Asian-Aust. J. Anim. Sci.
Vol. 21, No. 3 : 309 - 313
March 2008

www.ajas.info

Association Analysis between Insulin-like Growth Factor Binding Protein 3 (IGFBP3) Polymorphisms and Carcass Traits in Cattle

Hyun Sub Cheong, Du-Hak Yoon[1], Lyoung Hyo Kim, Byung Lae Park, Hye Won Lee, Sohg Namgoong Eun Mi Kim, Eui Ryong Chung[2], Il-Cheong Cheong[1] and Hyoung Doo Shin*

Department of Genetic Epidemiology, SNP Genetics, Inc., Seoul, Korea

ABSTRACT : The insulin-like growth factor binding protein 3 (IGFBP3) has been investigated as a candidate gene for growth promoting effects in beef cattle and a modulator of IGF bioactivity. Previously, we have reported twenty two sequence variants discovered in Korean native cattle (Hanwoo). In this study, we examined the association between gene-specific polymorphisms of IGFBP3 and cold carcass weight (CW) and marbling score (MS) among Korean native cattle. Among twenty two polymorphisms, four common polymorphic sites (-854G>C, -100G>A, +421G>T and +3863C>A) were genotyped in our beef cattle (n = 437). Statistical analysis revealed that one common polymorphism in the promoter region (-854G>C) showed putative associations with MS ($p = 0.03$). IGFBP3 variation/haplotype information analyzed in this study will provide valuable information into strategies for the production of a commercial line of beef cattle. (**Key Words :** IGFBP3, Cold Carcass Weight, Marbling Score, Polymorphism)

INTRODUCTION

Insulin-like growth factor-I (IGF-I) has important roles in growth and milk synthesis (Colick, 1998). Because of the complexity of IGF interactions in tissues, the complex has been termed the IGF system. The IGF system consists of ligands (insulin, IGF-I and IGF-II), receptors (insulin-R, IGF-R1 and IGF-R2), six IGF binding proteins (IGFBP1 to 6) and proteases that may alter IGFBPs and change IGF and IGFBPs affinities (Giudice et al., 1990). Even though all cells seem to synthesize IGFBPs, each has the potential for different synthesis patterns (Clemmons, 1997). Numerous studies indicate that IGFBPs are synthesized and secreted by mammary cells from breast cancer cell lines (Yee, 2002), mouse cells (Baumrucker et al., 1993; Skaar and Baumrucker, 1993) and bovine cells (Campbell et al., 1991; Woodward et al., 1996; Baumrucker et al., 2003).

Insulin-like growth factor binding protein-3 (IGFBP3) is one of a family of IGFBPs. IGFBPs are multifunctional proteins that transport and stabilize IGF in the circulation and modulate the effects of IGF on a variety of cellular functions. IGFBPs may also regulate cells by IGF independent mechanisms. IGFBP3, a 40-45 kDa glycoprotein, is by far the most abundant IGFBP in the circulation, where it has a central role in regulating IGF bioavailability to the tissues by forming heterotrimeric complexes with IGF-I or IGF-II and an 85 kDa glycoprotein (Baxter, 2001).

In some cases, IGFBP3 is proposed to act by inhibiting the access of IGF to the IGF-I receptor, which mediates most of the actions of IGF. However, the effects of IGFBP3 on IGF dependent cellular functions are complex, with both stimulatory and inhibitory actions reported (De Mellow and Baxter, 1988). In recent years, important studies with cells lacking IGF-I receptor have revealed that IGFBP3 can be inhibitory to cell growth even in the absence of IGF signaling (Valentinis et al., 1995). But there are no reported QTL around IGFBP3 genomic position.

In our previous study, twenty two sequence variants were identified: one in the promoter region, twenty in introns and one in exons in IGFBP3 in Korean cattle (Kim et al., 2004). In this study, we examined IGFBP3 gene as one of most promising candidate genes in meat production and quality in cattle. Here, we present four polymorphisms (-854G>C, -100G>A, +421G>T and +3863C>A) genotyped in IGFBP3 and the results of association study with meat quantity and quality in Korean cattle (Hanwoo, *Bos taurus coreanae*).

* Corresponding Author: H. D. Shin. Tel: +82-2-2026-4288, Fax: +82-2-2026-4299, E-mail: hdshin@snp-genetics.com
[1] National Institute of Animal Science, RDA, Suwon, Korea.
[2] Department of Biotechnology, Sangji University, Wonju, Korea.
Received August 9, 2006; Accepted April 7, 2007

Table 1. Genotypes and allele frequencies of 4 polymorphisms in IGFBP3

Name	Position	Genotype				Frequency	Heterozygosity
-854G>C	Promoter	G	CG	C	N	0.371	0.467
		173	195	62	430		
-100A>G	5'UTR	A	AG	G	N	0.470	0.498
		124	208	98	430		
+421G>T	Intron1	G	GT	T	N	0.322	0.436
		174	245	18	437		
+3863C>A	Intron2	C	AC	A	N	0.230	0.354
		248	171	14	433		

Rare allele frequencies, heterozygosity calculated in Korean male beef cattle.

MATERIALS AND METHODS

Animals and phenotypic data

The Korean native cattle genomic DNA samples were obtained from 437 steers produced from 76 sires used in progeny testing program of National Livestock Research Institute (NLRI) of Korea. The dams were inseminated randomly with young sires. All steers were fed for 731.39±16.53 days period under tightly controlled feeding program in Daekwanryeong and Namwon branch of NLRI. Live weights were determined before slaughter. Mean of live weights was 539.93±51.96 kg. Yield grades for carcasses were determined by cold carcass weight (CW). After a 24-h chill, CW weights were measured, and then the left side of each carcass was cut between the last rib and the first lumbar vertebrae to determine marbling score (MS). Mean of CW was 311.47±33.39 kg. MS was determined by assessing the degree of marbling in the cut surface of the ribeye. The degree of marbling was evaluated according to the Korean Beef Marbling Standard (1 = trace, 7 = very abundant) (APGS, 1995). Mean of MS was 2.25±1.36.

Genotyping by single-base extension and electrophoresis

For genotyping of polymorphic sites, amplifying and extension primers were designed for single-base extension (SBE) (Vreeland et al., 2002). Primer extension reactions were performed with the SNaPshot ddNTP Primer Extension Kit (Applied Biosystems). To clean up the primer extension reaction, one unit of SAP (shrimp alkaline phosphatase) was added to the reaction mixture, and the mixture was incubated at 37°C for 1 h, followed by 15 min at 72°C for enzyme inactivation. The DNA samples, containing extension products and Genescan 120 Liz size standard solution were added to Hi-Di formamide (Applied Biosystems) according to the recommendation of the manufacturer. The mixture was incubated at 95°C for 5 min, followed by 5 min on ice, and then electrophoresis was performed using the ABI Prism 3100 Genetic Analyzer. The results were analyzed using the program of ABI Prism GeneScan and Genotyper (Applied Biosystems). Information regarding the primers is available on our website (http://www.snp-genetics.com/user/additional_list.asp).

Statistics

We examined a widely used measure of linkage disequilibrium between all pairs of biallelic loci, lewontin's d' (|D'|) (Hedrick, 1987), and r^2. Haplotypes and their frequencies were inferred using the algorithm developed by Stephens et al. (Stephens et al., 2001). Phase probabilities of each site were calculated for each individual using this software. Association analyses with carcass traits (CW and MS) were performed using a mixed effect model treating "sire" as a random effect. Age at slaughter was also included in the model. Other covariates were not available for this analysis. We fit a full model that includes all four polymorphisms in the model. We think the full model is more appropriate for controlling the closely linked polymorphisms more effectively. Putative transcription factor binding sites were examined using the software-TFSEARCH: Searching Transcription Factor Binding Sites (version 1.3, http://www.cbrc.jp/research/db/TFSEARCH.html) (putative score>0.9) based on TRANSFAC database (Heinemeyer et al., 1998).

RESULTS AND DISCUSSION

Among previously identified twenty two polymorphisms (Kim et al., 2004), four polymorphisms (-854G>C, -100G>A, +421G>T and +3863C>A) were selected for larger-scale genotyping based on LDs (only one polymorphism if there are absolute LDs (r^2 = 1) and frequencies in IGFBP3. The minor allele frequencies of genotyped polymorphisms were 0.371 (-854G>C), 0.470 (-100G>A), 0.322 (+421G>T) and 0.230 (+3863C>A) respectively, in Korean cattle (Table 1 and Figure 1). By pair-wise linkage analysis with 437 Korean cattle, which were used for genotyping, we have found that four sets of polymorphisms in strong LDs were identified (Table 2), and four major haplotypes (freq.>0.1) were constructed among these four polymorphisms (Table 3).

Associations of IGFBP3 polymorphisms with carcass

Table 2. Linkage disequilibrium coefficient ($|D'|$ and r^2) among *IGFBP3* polymorphisms

	SNPs	$\|D'\|$			
		-854G>C	-100G>A	+421G>T	+3863C>A
r^2	-854G>C	-	0.973	0.714	0.936
	-100G>A	0.501	-	0.947	0.957
	+421G>T	0.415	0.386	-	0.431
	+3863C>A	0.443	0.245	0.115	-

Table 3. Haplotypes of IGFBP3

Haplotype	-854G>C	-100G>A	+421G>T	+3863C>A	Frequency
ht1	G	G	G	C	0.453
ht2	C	A	T	A	0.143
ht3	C	A	T	C	0.125
ht4	G	A	G	C	0.106
ht5	C	A	G	A	0.079
ht6	G	A	T	C	0.050
Others	-	-	-	-	0.043

Others contain rare haplotypes: *CAGC*, *GGTC*, *GAGA*, *GGGA* and *CGGC*.

Table 4. Association analyses of the IGFBP3 polymorphisms with carcass traits (CW and MS) among Korean native cattle

Trait	Loci	Location	Genotype			p
			C/C*	C/R*	R/R*	
CW	-854G>C	Promoter	173(309.78±33.61)	195(312.02±31.00)	62(312.94±38.30)	0.51
	-100A>G	5'UTR	124(309.10±35.33)	208(312.51±33.94)	98(312.62±29.89)	0.33
	+421G>T	Intron1	174(312.05±32.98)	245(311.21±34.17)	18(309.33±27.60)	0.99
	+3863C>A	Intron2	248(310.11±33.56)	171(313.28±32.42)	14(313.07±41.55)	0.45
	ht2	-	306(311.30±34.17)	122(311.57±31.19)		0.77
	ht3	-	323(311.29±32.71)	103(311.59±35.36)	2(313.50±41.72)	0.97
	ht4	-	341(312.23±33.04)	83(309.95±33.96)	4(268.00±9.02)	0.08
MS	-854G>C	Promoter	173(2.32±1.40)	195(2.20±1.31)	62(1.82±1.03)	**0.03**
	-100A>G	5'UTR	124(1.99±1.20)	208(2.30±1.37)	98(2.26±1.36)	0.12
	+421G>T	Intron1	174(2.20±1.31)	245(2.22±1.36)	18(1.67±0.59)	0.38
	+3863C>A	Intron2	248(2.31±1.37)	171(2.04±1.26)	14(2.14±1.17)	0.08
	ht2	-	306(2.16±1.30)	122(2.25±1.36)		0.57
	ht3	-	323(2.24±1.36)	103(2.03±1.20)	2(2.00±0.00)	0.17
	ht4	-	341(2.14±1.30)	83(2.41±1.41)	4(1.75±0.96)	0.23

Genotype and haplotype distributions, means, standard deviations (SD), p values controlling for sire and age at slaughter as covariates was shown.
*C/C, C/R, and R/R represent the common allele, heterozygotes and homozygotes for the rare allele, respectively.
Significant associations are shown in boldface.

traits were analyzed using the mixed-effect model with sire and age as covariates. Sire was treated as a random effect and age a fixed effect. Among four major haplotypes (freq.>0.1) identified, *ht1* was not used for further analysis because it was almost (>95%) tagged by a single SNP, -100G>A (Table 3).

Statistical analysis revealed that polymorphisms in IGFBP3 showed no significant association with cold carcass weight (CW) and marbling score (MS). One common polymorphism in promoter region (-854G>C) showed putative associations with MS (p = 0.03). The highest MS was detected in G allele homozygotes (MS = 2.32), intermediate in C/G heterozygotes (MS = 2.20) and lowest in C allele homozygotes (MS = 1.82). In further haplotype association analysis, no possible association was detected with carcass traits (Table 4). But when Bonferroni corrections were strictly adopted, associated p value could not retain the significance (the threshold of significance would be 0.0063 (4 polymorphisms and 2 phenotypes analyzed).

By statistical analysis, it would be hard to tell that -854G>C is critical for the carcass quality grade, MS. But the promoter SNP, -854G>C, was analyzed as located in the consensus sequence of E2F binding site by software, TFSEARCH (putative score>0.90) (Figure 1). Moreover, the expression of IGF-I transported and stabilized by IGFBP3 is negatively regulated by an E2F binding site in IGF-I promoter region (Li and Baserga, 1996; Porcu et al., 1994). The possibility that -854G>C may alter IGFBP3 protein regulation needs to be evaluated in future studies.

It has been shown that SNPs in the UTR of genes can affect gene expression (Pesole et al., 2001), and it is possible that -100A>G variant in the 5'UTR of IGFBP3 might have an influence on gene expression. Although our data show that the 5'UTR polymorphism of IGFBP3 is not associated with CW and MS, it may have some implications for other IGFBP3 functions in cattle.

As one of six related proteins that modify IGF bioactivity in complex (Shimasaki and Ling, 1991; Jones and Clemmons, 1995; Zapf, 1995), both inhibiting and enhancing effects of IGFBP3 on IGF-I action have been reported (De Mellow and Baxter, 1988; Gopinath et al., 1989; Ernst and Rodan, 1990; Marcelli et al., 1995). Therefore concentrations of IGFBPs are potential indicators of IGF-I status and may provide insight into an animal's growth or carcass potential. Moreover, production of IGFBP3 is stimulated by growth hormone (GH) (Cohick et al., 1992; Assy et al., 1997), suggesting that animals with increased body weight may exhibit increased IGFBP3 due to endogenous GH activity. But in previous studies, IGFBP3 was not related to carcass backfat, intramuscular fat, *longissimus dorsi* area and hip height (Connor et al., 2000). IGFBP3 activity also revealed no differences in gilts selected for daily gain estimation (Clutter et al., 1995) and in obese sheep compared with lean sheep (McCann et al., 1997). In the present study, four polymorphisms (-854G>C, -100G>A, +421G>T and +3863C>A) in IGFBP3 also showed no strong associations with the quantity trait (CW) and the quality trait (MS) in Korean cattle in exception of a putative association of -854G>C with MS. Although it would be difficult to explain the reason of association with marbling score but not carcass weight, our results suggest that IGFBP3 has some other function involved in fat metabolism and/or fat contents of muscle.

In summary, we found that polymorphisms (-854G>C, -100G>A, +421G>T and +3863C>A) in IGFBP3 might be not associated with carcass traits of Korean cattle. The putative association of -854G>C with the carcass quality (MS) will be needed further statistical and functional studies.

ACKNOWLEDGMENT

This Study was supported by Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

REFERENCES

APGS. 1995. Report of business for animal products grading. Animal products grading system, national livestock co-operatives federation, korea.

Assy, N., Z. Hochberg, T. Amit, Z. Shen-Orr, R. Enat and Y. Baruch. 1997. Growth hormone-stimulated insulin-like growth factor (IGF) I and IGF-binding protein-3 in liver cirrhosis. J. Hepatol. 27:796-802.

Baumrucker, C. R., W. M. Campana, C. A. Gibson and D. E. Kerr. 1993. Insulin-like growth factors (IGF) and IGF binding proteins in milk; sources and functions. Endocr. Regul. 27:157-172.

Baumrucker, C. R., C. A. Gibson and F. L. Schanbacher. 2003. Bovine lactoferrin binds to insulin-like growth factor-binding protein-3. Domest Anim. Endocrinol. 24:287-303.

Baxter, R. C. 2001. Signalling pathways involved in antiproliferative effects of IGFBP-3: A review. Mol. Pathol. 54:145-148.

Campbell, P. G., T. C. Skaar, J. R. Vega and C. R. Baumrucker. 1991. Secretion of insulin-like growth factor-I (IGF-I) and IGF-binding proteins from bovine mammary tissue *in vitro*. J. Endocrinol. 128:219-228.

Clemmons, D. R. 1997. Insulin-like growth factor binding proteins and their role in controlling IGF actions. Cytokine Growth Factor Rev. 8:45-62.

Clutter, A. C., L. J. Spicer, M. D. Woltmann, R. W. Grimes, J. M. Hammond and D. S. Buchanan. 1995. Plasma growth hormone, insulin-like growth factor I, and insulin-like growth factor binding proteins in pigs with divergent genetic merit for postweaning average daily gain. J. Anim. Sci. 73:1776-1783.

Cohick, W. S. 1998. Role of the insulin-like growth factors and their binding proteins in lactation. J. Dairy Sci. 81:1769-1777.

Cohick, W. S., M. A. McGuire, D. R. Clemmons and D. E. Bauman. 1992. Regulation of insulin-like growth factor-binding proteins in serum and lymph of lactating cows by somatotropin. Endocrinol. 130:1508-1514.

Connor, E. E., S. M. Barao, A. S. Kimrey, A. B. Parlier, L. W. Douglass and G. E. Dahl. 2000. Predicting growth in angus bulls: The use of ghrh challenge, insulin-like growth factor-I, and insulin-like growth factor binding proteins. J. Anim. Sci. 78:2913-2918.

De Mellow, J. S. and R. C. Baxter. 1988. Growth hormone-dependent insulin-like growth factor (IGF) binding protein both inhibits and potentiates IGF-I-stimulated DNA synthesis in human skin fibroblasts. Biochem. Biophys. Res. Commun. 156:199-204.

Ernst, M. and G. A. Rodan. 1990. Increased activity of insulin-like growth factor (IGF) in osteoblastic cells in the presence of growth hormone (GH): Positive correlation with the presence of the GH-induced IGF-binding protein BP-3. Endocrinol. 127:807-814.

Giudice, L. C., E. M. Farrell, H. Pham and R. G. Rosenfeld. 1990. Identification of insulin-like growth factor-binding protein-3 (IGFBP-3) and IGFBP-2 in human follicular fluid. J. Clin. Endocrinol. Metab. 71:1330-1338.

Gopinath, R., P. E. Walton and T. D. Etherton. 1989. An acid-stable insulin-like growth factor (IGF)-binding protein from pig serum inhibits binding of IGF-I and IGF-II to vascular endothelial cells. J. Endocrinol. 120:231-236.

Hedrick, P. W. 1987. Gametic disequilibrium measures: Proceed with caution. Genet. 117:331-341.

Heinemeyer, T., E. Wingender, I. Reuter, H. Hermjakob, A. E. Kel, O. V. Kel, E. V. Ignatieva, E. A. Ananko, O. A. Podkolodnaya, F. A. Kolpakov, N. L. Podkolodny and N. A. Kolchanov. 1998. Databases on transcriptional regulation: Transfac, trrd and compel. Nucleic Acids Res. 26:362-367.

Jones, J. I. and D. R. Clemmons. 1995. Insulin-like growth factors and their binding proteins: Biological actions. Endocr. Rev. 16:3-34.

Kim, J. Y., D. H. Yoon, B. L. Park, L. H. Kim, K. J. Na, J. G. Choi, C. Y. Cho, H. K. Lee, E. R. Chung, B. C. Sang, I. J. Cheong, S. J. Oh and H. D. Shin. 2004. Identification of novel snps in bovine insulin-like growth factor binding protein-3 (IGFBP3) gene. Asian-Aust. J. Anim. Sci. 18:3-7.

Li, S. and R. Baserga. 1996. Epidermal growth factor and platelet-derived growth factor regulate the activity of the insulin-like growth factor I gene promoter. Exp. Gerontol. 31:195-206.

Marcelli, M., S. J. Haidacher, S. R. Plymate and R. S. Birnbaum. 1995. Altered growth and insulin-like growth factor-binding protein-3 production in pc3 prostate carcinoma cells stably transfected with a constitutively active androgen receptor complementary deoxyribonucleic acid. Endocrinol. 136:1040-1048.

McCann, J. P., S. C. Loo, D. L. Aalseth and T. Abribat. 1997. Differential effects of GH stimulation on fasting and prandial metabolism and plasma IGFs and IGF-binding proteins in lean and obese sheep. J. Endocrinol. 154:329-346.

Pesole, G., F. Mignone, C. Gissi, G. Grillo, F. Licciulli and S. Liuni. 2001. Structural and functional features of eukaryotic mrna untranslated regions. Gene 276:73-81.

Porcu, P., X. Grana, S. Li, J. Swantek, A. De Luca, A. Giordano and R. Baserga. 1994. An e2f binding sequence negatively regulates the response of the insulin-like growth factor 1 (IGF-I) promoter to simian virus 40t antigen and to serum. Oncogene 9:2125-2134.

Shimasaki, S. and N. Ling. 1991. Identification and molecular characterization of insulin-like growth factor binding proteins (IGFBP-1, -2, -3, -4, -5 and -6). Prog. Growth Factor Res. 3: 243-266.

Skaar, T. C. and C. R. Baumrucker. 1993. Regulation of insulin-like growth factor binding protein secretion by a murine mammary epithelial cell line. Exp. Cell Res. 209:183-188.

Stephens, M., N. J. Smith and P. Donnelly. 2001. A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. 68:978-989.

Valentinis, B., A. Bhala, T. DeAngelis, R. Baserga and P. Cohen. 1995. The human insulin-like growth factor (IGF) binding protein-3 inhibits the growth of fibroblasts with a targeted disruption of the IGF-I receptor gene. Mol. Endocrinol. 9:361-367.

Vreeland, W. N., R. J. Meagher and A. E. Barron. 2002. Multiplexed, high-throughput genotyping by single-base extension and end-labeled free-solution electrophoresis. Anal. Chem. 74:4328-4333.

Woodward, T. L., J. D. Turner, H. T. Hung and X. Zhao. 1996. Inhibition of cellular proliferation and modulation of insulin-like growth factor binding proteins by retinoids in a bovine mammary epithelial cell line. J. Cell Physiol. 167:488-499.

Yee, D. 2002. The insulin-like growth factor system as a treatment target in breast cancer. Semin. Oncol. 29:86-95.

Zapf, J. 1995. Physiological role of the insulin-like growth factor binding proteins. Eur. J. Endocrinol. 132:645-654.