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Associations between newly discovered polymorphisms in the *Bos taurus growth hormone receptor* gene and performance traits in Holstein–Friesian dairy cattle

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Summary

Variations in the *growth hormone receptor* (*GHR*) gene sequence are associated with performance traits in cattle. For example, the single nucleotide polymorphism (SNP) F279Y in transmembrane exon 8 has a strong association with milk yield. In this study, 32 previously unreported, putative novel SNPs (31 in the 5′ non-coding region) were identified by resequencing ~19 kb of the *GHR* gene in genomic DNA from 22 cattle of multiple breeds. A population of 848 Holstein–Friesian AI sires was subsequently genotyped for the 32 putative novel SNPs and seven published SNPs (including F279Y, one in exon 1A promoter and five in exon 10). Associations between each segregating SNP and genetic merit for performance were quantified in the 848 Holstein–Friesians using weighted animal linear mixed models. Six of the published SNPs and seven of the novel SNPs were associated with at least one of the traits – milk yield, fat yield, protein yield, fat percentage, protein percentage, somatic cell score, calving interval, survival and growth and size traits. Even when the allelic substitution effect ($P < 0.001$) of F279Y was accounted for, the allelic substitution effect of one of the novel SNPs (GHR4.2) in the 5′ non-coding region of *GHR* was associated with a lactation milk yield of 37.46 kg ($P < 0.001$). GHR4.2 and F279Y were not in linkage disequilibrium ($r^2 = 0.00$, $D' = 0.04$) in the 848 Holstein–Friesians, indicating that their association with milk yield was independent.

Keywords association, growth hormone receptor, Holstein–Friesian, single nucleotide polymorphism.

Introduction

Growth hormone (GH), also known as somatotrophin, stimulates important physiological processes in cattle, particularly post-natal growth and milk production (Etherton & Bauman 1998). The action of GH is mediated by the transmembrane GH receptor (GHR), which comprises a 24-amino acid single transmembrane domain, an extracellular binding domain and a long cytoplasmic domain (Kopchick & Andry 2000). Binding of GH to GHR activates an intracellular signalling pathway (Zhou & Jiang 2006) that induces the transcription of many genes including the *insulin like growth factor-1 (IGF-1)* gene (Jiang *et al.* 2007). The GH-IGF-1 system controls processes such as fertility and nutrient partitioning necessary for lactogenesis (Lucy *et al.* 2009).

The *GHR* gene is over 300 kb, with nine translated exons and a long 5′-untranslated region (5′-UTR) containing nine

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untranslated exons (numbered 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I) that are scattered over its long (~135 kb) 5′-non-coding region. These are alternately expressed from at least three different promoters (P1, P2 and P3) that are activated by several different transcription factors including hepatocyte nuclear binding factor-4γ (HNF-4γ) (Jiang & Lucy 2001; Jiang *et al.* 2005) and Sp1 (Jiang *et al.* 2000). The untranslated exons are then alternately spliced onto the 5′ end of exon 2 to generate multiple *GHR* mRNA variants that differ in the 5′-UTR (Jiang *et al.* 1998; Lucy *et al.* 1998; Jiang & Lucy 2001). These possibly have different translation efficiencies, e.g. the liver-specific *GHR* 5′-UTR variant 1A was shown to be efficiently translated whereas the variant 1B strongly inhibits translation (Jiang & Lucy 2001).

Variation in the *GHR* gene sequence is associated with a number of performance traits in cattle (Blott *et al.* 2003; Ge *et al.* 2003; Viitala *et al.* 2006; Garrett *et al.* 2008), the widest reported of which is the single nucleotide polymorphism (SNP) in exon 8 that causes a phenylalanine-to-tyrosine substitution (p.F279Y, hereafter referred to as F279Y) in the transmembrane domain of the GHR protein. This SNP has been shown to be associated with milk yield and composition (Blott *et al.* 2003; Viitala *et al.* 2006), as well as feed intake, feed conversion efficiency and body energy traits (Banos *et al.* 2008).

The objectives of this study were to identify novel SNPs in the *GHR* gene of dairy and beef cattle and to quantify associations of these novel SNPs and known *GHR* gene SNPs with performance and other traits in Holstein–Friesian dairy cattle.

Materials and methods

Resequencing and SNP identification

Genomic DNA samples, extracted from blood using a previously described method (Montgomery & Sise 1990), from 22 cattle of five different breeds (four Simmental, four Angus × Holstein, four Belgian Blue × Holstein, six Holstein and four Charolais) were used as templates for PCR amplification of ~1 kb surrounding 19 bovine *GHR* SNPs in the ENSEMBL database (http://www.ensembl.org/index.html; accessed March 2008). PCR amplicons were sequenced using the Sanger method and putative SNPs were identified by alignment, using CLUSTALW version 2.0 (http://www.ebi.ac.uk/Tools/clustalw2/index.html; Larkin *et al.* 2007). Chromatograms were analysed using CHROMAS LITE freeware version 2.01 (http://www.technelysium.com.au/chromas_lite.htm) to confirm identity of the SNPs and to determine whether they were homozygous or heterozygous in each of the 22 DNA samples.

In addition to the novel SNPs detected (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_viewBatch.cgi?sbid=1049006), seven previously published SNPs were identified from the literature (Table 1) that were not on the ENSEMBL *GHR* sequence (accession no. ENSBTAG00000001335). These were not screened for in the 22 animals.

Genotyping

Genotyping for each of the *GHR* SNPs was carried out on genomic DNA from 914 Holstein–Friesian sires with the Sequenom MASSARRAY® iPLEX Gold assay (Sequenom) using a chip-based matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometer (Leushner & Chiu 2000; Bray *et al.* 2001). Multiplex SNP assays were designed using SPECTRODESIGNER software and realSNP.com online tools (Sequenom). The 914 Holstein–Friesian sires had progeny in Ireland and were representative of the germplasm used in Irish dairy herds in past years.

Data for association analyses

Separate DNA samples of 25 Holstein–Friesian sires were genotyped twice for all SNPs. Concordance across all SNPs and all duplicates was 99%. Where discordance existed, the genotype for the sample in question was set to missing. Quality control of the genotypes was undertaken on all SNPs across all 914 individuals. An iterative algorithm was used to simultaneously discard both SNPs and individuals with poor genotype call rates. Initially SNPs with a call rate across all individuals that was lower than 75% were discarded, followed by individuals with a call rate across all remaining SNPs of less than 85%. The second iteration removed SNPs with a call rate across all remaining individuals of less than 90% and individuals with a call rate across all remaining SNPs of less than 90%. Genotypes of 21 individuals were discarded in the first iteration and 45 were discarded in the second iteration. No *GHR* SNPs were discarded for poor call rates. The average co-ancestry among the remaining 848 sires was 2.2%.

Summary statistics of SNPs in the *GHR* gene and allele frequency were derived from this edited dataset. A measure of linkage disequilibrium (r^2; Hill & Robertson 1968) was calculated between each pairwise combination of the segregating SNPs using the reconstructed haplotypes in Haploview (Barrett *et al.* 2005).

Daughter yield deviations (DYDs), expressed on the scale of predicted transmitting ability (PTA), as well as associated reliabilities for a range of performance traits evaluated by the Irish Cattle Breeding Federation in the January 2009 domestic genetic evaluations were available. Models used in genetic evaluations in Ireland, as well as variance components, are summarized in detail by Berry *et al.* (2007). DYDs for 305-day milk, fat and protein yield as well as geometric mean $\log_e$ somatic cell count [hereon referred to as somatic cell score (SCS)], are estimated in Ireland using a repeatability animal model across the first five lactations. PTA for calving interval and survival are estimated using a

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multi-trait animal model, including data from the first three lactations. PTAs for milk yield are used to adjust the PTAs for survival for differences in genetic merit of milk yield; hence, this survival trait is functional survival. PTAs for cull cow carcass weight, progeny carcass weight, progeny carcass fat score and progeny carcass conformation score, measured at slaughter, are estimated in a multi-trait animal model that includes weaning weight, live-weight of the animal between 300 and 600 days of age, feed intake and skeletal and muscular linear traits. Cows slaughtered between 875 and 4000 days of age are included in the evaluation of cull cow weight, while male progeny slaughtered between 300 and 1200 days of age and female progeny slaughtered between 300 and 875 days of age are included in the evaluation of the remaining three carcass traits. Genetic evaluations for linear type traits are undertaken as part of a joint evaluation in the UK and Ireland. The PTAs are standardized to the mean and standard deviation of the base population. All PTAs were deregressed using the procedure outlined by Berry *et al.* (2009). Parental contribution to the reliability of each DYD or PTA was removed using the approach of Harris & Johnson (1998), and only sires with a reliability, less parental contribution, of >60% were retained for inclusion in the association analyses. A total of 742 sires fulfilled these criteria for inclusion in the analysis of milk, fat and protein yield as well as milk fat and protein concentration; the number of sires included in the association analysis with SCS, calving interval and survival was 703, 501 and 477, respectively. The number of sires with a reliability of >60% for the carcass traits was 446 and the number of sires with a reliability of >60% for the linear type traits varied from 484 to 551.

Association analyses

The association between each SNP and performance was quantified using weighted linear models mixed in ASREML (Gilmour *et al.* 2009), with individual included as a random effect, and average expected relationships among individuals accounted for through the numerator relationship matrix. Year of birth (divided into 5 yearly intervals) and percent Holstein of the individual sire were included as fixed effects in the model. In all instances the dependent variable was DYD for milk yield, fat yield, protein yield, SCS, and de-regressed PTA for the remaining traits, weighted by their respective reliability less the parental contribution. Genotype was included in the analysis as a continuous variable coded as the number of copies of a given allele. A multiple regression mixed model was developed by backward elimination of the non-significant ($P > 0.05$) segregating SNPs.

Results

Resequencing and SNP discovery

Figure 1 shows the nucleotide (nt) positions of the 39 SNPs investigated in this study relative to exons in the bovine *GHR* gene. Seven of these SNPs were previously published and were all in exon 10, apart from F279Y which is in exon 8 (Table 1). The remaining 32 putative SNPs were discovered by sequencing 19 PCR amplicons (each ~1 kb) from different sections of the bovine *GHR* gene from the 22 animals of five different breeds. In the small number of animals tested, there did not appear to be a breed-specific bias towards one or other allele for any of the SNPs (data

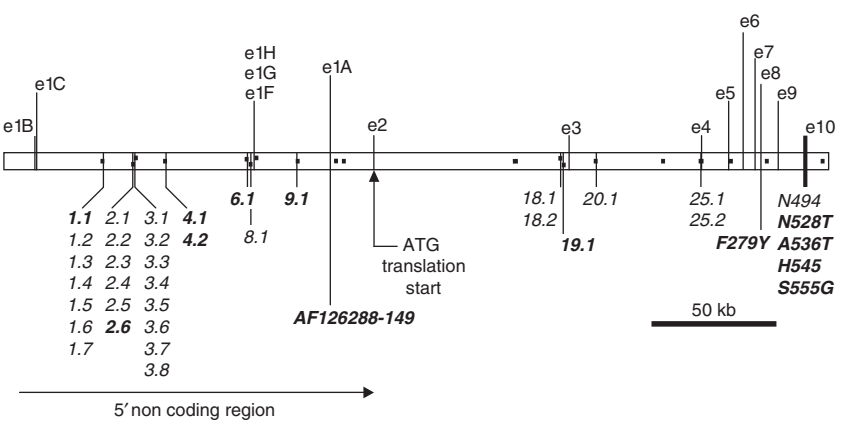

Figure 1 Positions (to scale) of the 39 SNPs (in italics) selected for this study relative to untranslated exons (e1A–e1F) and translated exons (e2–e10) on the bovine *GHR* gene (white box). The 5′ end of e1B is at g.34206563, and the 5′ end of e10 is at g.33897800 on bovine chromosome 20 (Btau_4.0, October 2007). Positions of untranslated exons 1A, B, C, D, E, F, G, H and I are derived from GenBank accession numbers AY249137, AF046861, AF046861, AF036292, AF036291, AF036294, AF036296, AF036297 and AF326349 respectively. Position on the *GHR* gene of PCR amplicons used for sequencing is indicated by black dashes in the white box. Putative novel SNPs are numbered (1.1–25.2; the prefix *GHR* is omitted for clarity) according to PCR amplicon in which they were identified (before decimal point) and the 5′–3′ order on the *GHR* gene (after decimal point). SNPs selected for association analysis are indicated in bold italic. N494 was previously published by Ge *et al.* (2000) at nucleotide position 76 on the sequence submitted under GenBank accession number AF140284.

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Table 1 Details of SNPs used in the association studies.

SNP name	Region of *GHR* gene	Chromosomal base position	SNPs, flanking nucleotide and corresponding amino acid sequence	ss number in dbSNP or GenBank accession number	Reference, designation therein
GHR1.1	5′	34178577	GTTCATACA[A/G]TTATGGAAG	ss159831010	Novel
GHR2.6	5′	34166944	CTTGTATCA[A/G]TCTGTTACA	ss159831011	Novel
GHR4.1	5′	34153375	CTACATGAT[G/T]CGWGATTGG	ss159831012	Novel
GHR4.2	5′	34153345	GATACAAAG[G/T]AACCACAAA	ss159831013	Novel
GHR6.1	5′	34121009	GCATCCTAC[A/G]TCTGTGCAG	ss159831014	Novel
GHR9.1	5′	34101240	AAAGTAATC[A/G]GAAATAAAG	ss159831015	Novel
AF126288:g.149	exon 1A promoter	34086084	AGGCAATGC[A/G]TTGTGTGCTC	AF126288 (nt position 149)	Ge *et al.* (2003), RP(1) Garrett *et al.* (2008), AF126288-149
GHR19.1	intron 2–3	33994639	GCGCCCTCT[A/C]GGTGCTGGC	ss159831016	Novel
F279Y	exon 8	33915502	GTG ACA TTA T[T/A]T TTA CTC V T L [F/Y] L L	AM161140	Blott *et al.* (2003), F279Y Viitala *et al.* (2006)
N528T	exon 10	33897150	ATC GTG GAC A[A/C]C GCT TAC I V D [N/T] A Y	AM161140	Blott *et al.* (2003), *N528T* Viitala *et al.* (2006)
A536T	exon 10	33897127	GAG GTA GAC [G/A]CC AAA AAG E V D [A/T] K K	AF140284 (nt position 200)	Ge *et al.* (2000), 200
H545	exon 10	33897098	GCC CCT CA[C/T] GTC GAG GCT A P [H/H] V E A	AF140284 (nt position 229)	Ge *et al.* (2000), 229 Blott *et al.* (2003), Nt1635 (C-T)
S555G	exon 10	33897070	GTA GAG CCA [A/G]GC TTT AAC V E P [S/G] F N	AF140284 (nt position 257) AM161140	Ge *et al.* (2000), 257 Viitala *et al.* (2006)

Sequences are written in the 5′ to 3′ direction of the *GHR* gene, which is on the minus strand of the published Btau_4.0 chromosome 20.
GHR, growth hormone receptor.

not shown). To the knowledge of the authors, the 32 putative SNPs discovered in this study by resequencing have not been described previously in the literature or dbSNP and are therefore novel. All 32 putative novel SNPs were biallelic and consisted of 30 transitions and two transversions.

SNP summary statistics from Holstein–Friesian population

The r^2 and D' values for LD between SNPs in this study are shown in Table S1. Several SNPs were in strong LD, particularly those in close physical proximity (within 200–500 bp) to PCR amplicons 1, 2 and 3 (Fig. 1). Unexpectedly, some SNPs that were in close physical proximity were not in complete LD; the r^2 between GHR4.1 and GHR4.2 was 0.73, even though these SNPs were only 30 bp apart. Of the 32 putative novel SNPs, nine were monomorphic in the 848 Holstein–Friesians: GHR1.6 (C/C), GHR3.4 (T/T), GHR3.5 (T/T), GHR3.6 (C/C), GHR3.7 (G/G), GHR3.8 (A/A), and GHR8.1 (A/A), GHR18.1 (C/C), GHR25.1 (T/T); two SNPs had a minor allele frequency of <2%: GHR18.2 (96% C/C; 3% C/G; 0.1% G/G) and GHR20.2 (96% T/T; 3% T/C and 0.1% C/C); and one SNP (GHR1.7) was coded as being 99.9% heterozygous. These 12 SNPs were excluded from the association analyses.

Because of the strong linkage disequilibrium between phases among the SNPs GHR1.3, GHR1.4, GHR1.5, GHR2.1, GHR2.2, GHR2.3, GHR2.4, GHR2.5, GHR2.6, GHR3.1, GHR3.2 and GHR3.3, only GHR2.6 (i.e. the SNP with the greatest call rate) was retained for the association analysis. Furthermore, SNP N494 was in complete LD with g.536A>T (Table S1), therefore only the latter was retained. Following the removal of all the aforementioned putative SNPs, a total of 13 segregating SNPs remained, details of which are summarized in Table 1. The genotype frequencies of the 13 SNPs are detailed in Table 2. Minor allele frequency (MAF) varied from 0.04 to 0.48. Two of the SNPs (AF126288:g.149 and GHR6.1) deviated ($P < 0.001$) from Hardy–Weinberg equilibrium; both were due to a greater frequency of heterozygotes than expected.

Association analyses

There were a number of strong associations with milk traits and somatic cell score for SNPs positioned on the *GHR* gene including GHR2.6 to F279Y (Table 3). The synonymous SNP H545 was the only SNP in exon 10 that was associated with a milk trait, albeit this was a weak association. As expected, F279Y showed strong associations with milk yield, milk fat content and milk protein content but not protein yield, and only a weak association with fat yield. There were also weak asso-

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Table 2 Genotype frequency, minor allele frequency (MAF) and significance of deviation for Hardy–Weinberg equilibrium (HWE) for the 13 segregating SNPs included in the association analysis.

SNP	Genotype	Genotype frequency (%)	MAF (%)	HWE
GHR1.1	A/A	0.35	0.40	0.18
	A/G	0.50		
	G/G	0.15		
GHR2.6[1]	A/A	0.17	0.40	0.11
	A/G	0.45		
	G/G	0.37		
GHR4.1	G/G	0.44	0.34	0.56
	G/T	0.44		
	T/T	0.12		
GHR4.2	G/G	0.19	0.41	0.05
	G/T	0.45		
	T/T	0.36		
GHR6.1	A/A	0.40	0.40	<0.0001
	A/G	0.40		
	G/G	0.20		
GHR9.1	A/A	0.11	0.33	0.45
	A/G	0.43		
	G/G	0.46		
AF126288:g.149	A/A	0.16	0.36	0.0005
	A/G	0.41		
	G/G	0.43		
GHR19.1	A/A	0.22	0.48	0.29
	A/C	0.52		
	C/C	0.26		
F279Y	T/T	0.75	0.13	0.87
	A/T	0.23		
	A/A	0.02		
N528T	A/A	0.76	0.13	0.29
	A/C	0.22		
	C/C	0.02		
A536T[2]	A/A	0.00	0.04	0.19
	A/G	0.07		
	G/G	0.93		
H545	C/C	0.03	0.17	0.40
	C/T	0.27		
	T/T	0.69		
S555G	A/A	0.78	0.12	0.99
	A/G	0.21		
	G/G	0.01		

[1]Because of strong linkage disequilibrium between phases among the SNPs GHR1.3, GHR1.4, GHR1.5, GHR2.1, GHR2.2, GHR2.3, GHR2.4, GHR2.5, GHR2.6, GHR3.1, GHR3.2 and GHR3.3, only GHR2.6 was retained for the association analysis.

[2]N494 was in complete LD with A536T.

Table 3 Estimated allele substitution effect (standard error in parenthesis) between 13 SNPs and milk performance, SCS, fertility and survival.

SNP	Allele substitution	Milk yield (kg)	Fat yield (kg)	Protein yield (kg)	Milk fat content[1] (%*100)	Milk protein content[1] (%*100)	SCS (units*100)	Calving interval (days)	Survival[1] (%*100)
GHR1.1	A>G	11.27 (10.08)	−0.49 (0.36)	−0.40 (0.28)	−1.52* (0.75)	−1.37*** (0.36)	−0.78 (0.61)	0.40 (0.26)	−11.83 (14.3)
GHR2.6[2]	A>G	−21.89* (10.62)	−1.22** (0.37)	−1.23*** (0.29)	−0.43 (0.80)	−0.71† (0.39)	−1.43* (0.64)	−0.24 (0.28)	11.65 (15.21)
GHR4.1	G>T	−29.36** (10.01)	−0.81* (0.36)	−0.77** (0.28)	0.61 (0.76)	0.43 (0.37)	−0.47 (0.60)	−0.19 (0.26)	9.64 (14.2)
GHR4.2	G>T	37.46*** (10.03)	0.90* (0.35)	0.88** (0.28)	−0.94 (0.76)	−0.78* (0.37)	0.43 (0.61)	0.31 (0.26)	−12.24 (14.42)
GHR6.1	A>G	10.02 (10.36)	−1.29*** (0.36)	−0.79** (0.29)	−2.68*** (0.78)	−1.87*** (0.37)	−1.67** (0.63)	−0.16 (0.27)	6.70 (14.88)
GHR9.1	A>G	−19.47† (10.92)	−1.13** (0.39)	−1.24*** (0.30)	−0.50 (0.82)	−0.92* (0.40)	−1.03 (0.65)	−0.42 (0.28)	15.39 (15.82)
AF-126288-149	A>G	−13.62 (10.57)	1.49*** (0.37)	0.83** (0.29)	3.35*** (0.79)	2.18*** (0.38)	1.60* (0.64)	0.13 (0.27)	−1.51 (14.93)
GHR19.1	A>C	7.48 (9.87)	1.07** (0.35)	0.91*** (0.27)	1.25† (0.74)	1.07*** (0.36)	1.57** (0.59)	−0.03 (0.25)	8.61 (13.92)
F279Y	T>A	73.8*** (13.49)	−1.08* (0.49)	−0.20 (0.38)	−6.62*** (1.01)	−4.76*** (0.48)	−1.35 (0.83)	0.89* (0.36)	−28.70 (19.41)
N528T	A>C	−11.53 (14.32)	−0.55 (0.51)	−0.59 (0.40)	0.00 (1.07)	−0.27 (0.53)	−1.22 (0.86)	−0.46 (0.36)	40.53* (20.02)
A536T[3]	A>G	42.74† (24.44)	0.24 (0.87)	0.73 (0.68)	−2.70 (1.83)	−1.57† (0.89)	−1.34 (1.50)	1.06† (0.58)	−56.01† (32.22)
H545	C>T	24.83† (13.42)	0.61 (0.48)	0.78* (0.37)	−0.83 (1.00)	−0.24 (0.49)	0.82 (0.80)	0.78* (0.33)	−50.11** (18.29)
S555G	A>G	−10.36 (15.16)	−0.57 (0.54)	−0.62 (0.42)	−0.12 (1.13)	−0.44 (0.56)	−0.99 (0.91)	−0.54 (0.39)	43.17* (21.58)

Significance of difference from zero †$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
SCS, somatic cell score.
[1]A value of 1, prior to multiplication by 100, equates to 1 percentage unit.
[2]Because of strong linkage disequilibrium between phases among the SNPs GHR1.3, GHR1.4, GHR1.5, GHR2.1, GHR2.2, GHR2.3, GHR2.4, GHR2.5, GHR2.6, GHR3.1, GHR3.2 and GHR3.3, only GHR2.6 was retained for the association analysis.
[3]N494 was in complete LD with A536T.

ciations with calving interval and survival between SNPs F279Y and S555G in the coding region of the *GHR* gene.

The *GHR* SNPs in this study were also associated with growth performance and size traits (Table 4). There were several strong associations between SNPs in the 5′ non-coding region and cow carcass weight and progeny carcass weight. There were also strong associations with stature, but these were not restricted to a particular region on the *GHR* gene. Mild to weak associations with body depth and

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Table 4 Estimated allele substitution effects (SE in parenthesis) of 13 SNPs in the bovine *GHR* gene on growth performance and size.

SNP	Allele substitution	Cow carcass weight (kg)	Progeny carcass weight (kg)	Progeny carcass conformation[1]	Progeny carcass fat[1]	Stature[2]	Chest width[2]	Body depth[2]	Rump angle[2]	Rump width[2]	Angularity[2]	Body condition score[2]
GHR1.1	A>G	−1.32 (1.01)	−0.31 (0.79)	6.51 (3.27)*	3.45 (2.84)	0.14 (0.89)	−0.18 (0.96)	0.48 (0.84)	−0.51 (0.89)	0.42 (0.93)	1.06 (0.93)	−0.01 (0.74)
GHR2.6[3]	A>G	−1.96** (0.65)	−1.67*** (0.50)	3.99 (2.14)†	3.62 (1.85)†	−2.94*** (0.90)	−0.49 (0.98)	−1.83* (0.85)	−0.51 (0.90)	−0.68 (0.95)	−1.60† (0.95)	0.07 (0.08)
GHR4.1	G>T	−0.24 (0.64)	−0.76 (0.50)	0.16 (2.09)	−1.31 (1.80)	−2.11* (0.92)	0.96 (1.00)	−0.60 (0.86)	0.19 (0.92)	−1.92* (0.96)	−2.22* (0.96)	0.16* (0.08)
GHR4.2	G>T	1.07 (0.65)	1.40** (0.50)	−0.77 (2.13)	−0.92 (1.83)	2.36* (0.92)	−0.22 (1.00)	1.35 (0.85)	−0.20 (0.92)	2.45* (0.95)	2.39* (0.95)	−0.11 (0.08)
GHR6.1	A>G	−2.12** (0.65)	−1.61** (0.50)	3.71 (2.12)†	4.38* (1.84)	−3.76*** (0.91)	0.49 (0.99)	−2.08* (0.86)	−0.97 (0.91)	0.02 (0.96)	−2.35* (0.95)	0.11 (0.08)
GHR9.1	A>G	−1.56* (0.67)	−1.25* (0.52)	3.09 (2.19)	3.60† (1.89)	−2.83** (0.94)	−0.86 (1.03)	−2.59** (0.88)	−1.11 (0.94)	−1.21 (0.99)	−1.89† (0.99)	0.05 (0.08)
AF126288:g.149	A>G	2.26*** (0.67)	1.71*** (0.52)	−3.09 (2.19)	−5.63* (1.89)	2.76** (0.95)	−0.89 (1.02)	1.72† (0.89)	1.32 (0.94)	−1.03 (0.99)	2.02* (0.98)	−0.11 (0.08)
GHR19.1	A>C	1.04 (0.63)	1.18* (0.49)	0.02 (2.05)	−1.92 (1.77)	0.94 (0.88)	−0.07 (0.95)	0.31 (0.83)	1.20 (0.87)	0.15 (0.92)	0.40 (0.92)	−0.05 (0.07)
F279Y	T>A	0.03 (0.89)	−0.13 (0.70)	−0.12 (2.88)	3.19 (2.50)	−0.39 (1.19)	2.59* (1.27)	0.98 (1.11)	0.69 (1.17)	0.68 (1.23)	−0.16 (1.23)	0.17 (0.10)
N528T	A>C	−1.25 (0.94)	−0.52 (0.73)	3.19 (3.07)	2.92 (2.65)	−4.66*** (1.32)	−0.05 (1.41)	−3.15* (1.26)	1.20 (1.31)	−3.65* (1.35)	−3.70** (1.39)	0.18 (0.11)
A536T[4]	A>G	1.91 (1.64)	0.41 (1.26)	−8.31 (5.32)	−6.56 (4.59)	2.50 (2.56)	0.48 (2.78)	3.48 (2.39)	−3.61 (2.59)	−1.71 (2.71)	0.53 (2.72)	0.03 (0.21)
H545	C>T	1.73* (0.88)	0.64 (0.68)	−5.65 (2.91)†	−4.61† (2.50)	4.91*** (1.23)	−0.10 (1.33)	3.64** (1.17)	−2.29† (1.23)	2.91* (1.28)	3.80** (1.30)	−0.18† (0.10)
S555G	A>G	−1.32 (1.01)	−0.31 (0.79)	6.51* (3.27)	3.45 (2.84)	−4.67** (1.43)	0.91 (1.54)	−2.69† (1.37)	1.67 (1.43)	−1.94 (1.49)	−3.53** (1.50)	0.20 (0.12)

Significance of difference from zero $^{\dagger}P < 0.10$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
[1]Scale 1–15 × 100).
[2]Expressed in genetic standard deviation units (*10).
[3]Because of strong linkage disequilibrium between phases among the SNPs GHR1.3, GHR1.4, GHR1.5, GHR2.1, GHR2.2, GHR2.3, GHR2.4, GHR2.5, GHR2.6, GHR3.1, GHR3.2 and GHR3.3, only GHR2.6 was retained for the association analysis.
[4]N494 was in complete LD with A536T.

angularity for SNPs in both the 5′ non-coding and coding region were also observed.

Mixed model regression analysis

Table 5 summarizes the SNPs with associations with each of the performance variables when estimated using multiple regression. Following multiple regression, only three SNPs (GHR4.2, GHR6.1 and F279Y) remained associated with milk traits. Of particular interest was the fact that even after adjusting for the F279Y genotype, GHR4.2 was associated with milk yield. Combined with the low LD ($r^2 = 0.00$) between GHR4.2 and F279Y, this provides strong evidence that the GHR4.2-milk yield association is independent of the F279Y-milk yield association. Following mixed model regression, GHR4.2 also remained associated with yield but not milk fat content. F279Y remained associated with milk fat and protein content but not yield of milk fat and protein. This difference further demonstrates that the associations of GHR4.2 and F279Y with milk traits are probably resulting from independent mutations.

Only AF126288:g.149 remained associated with both progeny and cow carcass weight following multiple regression. GHR4.2 remained associated with progeny carcass weight but not with cow carcass weight. Conversely, H545 remained associated with cow but not progeny carcass weight. Four SNPs (GHR2.6, GHR4.2, AF126288-149 and H545) remained associated with angularity, indicating the possible presence of several mutations across the length of the *GHR* gene that affect this trait. H545 remained associated with seven growth and size traits, indicating that there is probably a causative mutation in the vicinity of this synonymous SNP that affects growth.

Growth hormone receptor 19.1 was the only SNP to remain associated with somatic cell score, indicating that the putative causative mutation for this trait is most closely linked to this SNP. Two SNPs (F279Y and H545) remained associated with calving interval, indicating causative variance at two independent loci. H545 was the only SNP to remain associated with functional survival.

Discussion

The persistence of the association of GHR4.2 with milk production in a multiple regression model that also included F279Y signifies a possible additive effect of both SNPs. To the knowledge of the authors, the GHR4.2 SNP and its associations with milk production have not been reported previously. However, unlike F279Y, the GHR4.2 allele which positively associated with milk yield had a high frequency (36%) in the Holstein–Friesian population, indicating that it may not be antagonistically associated with other performance or fitness traits. This was substantiated by its lack of association with calving interval and functional survival, and it is therefore very interesting

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Table 5 Associations ($P < 0.05$) between *GHR* SNPs and performance when estimated using multiple regression models.

	GHR2.6	GHR4.1	GHR4.2	GHR6.1	GHR9.1	AF126288-149	GHR19.1	F279Y	H545	S555G
Allele substitution	A>G	G>T	G>T	A>G	A>G	A>G	A>C	T>A	C>T	A>G
Milk yield (kg)			41.11 (9.81)					78.74 (13.73)		
Fat yield (kg)			0.78 (0.35)	1.26 (0.38)						
Protein yield (kg)			0.82 (0.28)	0.68 (0.30)						
Milk fat content (%*100)[1]								−6.62 (1.01)		
Milk protein content (%*100)[1]			0.95 (0.35)					−4.62 (0.58)		
Somatic cell score (unit*100)							1.58 (0.59)			
Calving interval (days)								0.99 (0.36)	0.81 (0.33)	
Survival (%*100)									−47.26 (18.59)	
Progeny carcass weight (kg)			1.19 (0.50)			1.55 (0.53)				
Cow carcass weight (kg)						2.19 (0.66)			1.82 (0.87)	
Progeny carcass conformation score (scale 1–15)[3]										6.51 (3.27)
Progeny carcass fat (scale 1–15)[3]						−5.48 (1.89)			−4.70 (2.48)	
Stature[2]				−3.01 (0.92)					3.98 (1.25)	
Chest width[2]								2.59 (1.27)		
Body depth[2]					−1.99 (0.90)				3.09 (1.21)	
Rump angle[2]									−2.29 (1.23)	
Rump width[2]									2.91 (1.28)	
Angularity[2]	3.84 (1.76)		3.71 (1.43)			4.00 (1.44)			3.41 (1.45)	
Body condition score[2]		0.16 (0.08)								

[1]A value of 1, prior to multiplication by 100, equates to 1 percentage unit.
[2]Expressed in genetic standard deviation units (*10).
[3]Values are multiplied by 100.

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as a marker for increasing milk yield without compromising reproductive performance or functional survival.

The association between SCS and the novel SNP GHR19.1 in the intron 2349 bp upstream of exon 3 was not an artefact of dilution, because it was not associated with milk yield. Blott *et al.* (2003) also identified SNP g.33992301T>C and the deletion SNP g.33992352delT on Btau_4.0 chromosome 20, just upstream from exon 3 (exon 3 start is at g.33992290), but predicted that these SNPs were unlikely to affect GHR gene function and therefore did not report their individual associations with performance.

It is only possible to speculate whether any of the novel SNPs identified in the present study and segregating in the Holstein–Friesian population are causative mutations or are merely linked to the causative mutations. GHR1.1, 2.6, 4.1, 4.2, 6.1, 9.1 and 19.1 may have an effect on the complex regulation of *GHR* gene expression mediated by its long 5′-UTR. Although these SNPs are probably too far from the nine known untranslated exons to be involved in the regulation of their transcription, they may affect as-yet unidentified untranslated exons. MATINSPECTOR (Genomatix Software GmbH) analysis showed that GHR1.1, 2.6, 4.1, 4.2, 6.1 and 9.1 were putative binding sites for transcription factors (data not shown), and the alternative allele abolished the binding site in all 6 SNPs (Table S2). For example, MATINSPECTOR showed that GHR4.2 mutates a putative HNF-4. Mutation of a HNF-4 site 180 bp upstream from the start of *GHR* exon 1A was reported to abolish transactivation of GHR P1-mediated transcription in transfected cell cultures (Jiang & Lucy 2001). However, GHR4.2 is too far from a known promoter for this to be likely, and promoter prediction software failed to identify a putative promoter region within 2 kb of GHR4.2.

The association of the F279Y SNPs with milk yield in this study is in very close agreement with reported estimates in Dutch and New Zealand Holstein–Friesians (Blott *et al.* 2003). Blott *et al.* (2003) reported that heterozygous (F/Y) and homozygous (Y/Y) Dutch Holstein–Friesian sires for the Y allele of the F279Y SNP had a 67 kg and 128 kg greater DYD respectively, than sires with two copies of the F allele. Furthermore, Blott *et al.* (2003) clearly documented a SNP by breed interaction in which the association with the Y allele differed between Holsteins and Jersey animals. Although an association between the F279Y polymorphism and fat and protein yield was identified in some of the populations studied by Blott *et al.* (2003), the significance of the association was not consistent across all populations and was not strong ($P > 0.05$). This occured despite a strong association between the F279Y SNP and both concentrations of milk fat and protein (Blott *et al.* 2003). These associations corroborate the results of this study where there was no association between the F279Y SNP and either fat yield or protein yield in milk, although the Y allele was associated with reduced protein concentration and fat concentration in milk.

The low frequency (2%) of the F279Y Y/Y homozygote genotype (2%) in this study is consistent with other populations of Holstein–Friesian in the Netherlands (Blott *et al.* 2003), New Zealand (Blott *et al.* 2003), the UK (Banos *et al.* 2008) and Italy (Fontanesi *et al.* 2007). Paradoxically, despite its strong association with increased milk yield, the Y allele of F279Y, particularly the homozygote Y/Y, occurs at a low frequency in some Holstein populations, even though most of these populations have been aggressively selected for increased milk yield. In addition, Jersey animals tend to have milk with higher concentrations of fat and protein than Holsteins (Prendiville *et al.* 2009), yet the frequency of the Y allele does not differ between these two breeds (Blott *et al.* 2003). Although the low frequency of the Y allele is consistent across most dairy and dual purpose breeds investigated (Blott *et al.* 2003; Fontanesi *et al.* 2007), the opposite (i.e. low frequency of the F allele) is generally observed in beef animals (White *et al.* 2007). The unfavourable association between the Y allele and calving interval observed in this study could explain the low Y allele frequencies that are often observed in dairy breed populations. Although reproductive performance was only recently introduced into breeding objectives in major Holstein populations, the size of the association in this study is considerable and may be associated with 'fitness' rather than calving interval *per se*. Furthermore, because deregressed PTAs were used as the dependent variables in the present study, it was not possible to estimate dominance effects at this locus. It is therefore unclear whether the Y allele is the causative mutation or if it is linked to the causative mutation, possibly a semi-lethal recessive polymorphism, associated with reproductive performance. This would account for the very low frequency of Y/Y homozygotes in almost all dairy populations. In beef cattle, either the negative association between the Y allele and fertility is less important than in dairy breeds, or, if the Y allele is not the causative mutation, the Y allele and the causative allele might not be linked in beef cattle as a result of recombination.

The lack of an association between N528T and milk production found here agrees with the previous studies in Holstein cattle (Signorelli *et al.* 2009) and Finnish Ayrshires (Viitala *et al.* 2006). However, both SNPs were associated with functional survival in this study; functional survival was also associated with H545 ($P < 0.01$) and A536T ($P < 0.10$), two other previously reported SNPs in the *GHR* gene. The association of H545 and A536T (and N494 which was in complete linkage disequilibrium with A536T and therefore was not included in the analysis) with survival is probably mediated through their association with calving interval, and this was substantiated by the lack of an association between either SNP and functional survival when genetic merit for calving interval was included in the multiple regression model as a covariate. To our knowledge, no previous study has

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attempted to relate these SNPs to fertility or functional survival in dairy cattle. Nonetheless, all four SNPs N528T, A536T, H545 and S555G are in close proximity on exon 10 of the *GHR* gene and, with the exception of A536T, are in moderate to strong LD with each other. Therefore it is likely that only one of the SNPs, or a linked polymorphism not included in this study, is the causative mutation associated with survival. This is further substantiated by the fact that only one of the SNPs (H545) remained in the multiple regression model when survival was the dependent variable. As the C to T substitution in H545 does not result in an amino acid change, this is unlikely to be the causal mutation and instead is likely to be genetically linked to the mutation responsible.

H545 also showed strong associations with the growth and body size traits (Tables 4 & 5). Blott *et al.* (2003) reported that H545 (designated as c.1635C>T)) was associated with milk production, but they did not test for an association with growth. Linkage disequilibrium analysis and the mixed multiple regression model show that, despite their close proximity, A536T, H545 and S555G are only in moderate LD, so it is probable that the causative mutation linked to H545 is located somewhere between A536T and S555G, which is a phosphorylation pocket critical for GHR signalling via signal transducer and activator of transcription 5 (STAT5).

In the multiple regression model and after adjusting for the genotype of H545, the association between S555G and carcass conformation score persisted. To the knowledge of the authors, this is the first report of associations between A536T, H545 and S555G and growth and body size. Ge *et al.* (2003) found no significant association of A536T, H545 and S555G with IGF-1 concentration, weight gain or off-test hip height. Sherman *et al.* (2008) also found that there was no significant association of A536T and S555G with a number of growth traits, including slaughter weight, average daily gain and carcass weight. Possible reasons for differences among studies include 1) the frequency of the different alleles in the sample populations; 2) the statistical models used to undertake the association analyses, as well as the statistical models and data (e.g. number of parities) used to estimate the dependent variables, 3) the mean performance of the animals or the environment they were exposed to (i.e. genotype by environment interaction); and 4) the genetic background of the animals in the study and how that may affect interactions between the SNPs and background genes.

We found a strong association between the G allele of AF126288:g.149 and increased carcass weight. Ge *et al.* (2003) reported that AF126288:g.149 was associated with IGF-1 concentration but not with increased growth rate in 470 Angus cattle. Maj *et al.* (2004) reported that AF126288:g.149 (designated as RFLP-NsiI) was associated with daily dry matter intake and percentage of lean in valuable cuts, but was not associated with growth traits in 71 bulls of four beef breeds and one dual purpose breed. Furthermore, Garrett *et al.* (2008) reported that AF126288:g.149 was not a significant predictor of weaning weight or carcass weight in 553 Brangus bulls. Sherman *et al.* (2008), however, reported a weak association between AF126288:g.149 and metabolic live weight in 464 beef animals. Therefore, the associations with this SNP and body size are not consistent across studies and breeds, suggesting that the SNP itself is not functional, but may be linked to the functional mutation in some breeds. Moreover, Zhou & Jiang (2005) reported that AF126288:g.149 did not affect promoter activity *in vitro*.

Growth hormone receptor 4.2 is associated with milk yield but not carcass weight, and AF126288:g.149 is associated with carcass weight but not milk yield. Assuming the causative mutations linked to these SNPs are both in the 5′-UTR, the reason why two mutations, despite both being in the *GHR* 5′-UTR, have different effects on traits, could be because they affect the amount of *GHR* transcript, and thus GHR protein to different extents. In view of the complex interactions between the myriad genes and metabolic pathways either directly or indirectly affected by GH/GHR binding, it would be no surprise that even small differences in amount of GHR protein available for GH binding at the cell surface may lead to very different effects on traits.

To conclude, the new bovine *GHR* SNP associations found in this study indicate that there may be numerous mutations in the *GHR* gene sequence of cattle, particularly in the complex 5′ non-coding region, that affect production traits. That different SNPs are associated with different traits is an indication that there are varying causative mutations with diverse effects on the quantity or function of GHR protein. Surprisingly, despite the large number of studies on variation in this pivotal gene, there remains a paucity of information on defining and characterizing the causative polymorphisms in the *GHR* gene of cattle. For example, so far the biological mechanism leading to the effect of F279Y on milk is not known; it is not even known whether this SNP is causative. A future approach which may help to pinpoint these causative polymorphisms would be to resequence the entire bovine *GHR* gene, including the 5′ and 3′ regions and introns, and analyse the detailed transcriptome of animals divergent for genetic merit of the traits included in the analysis. Using next generation sequencing technologies, the cost and technical logistics of sequencing large genes such as *GHR* in many different animals is no longer a hurdle.

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References

Banos G., Woolliams J.A., Woodward B.W., Forbes A.B. & Coffey M.P. (2008) Impact of single nucleotide polymorphisms in *leptin, leptin receptor, growth hormone receptor*, and *diacylglycerol acyltransferase (DGAT1)* gene loci on milk production, feed, and body energy traits of UK dairy cows. *Journal of Dairy Science* **91,** 3190–200.

Barrett J.C., Fry B., Maller J. & Daly M.J. (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21,** 263–5.

Berry D.P., Shalloo L., Cromie A.R., Veerkamp R.F., Dillion P., Amer P.R., Kearney J.F., Evans R.D. & Wickham B. (2007) The economic breeding index: a generation on. Technical report to the Irish Cattle Breeding Federation, February 2007, pp. 29–34.

Berry D., Kearney F. & Harris B. (2009) Genomic selection in Ireland. In: *Proceedings of the Interbull International Workshop – Genomic Information in Genetics Evaluations*, January 26–29, 2009, pp. 29–34. Uppsala, Sweden.

Blott S., Kim J.J., Moisio S. *et al.* (2003) Molecular dissection of a quantitative trait locus: a phenylalanine-to-tyrosine substitution in the transmembrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition. *Genetics* **163,** 253–66.

Bray M.S., Boerwinkle E. & Doris P.A. (2001) High-throughput multiplex SNP genotyping with MALDI-TOF mass spectrometry: practice, problems and promise. *Human Mutation* **17,** 296–304.

Etherton T.D. & Bauman D.E. (1998) Biology of somatotropin in growth and lactation of domestic animals. *Physiological Reviews* **78,** 745–61.

Fontanesi L., Scotti E., Dolezal M., Lipkin E., Dall'Olio S., Zambonelli P., Bigi D., Davoli R., Soller M. & Russo V. (2007) Bovine chromosome 20: milk production QTL and candidate gene analysis in the Italian Holstein-Friesian breed. *Italian Journal of Animal Science* **6,** 133–5.

Garrett A.J., Rincon G., Medrano J.F., Elzo M.A., Silver G.A. & Thomas M.G. (2008) Promoter region of the bovine *growth hormone receptor* gene: single nucleotide polymorphism discovery in cattle and association with performance in Brangus bulls. *Journal of Animal Science* **86,** 3315–23.

Ge W., Davis M.E., Hines H.C. & Irvin K.M. (2000) Rapid communication: single nucleotide polymorphisms detected in exon 10 of the bovine *growth hormone receptor* gene. *Journal of Animal Science* **78,** 2229–30.

Ge W., Davis M.E., Hines H.C., Irvin K.M. & Simmen R.C. (2003) Association of single nucleotide polymorphisms in the *growth hormone* and *growth hormone receptor* genes with blood serum insulin-like growth factor I concentration and growth traits in Angus cattle. *Journal of Animal Science* **81,** 641–8.

Gilmour A.R., Cullis B.R., Welham S.J. & Thompson R. (2009) *ASREML Reference Manual*. New South Wales Agriculture, Orange Agricultural Institute, Orange, NSW, Australia.

Harris B. & Johnson D. (1998) Approximate reliability of genetic evaluations under an animal model. *Journal of Dairy Science* **81,** 2723–8.

Hill W.G. & Robertson A. (1968) Linkage disequilibrium in finite populations. *Theoretical and Applied Genetics* **38,** 226–31.

Jiang H. & Lucy M.C. (2001) Variants of the 5′-untranslated region of the bovine *growth hormone receptor* mRNA: isolation, expression and effects on translational efficiency. *Gene* **265,** 45–53.

Jiang S.W., Zhang Z. & Eberhardt N.L. (1998) Genomic integration of GHF-1 binding sites inhibited growth hormone expression. *FASEB Journal* **12,** A319.

Jiang H., Okamura C.S., Boyd C.K. & Lucy M.C. (2000) Identification of Sp1 as the transcription factor for the alternative promoter P2 of the bovine *growth hormone receptor* gene. *Journal of Molecular Endocrinology* **24,** 203–14.

Jiang H., Lucy M.C., Crooker B.A. & Beal W.E. (2005) Expression of *growth hormone receptor 1A* mRNA is decreased in dairy cows but not in beef cows at parturition. *Journal of Dairy Science* **88,** 1370–7.

Jiang H.L., Wang Y., Wu M.Z., Gu Z.L., Frank S.J. & Torres-Diaz R. (2007) Growth hormone stimulates hepatic expression of bovine *growth hormone receptor* messenger ribonucleic acid through signal transducer and activator of transcription 5 activation of a major growth hormone receptor gene promoter. *Endocrinology* **148,** 3307–15.

Kopchick J.J. & Andry J.M. (2000) Growth hormone (GH), GH receptor, and signal transduction. *Molecular Genetics and Metabolism* **71,** 293–314.

Larkin M.A., Blackshields G., Brown N.P. *et al.* (2007) Clustal W and clustal X version 2.0. *Bioinformatics* **23,** 2947–8.

Leushner J. & Chiu N.H. (2000) Automated mass spectrometry: a revolutionary technology for clinical diagnostics. *Molecular Diagnosis* **5,** 341–8.

Lucy M.C., Boyd C.K., Koenigsfeld A.T. & Okamura C.S. (1998) Expression of *somatotropin receptor* messenger ribonucleic acid in bovine tissues. *Journal of Dairy Science* **81,** 1889–95.

Lucy M.C., Verkerk G.A., Whyte B.E., Macdonald K.A., Burton L., Cursons R.T., Roche J.R. & Holmes C.W. (2009) Somatotropic axis components and nutrient partitioning in genetically diverse dairy cows managed under different feed allowances in a pasture system. *Journal of Dairy Science* **92,** 526–39.

Maj A., Oprzadek J., Oprzadek A., Dymnicki E. & Zwierzchowski L. (2004) Polymorphism in the 5′-noncoding region of the bovine *growth hormone receptor* gene and its association with meat production traits. *Animal Research* **53,** 503–14.

Montgomery G.W. & Sise J.A. (1990) Extraction of DNA from sheep white blood cells. *New Zealand Journal of Agricultural Research* **33,** 437–41.

Prendiville R., Pierce K.M. & Buckley F. (2009) An evaluation of production efficiencies among lactating Holstein-Friesian, Jersey, and Jersey x Holstein-Friesian cows at pasture. *Journal of Dairy Science* **92,** 6176–85.

Sherman E.L., Nkrumah J.D., Murdoch B.M., Li C., Wang Z., Fu A. & Moore S.S. (2008) Polymorphisms and haplotypes in the bovine *neuropeptide Y, growth hormone receptor, ghrelin, insulin-like growth factor 2*, and *uncoupling proteins 2* and *3* genes and their associations with measures of growth, performance, feed efficiency, and carcass merit in beef cattle. *Journal of Animal Science* **86,** 1–16.

Signorelli F., Orru L., Napolitano F., De Matteis G., Scata M.C., Catillo G., Marchitelli C. & Moioli B. (2009) Exploring polymorphisms and effects on milk traits of the *DGAT1, SCD1*

doi: 10.1111/j.1365-2052.2010.02087.x

and *GHR* genes in four cattle breeds. *Livestock Science* **125,** 74–9.

Viitala S., Szyda J., Blott S., Schulman N., Lidauer M., Maki-Tanila A., Georges M. & Vilkki J. (2006) The role of the bovine *growth hormone receptor* and *prolactin* receptor genes in milk, fat and protein production in Finnish Ayrshire dairy cattle. *Genetics* **173,** 2151–64.

White S.N., Casas E., Allan M.F., Keele J.W., Snelling W.M., Wheeler T.L., Shackelford S.D., Koohmaraie M. & Smith T.P.L. (2007) Evaluation in beef cattle of six deoxyribonucleic acid markers developed for dairy traits reveals an osteopontin polymorphism associated with postweaning growth. *Journal of Animal Science* **85,** 1–10.

Zhou Y. & Jiang H. (2005) Trait-associated sequence variation in the bovine *growth hormone receptor 1A* promoter does not affect promoter activity in vitro. *Animal Genetics* **36,** 156–9.

Zhou Y. & Jiang H. (2006) A milk trait-associated polymorphism in the bovine *growth hormone receptor* gene does not affect receptor signalling. *Journal of Dairy Science* **89,** 1761–4.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Linkage disequilibrium between the 25 segregating SNPs in the bovine *GHR* gene.

Table S2 MatInspector transcription factor binding site analysis of novel SNPs in the 5′ non-coding region of the bovine *GHR* gene.

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