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# Correlation Analysis between the Breeding Value of Carcass Traits in Hanwoo (Korean Brown Cattle), *Bos Taurus*, L. and Spot Intensity on Two-dimensional Gel Electrophoresis

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**ABSTRACT :** In order to investigate the genetic marker associated with economic performance in Hanwoo (Korean Brown Cattle), proteomic approach was used. Breeding values were estimated from performance tested steers. The top 20 and bottom 19 steers based on carcass weight (CW), eye muscle area (EMA), backfat thickness (BF) and marbling score (MS) evaluation for one progeny testing period was used. Meat samples dissected from longissimus dorsi muscles were taken from the slaughter house and analyzed for two-dimensional gel electrophoresis. A total of 102 significant spots out of total 146 on each gel were detected and compared with the reference gel (synthetic gel) to be evaluated. Four candidate spots for marbling score were identified: 205, 84, 204 and 198. The study confirmed the relationship between breeding values of economic traits of Hanwoo cattle and spot intensity. (**Key Words :** Hanwoo, Proteomics, Spot Intensity, Two-Dimensional Gel Electrophoresis, Breeding Value)

## INTRODUCTION

Most quantitative traits are exceptionally complex with varying contributions of genetic susceptibility and interacting environmental factors. Predisposition to a phenotypic range for a complex trait such as carcass weight (CW) resulted from combinations of relatively small effects of DNA variations within a large number of unidentified polygene, known as quantitative trait loci (QTL). Although molecular biology has yielded significant gains in understanding complex traits, it is very difficult to find the genetic marker of major gene or QTL location for economic traits. New analytical approach like proteomics allows the simultaneous investigation of gene effects with protein structure and function. It involves the global analysis of cellular proteins using diverse technologies, such as 2-D gel electrophoresis, mass spectrometry and bioinformatics (Gorg et al., 2000; Friedman et al., 2004; Xu et al., 2004). Proteomics is the study of the expression pattern of genes within a tissue by the analysis of proteins that result from transcription and translation of DNA. Recently, researches concerned with diseases, especially cancer and obesity, have been studied by using proteomics in human and mouse (Houtman et al., 2003; Butterfield et al., 2003). However, proteomics approach for tissue-specific proteins in muscle was not developed very well until now.

Inconsistency in meat tenderness has been identified as one of the major problems facing the beef industry (Morgan et al., 1991; Boleman et al., 1998). Extensive knowledge of meat tenderness variation and meat palatability has been developed for the longissimus dorsi muscle which has low variation in sensory-detectable connective tissues and sarcomere length. Most of the variation in tenderness resulted from the variation in the extent of proteolysis of myofibrillar and cytoskeletal proteins (Koohmaraie, 1992; 1994). The importance of longissimus dorsi muscles development to the economics of beef production is well established. It contains intramuscular fat which is an important factor for evaluating beef quality. The identification of protein biomarkers will be helpful for improving production performance in cattle so that the animals which produce this protein could be identified and if that expressions is inherited by its offsprings that would lead to better selection tools for improving marbling.

Progeny testing program had been implemented in

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**Table 1.** Correlation coefficient between spot intensity and breeding values of Hanwoo

| Spot No. | No. of animals with spots | Carcass weight (EW) | Eye muscle area (EMA) | Backfat (BF) | Marbling Score (MS) |
|---|---|---|---|---|---|
| 24 | 4 | NS | NS | 0.944* | NS |
| 25 | 17 | NS | NS | 0.583* | NS |
| 42 | 13 | NS | 0.487* | NS | NS |
| 69 | 24 | NS | NS | -0.436* | NS |
| 75 | 12 | NS | 0.454* | NS | NS |
| 77 | 35 | NS | NS | -0.441* | NS |
| 84 | 9 | NS | NS | 0.884* | -0.516* |
| 91 | 26 | 0.479* | NS | NS | NS |
| 96 | 12 | NS | 0.574** | NS | NS |
| 158 | 4 | 0.622** | NS | NS | NS |
| 166 | 14 | NS | NS | 0.471* | NS |
| 198 | 22 | NS | NS | NS | 0.484* |
| 204 | 9 | NS | NS | NS | -0.514* |
| 205 | 14 | NS | NS | -0.607* | -0.531* |
| 258 | 30 | NS | 0.324** | NS | NS |
| 261 | 15 | NS | NS | 0.564* | NS |
| 262 | 5 | 0.864** | NS | NS | NS |
| 316 | 8 | NS | NS | -0.527* | NS |
| 318 | 9 | NS | 0.697** | NS | NS |

NS: not significant, ** Significant, p<0.01, * Significant, p<0.05.

Korea since1982 in order to evaluate candidate sires based on the traits which are considered vital to Hanwoo beef cattle production. The candidate young bulls were evaluated in terms of breeding value (BV) for each trait under consideration. Breeding value is defined the value of an individual as a contributor of genes to next generation. That is, breeding value represents the overall effects of an individual's genes for those traits. High coefficient between breeding value and spot intensity represents high possibility as a candidate marker for improving economic performance. Therefore, the objective of this study was to find correlation between breeding values and spot intensity for various carcass traits of Hanwoo. The study aimed to detect candidate protein spots that could be used as potential breeding markers.

## MATERIALS AND METHOD

### Estimation of breeding value for economic traits and correlation analysis

The data used in this study were collected at National Livestock Research Institute in Cheonan City, Korea. The number of records of steers on progeny test from 1998 up to 2003 were 1,676 heads while the total number of animals in the pedigree file was 35,140. This study used the data of the 33rd progeny testing using 39 steers.

The progeny test scheme started with the insemination of candidate sires in order to produce 50 calves for each sire. Since the cows inseminated with the candidate sires were owned by farmers, male calves produced were purchased at 130% of the prevailing market price. Prior to purchase the calf had to free from diseases and parentage check through DNA testing. Ten heads from each sire were purchased and brought to the performance testing centers owned by the institute. Testing period was from 6 to 24 months of age. Thereafter, the animals were slaughtered and carcass characterizations were conducted. The carcass traits considered in this study were cold carcass weight (CW), eye muscle area (EMA), back-fat thickness (BF) and marbling score (Intramuscular Fat, MS). For the measurements of carcass traits, the weight before slaughter was measured and CW was measured after cold storage over 24 h. EMA and BF were measured at the 13th rib-first lumbar section. The marbling score was measured on the same region and classified into seven levels (1-7).

The animals were ranked based on the carcass traits measured following the Animal Model with BLUP. Based on the performance from the Estimated Breeding Values (EBV) of marbling score, total 39 samples were divided into two groups consisting of the top 20 and bottom 19 animals. Correlation ananlysis between breeding values and spot intensity was undertaken. T-test was carried out to test the significance between high EBV animals and the low EBV animals.

The Animal Model with BLUP as a mixed linear model estimated individual breeding value using the following model:

$$Y_{ijkl} = \mu_i + YS_{ij} + L_{ik} + A_{ijkl} + b_i D_{ijkl} + e_{ijkl}$$

where, $Y_{ijkl}$ is observation $_{ijkl}$ for the traits, $\mu_i$ is the population mean for the *i*th trait, $YS_{ij}$ is the fixed effect of *j*th year-season for the *i*th trait, $L_{ik}$ is the fixed effect of *k*th location of birth for the *i*th trait, $A_{ijkl}$ is the random additive genetic effect of individual for the *i*th trait, $b_i$ is the linear regression coefficients for the *i*th trait, $D_{ijkl}$ is the age at slaughter in day for the *i*th carcass trait and $e_{ijkl}$ is the random residual associated with observation *ijkl*.

### Sample preparation of longissimus dorsi muscle

The bovine longissimus dorsi was obtained from Garak dong slaughterhouse in Seoul. Bovine longissimus dorsi was frozen directly after dissection and stored at -196°C until protein analysis. Before homogenization bovine longissmus dorsi and rump tissues were weighed and thawed in the buffer (7 M urea, 2 M thiourea, 4% CHAPS, 50 mM DTT). The homogenization (100 mg/ml) was performed at room temperature with a rotor blade homogenizer, 4,000 rpm, followed by centrifugation at 13,000 rpm, 4°C, for 30 min. Aliquot was taken from the supernatant and stored at -80°C until the 2-D analysis was

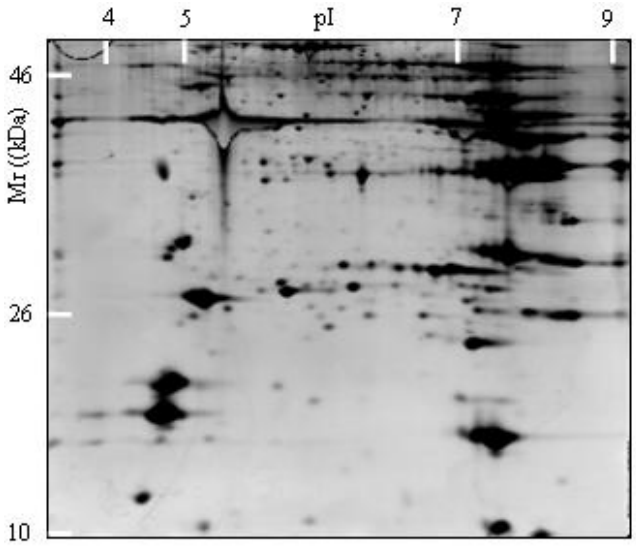


**Figure 1.** Two-dimensional electrophoresis analysis of top estimated breeding value (EBV) in bovine longissimus dorsi.

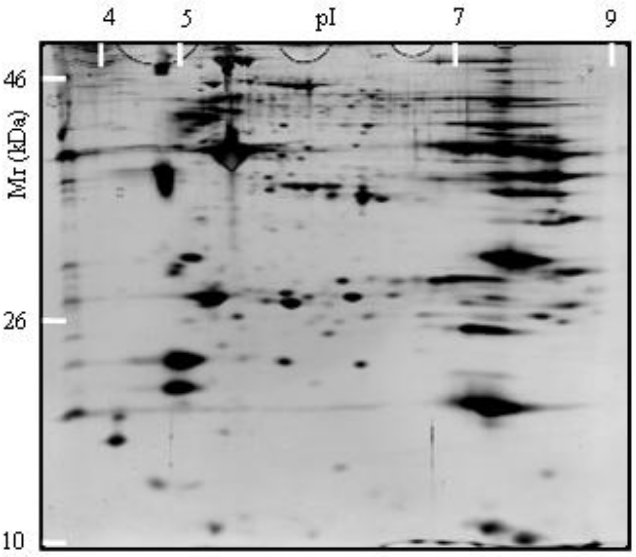


**Figure 2.** Two-dimensional electrophoresis analysis of low estimated breeding value (EBV) in bovine longissimus dorsi.

performed.

**Two-dimensional polyacrylamide gel electrophoresis**

Protein extracts (100 μg) were applied in IPG strips pH 3-10 L (Amersham biosciences). Isoelectrofocusing was conducted using pH 3-10 Pharmalytes at 12 h rehydration, 1 h at 500 V gradient, 1 h at 8,000 V gradient, and 13 h at 8,000 V steady-state level. Upon completion of the first dimension, strips were incubated with gentle shaking in an equilibration buffer (50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, a trace of bromophenol blue) containing 1% DTT for 15 min, and 2.5% iodoacetamide for 15 min. For second dimension, strips were transferred to the tops of 12.5% polyacrylamide gels containing SDS.

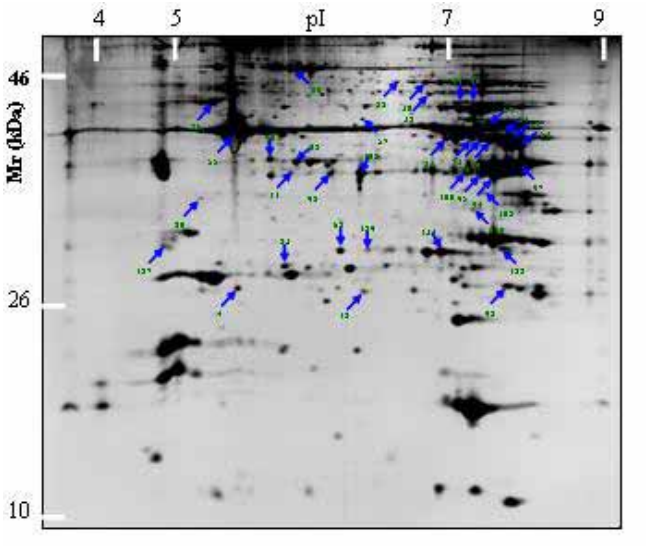


**Figure 3.** Silver-stained two-dimensional gel electrophoresis map of bovine longissimus dorsi muscle. The arrows indicate differential protein spots related with a correlation coefficient and statistical significance.

Gels were run for 12 h. After fixation, gels were stained with silver stain kit, and scanned using ImageScanner flatbed scanner. Protein spots were analyzed using ImageMaster 2-D Elite software (Amersham Biosciences Inc., Sweden).

**Image acquisition and data analysis**

Gel was scanned using an ImageScanner flatbed scanner. Computer-assisted image analysis was performed using ImageMaster 2-D Elite software package (Amersham Biosciences Inc., Sweden). The relative spot volume was directly related to protein concentration. All master images reported below were obtained from 2-DE analysis of real samples from a different tissue.

## RESULTS AND DISCUSSION

A total of 146 spots by two-dimensional electrophoresis were detected and compared with the reference gel (synthetic gel) for evaluation. To identify the changes in the protein components between top and low breeding values, the two-dimensional gel electrophoresis was used (Chatterjee and Chatterjee, 2005). Figure 1 presents two-dimensional electrophoresis gel of the top estimated breeding value (EBV) animal while Figure 2 shows the low EBV animal. Protein spots were compared and matched by ImageMaster 2-D Elite Software. Based on the statistical comparison of spot intensity among gels, there were 102 significant spots selected (Figure 3) and correlation analysis with the breeding value of carcass weight, eye muscle area, back-fat thickness and marbling score were conducted. High correlation coefficient between spot intensity and

breeding value represents high possibility as a candidate marker for improving economic performance.

For the trait cold carcass weight, spot no. 262 had the highest positive correlation between BV and spot intensity which was 0.864 ($p<0.01$) although the spot was detected in 5 animals only. Other significant spots were 158 and 91. For the trait EMA, the following spots revealed significant correlation between BV and spot intensity ($p<0.01$) spot numbers 318, 96, 258, 42 and 75. As far as BF was concerned, spot no. 24 had correlation coefficient of 0.944 ($p<0.05$) although it was revealed in 4 animals only followed by spot no. 84 with 0.884 shown in 9 animals. For the trait MS, the following spots revealed significant correlation coefficients ($p<0.01$); 205, 84, 204 and 198. Since the major concern for palatability is expressed in marbling score, the candidate spots were 205, 84, 204 and 198. Furthermore, spots no. 84 and 205 interacted with the trait BF and MS implying the presence of associated genes that could cause genetic interaction. Also, it might suggest the presence of major gene that was expressed in both traits although it required proteomics approach to elucidate the sequence of the gene.

The relationship of marbling score to beef palatability had been the subject of numerous investigations since marbling score estimation on the level of intramuscular fat deposition at the 12 to 13$^{th}$ rib region of the longissimus. A study conducted by Brendan et al. (2004) on a pilot project that demonstrated the feasibility and potential proteomics based technology in the study of protein expression in beef muscles. They found 136 protein spots which have altered protein expression levels as a consequence of change in breed, age and time after slaughter. Several QTL's were detected between centimorgans 46 and 76 for marbling score on chromosome 9 as reported by Casas et al., 2003. Likewise, in 2000 his group detected QTL on the telomeric region of chromosome 27 and same region in chromosome 10. Evidence suggested that one QTL affecting marbling score on the same chromosome in different studies. These results suggested that some proteins selected by proteomic analysis would be useful candidate markers for improving the economic performance of cattle.

Use of markers in pre-selection, as for the entry of young bulls into progeny test programs, does provide opportunities to assess the success of marker assisted selection. For example, by correlating EBV following progeny test with the pre-selection criterion, or by comparing progeny test EBV of pre-selected bulls to those of their full brothers, which may have been progeny tested by other organizations. Currently, Cowan et al. (1997) found an increase in mean EBV and in number of progeny tested dairy bulls. When two EBV's are estimated with perfect accuracy and based on independent sources of information, the expected value of the simple correlation between those two EBV is equal to the genetic correlation between the two traits. With less than perfectly estimated EBV, the expected simple correlation is a function of accuracy and genetic correlation (L. D. Van Vleck, personal communication). In general rank correlations do not have this expectation. However, rank correlations are preferable when EBV based on subsets of related data were compared.

Despite the power of statistical methods, and the wealth of genetic markers, there are few examples in which the genetic basis of a quantitative trait has been thoroughly dissected. Lessons learned from the statistical (QTL) analysis of complex phenotypes have the potential to be applied to an analysis of the variation of gene expression, and in this way a more complete understanding of functional networks of genes and other action might arise. This knowledge could benefit our current ability to understand the connection between phenotype and genotype.

## IMPLICATION

The study confirmed the relationship between breeding values of economic traits of Hanwoo cattle and spot intensity. Four candidate spots for marbling score were identified: 205, 84, 204 and 198. Therefore, the identification of these spots by proteomics needs to be undertaken. Once the enzymes had been identified, the animals that produce this enzyme to a higher degree and determine the mode of expression to its offsprings which would lead to better selection tools for marbling score in Hanwoo.

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