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Short Communication

ASSOCIATION OF A SINGLE NUCLEOTIDE POLYMORPHISM IN THE CALNEURON 1 GENE ON MEAT QUALITY AND CARCASS TRAITS IN HANWOO (*BOS TAURUS COREANAE*)

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ABSTRACT

The distribution and amount of intramuscular fat, collectively scored as marbling, is an economically important trait in beef cattle. Hanwoo (*Bos Taurus coreanae*) is the premium beef cattle in Korea.A previous GWAS analysis on Hanwoo had showed that an SNP located in the *CALN1* gene had a very strong association with Marbling. This SNP referred to as g.29172875A>G, was genotyped on Hanwoo tissue samples (n = 1103) collected from throughout Korea. The genotype frequencies were 0.152, 0.439 and 0.408 for AA, AG and GG, respectively. The association study confirmed significant association with marbling (P <0.0001) and loin eye muscle area (P = 0.001), with strong additive genetic effects. Significant departure from Hardy-Weinberg equilibrium was not detected. The findings suggest that the *CALN1* SNP polymorphism is strongly associated with marbling and loin eye muscle area and can be used as a robust marker for marker based selective breeding programs in Hanwoo.

Key words: Intramuscular Fat, *CALN*, SNP, Genetic association, Hanwoo.

INTRODUCTION

Hanwoo is the premium beef cattle of Korea (Lee *et al*. 2013). In Korea beef quality is graded based on intramuscular fat or marbling (MAR), meat color (MCO), fat color (FCO), maturity (MAT), texture (TXT), and meat yield grades such as carcass weight (CAW), back fat thickness (BFT) and loin eye muscle area (LEA). Meat quality and carcass traits are economically important traits in the beef cattle industry, particularly marbling (intramuscular fat) and is the major trait that contributes to the juiciness and flavor in beef, and higher levels of marbling improves the palatability and acceptability of beef by improving the taste and tenderness of the meat (Busboom *et al*. 1993, Sasaki *et al*. 2009). In the Korean beef market marbling contributes the most economic value for the producers, and due to the economics attached with marbling there has been a huge emphasis on improving it through selective breeding. However, genetic improvement of these traits through selective breeding is expensive and difficult due to the difficulties in collecting phenotypic data in live animals (Shin and Chung 2007).Marker assisted selection (MAS) is a promising approach for genetic improvement of meat quality traits. Studying the association of polymorphisms in genes involved in physiological or biological processes connected to a quantitative trait like carcass traits can be an effective approach for developing markers for selective breeding (Shin and Chung 2007). Korean beef industry lays a strong emphasis on meat yield and quality traits. Especially, in the commercial beef market in Korea, the quality of Hanwoo beef is directly influenced by the abundance of marbling. Therefore any SNP identified to have a significant association with carcass traits could be used in the national animal breeding program for selective breeding programs (Lee *et al*. 2014).

A GWAS (Genome wide association study) analysis (Srikanth *et al*., unpublished) showed a strong association between an SNP (g.29172875A>G) located in *CALN1* (BTA 25) with marbling trait in Hanwoo cattle. *CALN1* (Calneuron 1) is a calmodulin-like protein that contains two functional EF-hand motifs at the N-terminal and a hydrophobic segment at the C-terminal, and is 261 amino acids long. *CALN1* is localized in the plasma membrane, and plays an inhibitory role in neurotransmitter release and Ca^{2+} signaling (Shih *et al*. 2009). Being very similar to calmodulin it was suggested to have a potential role in signal transduction (Wu *et al*. 2001). The aim of this study was to verify and validate the genetic effect of the SNP g.29172875A>G on carcass traits in a nationwide randomly sampled Hanwoo cattle population, so that this SNP can be used as a standardized molecular marker for selective breeding of animals with high marbling trait.

MATERIALS AND METHODS

Animal and carcass traits: In all, 1103 muscle tissues of Hanwoo cattle were collected from the packing facilities of the Korean Animal Products Evaluation (KAPE) centers located throughout Korea. KAPE, the official meat quality grading agency of Korea graded meat quality based on an approved grading system

(http://www.ekape.or.kr/view/eng/system/beef.asp). The samples were collected 24 hours after slaughter, from the *longissimus thoracis* muscle between the 12th and 13th rib and stored immediately at -70˚C. All the samples were given an ID and their corresponding quality grade data were provided by KAPE. The data collected were (MAR, ranging from 1 (for poor) to 9 (highest quality), loin eye muscle area (LEA, cm^2) and backfat thickness (BFT, cm) and carcass weight (CAW, kg). The carcass traits of animals used in this study are summarized in Table 1.

Genomic DNA isolation: Approximately, 1 gram of tissue samples was used for DNA isolation using a commercial kit (Wizard DNA extraction kit, Promega) following the manufactures guidelines. The integrity of the DNA was checked by Agarose gel electrophoresis. The DNA quality and quantity was checked on a NanoDrop 1000 spectrophotometer (Thermo Scientific, USA). The genomic DNAs were then stored at -70˚C until further use.

Genotyping: Allele-specific PCR (AS-PCR) approach was followed as described by (Ye *et al*. 2001) for genotyping the samples. The primers were designed using the primer select program in DNAstar software (version 6.0), with options of amplification lengths (500 bp) and 60% GC contents. The allele-specific primers were modified to include a mismatch at -2 base from the 3' end. The PCR was carried out using outer common primer, Forward–F0 (TTAT GGAA TTTG GGG AAT GG) and Reverse-R0 (TTCT CCCC ATAC CAGT TTGC) which amplified a 412 bps fragment and inner allele specific primers F1 (TCTC TCAT CTAG TTAA GGAT TGCA GTC***G***) and F2 (TCTC TCAT CTAG TTA AGGA TTG CAGT C***A***) ~~**Hi**~~ in separate reactions, amplifying a 252 bp fragment.

The PCR reaction included 50 ng genomic DNA, 1x reaction buffer, 2.5mM dNTP, 10 pmoles of each outer primers and 8 pmoles of allele specific primer, 1 unit *Taq* DNA polymerase (Genetbio, Korea) in a 20 µl reaction. The PCR condition was as follows; an initial-denaturation at 95˚C for 5 min, followed by 35 cycles of denaturation at 95˚C for 45sec, annealing at 59℃ for 30sec and extension at 72˚C for 1 min and a final extension at 72˚C for 5 mins on a thermal cycler (Veriti®96-well, Applied Biosystems, USA). 5µl of the PCR product was visualized on an agarose gel stained with a fluorescent dye (Morning bio, Korea) under UV light. So the PCR reaction had either one of the following combination of primers F0+F1+R0 or F0+F2+RO. The presence or absence of the band was scored and the data was then used for association studies.

Data analysis: After cleaning the data for missing phenotypic information and genotypic information, 1103 samples were used for the statistical analysis.

The statistical analyses were performed using Statistical Analysis System (Institute 1999). Analysis of Variance (ANOVA) based on general linear model (GLM) was performed to verify the genotype effects on carcass traits. The model used was as follows

$Y = \mu + G + S + bA + e$,

Where; Y = observed for the target trait, μ = overall mean of the target trait, G = genotype effect, S = sex effect, b = the regression coefficient for age, A = age (covariate) and e = random error. Least squares means were compared using Fisher's least significant difference tests with comparison error rate of 0.005. Additive genetic effects were estimated by the difference between estimates for the two homozygous genotypes and the dominance deviation was estimated by the difference between the solution for the heterozygous genotype and the average of the solutions for the two homozygous.

RESULTS

Genotype analysis: In this study 1103 samples collected from throughout the country were used for genotyping to verify and validate the genetic effect of SNP g.29172875A>G, so that they can be used as a standardized marker for marbling trait.

Genotyping was performed using Allele Specific PCR that included two outer and one inner allele specific primers. The outer primers amplified a standard band (412 bp) and the allele specific primers amplified only if the target allele was present (252 bp) (Figure No. 1). The genotypes of the SNP g.29172875A>G were scored based on the presence or absence of the band. The genotype frequencies (Table 2) for AA, AG and GG were 0.152, 0.439 and 0.408, respectively. The allele frequency (Table 2) for A and G were estimated to be 0.3722 and 0.6278, respectively. No significant departure from Hardy-Weinberg equilibrium (HWE) was detected.

Association analysis: As shown in Table 2, the genotype orders for MAR and LEA were as follows AA > GG > AG. The association between genotypes and marbling was significant ($P<0.0001$), showing that genotypes AA (5.866 ± 0.15) had a more significant effect on MAR followed by GG (5.325 ± 0.08) and AG (5.034 ± 0.09), with significant additive genetic effects ($P<0.001$). The genotypes AA (91.372 ± 0.85) and GG (90.869 ± 0.50) had a more significant effect on LEA ($P<0.001$) than AG (88.473 ± 0.52) with significant additive effects ($P<0.010$). However, no significant association with BFT and CAW was detected in this analysis.

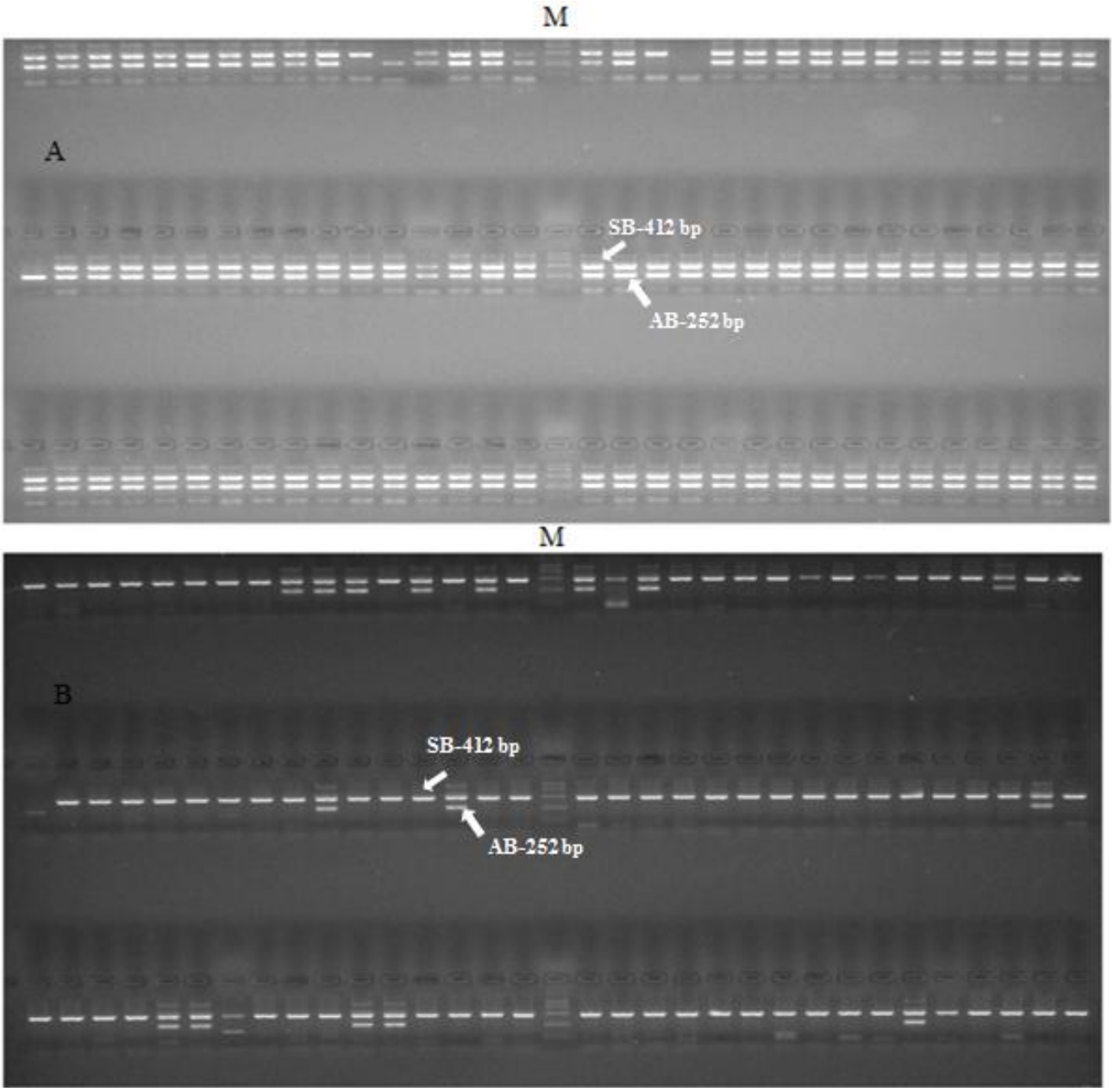


Figure 1. Agarose gel electrophoresis image showing the amplification of the target region using Allele specific PCR approach. The larger size band (412 bp) in both A and B is that of the common Forward and Reverse primer and the smaller sized band (252 bp) is that of the allele specific primers. M - 1Kb Marker.SB –Standard band, AB- Allele Specific Band.

Table 1. Descriptive summaries of carcass traits for Hanwoo cattle used in this study.

	MAR (1-9)	BFT (mm)	LEA (cm^2)	CAW (kg)
Mean	5.3	12.5	90.0	427.9
SD	2.1	5.7	11.3	50.0
Minimum	1	1	37	194
Maximum	9	49	133	623

MAR: Marbling score (1, low to 9, high), BFT: Backfat thickness, LEA: Loin eye area, CAW: Carcass weight

Table 2. Least squares means and standard errors for carcass traits of genotypes in *CALN* and their Genotype and Allele frequencies.

SNP	Genotypes		Alleles		MAR	BFT	LEA	CAW
	No	Frequency (%)	No	Frequency(%)	(1-9)	(mm)	(cm^2)	(kg)
					P = < 0.0001	P = 0.7718	P = 0.0010	P = 0.7544
AA(A)	168	0.152	821	0.3722	5.866 ± 0.15[a]	12.458 ± 0.42[a]	91.371 ± 0.85[a]	425.250 ± 3.78[b]
AG	485	0.439			5.034 ± 0.09[c]	12.562 ± 0.26[a]	88.473 ± 0.52[b]	428.381 ± 2.31[a]
GG (G)	450	0.408	1385	0.6278	5.325 ±0.08[b]	12.300 ± 0.25[a]	90.869 ± 0.50[a]	428.290 ± 2.23[a]
Effect								
Additive					0.831 ±0.17***	-01.03 ± 0.50	2.897 ± 1.00**	3.131 ± 4.43
Dominance					-0.248 ± 0.25	0.419 ± 0.71	1.893 ± 1.42	2.948 ± 6.29

[a, bandc] Different letters denote statistically significant differences between genotypes. **P < 0.01, ***P < 0.001.
MAR: Marbling score, BFT: Backfat thickness, LEA: Loin eye area, CAW: Carcass weight.

DISCUSSION

Hanwoo beef with high quality attributes like marbling is in huge demand in Korea, due to consumer preferences regarding meat quality (Kim and Boyd 2004). Starting from the 1970's the quality of Hanwoo has been hugely improved (Kim *et al*. 2014). There has been a concerted effort to constantly keep improving the breed for high quality traits through marker assisted selection program (MAS). In order to aid in this path, effective grading mechanisms were put in place by 1994 and therefore the improvement achieved could be effectively measured. By the year 2010 huge improvements in trait measurements were achieved reaching 407 kg CAW, MAR score of 5.0, 13 mm BFT, 86 cm^2 LEA. The future livestock improvement goal announced in 2013 has targeted to achieve a MAR score of 6.5 and LEA of 90 cm^2 for Hanwoo (http://ebook.mifaff.go.kr/ preview/ viewer/main.php?site=2&menuno=2&previewno=1381& iframe=0&dlbt=). To achieve this, markers with strong association for MAR and LEA has to be identified for marker based selection programs.

Using GWAS analysis we previously identified the SNP g.29172875A>G located in the intron of *CALN* gene to have a significant association with MAR and LEA. Previous studies however, have not reported the effect of this gene (*CALN)* or its variants on carcass traits in beef cattle. *CALN* is a calmodulin like protein that has been shown to play an inhibitory role in the release of neurotransmitter and Ca^{2+} signalling (Shih et al 2009). The genotypes showed a high association with MAR and LEA with significant additive effects. Since the population genotyped was different to the one used in the GWAS analysis, they are fit to be used as a robust molecular marker for marbling and loin eye muscle area in Hanwoo cattle.

In conclusion, 1,103 Hanwoo tissue samples were collected from KAPE throughout Korea, to check genetic effects of the SNP (g.29172875A>G) located in the *CALN* gene on carcass traits. Association analyses found significant associations for the SNP with MAR and LEA showing significant additive effects.

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