# Association of SNP Marker in *IGF-I* and *MYF5* Candidate Genes with Growth Traits in Korean Cattle

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**ABSTRACT :** Growth rate is one of the economically important quantitative traits that affect carcass quantity in beef cattle. Two genes, bovine insulin-like growth factor I (*IGF-I*) and myogenic factor 5 (*MYF5*), were chosen as candidate genes for growth traits due to their important role in growth and development of mammals. The objectives of this study were to determine gene-specific single nucleotide polymorphism (SNP) markers of the *IGF-I* and *MYF5* positional candidate genes and to investigate their associations with growth traits in Korean cattle. Genotyping of the SNP markers in these candidate genes was carried out using the single strand conformation polymorphism (SSCP) analysis. The frequencies of A and B alleles were 0.72 and 0.28 for *IGF-I* gene and 0.39 and 0.61 for *MYF5* gene, respectively, in Korean cattle population examined. The gene-specific SNP marker association analysis indicated that the SNP genotype in *IGF-I* gene showed a significant association ($p<0.05$) with weight at 3 months (W3), and cows with AB genotype had higher W3 than BB genotype cows. The SNP genotype of *MYF5* gene was found to have a significant effect ($p<0.05$) on the weight at 12 months (W12) and average daily gain (ADG), and cows with BB and AB genotypes had higher W12 and ADG compared with cows with AA genotype, respectively. However, no significant association between the SNP genotypes and any other growth traits was detected. The gene-specific SNP markers in the *IGF-I* and *MYF5* candidate genes may be useful for selection on growth traits in Korean cattle. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 8 : 1061-1065)***

**Key Words** : *IGF-I*, *MYF5*, Candidate Gene, Growth Traits, Korean Cattle

## INTRODUCTION

Korean cattle (Hanwoo) as an indigenous beef cattle are known to have an inferior ability to produce meat because of a low milk producing capacity and slow growth rate, while having a relatively favorable meat quality (Kim and Lee, 2000). Thus, the Korean cattle industry aimed to increase meat production and to produce high quality meat to meet the increasing demand of the consumer for high quality beef. Genetic improvement of Korean cattle has been accomplished using traditional methods of selection based on phenotypic information. Even though the nature of genes influencing economically important traits is in general not known, genetic selection in this way has been very successful.

Recent developments in molecular genetic techniques have made it possible to identify genetic variation at specific loci and the association between variation at gene affecting quantitative traits (quantitative trait loci, QTL) and production traits. Gene or marker-assisted selection (MAS) is a promising strategy for genetic improvement of economically important quantitative traits such as growth and carcass traits in beef cattle (Gerbens et al., 2000). Molecular marker-assisted selection will first require identification of candidate genes or anonymous genetic markers associated with the traits of interest. The candidate gene approach was proposed by many geneticists as a produce to identify genes with significant phenotypic performance effects for possible use in genetic improvement programs (Rothschild and Soller, 1997; Jiang et al., 2002a, b). Candidate genes are selected on the basis of known relationship between physiological or biochemical processes and quantitative traits of interest. Insulin-like growth factor I (*IGF-I*) plays an important physiological role in the growth and development of mammals by acting locally in specific organs or globally through circulating *IGF-I* (Werner et al., 1994; Ge et al., 2001). In meat producing animals like cattle and pigs, myofiber numbers have been related to growth capacity (Soumillion et al., 1997). Myogenic factor 5 (*MYF5*) gene plays a role in myogenic lineage determination and/or myocyte differentiation (Braun et al., 1989; Li et al., 2004). Therefore, the *IGF-I* and *MYF5* genes are important candidate genes for the identification of genetic markers for growth and carcass traits in livestock. The objectives of this study were to determine gene-specific single nucleotide polymorphisms (SNP) of these two candidate genes and to investigate their associations to growth traits in Korean cattle.

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Received September 22, 2004; Accepted February 19, 2005

## MATERIALS AND METHODS

### Animals and phenotypic data

The population of Korean cattle sampled in this study was taken at the Hanwoo Experiment Station of National Livestock Research Institute in Rural Development Administration of Korea. Data were collected from a total

**Table 1.** Primer sequences, amplified region and fragment size for PCR amplification in SSCP analysis of the *IGF-I* and *MYF5* genes

| Candidate gene | Primer sequences (5′ to 3′) | Nucleotide change | Amplified region | Fragment size (bp) | GenBank accession no. |
|---|---|---|---|---|---|
| *IGF-I* | ATTACAAAGCTGCCTGCCCC<br>ACCTTACCCGTATGAAAGGAATATTCGT | T/C | Exon 1 | 248 | AF210383 |
| *MYF5* | CTTCATCCCCATGAATTGCT<br>CCAGGTTGCTCTGAGTTGGT | A/G | Intron 2 -Exon 3 | 243 | M95684 |

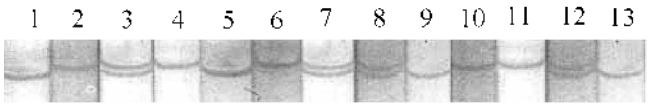


**Figure 1.** PCR-SSCP analysis of the *IGF-I* gene in Korean cattle. The PCR-amplified 248 bp DNA fragments were denaturated by heating and separated by 12% polyacrylamide gel. The SSCP bands were detected by silver staining. Lanes 2, 4, 6, 10 and 11 were of AA genotype; lanes 3, 7, 8 and 12 were of AB genotype; and lanes 1, 5, 9 and 13 were of BB genotype.

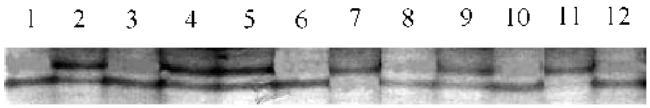


**Figure 2.** PCR-SSCP analysis of the *MYF5* gene in Korean cattle. The PCR-amplified 243 bp DNA fragments were denaturated by heating and separated by 12% polyacrylamide gel. The SSCP bands were detected by silver staining. Lanes 7 and 11 were of AA genotype; lanes 2, 4, 5 and 9 were of AB genotype; and lanes 1, 3, 6, 8, 10 and 12 were of BB genotype.

of 280 cows registered in the official performance-testing program. The data included weight at 3 months (W3), weight at 6 months (W6), weight at 12 months (W12) and average daily gain (ADG) of Korean cattle.

**SNP marker genotyping**

For each animal, genomic DNA was extracted from blood peripheral lymphocytes using a salting out procedure by Miller et al. (1988). Genotyping of the gene-specific SNP marker for the *IGF-I* and *MYF5* was carried out using single strand conformation polymorphism (SSCP) analysis. Based on the published nucleotide sequence information of the bovine *IGF-I* (GenBank no. AF210383) and *MYF5* (GenBank no. M95684) genes, pairs of oligonucleotide primers were synthesized to amplify *IGF-I* and *MYF5* fragments, respectively. The primer sequences, location and size of the amplified fragments are shown in Table 1. PCR was performed in a reaction volume of 20 μl using 50 ng of genomic DNA, 10 pmol of each primer, 1×PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP and 1.0 unit *Taq* polymerase. Amplification conditions were 94°C for 3 min followed by 35 cycles of 94°C for 1 min, 58°C for 1 min and 72°C for 1 min, with a final extension at 72°C for 5 min.

For SSCP analysis, 4 μl of the PCR product was mixed with 16 μl of gel loading solution containing 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol. The mixture was then denaturated at 96°C for 7 min, cooled in ice for 5 min and loaded on a nondenaturing 12% polyacrylamide gels (49:1 acrylamide to bis-acrylamide). Electrophoresis was performed in 1× Tris borate (pH 8.3)-EDTA buffer at 250 V for 4 h at room temperature. After electrophoresis, the DNA fragments in the gel were detected by silver staining. SSCP genotypes were identified by differential migration due to fragment size.

**Statistical analysis**

Allele frequencies at the two candidate loci were calculated by simple gene counting method. Hardy-Weinberg equilibrium in a 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 *IGF-I* or *MYF5* candidate gene and growth traits. The linear model used was as follows:

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

Where $Y_{ijkl}$ is the observation of the growth traits (BW, W3, W6, W12 and ADG), 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 effect of $j_{th}$ parity, $G_k$ is the fixed effect of $K_{th}$ *IGF-I* genotype (k = AA, AB, BB) or *MYF5* genotype (k = AA, AB, BB) and $e_{ijkl}$ is the random residual effect.

Additive genetic effects were estimated by pair-wise comparison of the two homozygous genotypes and the dominance effects were calculated as the deviation of the heterozygote effect from the average of the two homozygous genotypes. The estimated effects were tested for significance using the t-test.

## RESULTS

Figure 1 and 2 show the SSCP patterns of the *IGF-I* and *MYF5* genes, respectively. Two alleles were identified and designated as A and B, respectively on the basis of electrophoretic mobility in the gel. All three possible genotypes, AA, AB and BB were observed in the two candidate genes, respectively. The allele and genotype frequencies of *IGF-I* and *MYF5* genes in Korean cattle are

**Table 2.** Genotype distribution and allele frequency for *IGF-I* and *MYF5* genes in Korean cattle population (n = 280)

| Candidate gene | Genotype Frequencies | | | Allele Frequencies | | $X^2$ |
|---|---|---|---|---|---|---|
| | AA | AB | BB | A | B | |
| *IGF-I* | 164 (58.6)[1] | 74 (26.4) | 42 (15.0) | 0.72±0.02[2] | 0.28±0.02 | 34.27** |
| *MYF5* | 25 (8.9) | 137 (48.9) | 118 (42.2) | 0.33±0.02 | 0.67±0.02 | 2.86 |

[1] Figures within parenthese are the percentage. [2] Standard error. ** $p<0.01$.

**Table 3.** Association between *IGF-I* genotype and growth traits and estimates of additive and dominance effects

| Growth traits[1] | *IGF-I* genotype (LSM±SE) | | | | |
|---|---|---|---|---|---|
| | AA | AB | BB | Additive effect | Dominance effect |
| BW (kg) | 23.46±1.41 | 22.85±1.38 | 22.38±1.50 | -1.13±0.87 | 0.87±0.62 |
| W3 (kg) | 70.72±6.71[ab] | 75.05±5.06[a] | 62.74±4.46[b] | -3.16±0.95* | 0.46±0.43 |
| W6 (kg) | 124.56±11.31 | 128.72±7.01 | 123.62±7.53 | -0.91±0.84 | 0.20±0.17 |
| W12 (kg) | 186.52±18.09 | 191.33±11.02 | 189.62±12.65 | -0.87±0.46 | 0.32±0.31 |
| ADG (g) | 0.51±0.07 | 0.52±0.06 | 0.52±0.04 | -0.03±0.02 | -0.01±0.04 |

[a,b] Means with different superscripts within same column are significantly different ($p<0.05$).

* The effect was significant at $p<0.05$.

[1] BW: birth weight; W3: weight at 3 months; W6: weight at 6 months; W12: weight at 12 months; ADG: average daily gain.

**Table 4.** Association between *MYF5* genotype and growth traits and estimates of additive and dominance effects

| Growth | *MYF5* genotype (LSM±SE) | | | | |
|---|---|---|---|---|---|
| | AA | AB | BB | Additive effect | Dominance effect |
| BW (kg) | 22.26±1.87 | 22.74±1.46 | 22.76±1.29 | -0.56±0.66 | 0.23±0.18 |
| W3 (kg) | 68.89±6.02 | 70.38±5.33 | 74.24±4.76 | -0.88±0.76 | -0.19±0.24 |
| W6 (kg) | 120.60±10.21 | 125.21±8.95 | 129.17±8.83 | -0.43±0.38 | 0.31±0.36 |
| W12 (kg) | 186.37±13.38[a] | 198.14±12.73[b] | 200.86±14.21[b] | -1.13±0.92* | 0.45±0.63 |
| ADG (g) | 0.51±0.08[a] | 0.54±0.03[b] | 0.55±0.06[b] | -0.04±0.08* | 0.01±0.03 |

[a,b] Means with different superscripts within same column are significantly different ($p<0.05$). * The effect were significant at $p<0.05$.

[1] BW: birth weight; W3: weight at 3 months; W6: weight at 6 months; W12: weight at 12 months; ADG: average daily gain.

shown in Table 2. At the *IGF-I* gene, the genotype frequencies were 58.6, 26.4 and 15.0% for AA, AB and BB, respectively, and the allele frequencies for the A and B were 0.72 and 0.28, respectively. This locus was found to be in Hardy-Weinberg equilibrium. For the *MYF5* gene, frequencies of the AA, AB and BB genotypes were 8.9, 48.9 and 42.2%, respectively. The frequency of the A allele was 0.33 and that of the B allele was 0.67. However, this locus exhibited a larger number of animals with the AA and BB genotypes than was expected from the Hardy-Weinberg principle.

Results of the gene-specific SNP marker association analysis for the *IGF-I* and *MYF5* genes are presented Table 3 and 4, respectively. The effects of the genotypes and additive and dominance effects are also shown in Table 3 and 4. At the SNP marker of *IGF-I* gene, there was a significant additive effect on the W3 and allele A was associated with increases in the trait value. Cows with AB genotype had higher W3 than cows with BB genotype ($p<0.05$). Calves with the genotype AB gained 12.31 kg more than calves with the BB genotype. However, there were no significant effects of *IGF-I* genotypes on BW, W6, W12 and ADG. For the SNP marker of *MYF5* gene, significant additive effects on the W12 and ADG were observed. Cows with the genotype BB or AB had higher W12 and ADG compared with cows with AA genotype ($p<0.05$). Cows with the genotype AA had W12 and ADG that were 84.58 kg and 0.035 g lower than the average W12 and ADG of cows with the genotype AB or BB. No associations were found between the genotypes of the *MYF5* gene and other growth traits (BW and W6).

## DISCUSSION

Growth traits are one of the most economically important traits in beef cattle production. Therefore, breeding for faster growth 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). In previous studies (Li et al., 2002a, b), *IGF-I* and *MYF5* genes were both considered to be positional candidate genes underlying two of the three chromosomal regions (0 to 30 cM and 70 to 80 cM) as they were mapped at the chromosomal locations of 19.0 cM and 73.5 cM, respectively (Grosse et al., 1999). In this study, we examined the association between the two SNP marker genotypes of *IGF-I* and *MYF5* candidate genes and the

growth traits in Korean cattle. In bovine, a SNP (T to C transition) was detected at 512 bp 5′ to the first codon of the first exon of the *IGF-I* gene (Ge et al., 1997). A SNP by a point mutation (A→G) was identified at the 1,948 bp position of the intron 2 region for the *MYF5* gene (Drogemuller and Kempers, 2000).

Using the PCR-SSCP analysis, we detected two SNP allelels in *IGF-I* and *MYF5* genes of Korean cattle, respectively. Our results showed that PCR-SSCP analysis is useful technique to screen SNP genotype of candidate genes and can be used instead of RFLP and sequence analysis.

Ge et al. (2001) reported that allele frequencies of A and B for *IGF-I* gene in Angus beef cattle were 0.64 and 0.36, respectively, and genotype frequencies of AA, AB and BB were 43.3, 41.3 and 15.4%, respectively. The difference in allele frequencies between the high and low *IGF-I* lines (A allele 0.75 in high line vs. 0.52 in low line) was highly significant. The genotype frequencies of Korean cattle were different from the data given by Angus cattle, but the allele frequencies of Korean cattle were very similar to those observed in high *IGF-I* line of Angus cattle. At the *MYF5* gene, Drogemuller and Kempers (2000) reported that allele frequencies of A and B were 0.32 and 0.68 in 48 unrelated German Holstein cattle, respectively. This result was very similar to those of Korean cattle.

Genetic improvement of meat-producing animals focuses on growth and lean meat deposition. Several candidate genes might be selected as potentially affecting the meat content of carcass on the basis of analysis of molecular mechanisms controlling muscle development. The *IGF-I* gene has been considered to play an important role in growth and development of mammals. A dinucleotide (CA) repeat polymorphism in the 5′-flanking region of the *IGF-I* gene has been identified in cattle and swine (Kirkpatrick, 1992). In beef cattle, Moody et al. (1996) reported that *IGF-I* was significantly associated with weaning weight, yearling weight and birth weight. Ge et al. (2001) also reported that the genotype BB of *IGF-I* was found to have higher weight gain during the first 20 d after weaning and higher on-test weight. In our study, genotype effect of the SNP of *IGF-I* on the weight at 3 months was observed in Korean cattle. Recently, Li et al. (2004) reported that no significant association between the SNP of *IGF-I* and the growth traits was detected in two commercial lines of *Bos taurus*, whereas there was only a significant dominance effect on birth weight when the data from the two commercial lines were pooled.

Growth traits are muscle cell and tissue-related traits. The number of muscle fibers at birth appears to determine the maximal lean meat growth capacity in cattle and pigs (Handel and Stickland, 1988; Hanset et al., 1982). Development of muscle fibers is regulated by the *MyoD* gene family consisting of myogenin gene (*MYOG*), MYF3 (*MYOD1*), *MYF5* and MYF6. The *MyoD* family genes coding for muscle regulatory factors, seem to be the proper candidate genes for meat deposition in carcass. They control entire muscle development from commitment and proliferation of muscle precursor cells, through muscle fibre formation and their postnatal maturation and functions (Te Pas and Visscher, 1994; Hughes and Schiaffino, 1999). *MYF5* and *MYOD1* are expressed during proliferation of myoblasts (Te Pas et al., 1999). Therefore, the *MYF5* gene has also been considered to play an important role in growth and development of mammals. Knockout mouse experiments demonstrated that the gene *MYF5* had an effect on muscle development (Braun et al., 1994). Li et al. (2004) reported that the SNP in *MYF5* showed a significant association with preweaning average daily gain (PWADG) and with average daily gain on feed (ADGF). The significant association between the SNP of *MYF5* gene and the W12 and ADG were also found in Korean cattle. These results suggest that the gene may be one of the causative genes that control growth traits in beef cattle or that the gene is very close to the causative gene(s) (Li et al., 2004). The SNP examined here was located in one of the intron region. An association study using more SNP of *MYF5* including SNP in the promoter and exon regions and further functional tests of candidate genes may lead to the identification of causative mutation(s) in the gene(s) that controls growth traits in beef cattle. Consequently, our results indicate that the *IGF-I* and *MYF5* are candidate genes that influences some growth traits in Korean cattle and these candidate genes could be used in a marker assisted selection program to improve growth traits in Korean cattle. Further investigations are needed in other populations of Korean cattle to verify the associated effects of the gene-specific SNP marker, as well as the effect of the other DNA polymorphisms in positional candidate genes.

## ACKNOWLEDGEMENTS

This study was supported by a grant from BioGreen 21 Program, Rural Development Administration, Republic of Korea.

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