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

Synergistic and threshold effects of *GH1* and *GHR* promoter size variation on body growth and fat accrual in young Nelore (*Bos indicus*) bulls

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Genet. Mol. Res. 11 (2): 1783-1798 (2012)
Received March 13, 2012
Accepted June 1, 2012
Published June 29, 2012
DOI http://dx.doi.org/10.4238/2012.June.29.11

ABSTRACT. A synergistic effect in the somatotropic axis (*GH1-GHR-IGF1*) was observed in 736 young Nelore (*Bos indicus*) bulls under *ad libitum* grass feeding conditions on irrigated pasture in central Brazil. Stepwise substitution of shorter alleles of the promoter region of the growth hormone gene (*GH1*) and the P1 promoter of the *GH1* receptor gene (*GHR*) with longer alleles was associated with significantly increased body weight gain (W550, weight at age 550 days; ADG, average daily gain) and fat accrual (FAT, rib eye fat thickness). A threshold effect on ADG was associated with allele size variation at the

GH1. A best fit model indicated a 3- to 6-fold effect of *GH1* variation on ADG, when compared to the variation at the *GHR* and a known microsatellite at the somatomedin gene (*IGF1*, insulin-like growth factor 1). A threshold effect on FAT was associated with substitution of the short *GHR* allele by the longer *GHR* alleles; the effect of the *GHR* variation on FAT was 10-fold that of the variation at the *GH1* and *IGF1* loci. Among the 10 *GH1-GHR-IGF1* multi-genotypes identified, the predominant genotype was homozygous for the large *GH1* promoter (long/long, *G2/G2* or domestic type), short *GHR* promoter (short/short or wild type), and short *IGF1* microsatellite (short/short or wild type). This predominant multi-genotype suggests that selection pressure in the Nelore breed has been directed towards high ADG and W550, and low FAT. Our results mirror previous findings in the oMtla-oGH transgenic mouse model, in which the level of somatotropic gene expression acts through a threshold mechanism, and low expression results in adipogenesis, while high expression increases body growth.

Key words: Somatotropic axis; Growth hormone gene; *Bos indicus*; Promoter region; Growth and fat accrual; Marker-assisted selection

INTRODUCTION

Growth hormone (GH1), GH1 receptor (GHR) and somatomedin (IGF1 - insulin-like growth factor 1) constitute the somatotropic axis, which controls metabolism, growth, development and aging in a wide range of animals (Berryman et al., 2008). In bovines, it has been shown that the somatotropic axis is functionally coordinated and sensitive to nutrient intake and GH1 (Smith et al., 2002; Butler et al., 2003; Radcliff et al., 2006). Mutations that reduce GH1 signaling have been shown to extend lifespan and increase longevity in similar ways as observed in diet restriction (DR) models (Shimokawa et al., 2003; Zha et al., 2008).

We have previously shown that the short, wild-type allele of the promoter region of the bovine *GH1* gene, *G1*, confers a thrifty phenotype under DR as observed in a drought season, as compared to the longer allele, *G2* (Dani et al., 2010). This observation prompted us to study the associations between the *GH1*, *GHR* and *IGF1* genotypes and a number of growth and carcass traits of 1269 young Nelore bulls (*Bos indicus*) fed *ad libitum* on irrigated pasture.

The biallelic *GH1* promoter polymorphism analyzed in this study is characterized by the presence of one (Rodrigues et al., 1998) or two (Gordon et al., 1983) AAG trinucleotides positioned, in tandem, 9 bp upstream of the TATA-box transcription control site of the *GH1* promoter region. The longer allele, *G2* has been originally described for *Bos taurus* cattle (Gordon et al., 1983), while the shorter allele, *G1*, has been observed in water buffaloes, *Bubalus bubalis*, and also in *B. indicus* and some tropically adapted *B. taurus* breeds (Rodrigues et al., 1998, 1999; Dani et al., 2010). Since molecular phylogenetics has shown that *Bos* and *Bubalus* share the same ancestor from some 5-8 million years ago (Ritz et al., 2000; MacEachern et al., 2009), we assume that *G1* is the ancient wild-type form, whereas *G2* is the mutated, domestic form.

The multiallelic *GHR* polymorphism studied here is a polymorphic TG-repeat mic-

rosatellite located 90 bp upstream of a major transcription start site in the P1 promoter of the *GHR* gene on bovine chromosome 20 (Lucy et al., 1998). A shorter allele with 11 consecutive TG [$(TG)_{11}$)] is common in *B. indicus* cattle, whereas longer [$(TG)_{>11}$], especially 16- to 20-TG-repeat alleles predominate in *B. taurus* breeds (Hale et al., 2000).

At the *IGF1* locus, the polymorphic microsatellite (CA)n located in the 5'-flanking region of the *IGF1* gene (Kirkpatrick, 1992) has been widely employed in studies targeted towards the localization of quantitative trait loci. Of the known 4 alleles, the shorter allele has never been observed in taurine breeds.

MATERIAL AND METHODS

Field conditions, feeding test

The experimental Nelore (*B. indicus)* herd was a commercial herd owned by Perdizes Farm (Quilombo E&P Ltda.) and other owners; it included 1065 standard Nelore and 204 polled Nelore bulls. Animals were handled and managed according to guidelines and standard practices of the annual feeding test at the Perdizes Farm in Jaraguari, MS, Brazil (20°22'29.78"S, 54°18'52.66"W). These included pasture-feeding *ad libitum* in an irrigated pasture on quartzarenic neosol, with water and mineral supplementation. Green mass was available all year long, although some seasonal variation in grass productivity apparently occurred. Animals entered the feeding test directly after weaning at ages varying between 8 and 10 months and were discharged 365 days later.

Growth and carcass traits

Measures of growth and carcass traits were obtained at 3-month intervals. Animals were weighed at entry into the trial. Average daily gain (ADG, in g) was calculated after an adaptation period of 56 days. Weight (W, in kg) was adjusted to 550 days of age according to Equation 1:

$$W550 = [((AW365 - BW) / AEFT) * 550 + BW], \quad \text{(Equation 1)}$$

with AW365 being actual weight, in kg, after the end of 365 days of feeding test; BW, birth weight, in kg; AEFT, actual age, in days, at the end of the feeding test. Rib eye area (REA, in cm^2), subcutaneous fat thickness (FAT, in mm) and marbling score (MARB) were obtained ultrasonographically. MARB information was available for 358 bulls, all of which were genotyped for *IGF1*. Scrotal circumference (SC, in cm) was obtained at final weighing.

Samples and genotyping

The bulls were genotyped with respect to the *GH1*, *GHR* and *IGF1* polymorphic sites as shown in Table 1. All bulls were genotyped for *GH1*, *GHR*, but only 460 bulls were genotyped for *IGF1*. Nasal swabs were collected in the field and stored in ethanol. DNA from 40 μL nasal swab was extracted using the DNA IQ System (Promega), according to manufacturer instructions. Extracted DNA was dissolved in deionized water to a final working concentra-

tion (50 ng/μL). PCR genotyping was carried out with the primer pairs as listed in Table 1 (forward primers were FAM-labeled). The PCR mixture included 2 mM $MgCl_2$, 0.375 mM of each dNTP, 1 pmoL of each forward and reverse primer, 1 U GenoTaq® (5 U/μL; Genon Ltda., Brazil) and 50 ng DNA template, in a 20-μL total reaction volume overlayed with mineral oil. PCR was carried out in a Mastercycler PCR thermocycler (Eppendorf, Germany) with the following profile: hot start at 94°C, 10 min; 35 cycles of 94°C for 1 min, 58°C for 1 min and 72°C for 1 min, and a final extension at 72°C for 4 min. PCR products were separated and analyzed by polyacrylamide gel electrophoresis (PAGE) in a MegaBACE 1000 capillary sequencer (GE Healthcare Life Sciences, USA). Electropherograms were analyzed using the Fragment Profiler software, and the results were deposited in our data bank for further statistical analyses. To rule out any confounding effects of the L127V variant site (Lucy et al., 1991) on production traits (Lucy et al., 1991; Schlee et al., 1994; Lee et al., 1996; Grochowska et al., 2001; Sorensen et al., 2002; Paz, 2002; Ge et al., 2003), we performed a PCR-RFLP genotyping of exon-5 of the GH1, according to Schlee and co-workers (1994) in a subset of 85 Nelore bulls representative of their respective sibling groups, and taken at random out of our series of experimental animals encompassing all three *G1* and *G2* genotypes. PCR-RFLP *Alu*I (10 U/μL; Gibco BRL) digests were separated by PAGE followed by silver staining as described by Sanguinetti and co-workers (1994). Possible confounding effects of the *GH1* gene L127V variant site on productive traits were ruled out because the whole experimental herd was monomorphic, *LL*. The fixation of the *LL* genotype in our herd confirms previous reports on *L* allele fixation in the Brazilian Nelore population (Rosa et al., 1996; Rosa, 1997) or the Zebu population - Brahman breed (DeNise and Regitano, 1996). Absence of the *V* allele in the Zebu population in Brazil can be attributed to the small number of animals that gave origin to the Brazilian herd and by the very low frequency of this allele (Mitra et al., 1995).

Table 1. Primer sequences, chromosomal localization, and allele nomenclature of selected regions of the bovine growth hormone gene (*GH1*), growth hormone receptor gene (*GHR*) and insulin-like growth factor-1 gene (*IGF1*).

Gene, region	Genotyping method and PCR primer sequences	Cromosomal location (chromosome number)	Alleles (sizes, in bp, or names)	References
GH1, promoter region	AFLP F: 5'-TGGCAGTGGAGACGGGATGATG-3' R: 5'-CCTCCCCAAATCAATTACATTTTCTC-3'	19	193 (wt) 196	Dani et al. (2010)
GH1, exon (L127V)	RFLP (*Alu*I) F: 5'-GTGGGCTTGGGGAGACAGAT-3' R: 5'-GTCGTCACTGCGCATGTTTG-3'	19	L (wt) V	Lucy et al. (1991); Schlee et al. (1994)
GHR, promoter region	AFLP F: 5'-CTCTAATCTTTTCTGGTACCAGG-3' R: 5'-CCTGCTGGGCCATTTTTATACC-3'	20	93 (wt) 103 105 107	Lucy et al. (1998)*
IGF1, microsatellite	AFLP F: 5'-GCTTGGATGGACCATGTTG-3' R: 5'-CACTTGAGGGGCAAATGATT-3'	5	225 (wt) 227 229	Bishop et al. (1994)

AFLP = amplified fragment length polymorphism; RFLP = restriction fragment length polymorphism; F = forward and R = reverse (F-primers used in AFLP analyses were FAM-labeled); wt = wild type. *The GHR-F primer has been shortened by three nucleotides (5'-GTG) as compared to the Lucy et al. (1998) primer. It follows the corresponding allele nomenclature change (Lucy et al., 1998; this paper): 94-(91)/(96)-93/104-(101)/106-103/108-105/(110)-107/112-(109) (in parentheses: alleles that have not been identified in the respective studies).

Statistical analyses

Gene frequency (x_i), for the *i* allele, and genotypic frequency (x_{ii}), for the *ii* genotype were estimated for the population, according to Equations 2 and 3:

$$x_i = [(2n_{ii} + \sum n_{ij}) / 2n], \quad \text{(Equation 2)}$$

$$x_{ii} = [n_{ii} / n], \quad \text{(Equation 3)}$$

where n_{ii} and n_{ij} refer to the number of homozygotes and heterozygotes observed for the allele *i*, respectively; *n* refers to the number of individuals. Growth traits were analyzed by the least squares method, using the GLM procedure of SAS® (Statistical Analysis System, 2000). Genetic association tests, analyses of variance and box and whisker plots were analyzed with IBM SPSS V19 (IBM Corp., Armonk, NY, USA). To rule out possible confounding effects due to paternity, analyses were performed within and between groups of the sires' half-siblings.

Definition of wild-type and domestic type alleles of *GH1*, *GHR* and *IGF1*

The short alleles of each *GH1*, *GHR* and *IGF1* were considered wild types, whereas the longer alleles were the domestic types. Rare longer alleles were pooled and regarded as domestic type irrespective of their size differences. The significance of the observed effect was measured by the Fisher exact test.

Best-fit model

The effect of an allele *a* on a carcass trait *t*, in the context of the alleles for *GH1*, *GHR*, and *IGF1* was studied by the best-fit values of *t* in the sets of bulls having 0, 1, or 2 copies of *a*, respectively, called $v_{a,t,0}$, $v_{a,t,1,}$ and $v_{2a,t,2}$ here. The best-fit model is the linear equation that minimizes the error between the values of *t* in the set.

RESULTS

We detected 10 *GH1-GHR-IGF1* multi-genotypes in our Nelore population, with the predominant multi-genotype being homozygous for the large *GH1* promoter (large/large, *G2/G2* or domestic type), short *GHR* promoter (short/short or wild-type), and short *IGF1* microsatellite (short/short or wild type). The L/L variant of the L127V *GH1* polymorphic site was fixed in the population. The single-allele frequencies are shown in Table 2, and the growth and carcass traits are shown in Table 3.

The Nelore bulls were either standard (N = 1065, 84%) or polled (N = 204, 16%). The polled trait is believed to have been introgressed into the Nelore population from cattle of European origin, and the polled Nelore group had a higher frequency of domestic alleles. Thus, we took this group as proxy to domestic cattle (*B. taurus*), whereas the standard Nelore was taken as a proxy to the semi-domesticated or wild-type cattle (*B. indicus*). Table 2 shows the genotypic differences between these 2 Nelore sub-groups.

A pairwise comparison showed that the polled Nelore differed from the standard

Table 2. Estimated allele frequencies (P) for the loci studied.

Gene	N	Allele	P	Standard Nelore	P	Polled Nelore	P
GH1 (promoter)	2538	193 (wt)	0.048	103	0.048	9	0.022
		196	0.956	2027	0.952	399	0.978
GH1 (L127V)	170	L (wt)	1.000	(2130)	1.000	(408)	1.000
		V	-	-	-	-	-
GHR (promoter)	2538	93 (wt)	0.914	1987	0.933	336	0.824
		103	0.003	6	0.003	1	0.002
		105	0.082	135	0.063	70	0.172
		107	0.001	2	0.001	1	0.002
IGF1 (microsatellite)	920	225 (wt)	0.834	650	0.827	119	0.875
		227	0.009	7	0.009	1	0.007
		229	0.158	129	0.164	16	0.118

wt = wild type.

Table 3. Carcass and growth traits of 1269 young Nelore bulls in the feeding test.

Traits	Minimum	Maximum	Median	Average	SD
W550	276.8	509.9	382.4	383.4	30.9
ADG	201	959	633	633	897
SC	20.5	38.2	28.9	28.8	3.1
REA	36.9	79.9	55.9	56.5	6.4
FAT	0.587	5.392	2.229	2.124	0.668
MARB	1.03	4.05	2.29	2.36	0.45
FAT/ADWG	0.778	8.958	3.338	3.409	1.133
FAT/W550	0.001	0.015	0.005	0.006	0.002
FAT/REA	0.009	0.104	0.036	0.038	0.013
REA/ADWG	55	209	89	91	16
REA/W550	0.105	0.198	0.147	0.148	0.0149

W550 = weight, in kg, adjusted to 550 days of age; ADG = average daily weight gain, in g/day; SC = scrotal circumpherence, in cm; REA = rib eye area, in cm^2; FAT = rib eye fat thickness by ultrasound, in mm; MARB = marbling score by ultrasound. SD = standard deviation.

Nelore in W550, SC and REA ($P < 0.001$), with the polled Nelore values significantly higher by 10.6 kg, 1.1 cm, and 2.5 cm^2, respectively, as compared to the standard Nelore. Also, there were significant genotypic differences in *GH1* and *GHR* allele frequencies ($P < 0.001$), although not in *IGF1* or *L127V* allele frequencies (Table 2). There were no significant differences in FAT between these groups. However, the relatively small number of polled Nelore bulls and the lower frequencies of wild-type alleles in this group hindered a comparison between distinct genotype classes. Within the standard Nelore group, a three-factorial analysis of variance showed that differences in FAT were significantly associated with *GHR* ($P = 0.001$) and the interaction *GH1 * GHR* ($P = 0.021$). Differences in MARB were weakly associated with the interaction *GH1 * GHR* ($P = 0.037$) and strongly associated with the interaction *GH1 * GHR * IGF1* ($P < 0.001$). However, the data did not show a normal distribution, and under the conditions of non-parametric tests, no significant differences were observed.

We then set out to normalize the data. Of the 1269 bulls, 766 (60%) were fathered by 20 sires, whereas 503 (40%) were fathered by 152 sires. The number of bulls/sires in the first subgroup varied from 14 to 112, whereas in the latter group it varied from 1 to 13 bulls. Sires were unknown for 99 bulls. Figure 1 shows the distribution of W550 of 736 bulls from 18 sires who fathered at least 18 bulls in the feeding test. The half-sib progeny of these sires differed significantly in W550, ADG, SC, REA, and FAT ($P < 0.001$) and MARB ($P = 0.004$) (one-way analysis of variance).

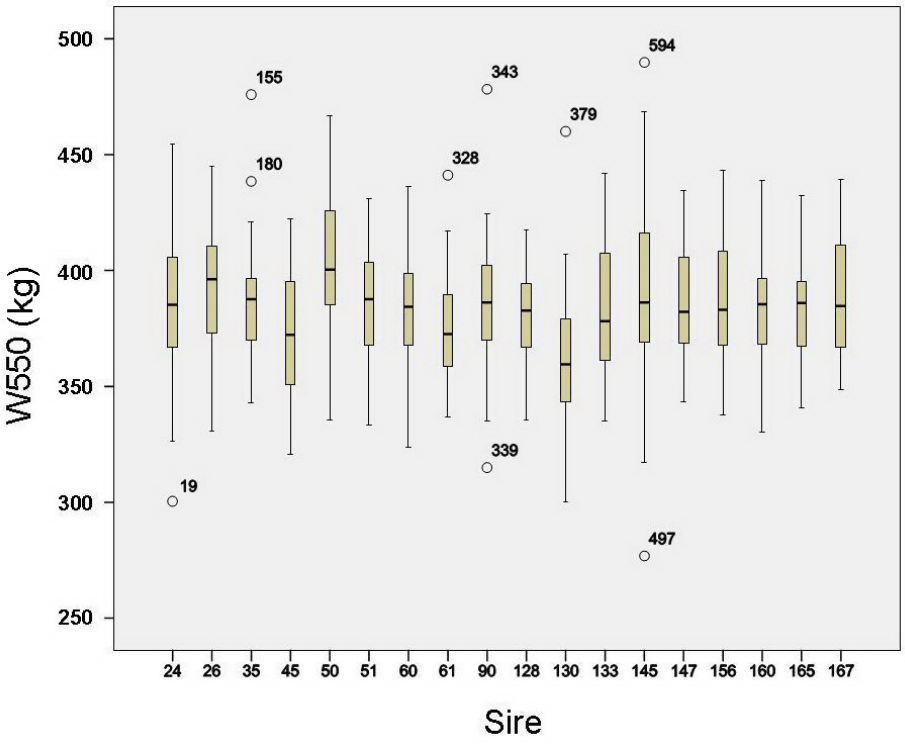

Figure 1. Average W550 distribution of 736 half-sib bulls grouped per sire progeny. Depicted is the progeny of sires that fathered at least 18 half-sib bulls in the feeding test.

To eliminate the bias due to parental origin, we distributed the bulls with no missing genotypic and phenotypic information into genotype classes, and we then calculated the average growth and carcass traits for each half-sib group within each pooled genotype class. The normalized genotype classes and respective mean values of selected growth and carcass traits are depicted in Table 4.

Table 4. Average of traits per normalized genotype classes.

Genotype classes		N (bulls/pools)	W550 (kg)	ADG (g/day)	FAT (mm)	MARB	SC (cm)	REA (cm^2)
1	s/s-s/s-s/s	7/4	386	634	1.71	2.6	29	57
2	s/l-s/s-s/s	41/17	381	630	1.87	2.3	28	57
3	s/l-s/s-s/l	23/7	382	676	1.83	2.6	29	56
4	s/l-s/l-s/s	4/4	410	644	2.43	2.4	29	55
5	s/l-s/l-s/l	3/3	362	589	2.75	1.5	28	54
6	l/l-s/s-s/s	198/48	391	658	1.84	2.3	28	57
7	l/l-s/s-s/l	69/25	384	650	1.95	2.3	29	56
8	l/l-s/s-l/l	8/8	392	657	1.76	2.4	28	53
9	l/l-s/l-s/s	73/28	382	660	2.08	2.1	28	55
10	l/l-s/l-s/l	33/20	396	646	2.07	2.3	29	57

s = short (wide type) allele; l = long (domestic) allele. For other abbreviations, see legend to Table 3.

A synergistic effect was observed in that the stepwise substitution of shorter alleles with longer alleles in the *GH1-GHR-IGF1* axis was associated with increased W550, ADG and FAT. A threshold effect on ADG was associated with size variation at the *GH1* locus. A simple best-fit model indicated a 3- to 6-fold effect of *GH1* variation on ADG, as compared to the variation at *GHR* and *IGF1* loci, respectively. A threshold effect on FAT was associated with substitution of the short *GHR* allele by the longer *GHR* alleles. The effect of the *GHR* variation on FAT was 10-fold that of the variation at the *GH1* and *IGF1* loci (Figure 2).

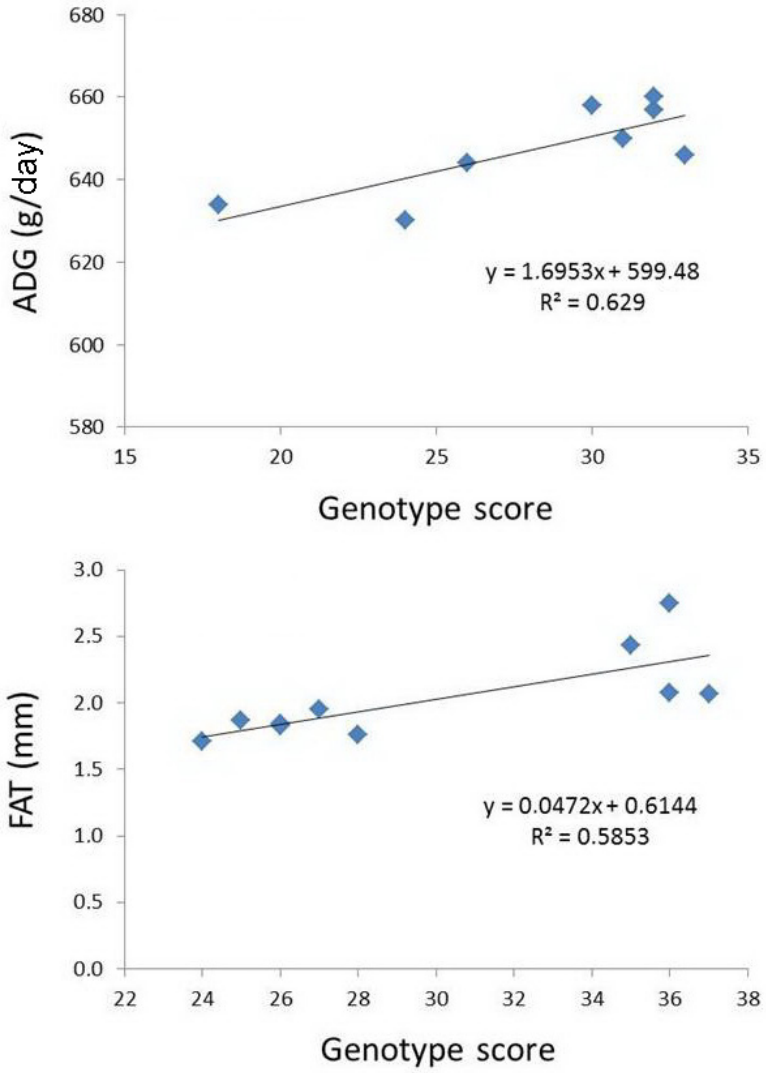

Figure 2. Best-fit models of genotype-phenotype associations in the *ad libitum* condition for average daily gain (ADG) [((GH1a+GH1b)*6) + ((GHRa+GHRb)*2) + ((IGF1a+IGF1b)*1)] and rib eye fat thickness (FAT) [((GH1a+GH1b)*1) + ((GHRa+GHRb)*10) + ((IGF1a+IGF1b)*1)]. Alleles a,b = 1, if short or wild type; a,b = 2, if long or domestic type.

MARB was inversely correlated with FAT, but no significant correlation was found between MARB and ADG, W550 or any particular *GH1-GHR-IGF1* genotype (Figure 3).

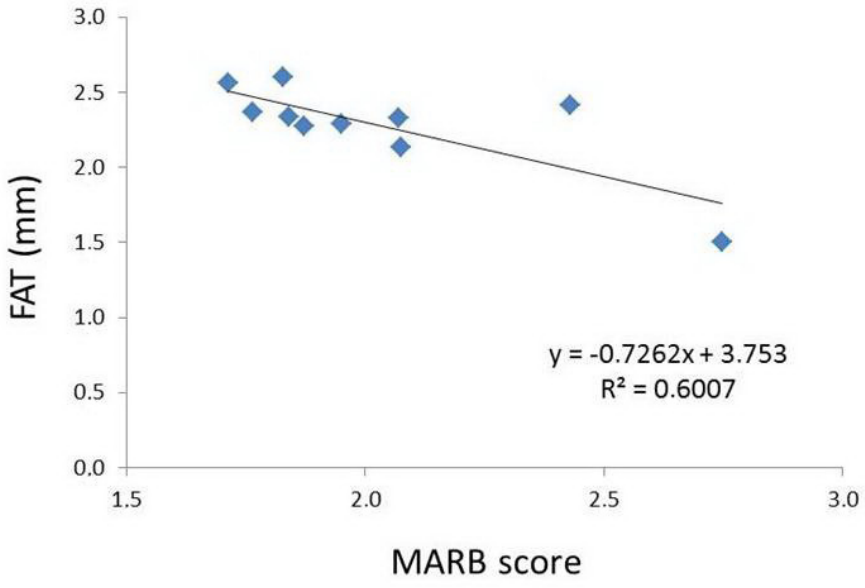

Figure 3. Rib eye fat thickness (FAT) and marbling score (MARB) are inversely correlated across 10 genotypes of the *GH1-GHR-IGF1* axis.

DISCUSSION

Associations of DNA polymorphisms and bovine growth and carcass traits have been reported for *GH1* (Schlee et al., 1994; Lee et al., 1996; Rosa et al., 1996; Grochowska et al., 2001; Sorensen et al., 2002; Paz, 2002; Lima, 2003; Katoh et al., 2008; Ardiyanti et al., 2009; Matsuhashi et al., 2011), *GHR* (Hale et al., 2000; Ge et al., 2003; Garrett et al., 2008) and *IGF1* (Ge et al., 2001; Li et al., 2004; Curi et al., 2005; Andrade et al., 2008; Maj et al., 2008; Kim et al., 2009; Islam et al., 2009; Reyna et al., 2010).

Most of these studies have been performed on European cattle (*B. taurus*), which is believed to have undergone a more intensive domestication as compared to Zebu cattle (*B. indicus*). Also, almost all of these studies have focused on isolated effects of *GH1*, *GHR* or *IGF1*, in different *ad libitum* conditions. In the present study, we examined the synergistic and threshold effects of the genotypic transitions in the *GH1-GHR-IGF1* axis in the Brazilian Nelore bulls fed *ad libitum* on irrigated pasture.

Although the Nelore breed is usually considered as *B. indicus*, it does not constitute a genetically homogeneous population - perhaps reflecting the longitudinal diverse environmental realities in Brazil and also the genetic mixing with *B. taurus* breeds and other *B. indicus* breeds. This genetic mixing that occurred in the process of formation of the Nelore breed has been advantageous in the context of the diverse Brazilian environments, because the crossbred *taurus-indicus* and *indicus-indicus* usually show hybrid vigor.

In a genetic similarity study, we found that the Nelore population is divided into at

least two genetically distinct populations, which we have classified as the "thrifty type" and the "demanding type" (Dani et al., 2008). At least one gene has been associated with the thrifty phenotype in DR conditions, namely the wild-type allele *G1* of the *GH1* promoter region (Dani et al., 2010).

In expanding our analyses to the *GH1-GHR-IGF1* axis in the present study, we found synergistic and threshold effects in that each stepwise substitution of a wild-type allele with a longer allele was associated with differentially increased weight gain and fat accrual in the *ad libitum* condition. Our results are in agreement with some isolated findings as reported in other studies, but also demonstrate novel aspects of regulation in the bovine somatotropic axis as follows.

GH1 substitution effects

Our own studies (Lima, 2003; Dani et al., 2010) indicate that substitution of *G1* with *G2* results in significantly increased weight gain and increased hip height in the *ad libitum* conditions of feedlot experiments. Homozygous *G2/G2* Nelore bulls in a feedlot had an extra 34 ± 20 g/day average weight gain during a 112 day feeding period, equivalent to an extra 10 ± 5 kg at 378 days of age, and were 2 cm taller as compared to *G1/G2* males at 378 days of age (Lima, 2003). Also, the results of a *G1/G2* x *G1/G2* breeding test indicated that *G1/G1* and *G2/G2* full siblings can differ in weight at 365 days of age by as much as 50 kg (Dani et al., 2010). However, homozygous *G2/G2* heifers at 550 days of age in the pasture were only 6 ± 4 kg heavier as compared to *G1/G2* heifers (Lima, 2003). Under more exacting DR, the *G1* allele sustained growth better than the *G2* in young bulls (Dani et al., 2010). In the latter study, the trend towards better performance of *G2/G2*, as observed in the more regular *ad libitum* feedlot model, disappears in the pasture feeding model. Under the more exacting DR conditions, *G1/G1* cattle (and *G1/G2* cattle to a lesser extent) average better in postweaning growth or, more appropriately, they are more resilient to DR-related decreases in growth rates than their *G2/G2* counterparts. Under exacting DR, two *G1* alleles provided for maximal adaptation - as growth resilience and thriftiness - while one or two *G2* alleles were detrimental or abiotropic.

The present study indicates that the thrifty effect of the shorter *GH1* allele can be at least partly explained by downregulated growth and upregulated fat accrual in DR conditions. In the present study, we found that size variation at the *GH1* locus was associated with a threshold effect on ADG. Our best-fit model indicated a 3- to 6-fold effect of *GH1* variation on ADG, as compared to the variation at *GHR* and *IGF1* loci, respectively. Our findings are in line with the observations made in the oMtla-oGH transgenic mouse model, where different levels of GH1 expression can be induced. In this model, chronic highly elevated expression of GH1 enhances overall body growth with minimal adipose accretion, while moderate levels of circulating GH1 fail to enhance body growth yet promote adipose deposition (Oberbauer et al., 2004). The lowest GH1 levels did not increase body size but did enlarge fat depots. The highest levels of circulating GH1 increased all forms of the GHR, IGF1, and hepatic lipoprotein lipase mRNA. The dissociation of GH1 effects on growth and adipogenesis as a function of circulating GH1 levels suggests that the level of GHR and IGF1 expression acts through a threshold mechanism and that low expression results in adipogenesis while high expression generates body growth.

GHR substitution effects

Our results indicating a direct effect of the longer *GHR* alleles on growth and carcass traits are in partial agreement with those of Hale et al. (2000). These authors found an association of the short *GHR* allele with decreased growth in Angus steers. Contrasts for long/long homozygotes *vs* short/long heterozygotes were significant for weaning weight and carcass weight. Approaching significance was the contrast for USDA marbling score, whereas no significant differences were detected for contrasts in birth weight, longissimus muscle area, or carcass fat depth. We did find an association of the large *GHR* alleles with W550, ADG and FAT, but this association was only visible after taking the synergistic effects of *GH1* and *IGF1* variation into account. The effect of *GHR* variation on FAT was 10-fold that of the variation at the *GH1* and *IGF1* loci (Figure 2). MARB was inversely correlated with FAT, but no significant correlation was found between MARB and ADG, W550 or any particular *GH1-GHR-IGF1* genotype (Figure 3). It has been shown that FAT is more accurate than MARB in predicting fat content (Bullock et al.,1991; Belk et al., 1991), perhaps reflecting the fact that marbling is more directly influenced by a set of genes outside the somatotropic axis. Also, it is known that different cattle breeds differ in their ability to partition fat between the internal and the carcass depots (Sprinkle et al., 1998). Fat deposition in ectopic depots such as skeletal muscle and liver can cause lipotoxicity and impair insulin action. Conversely, expansion of subcutaneous adipose tissue may confer protection from metabolic derangements by serving as a 'metabolic sink' to limit both systemic lipids and the accrual of visceral and ectopic fat (Huffman and Barzilai, 2010).

IGF1 substitution effects

The effects of the region located on chromosome 5 on growth traits have been documented for the interval between the TEXAN15 and BMS1248 microsatellite markers located 4.2 and 15.4 cM from the *IGF1* candidate gene, respectively (Davis et al., 1998; Stone et al., 1999; Casas et al., 2000). Moody et al. (1996) found a significant effect of this polymorphism on weaning weight of selected and control populations of Hereford cattle, but they were careful in the interpretation of these results because the structure of the study population was not ideal. On the basis of results obtained with two different Canchim (Nelore x Charolais) lines, Machado and co-workers (2003) concluded that the *IGF1* microsatellite is not directly responsible for variations in growth traits. In another study with Canchim cattle, the *IGF1* polymorphism was found to be associated with phenotypic variation and breeding values only in the early phase of growth, from birth to 240 days (Andrade et al., 2008). Curi et al. (2005) found no effect of the substitutions of the *IGF1* alleles on growth and carcass traits in Nelore or Angus-Nelore crossbreds. In the present study, we found no isolated effect of *IFG1* on growth and carcass traits, but we did find a weak synergistic effect of *IGF1* on the *GH1-GHR-IGF1* axis. This effect appeared to be more related to the *GH1* variation than to *GHR* variation. Future studies should explore associations with factors other than *GH1* or *GHR* such as insulin and thyroid hormones, which may affect *IGF1* signaling (Ronge and Blum, 1989).

Regulation of growth and fat accrual at the somatotropic axis: an overall view

The observed threshold of response of growth and fat accrual to allele substitutions in

the bovine somatotropic axis may be attributed to differences in half-lives of GH1 and GHR. The half-life of GH1 is shorter than that of GHR, and GHR have ligands and modulators other than GH1 that modify GHR expression, surface availability and signaling (Birzniece et al., 2009). Also, GH1 autofeedback signaling is significantly affected by GHR amount, processing, maturation, ligand binding and signaling (Asa et al., 2007).

As a consequence, only a sustained increase in GH1 synthesis would overcome the thresholds for the different GH1 actions. It has been shown, for example, that GH1 treatment (1.5-2 h) of Chinese hamster ovary cells, stably transfected with GH1 receptor cDNA (CHO4), resulted in increased cellular lipid synthesis (240% of control). However, if GH1 treatment of CHO4 cells was prolonged (16 h), this instead decreased cellular lipid synthesis (128% of control) (Möller et al., 1994). This pattern of change correlates with the changes observed in the transition from the shorter *GH1* allele to the longer *GH1* allele.

Circulating GH1 levels rise in response to nutrient deprivation and fall in states of nutrient excess (reviewed in Luque et al., 2011). A classical example is the seasonal regulation of the GH1/IGF1 axis in bears, in which diminished GH1/IGF1 activity by overeating promotes fat storage in preparation for winter denning (Blumenthal et al., 2011). Low GH1 secretion is associated with features of the metabolic syndrome, in particular increased visceral body fat and decreased lean body mass. The combination of fasting and GH1 exposure translates into enhanced lipolysis and reduced IGF1 activity in healthy men (Möller et al., 2009a). Yet, the GH1-mediated reduction in adipose tissue growth is due to a reduction in lipogenesis, which is the consequence of GH1 blunting the effects of many insulin-dependent events; lipolysis is not directly affected (Etherton et al., 1993; Etherton, 2000). Plasma IGF1 concentrations are exclusively dependent on basal GH1 levels, and GH1 pulses do not determine plasma IGF1 concentrations (Faje and Barkan, 2010). Release of GH1 is regulated by somatostatin (Luque et al., 2008) and ghrelin (Zhao et al., 2010).

An increase in the amount of GHR relative to GH1 and the consequences thereof would be the correlate of the substitution of the shorter *GHR* allele with the longer ones. We saw that this transition had a direct effect on ADG, and a threshold effect on fat accrual. Adipose tissue is a GH1-responsive tissue in which GH1 regulates energy metabolism by interacting with its specific receptor, GHR. Several lines of evidence indicate that changes in the expression levels of *GHR* dramatically affect adiposity: i) *GHR* gene expression is dramatically upregulated during preadipocyte-adipocyte differentiation (Zou et al., 1997); ii) *GHR* gene polymorphisms modulate adiposity and IGF1 activity in adolescents (Mong et al., 2010); iii) growth hormone receptor gene-disrupted ($GHR^{-/-}$) mice exhibit increased lifespan and adipose tissue mass (Berryman et al., 2010); iv) the functional inactivation of two copies of the GHR by mutations results in primary GH1 insensitivity in humans with short stature and progressive and marked obesity (Laron syndrome), whereas the presence of one wild-type allele is associated with varying levels of normality in the GH1-induced action, with symptoms ranging from short stature to Laron syndrome (Shevah et al., 2004; Fang et al., 2007; Aalbers et al., 2009), and v) treatment of Laron syndrome patients with IGF1 reduces their body fat mass, indicating that IGF1 exerts a direct effect on adipose tissue metabolism (Laron and Klinger, 1993). However, GHR blockade by pegvisomant without changes in circulating or tissue IGF1 levels, selectively suppresses lipid mobilization and oxidation after short-term fasting in healthy young men (Möller et al., 2009b). This supports the notion that decreased lipogenesis during fasting is a primary and important effect of GH1.

The observed effects of genotypic transitions in the *GH1-GHR-IGF1* axis are likely to be directly related to allele substitution effects on gene transcriptional dynamics at the *GH1* and *GHR* sites and, consequently on metabolic regulation of the phenotypic traits. The *G1* allele, characterized by one AAG trinucleotide is located in the *GH1* promoter region, 9 bp upstream of the TATA-box transcription control site. Any change in the sequence of this site may affect the transcriptional efficiency of the locus. The AAG tandem repeat, which characterizes the *G2* allele, presumably increases transcriptional efficiency in the *GH1*, as suggested by the better growth performance of *G2/G2* animals. Available data support the view that the short, wild-type *GH1* allele is associated with downregulated growth and upregulated fat accrual in DR, as compared to the longer allele. Yet, the short, wild-type *GHR* allele is associated with downregulated growth and downregulated fat accrual in the *ad libitum* condition.

Complex patterns of metabolic regulation arising from distinct genotypic combinations of *GH1*, *GHR* and *IGF1* in different environmental conditions are likely to occur. These should be better examined by breeding appropriate parental lines of cattle, which still do not exist, and observing these animals in different environmental conditions. The development of experimental parental lines is needed because the analysis of genetic-phenotypic associations is often affected by genotypic sampling bias. For example, homozygous *G1/G1* animals are very rare in commercial *B. indicus* herds, and absent in *B. taurus* herds. The short *GHR* allele is very common in *B. indicus*, but absent in *B. taurus*. Of the ten *GH1-GHR-IGF1* multi-genotypes identified in our Nelore herd, the predominant multi-genotype was homozygous for the large *GH1* promoter (long/long, *G2/G2* or domestic type), short *GHR* promoter (short/short or wild-type), and short *IGF1* microsatellite (short/short or wild-type). The homozygous L variant of the L127V *GH1* polymorphic site was fixed in the Nelore population. The predominant multi-genotype of the Nelore breed suggests that selection pressure in this breed has been directed towards high ADG and W550, and low FAT, under *ad libitum* conditions.

Our results suggest that the thrifty phenotype of cattle carrying the shorter promoter regions of *GH1* and *GHR* is at least partly explained by downregulated growth and upregulated fat accrual. Future study should be aimed at determining the transcriptional activities of the *GH1* and *GHR* coding sequences in different allele combinations, and the effects thereof in different nutritional conditions.

ACKNOWLEDGMENTS

Research supported by Excegen Genética S.A., Genon Genética Ltda., and grants from FAPESP (Genon, S.U. Dani), SEBRAE-MG (Excegen), FAPEMIG (Excegen, M.A.C. Dani and S.U. Dani), and CNPq (M.A.C. Dani and S.U. Dani). We thank the following individuals: C. Trancoso, D.V. Arruda, and L.M. Souza for laboratory assistance; F.S. Teles, S. Campos and A.F. Campos for assistance in setting up the data bank; P. Jardim and J.L.F. Spelta for secretarial assistance; J.H. Rabelo for field assistance; E.K. Pedroso for help establishing a collaboration with Perdizes Farm in Jaraguari (Quilombo Empreendimentos & Participações Ltda., and A.S. Ferreira); co-workers of the Perdizes Farm for field assistance, and O.S. da Silva Filho for support. M.A.C. Dani and S.U. Dani hold the following patents with Excegen Genética S.A. that are related to the subject of this study: PI 0403770-7 A (2006) and PI 0503427-2 (2007), INPI, Brazil.

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