GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Polymorphism in the 5'-UTR of the insulin-like growth factor I gene associated with production traits in Chinese cattle

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Genet. Mol. Res. 13 (3): 6899-6905 (2014)
Received July 12, 2013
Accepted October 29, 2013
Published August 29, 2014
DOI http://dx.doi.org/10.4238/2014.August.29.12

ABSTRACT. The insulin-like growth factor I (IGF-1) gene plays important roles in the growth and body composition of animals. Serum IGF1 concentration has been associated with growth traits in many livestock species. We found a polymorphism of cattle *IGF1-Tas*I locus and analyzed the distribution of alleles in three cattle breeds, including Qinchuan, Nanyang, and Chinese Holstein. PCR-RFLP analysis showed that allele A was the dominant allele. The frequencies of allele A varied from 0.84 to 0.97. Distributions of genotypic and allelic frequencies were significantly different among breeds. Polymorphism of the *IGF1* gene was significantly affecting hucklebone width at 6 months in the Nanyang breed and protein and fat yield of the third lactation in Chinese Holstein cattle. Individuals with allele C had a significantly higher performance in production traits.

Key words: *IGF1* gene; PCR-RFLP; Dairy performance; Chinese cattle

INTRODUCTION

Insulin-like growth factor I (IGF1) is a member of the large family of insulin-related peptides including insulin, IGF1, and IGF2 (Daughaday and Rotwein, 1989), which play important roles in the regulation of cellular metabolism and growth of vertebrates. They also act as potent mitogens, regulating the balance of cellular proliferation, differentiation, and survival (Singh and Rubin., 1993), and they play important roles in the growth and body composition of animals (Yu et al., 1995). The serum IGF1 concentration was associated with growth traits in many livestock species. Selection studies in Angus cattle demonstrated a high heritability of mean serum IGF1 concentration during the post-weaning period, which suggested a strong additive genetic control of this growth factor (Davis and Simmen, 1997). In addition, the *IGF1* gene has also been used to selection experiments of some farm animal species as well as in beef cattle (Davis et al., 1995).

In humans, a multiple cytosine-adenine (CA) dinucleotide repeat motif, located 969-bp upstream of the transcription start site of the *IGF1* gene, was studied in colorectal, breast, and prostate cancers (Morimoto et al., 2005; Schildkraut et al., 2005; Wong et al., 2005). The microsatellite (CA) repeats of the *IGF1* gene had been studied in domestic animals. In pig, the microsatellite was significantly associated with different quantitative traits (Faria et al., 2009). In goats, the microsatellite had effects on birth weight (Wang et al., 2011). In cattle, this microsatellite was associated with birth weight and weaning weight in the early growth phase (Andrade et al., 2008).

Recently, an A→C transversion in the P1 promoter region 977-bp upstream of the bovine *IGF1* gene was found associated with milk, fat, and protein yield (Szewczuk et al., 2011). The objective of this study was to detect the A→C polymorphism in the 5'-flanking region of the *IGF1* gene in Chinese indigenous cattle and to determine associations between the polymorphism and production traits.

MATERIAL AND METHODS

DNA samples and data information

Genomic DNA samples were collected from 406 cattle belonging to three breeds: Qinchuan (QC, N = 95), Nanyang (NY, N = 216), and Chinese Holsteins (CH, N = 95). The QC animals were from the reserved farm (Weinan city, Shaanxi Province, China). The NY animals were from the breeding center of Nanyang cattle (Nanyang city, Henan Province, China), and the growth records (including birth weight, body weight, average daily gain, and body measurement) of Nanyang cattle in different growth periods (6, 12, 18, and 24 months) were collected for statistical analysis. The Chinese Holsteins were from the breeding farm of milk cattle (Xi'an city, Shaanxi Province, China). The performance records of Chinese Holsteins (including fat percentage, protein percentage, fat/protein percentage, fat yield, protein yield, and milk yield) during the third lactation was collected for statistical analysis.

DNA was isolated from 2% heparin-treated blood samples (stored at -80°C) using the standard phenol-chloroform extraction protocol (Sambrook et al., 1989). The content of DNA were estimated by spectrophotometry and diluted to 50 ng/μL. All DNA samples were stored at -20°C for subsequent experiments.

Genotyping of the *Tas*I *IGF1* allele by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

The primers of the amplification created restriction site-PCR (ACRS-PCR) were used to amplify the 5'-untranslated region (5'-UTR) of the bovine *IGF1* gene. The primers (IGF1P F: 5'-TCATCCAGCTGAGAGATTTGAAT-3' and IGF1P R: 5'-TGTGTGTGTGTGTGTGTG TGAAT-3') were from Zych et al. (2007).

The 20-μL PCR mixture contained the following: 50 ng bovine genomic DNA, 0.4 μL each primer (10 pmol), 200 μM dNTP, 10X buffer (including 1.5 mM $MgCl_2$), and 0.35 U *Taq* DNA polymerase (MBI, Vilnius, Lithuania). The thermal cycle program performed was as follows: pre-denaturation at 94°C for 5 min; 34 cycles of 94°C for 45 s, annealing at 58°C for 60 s, and 72°C for 30 s; and a final extension at 72°C for 7 min.

PCR products were digested with 2 U *Tas*I endonuclease (MBI, Vilnius, Lithuania) for 8 h at 65°C following manufacturer directions, and the digested products were subjected to 10% polyacrylamide gel electrophoresis (80 x 73 x 0.75 mm) in 1X Tris borate, ethylene-diaminetetraacetic acid buffer and at constant voltage (200 V) for 40 min. The gel was stained with 0.1% silver nitrate (Zhang et al., 2007).

Data analyses

Genotypic frequencies, allelic frequencies, and Hardy-Weinberg equilibrium were directly calculated. Differences in these frequencies at the *IGF1-Tas*I locus among populations were analyzed using a χ^2-test, which was performed using the SPSS software (version 13.0). Population genetic indexes, gene homozygosity, gene heterozygosity, effective allele numbers, and polymorphism information content (PIC), were calculated using the methods Nei and Li (Nei and Li., 1979).

The adjusted linear model (SPSS software, version 13.0) with fixed effects was used to evaluated the relationship between genotypes and growth traits. Analyses were performed through two steps, first using a full animal model and then using a reduced animal model. The full animal model included fixed effects of the marker genotype, birth year, season of birth (spring *vs* fall), age of dam, sire, farm, sex, and random effects (permanent environment, animal, and residual). The reduced model used in the final analysis (Boldman et al., 1995; Huang et al., 2011).

In the model for Nanyang cattle, the effects of age, genotypes, and the interaction between age and genotypes were included. The following adjusted linear model was used: $Y_{ij} = \mu + A_i + G_j + (AG)_{ij} + E_{ij}$, where Y_{ij} was the performance measured on each of the $_{ij}$, μ was the overall population mean, A_i was the fixed effect due to the i_{th} age, G_j was the fixed effect association with the j_{th} genotype (AA, AC, and CC genotype), $(AG)_{ij}$ was the interaction between the i_{th} age and the j_{th} genotype, and E_{ij} was the random error.

In the model for the Chinese Holsteins, the effects of genotypes and the season at the start of lactating were included. The following adjusted linear model was applied: $Y_{ij} = \mu + R_i + G_j + E_{ij}$, where Y_{ij} was the performance measured on each of the $_{ij}$, μ was the overall population mean, R_i was the fixed effect of the season at the start of lactating, G_j was the fixed effect association with the j_{th} genotype (AA, AC, and CC genotype), and E_{ij} was the random error.

RESULTS AND DISCUSSION

IGF1 stimulates protein metabolism and plays important roles in the regulation of cell proliferation and differentiation (Davis and Simmen, 1997). The *IGF1* gene is a candidate gene for growth in bovine because it plays a key role in growth regulation and development (Hossner et al., 1997; Breier, 1999). The AATA interrupt within the $(CA)_n$ microsatellite in the 5'-flanking region of the *IGF1* gene existed in some Artiodactyl species (including goats, sheep, cattle, and deer), but it did not exist in horses, pigs, and camels; additionally, it was also absent from humans (Reza and Moran, 2000). Because the $(CA)_n$ microsatellite is close to the transcription site and the CA repeats can form a Z-DNA structure, mutations in the CA repeat region may influence the expression of IGF1 protein. Therefore, the association between the CA repeat polymorphisms and growth traits have been reported (Nordheim and Rich, 1983; Hamada et al., 1984).

Polymorphisms of the 5'-UTR of the *IGF1* gene in Chinese cattle populations

In this study, we inserted the A→C mutation at the AATA interrupt within the $(CA)_n$ microsatellite in the 5'-flanking region of the Chinese cattle *IGF1* gene by the ACRS-PCR method. The electrophoretic patterns of digested products are shown in Figure 1. The distributions of genotypic and allelic frequencies were shown in Table 1, the Holstein-Friesian (HF) which had the same mutation in the locus (Szewczuk et al, 2011) was also included in our analysis. Genotypic frequencies for the polymorphisms were found to be significantly different among the four populations based on a χ^2 test ($\chi^2 = 30.54$, d.f. = 6, $P < 0.001$). Significant differences in allelic frequencies among the populations were also revealed ($\chi^2 = 10.51$, d.f. = 3, $0.01 < P < 0.05$) in populations. Moreover, there were significant differences in genotypic frequencies between the QC, NY, CH, and HF populations ($P < 0.05$) (Table 1). It was obvious that the breed factor significantly affected the distribution of genotypic and allelic frequencies at the cattle *IGF1-Tas*I locus.

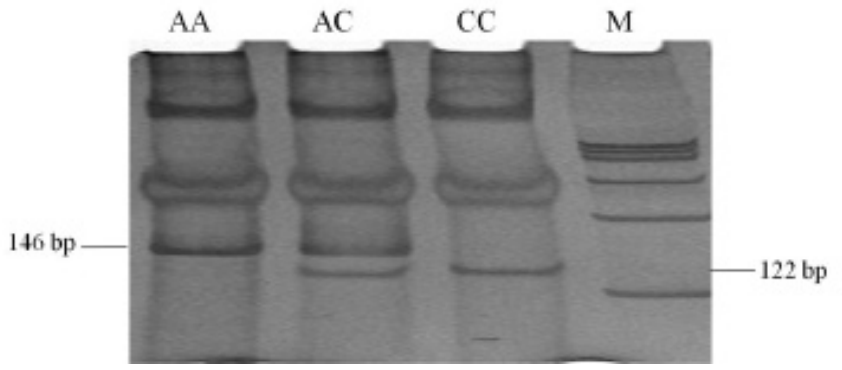

Figure 1. Electrophoretic patterns on 12% polyacrylamide gel electrophoresis (PAGE) after digestion by *Tas*I. Polymerase chain reaction products were digested by *Tas*I and analyzed by 12% PAGE. Digested products demonstrated three genotypes (WW, WI, and II). *Lane 1* = genotype AA (146 bp); *lane 2* = genotype AC (146, 122, and 24 bp); *lane 3* = genotype CC (122 and 24 bp); and *lane M* = marker I (from top to bottom: 600, 500, 400, 300, 200, and 100 bp). The 24-bp fragment was invisible in the AC and CC genotypes.

The diversity parameters (H_O, H_E, N_E, and PIC) are shown in Table 1. Comparisons of the genetic diversity of breeds demonstrated that HC had the highest homozygosity and the lowest PIC. According to the classification of the PIC values (PIC value < 0.25, low polymorphism; 0.25 < PIC value < 0.5, intermediate polymorphism; and PIC value > 0.5, high polymorphism), all breeds possessed low genetic diversity at the locus. At this locus, the NY and QC populations were in Hardy-Weinberg disequilibrium (P < 0.01); this suggested that selection might have been sufficient to perturb the locus in the QC and NY breeds.

Table 1. Genotype frequencies and genetic diversity parameters at the *IGF1-Tas*I locus.

Breeds	Observed genotypes			χ^2 (genotypic frequencies)[2]	Allelic frequencies		χ^2 (HWE)[3]	Diversity parameters[4]			
	AA	AC	CC		A	C		H_O	H_E	N_E	PIC
Chinese Holstein (CH)	0.94	0.06	0	17.68**	0.97	0.03	0.07	0.95	0.05	1.06	0.05
Qinchuan (QC)	0.88	0.08	0.04	8.65*	0.92	0.08	14.13**	0.85	0.15	1.79**	0.14
Nanyang (NY)	0.84	0.10	0.06	11.74**	0.89	0.11	47.52**	0.80	0.20	1.25**	0.18
Holstein-Friesian (HF)[1]	0.77	0.21	0.02		0.87	0.13					

[1]These results were reported by Szewczuk et al., 2011. [2]χ^2 (genotypic frequencies) = χ^2 value of genotypic frequencies between Chinese cattle breeds and HF breeds (*P < 0.05; **P < 0.01). [3]χ^2 (HWE) = Hardy-Weinberg equilibrium χ^2 value (*P < 0.05; **P < 0.01). [4]H_O = gene homozygosity, H_E = gene heterozygosity, N_E = effective allele numbers, and PIC = polymorphism information content.

Associations between different genotypes of the *IGF1* gene and production traits in Chinese cattle

It was reported that the number of CA repeats in the 5'-flanking region of the AATA interrupt was the same [$(CA)_6(CA)_n$] in interrupt-containing species, and that it served as a type of anchor point, impeding replication slippage in the 5'-flanking region and preventing variation in the repeat number in this block (Reza and Moran et al., 2000). In Canchim cattle, this microsatellite was associated with birth weight and weaning weight in the early growth phase (Andrade et al., 2008). In swine, the microsatellite significantly affected average daily gain (Casas-Carrillo et al., 1997). The A to C transversion changed the repeat numbers, and in Polish Holstein Friesian, the mutation was associated with the milk, fat, and protein yield (Siadkowska et al., 2006). Therefore, we analyzed the association between the polymorphism and performance of the Nanyang and Chinese Holstein populations. Significant differences were found between the hucklebone width at 6 months and genotypes in Nanyang cattle (Table 2); this result suggested that the C allele had a positive effect on the hucklebone width at 6 months. However, there was no significant difference between body weight (body weight and average daily gain) and genotypes. This divergence might be caused by the discrepancies between breeds. In the Chinese Holstein population, significant associations between the polymorphism and protein and fat yield (P < 0.05) were detected in the third lactation (Table 3). This implied that the C allele had a positive effect on protein and fat yield, which was similar to the study by Siadkowska et al. (2006). Because small populations were involved in the analysis, further investigations are essential to detect the polymorphism of this gene in more cattle breeds and larger populations.

Table 2. Associations between different genotypes of the *Tas*I polymorphism within the bovine 5'-UTR of the *IGF1* gene and growth traits in Nanyang cattle.

Traits	AA (N = 185) (Means ± SE)	AC (N = 19) (Means ± SE)	CC (N = 12) (Means ± SE)
Hucklebone width at 6 months (cm)	17.00 ± 0.65[a]	18.00 ± 0.49[b]	18.35 ± 1.32[b]

Least square means within the same row with no common superscript differ significantly ($P < 0.05$).

Table 3. Associations between different genotypes of the *Tas*I polymorphism within the bovine 5'-UTR of the *IGF1* gene and dairy performance in Chinese Holstein cattle.

Traits	AA (N = 90) (Means ± SE)	AC (N = 5) (Means ± SE)	CC (N= 0) (Means ± SE)	P
Fat yield (kg)	208.18 ± 13.62	213.12 ± 36.04	-	0.02
Protein yield (kg)	167.75 ± 10.67	179.68 ± 28.24	-	0.04

CONCLUSION

The A to C polymorphism within the 5'-UTR of the *IGF1* gene was analyzed in Chinese cattle in this study. The distributions of genotypic and allelic frequencies were significantly different in the different breeds. The association analysis indicated that the C allele had a positive effect on the body measurement of Nanyang cattle and the protein and fat yield of Chinese Holsteins.

ACKNOWLEDGMENTS

Research supported by the National Natural Science Foundation of China (Grant #31272408), Program of National Beef Cattle Industrial Technology System (CARS-38), Agricultural Science and Technology Innovation Projects of Shaanxi Province (#2012 NKC01-13), and Natural Science Foundation of Jiangsu Province (#BK2011206).

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