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Identification and Analysis of *PIT1* Polymorphisms and Its Association with Growth and Carcass Traits in Korean Cattles (Hanwoo)

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한우에서 Pituitary-specific Transcription Factor (*PIT1*) 유전자와 경제 형질과의 연관성 분석

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SUMMARY

Pituitary-specific transcription factor (*PIT1*) 유전자는 동물의 성장을 조절하고 근육 형성에 관여하는 유전자로서 최근에는 단일염기다형성 변이가 한우를 비롯한 동물에서 관찰되었으며, 한우의 경제 형질과 연관성이 보고되었다. 본 연구는 *PIT1* 유전자의 단일염기다형성 변이가 한우에서 성장 인자에 미치는 영향과 경제 형질에 대한 유전자형간 육종가와의 상관성에 대해 알아보고자 하였다. 도체 성적을 보유하고 있는 한우 후보종모우 집단 268두를 대상으로 *PIT1* 유전자 A1256G 다형성을 조사하여 유전자형의 빈도를 분석하였고 각각의 유전형에 따른 기본적인 검정 성적을 바탕으로 경제 형질과의 연관성을 비교 분석하였다. 268두의 한우에서 *PIT1* 유전자의 A1256G 유전자형 빈도는 *MseI* 제한 효소를 사용했을 때 A 유전자 빈도(0.37)보다 G 유전자 빈도(0.62)가 높게 나타났다. 통계적 분석을 통하여 각 유전자형에 대한 경제 형질과의 관련성을 분석한 결과, 각 유전자형간에 12개월령 체중 (body weight 12, BW12)에서 유의한 차이를 보였고, 등지방 두께 육종가 (Backfat thickness-estimated breeding value, BF-EBV)와도 유의한 차이가 있었지만 ($p<0.05$), marbling score (MS), carcass weight (CW), *M. longissimus dorsi* area (LDA) 등 다른 경제 형질과는 통계학적으로 유의한 차이가 없었다. *PIT1* 유전자의 A1256G 다형성은 한우의 성장과 도체체중에 관여하는 인자로 작용하는 것으로 보여진다.

(Key words : pituitary-specific transcription factor, single nucleotide polymorphisms, Korean cattles, economic traits, growth rate)

INTRODUCTION

Meat quality and carcass composition are the most economically important traits in beef cattle production. Carcass and meat quality traits are typical quantitative characteristics controlled by a number of genes. Mutations in their (sequences may alter animal performance as well as their breeding values. Recently, advances in molecular genetic techniques focused on genome analysis open new possibilities for genetic evaluation of economically important traits in farm animals. These molecular technologies allow the isolation and mapping of specific regions of the genome that influence quantitative traits. Marker or gene-assisted selection is a promising strategy for genetic improvement of such traits. The DNA markers could be particularly useful for genetic evaluation of economic traits for which phenotypic measurements or data are difficult or expensive to obtain. A large number of potential candidate genes in livestock have been recognize to date but relatively little is known about which markers could be useful in evaluation of specific traits (Shin and Chung, 2007). Some DNA polymorphisms may affect gene expression at ever, the effect of a single polymorphism may be masked by an interaction with environmental and genetic factors. Such interactions may mask the effects of a given gene through a genotypic disequilibrium among genes and/or a strong environmental influence (Franco *et al.*, 2005).

The candidate gene approach is justified when genes previously identified in the species of interest or other species have functions related to the traits of interest (Yu *et al.*, 1995). *Pituitary-specific transcription factor (PIT1)* was chosen as a

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candidate gene to investigate its associations with growth, meat quality and carcass composition traits in the cattle (Zhao *et al.*, 2004). *PIT1* is known as the pituitary-specific activator of the growth hormone in several mammals (Brunsch *et al.*, 2002). *PIT1* is such a candidate gene for growth and muscle development in the pig and cattle which belongs to the large *POU* domain family (Jiang *et al.*, 2004; Tanaka *et al.*, 1999). The POU-domain was originally identified as a highly conserved region of 150 to 160 amino acids found in three mammalian transcription factors, *Pit-1, Oct-1, Oct-2* and also in the product of the nematode gene *unc-86*. *PIT1* is the pituitary specific transcription factor 1 required for the expression of the growth hormone gene, the prolactin gene and the thyroid-stimulating hormone β subunit gene. Mutations in the *PIT1* gene were first observed in dwarf mice and later in human families with a combined pituitary hormone deficiency. Thus, dwarf individuals have been found due to the lack of the *PIT1* gene activity. Three *PIT1* polymorphims have been detected using the endonucleases *BamHI, MspI* respectively *RsaI*. A number of studies have shown that *PIT1* gene is associated with variation in growth and carcass traits in animals. An interval mapping study by Yu *et al.* (1999) confirmed a QTL for birth weight at the estimated *PIT1* position and detected evidence of a QTL for the to the first back fat thickness approximately 20cM away from the *PIT1* gene (Brunsch *et al.*, 2002; Yu *et al.*, 1999; Yu *et al.*, 1995).

The aim of the present study was to evaluate the association of SNPs in the *PIT1* gene with growth and economic traits in Korean cattle.

MATERIALS AND METHODS

1. Animals and Data Collection

Two hundred and sixth eight Korean cattles were used in this study. Cows from the base population were randomly assigned to the selection lines. The Korean native cattle genomic DNA was isolated from white blood cells and was used to genotype the *PIT1* genes.

The growth and economic traits assessed included body weight (BW), carcass weight (CW), *M. longissimus dorsi* area (LDA), backfat thickness (BF), breeding value of carcass weight (CW-EBV), breeding value of marbling score (MS-EBV), breeding value of backfat thickness (BF-EBV), breeding value of *M. longissimus dorsi* area (LDA-EBV) and marbling score (MS). The traits also involved in these analysis were 6 month weight (BW6), 12 month weight (BW12) and 18 month weight (BW18).

Live weights were determined before slaughter. Mean of live weights was 551.1±55.0 kg. Means of carcass traits, *M. longissimus dorsi* area and backfat thickness were 323.9±35.0 kg (CW), 75.9±8.3 cm (LDA) and 0.8±0.3 cm (BF). Birth weight, weight at slaughter corrected for slaughter age, carcass meat weight data and back fat thickness data were available. Birth weight was recorded within a day after birth; weight at slaughter was recorded as the carcass weight at slaughter age (Franco *et al.*, 2005). The carcass data included were CW, LDA, BF and MS. BF and LDA were measured at the 12th- and 13th- rib interface. MS for quality grade was evaluated on a cross section of the longissimus muscle at the 12th-to 13th-rib interface. MS is scored on a scale from 1 to 7 with 7 being associated with the most marbling (Table 1).

2. Sequencing

Sequencing of the genes were performed for a subset of 26 study subjects and gene-specific primer pairs were used to amplify the *PIT1* genes. Direct sequences were generated from both stands with ET terminator Cycle Sequencing Kit on a

Table 1. Overall means and standard deviation (SD), minimum (Min) and maximum (Max) of traits analyzed in this study (*n*=268)

Economic traits	Mean	SD	Min	Max
Body weight (kg) at slaughter	551.1	55.0	320.0	710.0
Carcass weight (kg)	323.9	35.0	187.0	423.0
M. longissimus dorsi area (cm)	75.9	8.3	30.0	99.0
Backfat thickness (cm)	0.8	0.3	0.3	2.1
Marbling score (1~7)	2.2	1.4	1.0	7.0
Carcass weight-EBV (kg)	2.4	10.2	−35.9	29.6
Backfat thickness-EBV (cm)	0.5	1.6	−3.6	8.8
M. longissimus dorsi area-EBV (cm^2)	0.6	2.7	−15.0	10.2
Marbling score-EBV (1~7)	0.03	0.6	−1.0	2.5
Body weight at 6 month	165.6	27.6	70.9	247.2
Body weight at 12 month	273.9	36.7	174.0	362.0
Body weight at 18 month	421.1	44.2	303.1	543.2

EBV, estimated breeding value.

PTC 200 peltier thermal cycler (MJ Research, USA). The extension reaction in a 10 μl volume was performed and extension products were electrophoresed on MegaBACE DNA Analysis System (Amersham Biosciences, USA). Searching sequence mutation was using the seqMAN II software (DNA Star Inc.) (Oh *et al.*, 2006).

3. *PIT1* Genotyping

A pair of primers was designed basing upon the bovine *PIT1* gene sequence using Primer 3 software (http:/www-genome.wi.mit.edu/cgi-bin/primer3-www.results.cgiv) (Table 2). The *PIT1* gene was genotyped by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism). The PCR was conducted in 10 μl volumes, each containing 100 ng of genomic DNA, 10× PCR buffer (100 mM Tris pH 8.9, 50 mM KCl, 15 mM MgCl, 0.01% gelatin, 0.1% Triton X-100, 10 mg/mlL BSA), 10 pmol of each primer, 40 μM of dNTPs and 0.5 unit Taq DNA polymerase (Promega, USA). The condition of PCR was a first denaturation step of 4 min at 94℃ followed by 35 cycles, each consisting of 40 sec at 94℃, 30 sec at 56.4℃, 2 min at 72℃ and then a final step of 10 min at 72℃ using Peltier Thermal Cycler 200 (MJ Research, USA). After amplification, 10 μL of the PCR amplicon were digested using 1 U of *MseI* restriction enzyme by incubation overnight at 37℃ and the bands were detected in 2% agarose gels stained with ethidium bromide (10 mg/ml) and photographed under UV light.

4. Statistical Analysis

Allele and genotype frequencies were calculated by simple allele counting method. Hardy-Weinberg equilibrium in examined population was tested by comparing expected and observed genotype frequencies using a chi-square test. The association between the genotypes of *PIT1* candidate gene and economic traits was evaluated with the least square method (GLM procedure of SAS software package, SAS Institute Inc., 2002) using following statistical linear model (Shin and Chung, 2007).

$$Y_{ijkl} = \mu + YS_i + P_j + M_k + e_{ijkl}$$

Where Y_{ijkl} is the observation of the carcass traits, μ is the overall mean for each trait, YS_i is the effect of i_{th} year and season of calving, P_j is the fixed effect of j_{th} parity, M_k is the fixed effect of K_{th} SNP genotype and e_{ijkl} is the random residual effect.

RESULTS

1. SNP Identification and Genotyping

DNA samples from twenty six unrelated Korean cattle were amplified and sequenced. We identified two polymorphic sites (SNPs) by sequencing analysis of the bovine DNA. The two primer pairs of *PIT1* gene were designed for the PCR-RFLP genotyping of these SNPs on genomic DNA samples. The primers amplified a 610 bp fragment in exon 2. These SNPs were confirmed by sequencing analysis of the PCR products corresponding to positions 1256 (A/G SNP) in exon 2 of the bovine *PIT1* gene. The sequences have been deposited in GenBank database with accession numbers Y15995. These transitions resulted in no changes to the amino acid substitution. These SNPs can be detected by PCR-RFLP using digestion of the amplified fragment with *Mse* I (T$^{\blacktriangledown}$TAA) for SNPs at sites 1256 in exon 2. Two alleles, A and G, showing three different genotypes AA, AG and GG were observed for each RFLP. For the A1256G SNP of *PIT1* gene, the A allele showed one fragment 610 bp, while the T allele showed three fragments.

The allele and genotype frequencies of the *PIT1* gene estimated for the Korean cattle population are shown in Table 3. In A1256G SNP, the frequencies of allele G (62.2) were higher than those of allele A (37.8). The genotype frequencies were as follows: 53.8% AG, 35.3% GG and 10.9% AA for the

Table 3. Distribution of geneotypes and alleles for *PIT1* gene in Korean

SNP	Genotype frequencies (%)			Allele frequencies (%)	
	G/G	A/G	A/A	G	A
A1256G	35.3	53.8	10.9	62.2	37.8

Table 2. Primer sequences, amplified region and fragment size for PCR amplification in SNP genotyping of *PIT1* gene

Primer	Primer sequence (5' - 3')	Nucleotide substitution	Amplified region	Product size (bp)	Restriction enzyme	GenBank accession No.
PIT1-F	AAACCATCATCTCCCTTCTT	A1256G	Exon2	610	*MseI*	Y15995
PIT1-R	AATGTACAATGTGCCTTCTGAG					

A1256G SNP in *PIT1*.

2. Gene-specific SNP Marker Association Analysis

By statistical analyses, at the SNP marker of A1256G in exon 2, there was a significant effect on the BF-EBV and BW12. Animals with the genotype GG had higher BF-EBV compared with AA genotype ($p<0.05$). This marker also showed a significant dominance effect for the BF-EBV ($p<0.05$) (data not shown). No significant associations were observed between A1256G SNP genotypes and other traits. Also, the A1256G genotypes were significantly associated with BW12 and were not significantly associated with most BW.

BF-EBV and BW12 traits were highest in "G" allele homozygotes (BF-EBV = 1.1±0.1 and BW12 = 279.1±3.7), intermediate in "A/G" heterozygotes (BF-EBV = 0.6±0.1 and BW 12 = 270.5±2.8), and lowest in "A" allele homozygotes (BF-EBV = 1.0±0.3 and BW12 = 272.7±8.1) ($p<0.05$). Results of the gene-specific SNP marker association analysis for the *PIT1* gene are presented Table 4.

Table 4. Least squares means and standard errors for economic traits of *PIT1* (A1256G) genotype in Korean cattle

Traits	SNP genotype		
	A/A	A/G	G/G
BW (kg)	564.9 ±13.0	549.2 ±4.6	558.2 ±6.0
BW6	168.6 ± 6.4	162.4 ±2.3	166.2 ±2.9
BW12	272.7 ± 8.1^{ab}	270.5 ±2.8^{b}	279.1 ±3.7^{a}
BW18	417.6 ±10.4	421.3 ±3.7	427.5 ±4.8
CW (kg)	332.5 ± 8.2	320.9 ±2.9	326.9 ±3.8
LDA (cm^2)	77.0 ± 1.9	76.6 ±0.6	76.7 ±0.9
BF (cm)	0.8 ± 0.07	0.8 ±0.02	0.8 ±0.03
MS (1~7)	2.6 ± 0.3	2.1 ±0.1	2.3 ±0.1
CW-EBV (kg)	6.4 ± 2.3	2.4 ±0.8	4.8 ±1.0
LDA-EBV (cm^2)	1.0 ± 0.6	0.9 ±0.2	0.7 ±0.2
BF-EBV (cm)	1.0 ± 0.3^{ab}	0.6 ±0.1^{b}	1.1 ±0.1^{a}
MS-EBV (cm)	0.07± 0.1	−0.00±0.0	−0.01±0.06

BW, body weight; CW, carcass weight; LDA, *M. longissimus dorsi* area; BF, backfat thickness; MS, marbling score; EBV, estimated breeding value. BW6, 12, 18, body weight at 6, 12, 18 month.

[a,b] Different superscripts within columns differ significantly ($p<0.05$).

DISCUSSION

Birth weight (BWT), preweaning average daily gain (PWADG) and average daily gain on feed (ADGF) are three growth traits that have an important impact on the profitability in the beef cattle industry. Therefore, breeding for optimal BWT and larger gains is a major consideration in beef cattle breeding programs. Mapping of QTL and identification of causative genes that affect growth traits will greatly enhance the progress towards this goal (Li *et al.*, 2004). The isolation and sequence analysis of the bovine *PIT1* gene described here have led to the characterization of genetic variation at this gene locus, which enabled us to study the association between one polymorphism at the *PIT1* gene locus and muscle development-related meat growth traits (Yu *et al.*, 1999).

The bovine *PIT1* genomic DNA sequence reported in this study allowed the identification of one SNP (A1256G in exon 2). This SNP creat a polymorphic *Mse* I restriction sites. In the case of exon 2, although the mutation is located in the coding region, it does not alter the amino acid sequence of the *PIT1* gene. The A1256G SNP may not be a causative or close to the causative mutation that affects the carcass and meat quality traits in the populations of Korean cattle examined in this study (Shin and Chung, 2007).

PIT1 is a pituitary-specific activator of the growth hormone gene. *PIT1* can activate the prolactin gene in cell culture and *PIT1* is involved in thyroid stimulating hormone (TSH-β, thyrotropin) expression (Turton *et al.*, 2005). *PIT1* gene mutations have been identified as the cause of genetic disorders resulting from multiple hormonal deficiencies in both rodents and humans (Turton *et al.*, 2005; Yu *et al.*, 1995). At least four hypotheses could explain the association between the *PIT1* polymorphism and economic traits: (1) This polymorphism could be involved in processing *PIT1* mRNA to generate an RNA molecule with variations covering exon 1,2 in this region; (2) This polymorphism can be involved in alternative transcript production to produce variations in other regions of the gene. Various transcripts have been described for the *PIT1* gene (3) This polymorphism could be involved in expressing its own *PIT1* gene (we have not tested the latter two hypotheses); and (4) The *PIT1* gene is linked to one or more genes that are also involved in these traits (Franco *et al.*, 2005). This hypothesis is supported by the polygenic inheritance of quantitative traits as indicated by Yu *et al* (Yu *et al.*, 1999). Based

on the known physiological effects of the *PIT1* gene and on the results of the present study, we suggest that the polymorphism within *PIT1* affects the traits studied here by controlling growth rate (Franco *et al.*, 2005). *PIT1* polymorphisms (A1256G) were significantly associated with cattle birth weight at 12 month and BF-EBV in Korean cattles. In the Edinburgh resource families *PIT1* polymorphisms were significantly associated with birth weight as well (Brunsch *et al.*, 2002). Yu *et al.* found an associations of *PIT1* haplotypes and the markers around *PIT1* with early weights and growth rate (Yu *et al.*, 1999). No agreement of results with provided associations between *PIT1* genotypes and back fat measures as well as birth weight could be established. Stancekova *et al.* (1999) found a significant effect of *PIT1/MspI* polymorphism on back fat thickness but no significant effect of *PIT1/RsaI* polymorphism on this trait. We found a significant effect of *PIT1/MseI* polymorphism on BF-EBV but no significant effect of back fat thickness. The reason for this is the extreme origin of the breeds. Additional research with commercial breeds is necessary to attribute the shaping of these traits to the *PIT1* genotype.

One possible limitation of the present study is a relatively small variation of the subjects and this lead to insufficient statistical power to detect the presumably modest effects in phenotypes that may be associated with *PIT1* variation. Further studies are needed to establish the mechanisms of action associated with the presence of this allelic variation. The exact molecular and physiological mechanisms underlying the association of the SNP with the growth traits reported in the present study are unknown. Although data sets for some of the individual SNP genotype were limited, these results indicate that *PIT1* or a closely linked gene to those genes may be important in growth and meat quality traits in Korean cattles. Thus, other SNP of *PIT1* or other genes in the chromosomal regions should be studied. Comparative genomics in combination with the use of functional information from different species will greatly improve the understanding of the genetic bases of complex biological processes influencing health and performance of domestic animals.

CONCLUSION

We have identified one polymorphisms in *PIT1* for genotyping in Korean native cattle. Statistical analysis revealed that *PIT1* A1256G showed significant association with cattle growth and carcass traits. Replication of our finding in an independent data set and/or functional validation of polymorphisms should be performed in the future.

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