



A novel polymorphism of the insulin-like growth factors binding protein-5 (*IGFBP-5*) gene and its association with body measurement traits in *Bos taurus*

MEI XUE¹, LINSSEN ZAN² and HONGBAO WANG³

Northwest Agriculture and Forest University, Yangling, Shaanxi, P R China

Received: 2 October 2010; Accepted: 19 December 2010

ABSTRACT

In this study, based on the PCR-SSCP and DNA sequencing technology, the association of the single nucleotide polymorphisms (SNP) in the bovine *IGFBP-5* gene with body measurement traits was first assessed in 779 individuals from 6 native Chinese cattle, namely Luxi, Luxi × Simmental crossbred, Nanyang, Xia'nan, Jiaxian red and Qinchuan breeds. As the SNP marker, there were the significant effects on the hucklebone width (HW) in six Chinese indigenous cattle breeds ($P < 0.05$). In all breeds, animals with the GG genotype had lower mean values for HW than these with the AA and AG genotype ($P < 0.05$). Least squares analysis also revealed significant effects of genotypes on chest circumference (CC) in Qinchuan (QC), Jiaxian red (JR) and Luxi (LX) breeds ($P < 0.05$), on body Length (BL) in JR breeds $P < 0.05$, on body height (BH) in LX breeds ($P < 0.05$) and on withers height (WH) in Nanyang (NY) breeds ($P < 0.05$). These results suggested that the *IGFBP-5* SNP could be an effective genetic marker.

Key words: Body measurement traits, Cattle, *IGFBP-5* gene, Polymorphism

Insulin-like growth factors binding proteins-5 (*IGFBP-5*) are members of the insulin-like growth factors (*IGFs*) system. The role of *IGFBP-5* in the osteoblast proliferation and bone accretion was recognized by Dimai *et al.* (1998), Address (2001), Amaar *et al.* (2002), and Firth and Baxter (2002). *IGFBP-5* is the most abundant *IGFBPs* stored in bone (Amaar *et al.* 2002). Due to its strong affinity for hydroxyapatite (Schneider *et al.* 2001), the mineral constituent of bone (Firth and Baxter 2002), it has the unique property of binding to bone matrix, thereby regulating storage of *IGFs* in bone and resulting in modulating proliferation, differentiation and apoptosis of osteoblasts (Amaar *et al.* 2002, Dimai *et al.* 1998). When injected *in vivo* into *IGF-1* knockout mice, recombinant human *IGFBP-5* increased local levels of alkaline phosphatase activity and osteocalcin, markers of bone formation (Firth and Baxter 2002) demonstrated that *IGFBP-5* was a stimulator of *IGF*-induced mouse bone cell proliferation. Address (2001) using ovariectomized mice also observed that administration of recombinant human *IGFBP-5* stimulated osteoblast activity and bone accretion in the femur and spine. Moreover, intact *IGFBP-5* (1–169) stimulates growth hormone (GH) receptor synthesis and activity in rat osteoblasts, resulting in the

enhancement of GH-stimulated mitogenesis in rat osteoblasts (Slootweg *et al.* 1996). Meanwhile, *IGFBP-5* itself is a growth factor that is not dependent on *IGFs* (Miyakoshi *et al.* 2001).

Until now, the association of *IGFBP-5* genetic variations with body measurement has not been reported in bovine. Therefore, the objective of this study is to detect SNPs in the bovine *IGFBP-5* gene and to explore their possible association with body measurement traits in native Chinese breed types.

MATERIALS AND METHODS

Sample collection and DNA isolation: Adult animals (779) were randomly selected from breeding populations, including Qinchuan (QC, $n=473$, Shaanxi province), Nanyang (NY, $n=48$, Henan province), Jiaxian red (JR, $n=73$, Henan province), Xia'nan (XN, $n=67$, Henan province), Luxi (LX, $n=65$, Shandong province), Simmental and Luxi crossbred steers (SL, $n=53$, Shandong province). The following traits were measured as described previously (Gilbert *et al.* 1993), including body length, body height, withers height, rump length, hip height, chest depth, chest circumference and hucklebone width. To minimize systematic error, single person was assigned to measure 1 of the 5 traits in all animals. DNA samples were extracted from leukocytes and tissue samples using a standard phenol-chloroform protocol (Mullenbach *et al.* 1989).

Present address: ^{1,2,3} (e mails: xuemei_19851021@163.com; zanls@yahoo.com.cn; wanghongbao001@yahoo.com.cn), College of Animal Science and Technology

PCR amplification: PCR was performed to amplify a 339 bp fragment of bovine *IGFBP-5* gene and its flanking region, using forward (5'AGAGGGAGATGGAGAGA 3') and reverse (5' AGGTTGGAGTTCTGGGAT 3') primers designed with primer 5.0 based upon bovine *IGFBP-5* gene sequence (Gene Bank accession No. NC_007300). The 15 µl PCR reaction mixture contained 50 ng genomic DNA, 10 pM of each primer, 1×buffer (including 1.5 mM MgCl₂), 200 µM dNTPs and 0.5 units of Taq DNA polymerase (MBI). The cycling protocol was 5 min at 95°C followed by 35 cycles of 94°C for 30s, 57.1°C annealing for 40s, 72°C for 40s, with a final extension at 72°C for 10 min. PCR products were electrophoresed on 1.5% agarose gels using 1×TBE buffer (89 mM Tris, 89 mM boric acid, 2 mMNa₂EDTA), containing 200 ng/ml ethidium bromide.

PCR-SSCP (Single stranded conformation polymorphism): Aliquots of 6µl PCR products were mixed with 10µl denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled on ice. Denatured DNA was subjected to PAGE in 1 × TBE buffer and constant voltage (250V) for 15 min, then 110V for 16 h. The gel was stained with 0.1% silver nitrate and visualized with 2% NaOH solution (supplied with 0.1% formaldehyde) according to Zhang *et al.* (2007).

DNA sequencing analysis: After the polymorphism was detected, the PCR products of difference electrophoresis patterns were sent to sequence in both directions in ABI PRIZM 3730 DNA sequencer and analyze the sequences with BioXM software (version 2.6).

Statistical analysis: The following items were statistically analyzed according to the previous approaches (Liu *et al.* 2010), including genotypic frequencies, allelic frequencies, the Hardy-Weinberg equilibriums and population genetic indexes:

He (gene heterozygosity); Ho (gene homozygosity); Ne effective allele numbers and PIC polymorphism information content. The association between SNP marker genotypes of the *IGFBP-5* gene and records of body measurement traits (BL, BH, WH, RL, HH, CD, CC and HW) was analyzed by the least-squares method as applied in the GLM procedure

of SPSS according to the following statistical linear model:

$$Y_{ijk} = \mu + A_i + G_j + S_k + E_{ijk}$$

where Y_{ijk} was the observation for the body measurement trait, μ was the overall population mean, A_i was the fixed effect of the i th age, G_j was the fixed effect of j th genotype (AA, AG and GG genotype), S_k is fixed effect of sex, E_{ijk} was the random environment error.

RESULTS AND DISCUSSION

After a 339 bp product of the *IGFBP-5* gene was amplified, 3 unique banding patterns were detected by PCR-SSCP analysis, the genotypes were named AA, AG and GG, respectively. Sequence analysis revealed a G>A mutation, a synonymous mutation of Ala, at position-3402 of the *IGFBP-5* gene (Fig.1).

Moreover, allele frequencies of the SNP were investigated and performed by χ^2 test in the all populations of bovine in our study (Table 1). The data demonstrated that allelic frequencies of *IGFBP-5*-T/C in 6 breeds were 0.4493/0.5507 (QC), 0.4328/0.5672; (XN), 0.4479/0.5521; (NY), 0.4384/

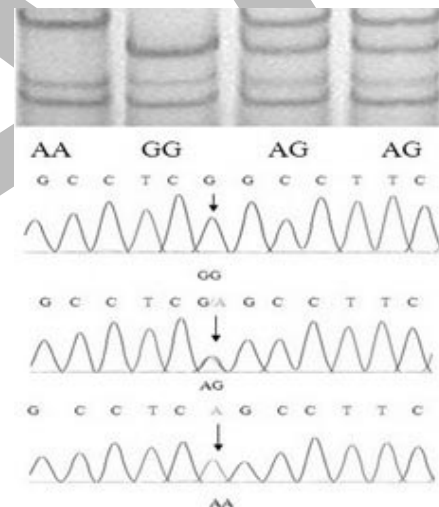


Fig 1. The PCR-SSCP patterns and the DNA sequencing maps of bovine *IGFBP-5* gene locus, three patterns (AA, GG, AG) were observed in 6 Chinese indigenous cattle. Sequencing map revealed a G>A mutation at -3402

Table 1. Genotype frequencies (%) at the *IGFBP-5* gene for the SNP in bovine populations

Breeds	Genotypic frequencies (number)			Sample size	Haplotype frequencies	
	AA	AG	GG		A	G
QC	0.0994 (47)	0.6998 (331)	0.2008 (95)	473	0.4493	0.5507
XN	0.1194 (8)	0.6269 (42)	0.2537 (17)	67	0.4328	0.5672
NY	0.1667 (8)	0.5625 (27)	0.2708 (13)	48	0.4479	0.5521
JR	0.1370 (10)	0.6027 (44)	0.2603 (19)	73	0.4384	0.5616
LX	0.2308 (15)	0.5077 (33)	0.2615 (17)	65	0.4846	0.5154
SL	0.1509 (8)	0.5849 (31)	0.2642 (14)	53	0.4434	0.5566
Total	0.1232 (96)	0.6521 (508)	0.2246 (175)	779	0.4493	0.5507

QC, Qinchuan; XN, Xia'nan; NY, Nan yang; JR, Jiaxian red; LX, Luxi; SL, Simmental and Luxi crossbred steers.

Table 2. Population genetic indexes at the *IGFBP-5* locus in bovine populations

Breeds	Ho	He	Ne	PIC	χ^2 test (HW*)
QC	0.5051	0.4949	1.9796	0.3724	81.1252**
XN	0.5090	0.4910	1.9694	0.3704	5.1323
NY	0.5054	0.4946	1.9785	0.3723	0.9054
JR	0.5076	0.4924	1.9701	0.3712	3.6656
LX	0.5005	0.4995	1.9981	0.3748	0.0174
SL	0.5064	0.4936	1.9747	0.3718	1.8139
Total	0.5051	0.4949	1.9796	0.3724	78.6710

Ho, Homozygosity; He, heterozygosity; Ne, effective allele numbers; PIC, polymorphic information content; HW, Hardy-Weinberg equilibrium; χ^2 0.052=5.991, χ^2 0.012=9.21, *means $P < 0.05$; for breed abbreviations, see legend to Table 1.

0.5616 (JR), 0.4846/0.5154 (LX) and 0.4434/0.5566 (SL). According to the χ^2 test, there was a significant difference in the allelic frequency in QC ($P < 0.05$) populations, suggesting that the QC were not in Hardy-Weinberg equilibrium. He, Ne and PIC of bovine *IGFBP-5* locus in 6 populations of *Bos taurus* breed varied from 0.4910 (XN) to 0.4995 (LX), 1.9694 (XN) to 1.9981 (LX), and 0.3704 (XN) to 0.3748 (LX), respectively (Table 2). Generally, PIC is classified into the following three types: low polymorphism (PIC value < 0.25), median polymorphism ($0.25 < \text{PIC value} < 0.5$), and high polymorphism (PIC value > 0.5). According to this classification of PIC, all *Bos taurus* population belongs to the median polymorphism level.

In healthy adults approximately 90% of *IGFBP-3* and 55% of *IGFBP-5* circulate in a ternary complex containing *IGFs*, *IGFBPs* and an acid labile subunit (Baxter *et al.* 2002). Of all the *IGFs* system, *IGFBP-5*, which is the most abundant *IGFBPs* stored in bone (Amaar *et al.* 2002), plays an important role in the osteoblast proliferation and bone accretion. So far, many studies focus on *IGFBP-3* gene variants, the available studies on *IGFBP-5* of bovine have never been reported. Zhao *et al.* (2009) used Nar I -PCR-RFLP analysis revealed that the *IGFBP-5* gene of pigs existed 3 different restricted nuclease polymorphic fragments. The significant effect of the *IGFBP-5* gene on birth weight has been detected by Zhao *et al.* (2009). Therefore, based on these results, considering the evolutionary conservation between cattle and pig, we apply the research results of pig *IGFBP-5* to analyze polymorphism and genetic effect on cattle *IGFBP-5* gene locus. And the present study firstly shows that the G>A SNP of bovine *IGFBP-5* is significantly associated with hip width (Table 3).

The novel alteration could result in a synonymous mutation at position 148. The degeneracy of the genetic code enables the same amino acid sequences to be encoded and translated in many different ways (Komar 2007). We know that the genome is highly redundant in terms of tRNA species for each amino acid but enigmatically under represents a number of specific codons (Shah 2008). Thus, in the synthesis of *IGFBP-5* protein, if the change of base at the third position of codon is not represented by a corresponding anti-codon within the nuclear tRNA, the rate of expression of the *IGFBP-*

Table 3. Associations of G3402 SNP genotypes with body measurement traits at bovine *IGFBP-5* gene

Breeds	Genotypes	Body measurement traits (cm, mean $\pm$ SE)				
		BL	BH	WH	CC	HW
QC	AA	129.770 $\pm$ 2.922	117.332 $\pm$ 1.920	121.348 $\pm$ 2.471	167.815 $\pm$ 3.697 ^a	25.542 $\pm$ 1.258 ^a
	AG	125.320 $\pm$ 1.439	117.255 $\pm$ 0.946	123.608 $\pm$ 1.218	159.365 $\pm$ 1.822 ^{ab}	22.856 $\pm$ 0.620 ^{ab}
	GG	124.014 $\pm$ 2.578	114.012 $\pm$ 1.694	120.613 $\pm$ 2.181	156.289 $\pm$ 3.263 ^b	21.818 $\pm$ 1.110 ^b
NY	AA	151.266 $\pm$ 1.731	133.488 $\pm$ 1.432	129.333 $\pm$ 1.451 ^a	185.608 $\pm$ 3.045	29.143 $\pm$ 0.641 ^a
	AG	149.424 $\pm$ 0.973	131.447 $\pm$ 0.805	130.239 $\pm$ 0.816 ^{ab}	182.364 $\pm$ 1.712	27.808 $\pm$ 0.360 ^{ab}
	GG	147.441 $\pm$ 1.334	130.096 $\pm$ 1.104	129.368 $\pm$ 1.118 ^b	180.179 $\pm$ 2.347	26.484 $\pm$ 0.494 ^b
XN	AA	144.177 $\pm$ 9.248	132.219 $\pm$ 3.890	137.124 $\pm$ 3.884	186.719 $\pm$ 9.910	26.951 $\pm$ 1.819 ^a
	AG	142.014 $\pm$ 5.208	129.649 $\pm$ 2.191	135.326 $\pm$ 2.187	179.188 $\pm$ 5.581	22.990 $\pm$ 1.025 ^b
	GG	140.991 $\pm$ 6.960	132.067 $\pm$ 2.928	136.116 $\pm$ 2.924	179.374 $\pm$ 7.459	23.007 $\pm$ 1.369 ^b
JR	AA	148.435 $\pm$ 4.052 ^a	130.833 $\pm$ 2.812	128.699 $\pm$ 3.305	177.721 $\pm$ 3.867 ^a	22.746 $\pm$ 1.139 ^a
	AG	138.244 $\pm$ 3.226 ^b	127.133 $\pm$ 2.238	124.997 $\pm$ 2.631	174.414 $\pm$ 3.078 ^{ab}	20.454 $\pm$ 0.906 ^b
	GG	137.107 $\pm$ 3.581 ^b	127.495 $\pm$ 2.485	125.311 $\pm$ 2.921	172.130 $\pm$ 3.417 ^b	19.347 $\pm$ 1.006 ^b
LX	AA	150.607 $\pm$ 3.428	134.149 $\pm$ 2.390 ^a	135.987 $\pm$ 2.439	188.067 $\pm$ 3.350 ^a	23.480 $\pm$ 0.601 ^a
	AG	142.915 $\pm$ 1.855	130.115 $\pm$ 1.293 ^{ab}	132.106 $\pm$ 1.320	183.613 $\pm$ 1.812 ^{ab}	22.286 $\pm$ 0.325 ^b
	GG	140.190 $\pm$ 2.668	127.153 $\pm$ 1.860 ^b	128.906 $\pm$ 1.898	175.916 $\pm$ 2.607 ^b	19.660 $\pm$ 0.468 ^c
SL	AA	128.750 $\pm$ 2.192	121.250 $\pm$ 2.832	128.000 $\pm$ 1.505	166.500 $\pm$ 2.774	24.875 $\pm$ 0.771 ^a
	AG	128.000 $\pm$ 1.114	121.226 $\pm$ 1.438	123.194 $\pm$ 0.765	161.839 $\pm$ 1.409	20.548 $\pm$ 0.391 ^b
	GG	129.714 $\pm$ 1.657	118.214 $\pm$ 2.141	122.643 $\pm$ 1.138	162.286 $\pm$ 2.097	19.929 $\pm$ 0.582 ^b

^{a,b} Means in columns with different superscripts were significantly different ($P < 0.05$).

BL, body length; BH, body height; WH, withers height; CC, chest circumference; HW, hucklebone width; For breed abbreviations, see legend to Table 1.

5 protein could change, which may affect bone formation.

In conclusion, we identified an SNP in the *IGFBP-5* gene and investigated its relevance in 6 native Chinese cattle. Our results provide evidence that the *IGFBP-5* gene may have potential effects on body measurement traits in native Chinese cattle. Therefore, further study will be necessary before using this SNP for marker-assisted selection in larger populations. It is also important to determine whether the *IGFBP-5* gene plays a role in the development of those traits and whether it is involved in linkage disequilibrium with other causative mutations.

ACKNOWLEDGMENTS

Research supported by the China National 863 Program (No.2008AA1010 and No.2006AA10Z1A1), the National Eleventh "Five Year" Science and Technology Support Project (No.2006BAD01A10-3), and GMO new varieties major project (No.2008ZX08007-002). Moreover, the cattle populations were supported by the Qinchuan beef cattle breeding center of Shaanxi province, Nanyang, Jiaxian and Xianan cattle breeding center of Henan province and Luxi cattle breeding center of Shandong province (P.R. China).

REFERENCES

- Amaar Y, Thompson G, Linkhart T, Chen S, Baylink D and Mohan S. 2002. Insulin-like growth factor-binding protein 5 (IGFBP-5) interacts with a four and a half LIM protein 2 (FHL2). *Journal of Biological Chemistry* **277**: 12053–60.
- Andress D. 2001. IGF-binding protein-5 stimulates osteoblast activity and bone accretion in ovariectomized mice. *American Journal of Physiology- Endocrinology and Metabolism* **281**: E2 83–88.
- Baxter R, Meka S and Firth S. 2002. Molecular distribution of IGF binding protein-5 in human serum. *Journal of Clinical Endocrinology and Metabolism* **87**: 271–76.
- Dimai H, Linkhart T, Linkhart S, Donahue L, Beamer W, Rosen C, Farley J and Baylink D. 1998. Alkaline phosphatase levels and osteoprogenitor cell numbers suggest bone formation may contribute to peak bone density differences between two inbred strains of mice. *Bone* **22**: 211–16.
- Firth S and Baxter R. 2002. Cellular actions of the insulin-like growth factor binding proteins. *Endocrine Reviews* **23**: 824–54.
- Gilbert R, Bailey D and Shannon N. 1993. Linear body measurements of cattle before and after 20 years of selection for postweaning gain when fed two different diets. *Journal of Animal Science* **71**: 1712–20.
- Komar A. 2007. Silent SNPs: impact on gene function and phenotype. *Pharmacogenomics* **8**: 1075–80.
- Kurland C. 1991. Codon bias and gene expression. *Federation of European Biochemical Societies letters* **285**: 165–69.
- Liu Y F, Zan L S, Li K and Zhao S P. 2010. A novel polymorphism of GDF5 gene and its association with body measurement traits in *Bos taurus* and *Bos indicus* breeds. *Molecular Biology Reports* **37**: 429–34.
- Miyakoshi N, Qin X, Kasukawa Y, Richman C, Srivastava A, Baylink D and Mohan S. 2001. Systemic administration of insulin-like growth factor (IGF)-binding protein-4 (IGFBP-4) increases bone formation parameters in mice by increasing IGF bioavailability via an IGFBP-4 protease-dependent mechanism. *Endocrinology* **142**: 2641–48.
- Mullenbach R, Lagoda P J and Welter C. 1989. An efficient salt-chloroform extraction of DNA from blood and tissues. *Trends Genetics* **5**: 391.
- Shah J H, Maguire D J, Munce T B and Cotterill A. 2008. Alanine in HI: a silent mutation cries out! *Advances in cirrhosis, hyperammonemia, and hepatic encephalopathy*. **614**: 145–50.
- Schneider M, Zhou R, Hoefflich A, Krebs O, Schmidt J, Mohan S, Wolf E and Lahm H. 2001. Insulin-like growth factor-binding protein-5 inhibits growth and induces differentiation of mouse osteosarcoma cells. *Biochemical and Biophysical Research Communications* **288**: 435–42.
- Slootweg M, Ohlsson C, Van Elk E, Netelenbos J and Andress D. 1996. Growth hormone receptor activity is stimulated by insulin-like growth factor binding protein 5 in rat osteosarcoma cells. *Growth regulation* **6**: 238–46.
- Zhao Xiao-feng, Ou Yang-ke, Zhang Yang and Xu Ning-ying. 2009. Quick screening SNP on partial coding sequence of insulin-like growth factor binding protein 5 gene (IGFBP5) and effect of its polymorphism on production performance in pigs. *Journal of Agricultural Biotechnology* **17**: 758–62.
- Zhang C L, Wang Y, Chen H and Lan X Y. 2007. Enhance the efficiency of single-strand conformation polymorphism analysis by short polyacrylamide gel and modified silver staining. *Analytical Biochemistry* **365**: 286–87.