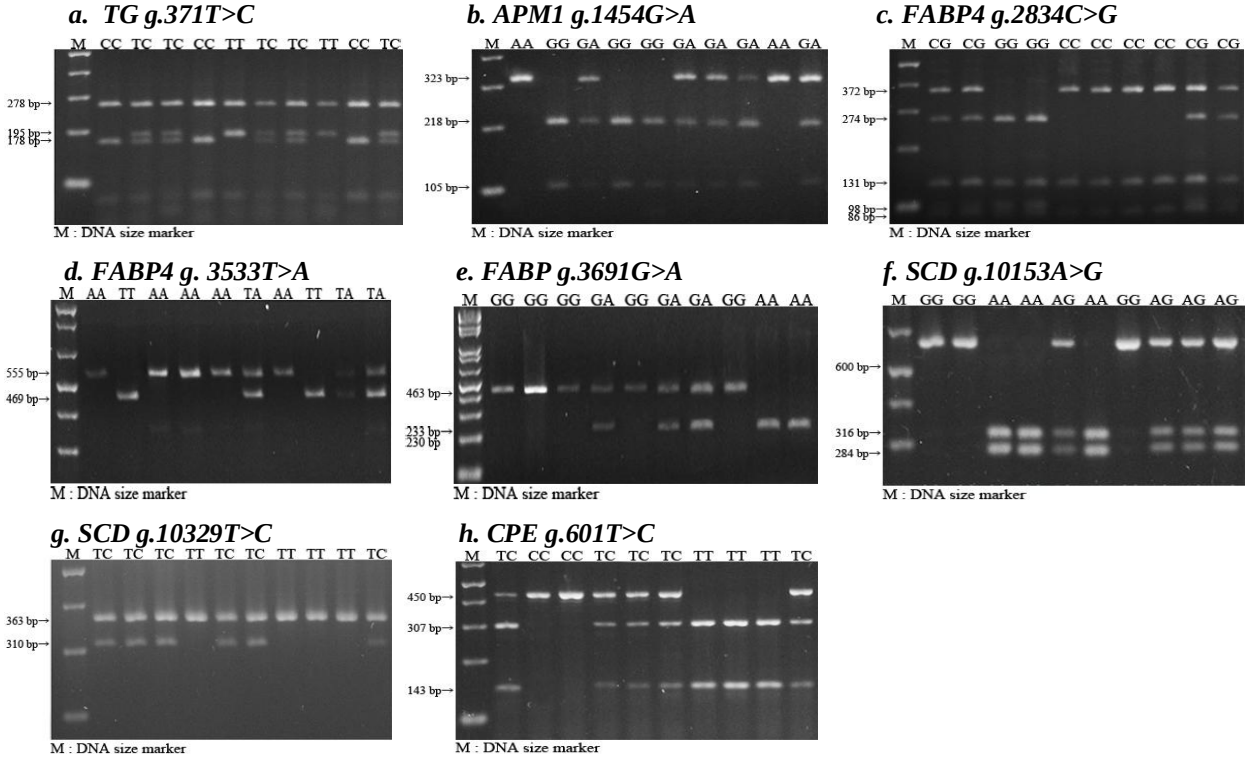

Figure 1. SNP marker genotyping using PCR-RFLP analysis.

Table2. Association test between SNP marker genotypes of the candidate genes and carcass traits in commercial Hanwoo steers

Traits		P-value of SNP markers (n=586)						
		TG g.371T>C	***APM1 g.1454G>A***	***FABP4 g.2834C>G***	***FABP4 g.3533T>A***	***FABP4 g.3691G>A***	***SCD g.10153A>G***	***CPE g.601T>C***
Meat quality	**MS**	0.541	0.948	0.496	0.749	**0.020**	**0.006**	0.140
	MC	0.141	**0.025**	0.347	0.545	0.582	0.069	0.651
	FC	0.800	**0.028**	0.453	0.432	0.959	0.492	0.459
	MT	0.569	0.827	0.528	0.706	0.173	**0.013**	0.334
	MA	0.483	0.534	0.852	0.727	0.655	0.678	0.857
	MG	0.502	0.988	0.533	0.548	**0.025**	**0.018**	0.111
Meat quantity	**BF**	0.163	**0.002**	0.210	0.196	0.092	0.973	0.066
	EMA	0.456	0.426	0.367	0.132	0.829	0.928	0.282
	CW	0.198	0.063	0.507	0.184	0.090	0.113	**0.042**
	MI	0.388	**0.003**	0.584	0.454	**0.038**	0.767	**0.032**
	QG	0.083	**0.003**	0.464	0.523	**0.038**	0.881	0.121

MS, marbling score; MC, meat color; FC, fat color; MT, meat texture; MA, maturity score; MG, grade of meat quality; BF, backfat thickness; EMA, M. *Longissimus dori area*; CW, carcass weight; MI, meat quantity index; QG, grade of meat quantity.

Table 3. Frequency of haplotypes by three SNP loci using the PHASE program

Haplotype	*APM1 g.1454G>A*	*SCD g.10153A>G*	*CPE g.601T>C*	Frequency
ht1	G	G	T	0.715
ht2	G	G	C	0.147
ht3	G	A	T	0.073
ht4	A	G	T	0.036
ht5	G	A	C	0.015
ht6	A	A	C	0.009
ht7	A	A	T	0.005

Table 4. Association test between haplotype and meat quantity traits in commercial Hanwoo steers

Traits		Haplotype (n=586)				P-value
		GGT	GAT	AGT	GGC	
Meat quantity	**BF**	12.800±0.618[b]	12.655±0.852[b]	15.804±1.076[a]	12.702±0.732[b]	**0.013**
	EMA	90.400±1.466	89.854±2.021	93.131±2.552	88.796±1.736	0.282
	CW	424.931±6.886	430.423±9.495	446.828±11.989	417.686±8.153	0.062
	MI	64.962±0.467[a]	64.850±0.644[a]	62.914±0.813[b]	64.988±0.553[a]	**0.035**
	QG	2.035±0.092[a]	2.066±0.127[a]	1.634±0.160[b]	2.063±0.109[a]	**0.030**

BF, backfat thickness; EMA, M. *Longissimus dori area*; CW, carcass weight; MI, meat quantity index; QG, grade of meat quantity. [a,b] Within a row, means with different superscript letter differ (P<0.05).

IV. CONCLUSION

According to the result of this validation study, At the *AMP1 g.1454G>A, FABP g.3691G>A, SCD g.10153A>G, CPE g.601T>C* SNP markers, there were a significant effect of the on carcass traits (MC, FC, BF, MI, QG, MT and CW, respectively), but no significant associations were observed between effect of the *TG g.371T>C, FABP4 g.2834C>G, FABP4 g. 3533T>A SNP and carcass trait in Hanwoo steers.* Of seven reconstructed haplotypes, four haplotypes (GGT, GGC, GAT and AGT) were predominant in the 586 Hanwoo steers, the haplotypes were significantly associated with BF, MI and QG. The multiple DNA marker by haplotype of available molecular diagnostic markers for carcass traits may be useful as a genetic marker for Hanwoo breeding using marker assisted selection.

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