Polymorphisms in the IGF1 gene and their effect on growth traits in Mexican beef cattle

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ABSTRACT. The IGF1 gene (insulin-like growth factor 1) is a candidate gene for marker-assisted selection strategies. A single nucleotide polymorphism in the promoter region (IGF1/*Sna*BI) has been reported to be associated with production traits in several cattle breeds. Here, we report its allelic frequencies in Charolais and Beefmaster breeds; we confirm its association with three growth traits: weaning weight, weaning weight adjusted to 210 days and preweaning weight gain in the Charolais breed. In addition, we designed a strategy to search these breeds for new polymorphisms in four coding regions of the gene. A C/A transversion was detected in intron 4, but it was not associated with the growth traits. A single nucleotide polymorphism (IGF1/S*na*BI) is proposed as a selection marker for Mexican Charolais cattle; validation of its association with weaning weight, weaning weight adjusted to 210 days and preweaning weight gain, could complement the genetic evaluations of this breed through marker-assisted management strategies.

Key words: IGF1; SNP IGF1/*Sna*BI; Polymorphism; Charolais; Beefmaster

INTRODUCTION

In vertebrates, the insulin-like growth factor 1 (IGF1) or somatomedin gene plays a key role in various physiological and metabolic processes, where IGF1 and growth hormone or somatotrophin are involved in the somatotropic axis. IGF1 is a mediator of many biological effects; for example, it increases the absorption of glucose, stimulates myogenesis, inhibits apoptosis, participates in the activation of cell cycle genes, increases the synthesis of lipids, stimulates the production of progesterone in granular cells, and intervenes in the synthesis of DNA, protein, RNA, and in cell proliferation (Etherton, 2004).

The bovine IGF1 gene was mapped on chromosome 5, in the centimorgan 73.5 (Grosse et al., 1999). The provisional nucleotide sequence is approximately 72 kb (ID number 281239). In humans, pigs, goats, rats, and chickens, the IGF1 nucleotide sequence is about 70-90 kb (Shimatsu and Rotwein, 1987; Kajimoto and Rotwein, 1991; Rose, 2002). Exon number is different between species; for example, goats, pigs and sheep have 1-6 exons (Mikawa et al., 1995), and humans and rats 1-5 (Rotwein et al., 1986; Shimatsu and Rotwein, 1987). Wang et al. (2003) reported that bovine IGF1 has 6 exons responsible for expressing heterogeneous mRNA. Like other species, depending on the exon leader present, two transcripts are identified, class 1 (exon 1 as the leader) or class 2 (exon 2 as the leader). In class 1 transcripts, there are three transcription start sites, whereas class 2 has one. Although the length and genetic structure between species is variable, the expressed protein of 70 amino acids is highly conserved in vertebrates (Upton et al., 1998). Fotsis et al. (1990), in a comparative study of the IGF-IA precursor between human and bovine, identified a conservation of around 93 and 96% in nucleotide and amino acid sequences, respectively. Due to its biological function, the IGF1 gene is considered to be a candidate gene for predicting growth and meat quality traits in animal genetic improvement schemes (Machado et al., 2003; Andrade et al., 2008). The bovine IGF1 gene has two polymorphisms located in the promoter region: a CA_n microsatellite (Kirkpatrick, 1992), and a T/C transition, also known as the single nucleotide polymorphism (SNP) IGF1/*Sna*BI (Ge et al., 1997, 2001). The microsatellite has been associated with birth weight and weaning in Hereford cattle, but these associations were not found in breeds such as Nellore, Canchim and Simental/Angus crosses studied by Curi et al. (2005a). After its evaluation with growth characteristics in Angus cattle, the SNP IGF1/*Sna*BI was considered to be a potential molecular marker associated with weight gain during the first 20 days after weaning (Ge et al., 2001). Curi et al. (2005b) studied some Nellore, Canchim and several population crosses and found a significant association of SNP with body weight and subcutaneous backfat. Li et al. (2004) analyzed the association between SNP and growth characteristics and found an unstable association between the BB genotype with birth weight, average daily gain and average daily preweaning in Beefbooster cattle. Although there is no evidence of a functional role in gene expression, these polymorphisms are considered to be potential molecular markers (Siadkowska et al., 2006), and it has even been proposed that the existence of other polymorphic sites linked with these markers will help to explain the effect of the IGF1 gene on phenotypic characteristics.

In this study, the effect of the SNP IGF1/*Sna*BI on growth characteristics was determined in Mexican beef cattle. In addition, we made a search for new polymorphisms in four coding regions of the IGF1 gene.

MATERIAL AND METHODS

Using Promega Wizard® (Promega Corporation, Madison, WI, USA), DNA was isolated from blood samples obtained from Charolais (group 1) and Beefmaster (group 2) breeds. Group 1 comprised 68 animals with different background, 25 dams from a nucleus herd that had been selected for improved lean beef and calving ease using founders from England, France and Ireland, while the other 43 corresponded to sires of 12 different herds and confined to be evaluated for feedlot response. These populations were located in Nuevo León and Coahuila, Mexico, respectively. Group 2 consisted of 25 Beefmaster sires from a herd located in Tamaulipas, Mexico.

Analysis of the IGF1/*Sna*BI polymorphism

The IGF1/*Sna*BI marker was analyzed using a modification of the procedure reported by Ge et al. (1997). Briefly, we used a nested-polymerase chain reaction (PCR) using the primers IGF1-E1 (Table 1). We then performed a nested-PCR with the previously reported primers (IGF677F 5'-ATTACAAAGCTGCCTGCCCC-3'; IGF897R 5'-ACCTTACCCGTATGAAAGGAATATACGT-3'). After enzymatic digestion with *Sna*BI, alleles A (223, 26 bp) and B (249 bp) were analyzed on 20% polyacrylamide gels (Sigma-Aldrich).

Detection of new polymorphisms in IGF1

Using the sequences reported in the NCBI AF210383, AF210384, AF210385, and AF210386 and Primer Select 4.03 (DNASTAR Inc.; Anonymous, 2008) primers were designed to amplify the adjacent regions of exons 1, 2, 3, and 4 of the gene (Table 1). To identify the polymorphic sites, we amplified the four gene regions using DNA from five different individuals of each breed. PCR products were cloned in the TOPO XL system (PCR Cloning Kit, Invitrogen). SequiTherm EXCEL™ II DNA Sequencing Kit (Epicentre Technologies, Madison, WI, USA) was used for bidirectional sequencing of plasmid DNA obtained individually in each clone. Sequence edition was done with the E-Seq program (LI-COR IR2 DNA Sequencer) and assembled with CAP3 (Huang and Madan, 1999). At least 3 different clones were sequenced from each fragment and breed.

Table 1. Primers used in the amplification of coding regions in the bovine IGF1 gene.

	Sequence 5'-3'	Sequence 3'-5'	PCR product
IGF-E1	GGGCAAAAAGCATGAGACAGT	GCTGATTTTTCCCATTGCTTCTGA	785 bp
IGF-E2	GCCAGCAGCTCACAAGCTGA	ACCATTTTTGTTGTTCCAGAT	390 bp
IGF-E3	TTGCACTCCTGGAAGGGGCATA	TCTTCGCACACTCCCCGGCAGTT	362 bp
IGF-E4	CCACTCTAAAGCTAGGCCTCTCTC	GAAGTCTATGAGGGTATGAAT	341 bp

PCR = polymerase chain reaction.

Multiple alignment of sequences was done with ClustalW2 (Larkin et al., 2007), comparing the new sequences with those previously reported (AF210383, AF210384, AF210385, and AF210386) and the provisional sequence reported for the bovine genome (ID number 281239).

For genotyping purposes, three different assays were designed and tested in Beefmaster and Charolais samples. Table 2 shows the PCR/RFLP (restriction fragment length

polymorphism), a restriction site insertion (RSI/RFLP) and allele specific PCR (PASA) tests. The PASA assay was performed in a final volume of 25 µL using two primer pairs, one showing a 3'-modified primer and the other 26 bp and GC-rich at the 5' end. The reaction mixtures for the three assays consisted of 100 ng DNA, 2.5 mM MgCl$_2$, 0.2 mM dNTPs, 5 µM of each primer and 1 U AmpliTaq® DNA Polymerase-Fragment Stoffel (Applied Biosystems). The PCR profile used in all assays was: 95°C for 5 min, 95°C for 45 s, 65°C for 45 s (two cycles), 95°C for 45 s, 65°C for 45 s , 72°C for 45 s (5 cycles decreasing 2°C in each cycle), 95°C for 45 s, 60°C for 45 s, 72°C for 45 s (25 cycles), and 72°C for 10 min. For the PCR-RFLP assay, we digested 150 ng PCR product with 5 U enzyme (Promega Corporation) in a final volume of 10 µL. The digestion pattern was visualized on 6% polyacrylamide gels (Sigma-Aldrich).

Table 2. Molecular assays designed for determination of polymorphisms.

	Sequence 5'-3'	Localization	PCR product	Technique
IGF-66F	GCTGCCTGCCCCTTTCCAaG	5'UTR	785 bp	RSI/*Alu*I
IGF- 66-95R	GGCGTGAAGACACACACATC			
IGF95NF	TGAGATCATTCCCCTCACTT			
IGF95MF	GCGGGCAGGGCGGCGGGGGCGGGGCC	5'UTR	240 bp	PASA
	TGAGATCATTCCCCTCACTc			
IGF66-95R	GGCGTGAAGACACACACATC			
IGF32NF	GAAGGGGCATAGTTTTGATTT	Intron 2	178 bp	PASA
IGF32MF	GCGGGCAGGGCGGCGGGGGCGGGGCC			
	GAAGGGGCATAGTTTTGATTa			
IGF32R	AAGCACAGGGCCAGATAGAA			
IGF-E4F	CCACTCTAAAGCTAGGCCTCTCTC	Intron 4	341 bp	PCR/*Nru*I
IGF-E4R	GAAGTCTATGAGGGTATGAAT			
IGF-270	TCGGCCATCTTCGCGcaAT	Intron 4	264 bp	RSI/*Mfe*I
IGF-E4R	GAAGTCTATGAGGGTATGAAT			

UTR = untranslated region; PCR = polymerase chain reaction; RSI = restriction site insertion; PASA = allele specific PCR.

Polymorphism association with growth traits

According to data availability from the Charolais and Beefmaster populations, we evaluated the association of IGF1 SNPs with growth traits including birth weight (BW), weaning weight (WW), weaning weight adjusted to 210 days (WW210), preweaning weight gain (WG), weight adjusted to 400 days (W400), postweaning weight gain (PWG), weight adjusted to 600 days (W600), and daily weight gain to 600 days (G600).

The procedure used for the analysis of the association was the general linear model of the statistics package SAS, with the following model:

$$Y_{ijk} = \mu + GS_i + GN_j + BW + \varepsilon_{ij} \qquad \text{(Equation 1)}$$

where Y_{ijk} = dependent variable (WW, WW210, WG, W400, PWG, W600, G600); μ = general average; GS_i = effect of the i-th genotype of IGF1/*Sna*BI (AA, AB, BB); GN_j = j-th genotype of IGF1/*Nru*I (AA, AB, BB); BW = birth weight effect as linear covariable for all variables except BW, and ε_{ij} = random error. The least square means were compared by the Tukey method in the same statistics package (SAS, 2000).

RESULTS

Allelic and genotypic frequencies of the IGF1/*Sna*BI polymorphism

Allelic and genotypic frequencies obtained in the two breeds studied are shown in Table 3. In the Beefmaster population, the favorable allele B was found at a high frequency (0.97). The two Charolais populations showed frequency differences according to their geographic origin and sex. In the sires from Coahuila, the favorable allele B (0.74) had a high frequency, while in the group of Charolais females (Nuevo Léon), the frequencies were 0.21, 0.50 and 0.29 for AA, AB and BB genotypes, respectively. In this last population, the allelic frequencies are approximately those expected for a population in equilibrium (allele A = 0.46 and allele B = 0.54).

Table 3. Allelic and genotypic frequencies of IGF1/S*na*BI observed in Charolais and Beefmaster breeds.

Population	Frequencies				
	A	B	AA	AB	BB
Charolais (Coahuila)	0.26	0.74	0.070	0.372	0.558
Charolais (Nuevo León)	0.46	0.54	0.208	0.500	0.292
Beefmaster (Tamaulipas)	0.03	0.97	0.00	0.067	0.933

Association analysis of the SNP IGF1/*Sna*BI was performed only in Charolais females, since the three genotypes were detected in HW proportions. The Beefmaster breed was omitted because allele B was practically fixed in the population studied. In this analysis, the SNP IGF1/*Sna*BI showed a significant effect ($P < 0.05$) on three production traits, WW ($P = 0.039$), WW210 ($P = 0.041$) and WG ($P = 0.0148$) (Table 4).

Table 4. Least square means ± standard error of the SNP IGF1/S*na*BI genotypes.

Growth trait	Genotypes		
	AA	AB	BB
BW	42.215 ± 3.19[a]	42.479 ± 2.21[a]	46.110 ± 2.55[a]
WW (kg)	214.517 ± 28.99[b]	301.201 ± 20.36[a]	268.949 ± 24.86[a,b]
WW210 (kg)	198.059 ± 21.22[b]	260.292 ± 15.16[a]	225.878 ± 18.12[a,b]
WG (kg)	0.692 ± 0.11[b]	1.058 ± 0.07[a]	0.832 ± 0.09[a,b]
W400 (kg)	340.129 ± 34.43[a]	392.373 ± 27.23[a]	361.014 ± 25.23[a]
PWG (g)	0.788 ± 0.23[a]	0.647 ± 0.17[a]	0.710 ± 0.16[a]
W600 (kg)	437.082 ± 18.80[a]	404.684 ± 17.93[a]	459.458 ± 10.78[a]
G600 (g)	0.783 ± 0.16[a]	0.817 ± 0.13[a]	0.553 ± 0.13[a]

Effects on growth traits in Charolais breed. BW = body weight; WW = weaning weight; WW210 = weaning weight adjusted to 210 days; WW = preweaning weight gain; W400 = weight adjusted to 400 days; PWG = postweaning weight gain; W600 = weight adjusted to 600 days; G600 = daily weight gain to 600 days; a,b,c: significantly different means between genotypes.

New polymorphic sites in IGF1

Five mutations were located in the IGF1 gene from the Charolais and Beefmaster breeds: two transitions in 5'UTR, 1 indel in intron 2, 1 transition and 1 transversion in intron 4 (Table 5). Genotyping results validated as a true polymorphism only the transversion C/T located in intron 4. As shown in Table 2, the PCR-RFLP assay relies on the use of restriction enzyme N*ru*I, which recognizes the sequence TCG/CGA; when the transversion is present, the

restriction site is modified and lost (TCG/AGA). The alleles were called A (nucleotide C) and B (nucleotide A). The frequency of the polymorphism was determined in the populations studied, as shown in Table 6. The Beefmaster population had higher frequencies of allele B (0.70), and contrasting allelic frequencies were found in the two Charolais populations, where allele A was more prevalent (0.72) in the Coahuila population and allele B in the Nuevo León population (0.52). The association analysis of the intron 4/N*ru*I polymorphism was not significant (P > 0.05) for the growth traits tested in either the Charolais or Beefmaster breed (Table 7).

Table 5. Nucleotide changes identified in the IGF1 gene in Charolais and Beefmaster cattle.

Contig position	Reference sequence position (ID number 281239)	Location	Nucleotide change
66	66	5'UTR	T/C
95	95	5'UTR	T/C
32	4632	Intron 2	T/-
264	56392	Intron 4	C/A
270	56398	Intron 4	T/C

Table 6. Allelic and genotypic frequencies of intron 4 T/C transversion in the IGF1 gene.

Population	Frequencies				
	A (264, 77 bp)	B (341 bp)	AA	AB	BB
Charolais (Coahuila)	0.48	0.52	0.442	0.070	0.488
Charolais (Nuevo León)	0.72	0.28	0.520	0.400	0.080
Beefmaster (Tamaulipas)	0.30	0.70	0.080	0.440	0.480

Table 7. Least square means ± standard error of the genotypic IGF1/N*ru*I polymorphism.

Growth trait	Genotypes		
	AA	AB	BB
BW	42.93 ± 1.88^a	42.56 ± 2.11^a	45.30 ± 4.52^a
WW	253.40 ± 17.41^a	290.90 ± 20.10^a	240.36 ± 41.40^a
WW210	215.78 ± 13.03^a	252.41 ± 14.71^a	216.04 ± 30.24^a
WG	0.819 ± 0.66^a	0.947 ± 0.07^a	0.816 ± 0.15^a
W400	365.66 ± 17.08^a	379.74 ± 21.04^a	348.12 ± 60.04^a
PWG	0.933 ± 0.11^a	0.663 ± 0.13^a	0.551 ± 0.39^a
W600	488.96 ± 8.30^a	416.28 ± 18.76^a	395.99 ± 27.22^a
G600	0.579 ± 0.09^a	0.978 ± 0.16^a	0.596 ± 0.18^a

Effects on growth traits in Charolais breed. BW = body weight; WW = weaning weight; WW210 = weaning weight adjusted to 210 days; WG = preweaning weight gain; W400 = weight adjusted to 400 days; PWG = postweaning weight gain; W600 = weight adjusted to 600 days; G600 = daily weight gain to 600 days. a,b,c: significantly different means between genotypes.

DISCUSSION

IGF1 is located on bovine chromosome 5, which to date harbors at least 73 quantitative trait loci (QTL) (Animal QTL Database, 2009; http://www.animalgenome.org/QTLdb/). Due to the high QTL number close to IGF1, this chromosomal region has been evaluated not only in different species of domestic animals but also between different breeds for example in cattle (Casas-Carrillo et al., 1997; Li et al., 2006; Bian et al., 2008; Stratikopoulos et al., 2008).

The SNP IGF1/*Sna*BI has been studied in more than 20 cattle breeds, including dairy and beef breeds. The allelic frequencies reported for the favorable B allele vary from 0.16 to

1.0 (Curi et al., 2005b; Li et al., 2006). There are contrasting allelic frequencies in Beefmaster and Charolais breeds. In the Beefmaster population, the B allele was almost fixed (0.97), and Curi et al. (2005b) proposed that allele B is characteristic of indicus populations because they found it fixed in a Nellore population. The high frequency in Beefmaster could therefore be due to its composition, which includes an indicus background. The Charolais populations evaluated had been selected mainly for improved growth traits. The frequency of the favorable allele B in the two Charolais groups evaluated was 0.54 and 0.74. This result is interesting since the greater frequency of the BB genotype (0.558) was observed in the population from Coahuila, represented by sires that could be considered as seed-stock for the Mexican Charolais Full-French in Mexico. Although the frequency of the favorable allele B was lower in the dam population (Nuevo León) than in the sires, the frequency of the heterozygote AB genotype was greater, where through adequate reproduction management it could be possible to increase the frequency of the homozygote BB genotype.

The association of the SNP IGF1/*Sna*BI was confirmed in the Charolais breed, where AB and BB genotypes showed a significant effect on WW, WW210 and WG. Our results are consistent with those previously reported in Angus, Nellore and Holstein-Friesian breeds, where a dominance effect of allele B over allele A was observed, affecting traits such as: PWG, body weight at sacrifice and daily weight gain (Ge et al., 2001; Curi et al., 2005b; Siadkowska et al., 2005). The SNP IGF1/*Sna*BI has shown inconsistencies in validation tests to probe its association with growth traits. It has been proposed that when a marker association discovery occurs in the Gene-SNP/trait direction, different factors should affect the association, for example, the genetic background of the animals studied, the selection, gene drift, linkage disequilibrium between genes of greater effect (positive or negative), linkage disequilibrium between marker/ trait, and epistatic interactions (Machado et al., 2003). The effect of these factors combined with the large number of QTL identified on chromosome 5, would help explain the possible inconsistencies of the association of the IGF/S*na*BI polymorphism with growth traits.

Nevertheless, for the genetic improvement of animals, this type of molecular marker has been integrated as part of the selection criteria to complement the genetic evaluations of production traits in cattle (Dekkers, 2004). When they are in linkage disequilibrium and in phase with functional genetic variation, they provide information on the genetic merit of the animals even after several generations (Stone et al., 2005). Ríos et al. (2007) reported the first data of heritability on growth characteristics in the Mexican Charolais breed: 0.22 for birth weight, 0.33 for adjusted weaning weight (205 days) and 0.45 for postweaning weight gain. The moderate heritability of these characteristics would be favored with the use of the SNP IGF/S*na*BI in programs of marker-assisted management. However, it is important to validate our association results in a greater number of Charolais populations. This approach could be also useful to test the C/A transversion located in intron 4 in Mexican Beefmaster and Charolais breeds. Although we did not probe any association of the new C/A polymorphism with growth traits, its potential as a marker is not discarded. Some polymorphic sites located in introns have been useful as molecular markers. For example, with regard to the μ-calpain gene (CAPN1), the predictive value of the CAPN1 4751 marker for shear force has been validated. This marker is found in intron 17, and its functional role has not yet been determined (White et al., 2005).

Due to the high degree of conservation in coding regions of the IGF1 gene, which was confirmed in our study, the search for causal polymorphisms has been extended to other genes of this system, including the two classes of growth factors (IGF1 and IGF2), the receptors

(IGF1R and IGF2R) and the six binding proteins (IGFBP1-6) (Machado et al., 2003). This global search is an alternative approach in genetic studies of Mexican cattle populations.

ACKNOWLEDGMENTS

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