ASSOCIATIONS OF AN *Aci*I POLYMORPHISM IN THE IGF-II GENE WITH GROWTH TRAITS IN BEEF CATTLE

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INTRODUCTION

Growth traits are economically important traits in livestock. It has been shown that these traits are under the control of multiple genes. Genetic marker information on related genes can be used to facilitate selection and breeding through marker assisted selection (MAS) in domestic animals. Insulin-like growth factor II (IGF-II) belongs to a family of structurally related polypeptides, which includes IGF-I, insulin and relaxin (Blundell and Humbel, 1980; Dafgard *et al*., 1985). IGF-II is important in the fetus (Han et al., 1987). It plays a key role in pre-adolescent growth, influencing fetal cell division and differentiation. IGF-II knockouts were shown to have significant fetal growth retardation, especially in the early stages of gestation (DeChiara *et al*., 1990). On the other hand, transgenic mice with overexpression of IGF-II were shown to have organ overgrowth and tumor formation (Ward *et al*., 1994, Rogler *et al.*, 1994). Therefore, the IGF-II gene may be a good candidate gene for growth. Detecting genetic variations in the IGF-II gene and relating them to growth rate could be helpful in development of marker-assisted selection (MAS) programs in animal breeding. This study was designed to examine the IGF-II gene for possible polymorphism and to test the association of the polymorphisms with growth traits in Angus beef cattle.

MATERIAL AND METHODS

Animals. Angus beef cattle, which were divergently selected for blood serum IGF-I concentration, were used as the experimental animals. Selection began in 1989 at the Eastern Ohio Resource Development Center (EORDC), using 100 spring-calving (50 high line and 50 low line) and 100 fall-calving (50 high line and 50 low line) purebred Angus cows with unknown IGF-I levels. Each year, four bull calves with the highest and four bull calves with the lowest IGF-I concentration were selected for breeding within the selection lines (Davis and Simmen, 1997). A total of 52 animals were genotyped in this study.

Methods. Genomic DNA was isolated from blood samples collected during the IGF-I selection experiment. A 280bp region within exon 9 of the IGF-II gene was amplified by polymerase chain reaction (PCR). The primers were designed based on ovine DNA sequences stored in GenBank. The sequences of the primers were 5' GGGCCCGCCTCTCGCTTCCTTCT 3' and 5' CTTGGCGGGGGCGGCACAGTAA 3'. The PCR was performed in a 30μl reaction volume containing 10pmol of forward primer and the same amount of reverse primer, 200μM dNTP's, 1x reaction buffer that contained 1.5mM $mgcl_2$, 1 unit of Taq-DNA polymerase and 100ng of genomic DNA as template. Conditions were 97°C for 2 min, followed by 35 cycles of 95°C for 45s, 66°C for 1 min, and 72°C for 60s. After 35 cycles, reactions were finished by an extension of 5 min at 72°C, and finally bulked at 4°C in a DNA thermal cycler. When

amplification was achieved, single strand conformation polymorphism (SSCP) was used to screen for mutations within the amplified segment. The reaction mixture, which included 10µl of PCR product, 10µl of ddH_2O and 12µl of loading dye, was denatured at 95°C for 5 minutes. The sample was put into ice for 10 minutes. The samples were loaded on 8% polyacrylamide gels, and run in 1x TBE buffer at 200 volts for 16 to 20 hours at a constant temperature of 10°C. Gels were stained by .01% Ethidium Bromide for 10 min and viewed under UV light. The PCR products of the two homozygous genotypes were sent to the Plant-Microbe Genomics Facility in the Ohio State University and sequenced to determine the nature of the polymorphism.

Statistical analysis. Associations of the animal genotypes with growth traits and IGF-I concentrations were determined by analysis of variance of quantitative traits, which included birth weight, weaning weight, preweaning gain, on-test weight, weight at d 28 and 56 of the 140-d postweaning test, off-test weight, weight gain during the 20-d period between weaning and the beginning the of postweaning test, postweaning gain, and serum IGF-I concentration on d 28, 42, and 56, and the mean serum IGF-I concentration, using General Linear Model (GLM) procedures in SAS. Fixed effects of genotypes, year, season of birth (spring vs fall), age of dam, sex, and IGF-I selection line (high vs low) were included as independent variables in the linear model. Age of calf was treated as a covariate in the model. Data were also analyzed separately within the high and low selection line using the same model, except selection line was deleted from the model, to test for differences in association between genotypes and the growth traits between the low and high selection lines.

RESULTS AND DISCUSSION

An *Aci*I polymorphism was found in exon 9 of the bovine IGF-II gene. Three genotypes, AA, AB and BB, were identified with frequencies of .10, .33, and .57, respectively, in 52 Angus cattle. Genotypic frequencies were not significantly different between the high and low IGF-I lines (Table 1).

Table 1. Genotypic frequencies for *Aci*I polymorphism in different IGF-I selection lines

Genotype	Low line	High line
AA	0.05	0.13
AB	0.38	0.29
BB	0.57	0.58

P value for χ^2 test was .54.

Sequencing results revealed that there is a transition from T to G in allele A, which can be recognized by digestion with restriction enzyme *Aci*I. No significant associations between the genotypes and growth traits were found. However, genotype AA tended to have higher values than genotype BB for every growth trait analyzed. A moderate association of genotypes with Gain20 (weight gain during the 20-d period between weaning and the beginning of the postweaning test) was found (P=.09). No significant relationships between these genotypes and IGF-I concentration were detected (Table 2) However, when animals in high and low IGF-I lines were analyzed separately, the IGF-I concentration at d 56 of the 140-d postweaning test

had a significant relationship with genotypes in the high IGF-I line. Genotype BB had a higher IGF-I concentration than genotype AA (P=.01) at d 56.

Table 2. Least-squares Means and Standard Errors for *Aci*I Polymorphism

	AA		AB		BB		P-value
Traits	LSM	SE	LSM	SE	LSM	SE	
Birth wt (kg)	36.43	1.83	33.74	1.12	33.96	0.80	0.36
Weaning wt (kg)	210.50	9.98	203.83	6.16	204.36	4.89	0.85
Prewean. gain (kg)	169.39	9.59	166.88	5.85	166.53	4.20	0.96
On-test wt (kg)	229.14	11.33	220.94	6.97	217.13	5.47	0.58
D 28 wt (kg)	257.47	11.81	245.84	7.20	245.32	5.17	0.58
D 56 wt (kg)	289.58	13.93	283.25	8.50	284.55	6.10	0.91
Off-test wt (kg)	393.14	19.19	379.40	11.70	371.87	8.40	0.54
Gain20[A] (kg)	18.81	3.68	17.71	2.24	12.68	1.61	0.09†
Postwean.gain(kg)	169.58	11.04	162.16	6.74	159.28	4.83	0.66
D28 IGF-I (ng/mL)	362.06	56.78	375.21	34.63	332.75	24.85	0.56
D42 IGF-I (ng/mL)	406.45	46.48	417.40	28.35	394.40	20.34	0.78
D56 IGF-I (ng/mL)	351.51	63.44	279.94	38.70	373.14	27.77	0.12
Mean IGF-I (ng/mL)	373.34	38.96	357.52	23.76	366.76	17.05	0.90

† .05< P value< .1.
[A] weight gain during the 20-d period between weaning and the beginning of the postweaning test

CONCLUSION

These results did not show any significant association between the *Aci*I polymorphism and growth traits in Angus beef cattle. However, genotype AA tended to have a higher value for growth traits than genotype BB. The only important relationship was with weight gain during the 20-d period between weaning and beginning of the postweaning test (P=.09). Genotype AA had higher weight gain than genotype BB. Allele A was the allele with lower frequency, and since allele A may be the favorable allele, this *Aci*I polymorphism could be useful in marker assisted selection in Angus beef cattle. More genotyping is needed to further study this polymorphism.

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